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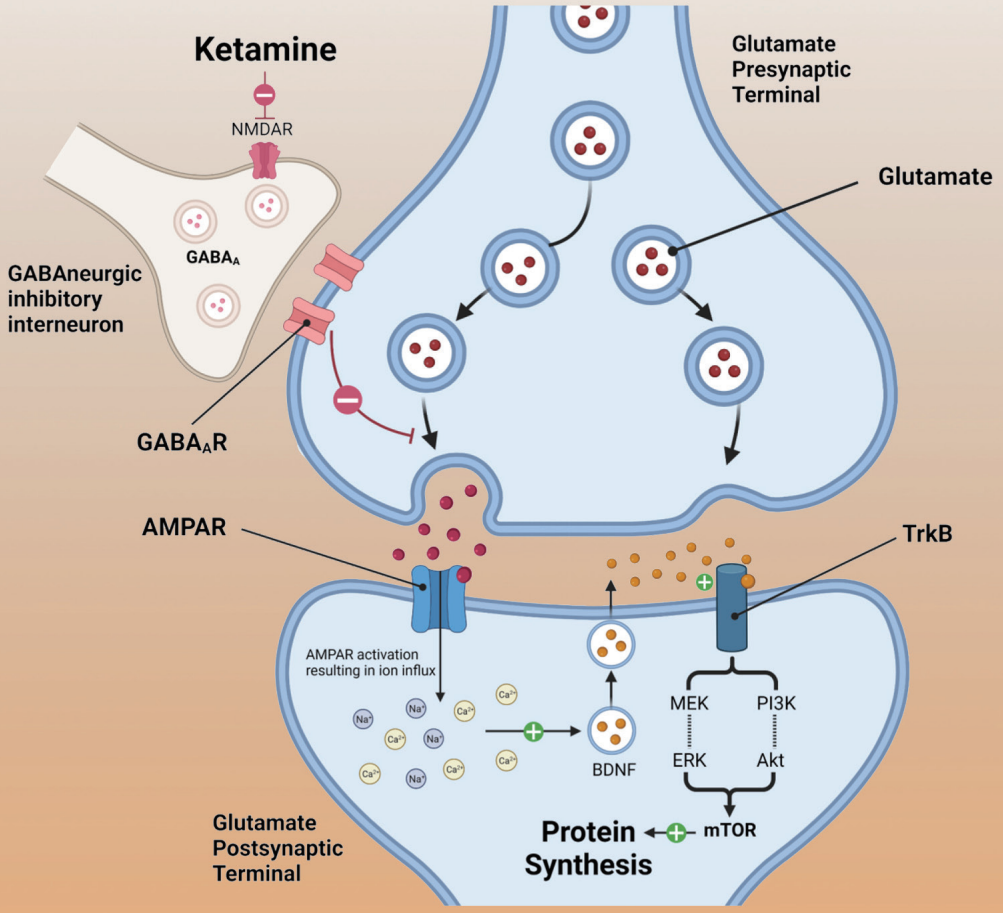

Utilization of Ketamine for Major Depression

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Aims and Scope

Journal of Exploratory Research in Pharmacology (JERP) publishes original innovative exploratory research articles, state-of-the-art reviews, editorials, short communications that focus on novel findings and the most recent advances in basic and clinical pharmacology, covering topics from drug research, drug development, clinical trials and application. Topics included, but not limited to the following areas will be considered for publication in JERP: drug composition and properties; synthesis and design of drugs and potential drugs; molecular/cellular and organ/system mechanisms; signal transduction/cellular communications/interactions; toxicology; chemical biology; molecular/biomarker diagnostics; therapeutics; medical applications; interventional (phases I-IV) clinical trials; observational (post-marketing) clinical studies on investigational new drugs; pharmacogenetics; pharmacoepigenetics; pharmacokinetics; pharmacodynamics; molecular pharmacology; transgenic models.

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Editorial

Neuronal Protective Effect of Nosustrophine in Cell Culture Models

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The role of nootropic supplements is becoming increasingly relevant in today's integrated therapeutic landscape.[1] The choice of adjunctive therapy is increasingly complex because of the increasingly elaborate nature of the drugs and, consequently, the potentially resulting drug interactions. The use of a nootropic, although it has an active component, turns out to be less disruptive overall because of the fewer interactions resulting from its very nature; another aspect not to be underestimated is the general propensity of the patient to take a product to which he or she does not attribute the exact nature as the drug generally used in therapy, and this allows the clinician to be able to administer an effective add-on with good compliance on the part of the patient.[2] Among nootropic supplements, Nosustrophine has an important role. The effects of this supplement are manifold, and currently, its use is found in the treatment of Alzheimer's disease (AD). Among pharmacological interventions, the most widely used in AD are natural products (25.6%), followed by anti-amyloid beta compounds (13%), neurotransmitter enhancers (11.4%), multitarget drugs (2.5%), and antitau drugs (2.3%).[3]

Nootropic supplements are composite preparations that help to enhance multiple areas of neuronal function, such as concentration, memory, cognitive attention, and motivation while strengthening cognitive functions in patients affected by multiple neurodegenerative diseases.[4] Delving deeper into the evaluation of the neurobiological aspects of nootropics, we can highlight that fractionated catecholamines and serotonin were found in the Nosustrophine extract using ultra-high performance liquid chromatography (UHPLC) with electrochemical detection (ECD).[5]

On deep biochemical evaluation, it emerges how Nosustrophine extracts contain brain-derived neurotrophic factors and multiple neurotransmitters, particularly dopamine, norepinephrine, and serotonin. It is well known that there is a correlation between the pathogenesis and course of AD and reduced brain concentrations of dopamine, norepinephrine, and serotonin.[6] Moreover, this emphasizes AD and a wide range of pathologies affecting the neurons and the brain, including psychiatric pathologies.

Laboratory data emphasizing the effects of Nosustrophine on microglia and multiple brain formations have shown that in aged mice, Nosustrophine promotes the expression of SIRT1. Furthermore, overexpression of SIRT1 may lead to neurodegeneration, with the implication of beta-amyloid and tau pathology potentially through deacetylation of histone H3 and dysfunction at the mitochondrial level.[7]

Various natural compounds protect against neurodegeneration and contain epinutraceutical properties[8] and, starting from this, the wide range of uses that could be fulfilled in neurobiology by nootropics, particularly by Nosustrophine, becomes apparent.

Furthermore, assessing the effects on neuronal plasticity reveals how Nosustrophine is responsible for effective regulatory activity of histone deacetylases with the improvement of neuroplasticity and consequent restoration of functions, such as learning and short- and long-term memory. In patients with AD, these neuroprotective aspects imply a regulatory and limiting role of processes aimed at microstructural degeneration of the neuron and its more refined functions.[9] It seems important to emphasize the role these findings might have in preventing and treating add-ons but not limited to all those diseases with a mnesic component, either from neurodegeneration or environmental demand overload, including multiple forms of depression and psychosis.

The reviewed article presents a strength in applying Nosustrophine to novel models, such as HepG2 hepatocarcinoma and SHSY5Y neuroblastoma cells. The highlighted therapeutic properties, particularly concerning dopaminergic neural leakage, and reduction of neuroinflammation, are accompanied by considerable evidence of increased neuroprotection, giving this compound possibilities in a future perspective as a protective factor not only in AD but also in other neurodegenerative diseases, such as Parkinson disease and multiple sclerosis.

Its limitations lie in the very nature of the work. However, the study was conducted with great rigor and precision. The applicability of the cell culture model necessitates confirmation of the data and a move to the following stages of experimentation up to human subjects. The article appears novel and significant in its scope by filling in the gaps in the literature inherent in the application of Nosustrophine, which has so far focused on alternative models. At present, there are no effective therapeutics available for neurodegenerative disorders.[10] It similarly shows how various studies address the usefulness of Nosustrophine, particularly Carrera *et al*.[11] highlight its usefulness as an effective therapy using nootropic supplements against degenerative diseases, while several authors delve into deeper cellular and molecular mechanisms,

Abbreviations: AD, Alzheimer's disease; UHPLC, ultra-high performance liquid chromatography; ECD, electrochemical detection.

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reflexively highlighting its validity as exerts substantial epigenetic effects against AD-related neurodegeneration.[12,13] Particularly in their work, Carrera *et al*.[14] effectively illustrate the neuronal protective effect of Nosustrophine in cell culture models by highlighting how this is an epigenetic bioproduct derived from the brain of *Sus scrofa* domesticus using nondenaturing biotechnological processes on the progression of neurodegeneration in human neuroblastoma cell line SH-SY5Y.

The data obtained in the laboratory, and particularly *in vitro*, show that Nosustrophine contributes to the prevention of dopaminergic neuron loss in the central nervous system, with an essential role at the neurobiological level in the course of diseases such as schizophrenia and psychotic spectrum disorders. The neuroprotective activity that is exercised directly and indirectly with the support of neuroplasticity, implies application for the prevention and treatment of neuroinflammation. This highlights how Nosustrophine may be useful for the prevention of toxic neuroinduction and environmental effects in the genesis of multiple psychopathological and neurological disorders.

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Conflict of interest

Dr. Massimo Tusconi has been an editorial board member of *Journal of Exploratory Research in Pharmacology* since July 2016. The author has no other conflict of interests to declare.

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Original Article

Effects of Liposomal Prostaglandin E1 on Coronary Stenosis and Restenosis after Percutaneous Coronary Intervention: A Prospective Clinical Trial

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Abstract

Background and objectives: Restenosis is a serious complication after percutaneous coronary intervention (PCI) for patients with coronary heart disease (CHD). This prospective clinical study was designed to investigate the effects of liposomal prostaglandin E1 (lipo-PGE1) on coronary stenosis and restenosis.

Methods: Sixty patients diagnosed with CHD and scheduled for PCI surgery in Guangdong Hospital of Traditional Chinese Medicine were enrolled in this study. The patients were divided into either the Control group (n = 30) or lipo-PGE1 treatment group (PGE group) (n = 30). Restenosis after PCI was the primary outcome, and newly increased stenosis was the secondary outcome.

Results: In total, 54 patients finished the follow-up and were included in the final analysis (n = 30 in the Control group and n = 24 in the PGE group). Baseline comparisons of stenosis location, stenosis degree, and the number of vessels in stenosis before PCI were comparable ($P > 0.05$). Comparisons of implanted stents showed similar features in stent diameter and stent length during PCI between the two groups ($P > 0.05$). For the primary outcome, there was no obvious difference in restenosis percentage ($\chi^2 = 1.520$, $P = 0.615$) nor number of vessels in restenosis ($\chi^2 = 0.070$, $P = 0.791$) in three arteries between groups. For the secondary outcome, although there was no significant difference in the number of non-culprit vessels in increased stenosis after PCI between groups ($\chi^2 = 3.902$, $P = 0.272$), the percentage of increased stenosis was much lower in the right coronary artery in the PGE group than the Control group ($U = 263.0$, $P = 0.048$).

Conclusions: This study demonstrated the lipo-PGE1 did not affect restenosis after PCI, but it may be effective in ameliorating newly increased stenosis in arteries.

Keywords: Coronary heart disease; Percutaneous coronary intervention; Nano liposomal prostaglandin E1; Restenosis; Newly increased stenosis.

Abbreviations: ACS, acute coronary syndrome; CCS, chronic coronary syndrome; CHD, coronary heart disease; DCB, drug-coated balloon; DES, drug-eluting stents; DM, diabetes mellitus; ISR, in-stent restenosis; lipo-PGE1, liposomal Prostaglandin E1; PCI, percutaneous coronary intervention; TLR, target lesion revascularization.

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Introduction

Patients with severe coronary heart disease (CHD) are commonly treated with percutaneous coronary intervention (PCI).[1] However, a loss in vessel lumen area of stented arteries is indicative of in-stent restenosis (ISR), which is a serious complication after PCI.[2] Although drug-eluting stents (DES) have dramatically decreased the incidence of ISR, the occurrence of ISR is still approximately 5–10% among CHD patients after PCI.[3,4] Therefore, there is a need to explore novel medications that can be administered in the peri-operative period of PCI to decrease the occurrence of restenosis or prevent ISR.

Liposomal prostaglandin E1 (lipo-PGE1) is a kind of nanolipid microsphere (liposome)-based PGE1. Previous studies showed

that lipo-PGE1 can decrease coronary restenosis in a canine thrombolysis model[5] and reduce the incidence of periprocedural myocardial injury both in patients[6] and porcine.[7] Lipo-PGE1 was also found to be effective for improving microcirculation.[8] The nanoliposome delivery system is also a popular method for targeted drug delivery,[9] and reviews have indicated that targeted nanoparticle-mediated delivery of multifunctional drugs could be a promising approach to prevent or treat restenosis.[10] Thus, this prospective clinical study was designed to investigate the effects of lipo-PGE1 on coronary stenosis and restenosis after PCI in CHD patients.

Methods

Ethical approval and informed consent

This study was approved by the Ethics Committee of Guangdong Provincial Hospital of Traditional Chinese Medicine (approval registration number BF2020-283). All samples were collected with appropriate participant informed consent in compliance with the Helsinki Declaration.

Patient source

Patients were enrolled into groups according to the diagnostic inclusion and exclusion criteria. Initially, 60 patients diagnosed with CHD scheduled for PCI surgery in Guangdong Hospital of Traditional Chinese Medicine from 2020 to December 2022 were enrolled and divided into two groups: basic medication for prevention and treatment of CHD (Control group, n = 30) and basic medication combined with lipo-PGE1 treatment (PGE group, n = 30).

Group treatments

For the Control group, basic medication normally included drugs for anti-platelet therapy, lipid lowering, controling ventricular rate, and controling hypertension or hyperglycemia. For the PGE group, nanolipid microspheres-based PGE (10 μg) (Penglai Nuokang Pharmaceutical Co., LTD) was added to 0.9% normal saline (NS) (250 ml) for intravenous injection, 20 gtt/min, once a day for 3 days during the peri-operative period of PCI. Basic medications were maintained in the two groups after discharge.

Diagnostic criteria

CHD diagnoses and the criteria for PCI followed the Guidelines for Percutaneous Coronary Intervention (2019) in China.

Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) The diagnosis fulfilled the criteria of CHD. (2) The condition conformed to the criteria for PCI. (3) The patients were able to complete the follow-up interview. (4) The patients voluntarily participated and signed informed consent.

Exclusion criteria included

(1) Patients with abnormal mental consciousness who could not cooperate, or patients with unstable vital signs. (2) Patients with related drug contraindications or allergies. (3) Those who participated in other clinical trials within 1 month. (4) Older than 80 years of age, pregnant or ready to be pregnant, lactating women, or infants.

Abscission criteria

(1) Patients who withdrew from the trial without adverse reactions or poor efficacy. (2) Those who lost connection during follow-up.

Termination criteria

(1) The researchers considered it medically necessary for the patients to terminate the trial. (2) Patients withdrew from the trial autonomously. (3) Those who suffered severe adverse reactions and could not insist on continuous treatments.

Primary and secondary outcomes

The rate of restenosis after PCI was the primary outcome, and the rate of newly increased stenosis was the secondary outcome. The measurement for restenosis and increased stenosis was performed using angiography or coronary computed tomography (CT), with or without transthoracic coronary doppler ultrasound. All outcomes were observed within 1.5 years after PCI.

Safety index monitoring

Adverse reactions were closely monitored when treatments were administered to all patients. All adverse reactions were observed, treated when necessary, and recorded.

Statistical analysis

A dataset was constructed and analyzed using SPSS (v26.0, Inc. USA) and R (v3.6.2, http://www.r-project.org) software. Continuous data are expressed as mean ± standard deviation, and the Kolmogorov-Smirnov test was used for normally distributed data. If the continuous data fit a normal distribution, comparisons between the two groups were performed using two independent sample Student's t-tests. Otherwise, the Mann-Whitney U test was used. Categorical variables are expressed in frequency and proportions (%). Chi-square (χ^2) tests with or without continuous correction or Fisher's exact test were used for comparisons between groups. $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics of patients

In total, 60 patients were enrolled based on the criteria, and 6 patients were lost during follow-up. Finally, 54 patients (Control group, n = 30; PGE group, n = 24) finished the follow-up and were included in the final analysis. There were no significant differences in sex, age, diagnosis subsets, comorbidities, and basic treatments between the Control and PGE groups (Table 1).

Baseline of vessel features in stenosis before PCI

We first compared the baseline of vessel features in stenosis before PCI between the Control and PGE groups. Stenosis location was defined as proximal, middle, and distant. We observed no significant difference in stenosis vessel features between the two groups ($P > 0.05$). The stenosis degree was also calculated by the area percent of occlusion and distinguished by total occlusion or not. The results showed no obvious differences between the two groups ($P > 0.05$) (Table 2). Comparisons of the number of vessels in stenosis before PCI were not statistically different ($\chi^2 = 5.982$, $P = 0.050$) (Table 3).

Characteristics of implanted stent features during PCI

As the characteristics of implanted stent features during PCI may affect the prognosis of restenosis,[11] we collected and compared the stent features. There were no statistical differences in the stent diameter and stent length between the two groups ($P > 0.05$) (Table 2). Comparisons of the number of stents implanted during PCI also

Table 1. Comparison of baseline characteristics between groups, [n(%)]

Variables	Control (n = 30)	PGE (n = 24)	*P*
Sex			0.210
female	5 (16.7%)	1 (4.17%)	
male	25 (83.3%)	23 (95.8%)	
Age	61.2 (11.0)	61.2 (9.21)	0.986
Diagnosis			0.063
ACS	7 (23.3%)	1 (4.17%)	
CCS	23 (76.7%)	23 (95.8%)	
Comorbidity			
hypertension	18 (60.0%)	14 (58.3%)	1.000
hyperlipidemia	9 (30.0%)	6 (25.0%)	0.919
DM	10 (33.3%)	10 (41.7%)	0.729
Other	0 (0.00%)	2 (0.08%)	0.193
Basic treatments			
anti-platelet	23 (76.7%)	23 (95.8%)	0.113
lipid-lowering	9 (30.0%)	6 (25.0%)	0.919
Number of comorbidities			0.261
0	2 (6.67%)	5 (20.8%)	
1	15 (50.0%)	8 (33.3%)	
2	8 (26.7%)	6 (25.0%)	
3	5 (16.7%)	3 (12.5%)	
4	0 (0.00%)	2 (8.33%)	

ACS, acute coronary syndrome; CCS, chronic coronary syndrome; DM, diabetes mellitus; PGE, prostaglandin E.

demonstrated no significant differences between the two groups ($\chi^2 = 1.520$, $P = 0.615$) (Table 4). These results showed that vessel features in stenosis before PCI and implanted stent features during PCI were similar between the Control and PGE groups. This, combined with the demographic characteristics of the patients, indicates that the two groups were comparable at baseline.

Effects of PGE on restenosis in culprit vessels after PCI

The percentage of restenosis was generally divided into less or more than 50% of the artery lumen area, and the number of restenosis in each vessel was calculated.[12,13] We found that restenosis in the LCX was the least severe, and the percentage of restenosis in the LCX was less than 50%. Statistical analysis showed no obvious difference in restenosis percentage of each of these three arteries between the Control and PGE groups ($\chi^2 = 1.520$, $P = 0.615$) (Table 5). Analysis of the restenosis type[14] of each vessel showed similar results, with no significant difference in each artery between the two groups ($P > 0.05$) (Table 6). Comparisons of the number of vessels in restenosis showed no statistical differences ($\chi^2 = 0.070$, $P = 0.791$) (Table 7). These data suggest that lipo-PGE1 has no significant effects on ameliorating restenosis after PCI.

Effects of PGE on newly increased stenosis in non-culprit vessels after PCI

As there was no obvious effect of lipo-PGE1 on restenosis, we further investigated the effect of PGE on newly increased stenosis after PCI, which was calculated by comparing the baseline of vessel stenosis and the stenosis in non-culprit vessels after PCI. The Kolmogorov-Smirnov test indicated abnormal distribution and the Mann-Whitney U test was used for comparison. Results showed that the percentage of increased stenosis of the RCA in non-culprit vessels was much lower after PCI in the PGE group compared to the Control group ($U = 263.0$, $P = 0.048$), while no significant differences were observed in the LAD and LCX arteries (Table 8). The number of non-culprit vessels in increased stenosis after PCI was also calculated; we found no significant differences between the Control and PGE groups ($\chi^2 = 3.902$, $P = 0.272$) (Table 9). These data suggest that lipo-PGE1 treatment may be effective in decreasing newly increased stenosis in non-culprit vessels after PCI.

Adverse reactions

The most frequently observed adverse reactions of lipo-PGE1 were phlebitis and anaphylaxis, and most of these adverse reactions disappeared after discontinuation of medication (Table 10). No severe adverse reactions were found with lipo-PGE1 treatment.

Discussion

This study examined the effects of nanolipid microspheres (liposome)-based PGE1 on coronary stenosis and restenosis after PCI using a prospective clinical trial design. We found that lipo-PGE1 treatment may be effective in decreasing newly increased

Table 2. Characteristics of vessels in stenosis and stents between groups, [n(%)] or [M(IQR)]

Variables	Control (n = 30)	PGE (n = 24)	*P*
Location of stenosis			
LAD			1.000
proximal	17 (56.7%)	15 (62.5%)	
middle	8 (26.7%)	6 (25.0%)	
distant	1 (3.33%)	0 (0.00%)	
none	4 (13.3%)	3 (12.5%)	
LCX			0.184
proximal	3 (10.0%)	5 (20.8%)	
middle	11 (36.7%)	11 (45.8%)	
distant	3 (10.0%)	4 (16.7%)	
none	13 (43.3%)	4 (16.7%)	
RCA			0.225
proximal	10 (33.3%)	7 (29.2%)	
middle	3 (10.0%)	7 (29.2%)	
distant	5 (16.7%)	5 (20.8%)	
none	12 (40.0%)	5 (20.8%)	
Stenosis in vessels (%)			
LAD	80.0 [50.0;90.0]	75.0 [57.5;86.2]	0.512
LCX	32.5 [0.00;72.5]	80.0 [37.5;90.0]	0.050
RCA	43.5 [0.00;90.0]	85.0 [36.2;90.0]	0.275
Total occlusion	12 (40.0%)	8 (33.3%)	0.825
Stent diameter (mm)			
LAD	2.75 [0.00;3.00]	2.00 [0.00;2.78]	0.130
LCX	0.00 [0.00;0.00]	0.00 [0.00;2.12]	0.572
RCA	0.00 [0.00;1.88]	0.00 [0.00;2.75]	0.921
Stent length (mm)			
LAD	16.5 [0.00;29.0]	22.5 [0.00;29.0]	0.843
LCX	0.00 [0.00;0.00]	0.00 [0.00;4.75]	0.747
RCA	0.00 [0.00;18.0]	0.00 [0.00;29.2]	0.499

LAD, left anterior descending; LCX, left circumflex artery; PGE, prostaglandin E; RCA, right coronary artery.

stenosis in non-culprit vessels after PCI.

Nanolipid microspheres (e.g. liposome) are a novel drug delivery system. It was reported that drug-loaded liposomes applied on a multilayer-coated balloon catheter improved the limitations of drug-eluting balloons (DEB) for the treatment of coronary artery disease.[15] A double-blind, randomized clinical trial (BLAST study) used liposomal Alendronate as a single intravenous bolus and showed that treatment with liposomal Alendronate could significantly decrease in-stent late loss in patients with baseline monocyte counts higher than the median value.[16] These data sug-

Table 3. Comparisons of the number of vessels in stenosis before PCI between groups, [n(expected)]

Groups	Number of vessels in stenosis			Total	χ^2	*P*
	1	2	3			
Control	9 (7.2)	11 (8.3)	10 (14.4)	30 (30.0)	5.982	0.050
PGE	4 (5.8)	4 (6.7)	16 (11.6)	24 (24.0)	5.982	0.050
Total	13 (13.0)	15 (15.0)	26 (26.0)	54 (54.0)		

Pearson χ^2 test.

Table 4. Comparisons of the number of stents in PCI between groups, [n(expected)]

Groups	Number of stents			Total	χ^2	*P*
	1	2	3			
Control	24 (22.8)	6 (6.7)	0 (0.6)	30 (30.0)	1.520	0.615
PGE	17 (18.2)	6 (5.3)	1 (0.4)	24 (24.0)	1.520	0.615
Total	41 (41.0)	12 (12.0)	1 (1.0)	54 (54.0)		

The minimum expected count was 0.44, used fisher's exact test.

Table 5. Characteristics of vessels in restenosis after PCI between groups, [n(%)]

Variables	Control (n = 30)	PGE (n = 24)	*P*
LAD			0.684
0	27 (90.0%)	21 (87.5%)	
−50	1 (3.33%)	2 (8.33%)	
50−	2 (6.67%)	1 (4.17%)	
LCX			1.000
0	29 (96.7%)	24 (100%)	
−50	1 (3.33%)	0 (0.00%)	
RCA			1.000
0	27 (90.0%)	23 (95.8%)	
-50	2 (6.67%)	0 (0.00%)	
50−	1 (3.33%)	1 (4.17%)	

−50; percentage of restenosis less than 50%, 50−; percentage of restenosis no less than 50%. Pearson χ^2 test or fisher's exact test.

gested that nanolipid microspheres could be a potential method for improving restenosis treatment.

Restenosis in coronary arteries after PCI has several underlying pathogenic causes, such as activation of the clotting system by injured endothelial cells and healing facilitated by vascular smooth muscle cell migration, proliferation, and synthetic activities.[4,14] The average time from restenosis occurrence after PCI has been reported to be within 12 months with drug-eluting stents (DES), and typically presents as recurrent angina.[17] Evaluation of staged, target lesions, and other unplanned revascularization procedures during the first year after PCI showed that target lesion revascularization (TLR) occurred with higher hazard rates between 2 to 9 months after PCI.[18] The commonly used technologies for restenosis treatment include bare metal stents, DES, conventional and cutting balloon angioplasty, drug-coated balloons (DCB), and atherectomy devices.[14,19] However, there is still a population of patients who suffer restenosis more than once even with suitable treatments. Thus, adjuvant medication becomes more important in the peri-operative period of PCI.

PGE1 (also named Alprostadil) has been used to treat chronic arterial obliterans (thromboangiitis obliterans, obliterans arteriosclerosis, etc.) and improve cardiovascular and cerebrovascular microcirculation disorders. A prospective, single-blind, randomized trial of 30 patients administered intravenous PGE-1 by hemodynamically based titration at a mean dosage of 10–20 ng/

Table 6. Characteristics of restenosis types after PCI between groups, [n(%)]

Variables	Control (n = 30)	PGE (n = 24)	*P*
LAD			0.805
none	27 (90.0%)	21 (87.5%)	
type1	2 (6.67%)	3 (12.5%)	
type2	1 (3.33%)	0 (0.00%)	
LCX			1.000
none	29 (96.7%)	23 (95.8%)	
type1	1 (3.33%)	1 (4.17%)	
RCA			0.747
none	27 (90.0%)	23 (95.8%)	
type1	2 (6.67%)	0 (0.00%)	
type3	1 (3.33%)	1 (4.17%)	

Pearson χ^2 test or fisher's exact test.

Table 7. Comparisons of the number of vessels in restenosis after PCI between groups, [n(expected)]

Groups	Number of vessels in restenosis		Total	χ^2	*P*
	0	1			
Control	23 (23.9)	7 (6.1)	30 (30.0)	0.070	0.791
PGE	20 (19.1)	4 (4.9)	24 (24.0)	0.070	0.791
Total	43 (43.0)	11 (11.0)	54 (54.0)		

Note The minimum expected count was 4.89, used continuous corrections χ^2 test.

Table 8. Characteristics of the percentage of increased stenosis after PCI between groups, [M(IQR)]

Variables	Control (n = 30)	PGE (n = 24)	Mann-Whitney U	*P*
LAD	0.00 [0.00;17.5]	0.00 [0.00;0.00]	313.0	0.288
LCX	0.00 [0.00;0.00]	0.00 [0.00;10.5]	340.5	0.659
RCA	0.00 [0.00;28.8]	0.00 [0.00;0.00]	263.0	0.048*

Kolmogorov-Smirnov test and Mann-Whitney U test. *$P < 0.05$ between groups.

Table 9. Comparisons of the number of vessels in increased stenosis after PCI between groups, [n(expected)]

Groups	Number of vessels in increased stenosis				Total	χ^2	*P*
	0	1	2	3			
Control	10 (12.8)	10 (9.4)	8 (5.6)	2 (2.2)	30 (30.0)	3.902	0.272
PGE	13 (10.2)	7 (7.6)	2 (4.4)	2 (1.8)	24 (24.0)	3.902	0.272
Total	23 (23.0)	17 (17.0)	10 (10.0)	4 (4.0)	54 (54.0)		

The minimum expected count was 1.78, used continuous corrections χ^2 test.

kg/min at 2 hours before angiography. The 6-month follow-up showed that restenosis occurrence was 17% in the PGE-1 treated group, compared with 33–50% in the control group which only received basic medication ($P < 0.05$). These data indicated that PGE-1 was effective in decreasing coronary restenosis at 6 months after percutaneous transluminal coronary angioplasty.[20] Since restenosis usually occurs during the first year after PCI,[17] we examined the effect of PGE-1 at 1.5 years after PCI initially to obtain a more comprehensive understanding of the role of PGE-1 in preventing restenosis occurrence. However, we did not find positive results. The reason may lie in the time point for outcome observation and relatively small sample size.

Although our data did not show significant effects of lipo-PGE1 treatment for restenosis after PCI, we did observe a decrease in restenosis percentages in each of the three arteries examined. Furthermore, the newly increased stenosis in vessels was affected by lipo-PGE1 treatment, and a significant difference was observed in the RCA artery. A previous randomized controlled trial indicated that intracoronary administration of Nicorandil and PGE1 was more effective in improving myocardial perfusion than Nitroglycerin.[21] Another randomized-controlled study administered lipo-PGE1 at 20 μg/day diluted in 10 ml of NS through an intravenous injection over 5 min, starting at 3 days before PCI and continuing for 4 days after PCI. The results suggested that the cardioprotective effects of lipo-PGE1 were associated with its anti-inflammatory properties and its ability to improve microvascular perfusion.[6] Another clinical study suggested a relationship between the microcirculation and restenosis, evidenced by the finding that lower coronary blood flow responded to an endothelium-dependent vasodilator stimulus and was associated with long-term recurrence of restenosis.[22] Thus, the anti-inflammatory and microvascular improvement effects of lipo-PGE1 may underlie the reduction of newly increased stenosis in arteries.

Future directions

The main limitation of this study was the relatively small sample size. Further studies with more subjects are needed to validate our conclusions. New studies can be designed to evaluate the treatment effect of lipo-PGE1 on restenosis, which can be assessed by quantifying the degree of restenosis before and after lipo-PGE1 treatment. Moreover, the underlying mechanisms of lipo-PGE1's cardioprotective effects should be done by examining endogenous plasma PGE1 levels from CHD patients before and after PCI.

Conclusions

The current study was designed to evaluate the protective effects of lipo-PGE1 on coronary stenosis and restenosis after PCI. Our study showed the lipo-PGE1 did not affect restenosis after PCI, but it may be effective in ameliorating newly increased stenosis in arteries.

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Table 10. Comparisons of the number of adverse reactions between groups, [n(expected)]

Groups	Number of adverse reaction		Total	χ^2	*P*
	No	Yes			
Control	30 (28.3)	0 (1.7)	30 (30.0)	1.946	0.163
PGE	21 (22.7)	3 (1.3)	24 (24.0)	1.946	0.163
Total	51 (51.0)	3 (3.0)	54 (54.0)		

The minimum expected count was 1.33, used continuous corrections χ^2 test.

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Conflict of interest

The authors declare that there is no conflict of interest in the authorship and publication of this contribution.

Author contributions

QL designed the study and finalized the manuscript. AZ, JQ, CW, PL, and CL collected patient information and constructed the dataset. RY and QL completed the first version of manuscript. QL finished manuscript corrections. QL, CW, and GL contributed to manuscript revision. All authors read, revised, and approved the final manuscript.

Ethical statement

This study was approved by the Ethics Committee of Guangdong Provincial Hospital of Traditional Chinese Medicine (approval registration number BF2020-283). All samples were collected with appropriate participant informed consent in compliance with the Helsinki Declaration.

Data sharing statement

The authors confirm that the data supporting the findings of this study are available within the article, and these data are also available from the corresponding author upon reasonable request.

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Original Article

Neuronal Protective Effect of Nosustrophine in Cell Culture Models

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Abstract

Background and objectives: Alzheimer's disease (AD) is characterized by the progressive degeneration of neurons and pathological activation of glial cells. The present study aims to investigate the potential protective effects of Nosustrophine, a nootropic supplement derived from young porcine (*Sus scrofa domesticus*) brains that contain neurotrophic factors, on the progression of neurodegeneration in the SH-SY5Y neuroblastoma cell line. Cellular screening, which focused on the trophic activity of neurons, astrocytes and microglia, was performed with different concentrations (10, 50 and 100 μg/mL) of Nosustrophine.

Methods: Lyophilized Nosustrophine extract (young porcine brain extract) was used for all cell culture experiments in order to determine the response to oxidative stress of neurons, astrocytes, and microglia at different concentrations.

Results: The cell viability of SH-SY5Y cells treated with low concentrations of Nosustrophine notably improved, when compared to control cells. In the HepG2 hepatocarcinoma cell line, Nosustrophine had a moderate, concentration-dependent impact on cell viability, with the most significant effects observed at concentrations greater than 1 mg/mL. However, Nosustrophine did not confer any toxic effects on human cell lines, sustain neuronal survivability rates, or significantly enhance the astroglial cell survival in mouse primary neuronal and glial cells. The protective effect of Nosustrophine on microglia was inversely correlated to the drug concentration in the culture medium. It was found that Nosustrophine is protective against Aβ1-42-induced neurodegeneration in mouse organotypic hippocampal slice cultures.

Conclusions: The present findings revealed that Nosustrophine has potent neuroprotective properties, enhances neural plasticity, and may be a potential therapeutic option for degenerative diseases.

Introduction

The gradual death of neurons in diverse parts of the nervous system is a feature of neurodegenerative diseases.[1] This loss of nerve cells leads to distinct neurological and cognitive symptoms that are specific to each condition. A prominent pathological feature in Alzheimer's disease (AD) is the loss of neurons and synapses in the cerebral cortex and subcortical regions.[2] This causes the atrophy of some affected areas due to pathological abnormalities, including the accumulation of β-amyloid protein and cellular material in thick, insoluble deposits around and outside of neurons. The accumulation of Aβ and tau proteins in the brain has led to the definition of AD as a condition that produces proteotoxic proteins.[3] Previous studies have attempted to identify new therapeutic approaches. However, effective medications that can slow or halt the progression of neurodegenerative diseases, such as AD, are yet to be discovered.[4–8]

In the majority of cases, the supplementation of culture media with serum or other substances is required. However, there are a number of drawbacks to using serum, including high costs, unknown composition, and greater risk of contamination with accidental chemicals. For these reasons, the porcine (*Sus scrofa domesticus*) brain is a reliable source of a number of growth factors.[9] Indeed, the growth factors released from neural tissues, such as those in the brain and retina, are powerful mitogens for mesoderm-

Keywords: Alzheimer's disease; Cellular screening; Nosustrophine; Neurodegeneration; Neuroprotection.

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid beta; BDNF, brain-derived neurotrophic factor; CA1-2, hippocampal subfields; GDNF, glial cell line-derived neurotrophic factor; HepG2, hepatocarcinoma cell line; LDH, lactate dehydrogenase; MHC, major histocompatibility complex; NST, Nosustrophine; OGD, oxygen and glucose deprivation; OHSCs, organotypic hippocampal slice cultures; PBE, porcine brain extract; SH-SY5Y, neuroblastoma cell line.

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derived cells, particularly for vascular endothelial cells and some ectoderm-derived cells.[10] These chemicals are known as pituitary and brain fibroblast growth factors, due to the ability to stimulate fibroblast proliferation.[11] A large number of sympathetic,[12] sensory,[13] and parasympathetic[14] neurons respond to neurotrophic chemicals by maturing, growing, and/or needing maintenance. The importance of substrate-binding neurite-promoting factors (NPFs), which are required under particular culture conditions for neurotrophic factors to affect peripheral neurons, has been highlighted.[14–18]

The number of astrocytes in the mammalian brain is consistently steady throughout maturity,[19,20] and this is most likely caused by the coexistence of specific mitogens and mitogen inhibitors.[21] Specific astroblast mitogen inhibitors exist in rat brain,[22,23] and one such inhibitor, neurostatin, was recently discovered in rat and bovine brain extracts.[24] Neurostatin shares epitopes with human blood types and the carbohydrate moiety of the epidermal growth factor (EGF) receptor.[22,23] The elimination of an experimental rat brain tumor *in vivo* was aided by neurostatin.[22,25,26] Furthermore, the artificial oligosaccharide counterparts of neurostatin can prevent astrocyte, glioma, and neuroblastoma cell division in culture. In addition, the brain-derived trophic peptides used to treat endogenous neurotrophic factor deficiencies can decrease the degeneration of neurological diseases.[27,28] In order to protect neurons from oxidative stress-induced degeneration, these peptides promote cell survival and possible cell growth, in addition to other beneficial effects, *in vitro* and *in vivo*.[29,30] Neurotrophic drugs may also improve neuronal metabolism and cell performance, which can restore synaptic plasticity through the growth of new axons, enhance cognitive function by increasing neural connectivity, and enhance long-term memory.[31] There is presently no effective treatment to stop the progressive degeneration of affected brain areas, and standard care typically concentrates on palliative medications to postpone dementia. Therefore, pharmacogenomic methods would directly contribute to improve pharmaceutical treatment responses for people with AD or other similar disorders.[32] Nosustrophine is a novel pleiotropic epigenetic bioproduct, which is a nootropic supplement produced from young porcine (*Sus scrofa domesticus*) brain through non-denaturing biotechnological methods.[33] The intended function of this formulation is to stimulate endogenous neuropeptide synthesis and release by activating neuro-enzymatic processes.

The present study aims to examine the neuroprotective effects of Nosustrophine against cellular degeneration, which lead to the development of neuropathologies. In order to determine the response of neurons, astrocytes, and microglia to Nosustrophine at different concentrations, cell culture models were used in the presence or absence of oxidative stress. The present findings revealed that Nosustrophine can reduce the activation of microglia, and has a neuroprotective effect on neurons and astrocytes in culture.

Materials and methods

Biochemical characterization of Nosustrophine

Nosustrophine is a biological extract and an epigenetic bioproduct[33] synthesized from the brain of *Sus scrofa domesticus* using non-denaturing biotechnological methods (Patent ID: P202230047/ES2547.5).

Compound analysis: The nutrient profile and analysis of the catecholamines, serotonin, L-dopa and neurotrophic factors of the powdered extract have been examined, and previously published.[33]

Experimental design

Treatment preparation: A stock solution (20 mg/mL) of lyophilized Nosustrophine extract (young porcine brain extract [PBE]) was sonicated in sterile filtered 0.9% NaCl, and centrifuged at 3,000 g for three minutes. Then, the supernatant was collected and used for all cell culture experiments.

For the analytic assays, 4.5×10^5 cells were grown for 24 hours in 6-well plates at 37°C. Then, these cells were exposed to 10 µg/mL and 50 µg/mL of Nosustrophine for 0, 3 and 24 hours.

Cell line culture assays

Cell lines: Human neuroblastoma SH-SY5Y and hepatocarcinoma HepG2 cell lines were maintained in Roswell Park Memorial Institute (RPMI, Gibco) or Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 1% penicillin/streptomycin (Gibco) and 10% heat-inactivated fetal bovine serum (Gibco). Then, the cells were incubated at 37°C in a humidified incubator with 5% CO_2. These cells were kindly provided by Dr. Ana Aranda (Instituto de Investigaciones Biomédicas, Madrid). The SH-SY5Y neuroblastoma cell line is a commonly used experimental model for studying the molecular mechanisms underlying AD, due to its ability to differentiate into neurons, low cost, and ease of handling.

Cell viability assay: Cell viability was determined by Presto-Blue Cell Viability assay (Thermo Fisher). Cells (1×10^4) were incubated with different concentrations of Nosustrophine (0.05–10.00 mg/mL) for 72 hours in 96-well plates. Then, the Presto Blue reagent (10 µL) was added to each well, and incubated for three hours. Afterwards, the absorbance was recorded at 570 nm, with the absorbance at 630 nm used as the reference. Eight replicates were performed for each condition, and the experiment was repeated twice.

Primary cultures of cortical neurons

Obtaining the cells: All experimental procedures were performed in accordance with the European Community Law (86/609/EEC), European Union Directive 2016/63/EU, and the Spanish Royal Decree (R.D. 1201/2005). Study procedures were reviewed and approved by the Ethics Committee of the International Center of Neuroscience and Genomic Medicine.

Before starting the dissociation process, the culture plates were treated with poly-L-lysine to enhance the cell adhesion to the surface. These cells were obtained from 17-day gestation Wistar rat fetuses. These rats were decapitated, and the fetuses were extracted and washed with washing buffer (150 mM of NaCl, 8 mM of $Na_2HPO_4 \cdot 2H_2O$, 2.7 mM of KCl, 1.45 mM of KH_2PO_4 and 2.6 mM of $NaHCO_3$, pH = 7.2). Then, the cerebral cortices were obtained by transferring the fetuses to a plate that contained commercial dissection medium (L-15). Next, the meninges were removed from the cortex before homogenization. Then, the cleaned cortex was transferred to a plate that contained the incubation medium, which consisted of 80% (v/v) Minimum Essential Medium (MEM), 10% (v/v) horse serum, 10% (v/v) fetal serum, 1.98 mM of glutamine, 3.3 mM of glucose, and 16 mg/L of gentamicin sulfate. Afterwards, the tissue was homogenized after mechanical disruption.

Sub-culturing: After homogenization, the number of obtained cells was counted. In order to count these cells, the Trypan Blue exclusion method was used in a Neubauer chamber. When the number of cells obtained was known, these were suspended in "incubation medium" up to the required density, which was 2×10^5 cells/cm^2 for the present study. Then, these were seeded in 12-unit multi-well Petri dishes (∅ = 2.2 cm).

Cell culture maintenance: During the first four days, the cells were maintained in the incubation medium. On the fourth day, this was changed to a growth medium (90% [v/v] MEM, 10% [v/v] horse serum, 1.98 mM of glutamine, 3.3 mM of glucose, and 16 mg/L of gentamicin sulfate), which included a cytostatic agent, cytosine arabinoside, in order to prevent the growth of different proliferating cells, such as glia. At one week after these were plated, the medium was changed to a new growth medium that excluded cytosine. With this treatment, a homogenous neuronal culture was obtained that contained an approximately 5% glial cell population.

Primary cultures of glial cells

Obtaining the glial cells: In order to obtain a culture with a mainly glial cell population, the procedure performed was similar to that for obtaining neurons with some modifications. The seeding density was 5×10^4 cells/cm^2, and no cytosine arabinoside was added on the fourth day after seeding. In this manner, the proliferation and growth of the glia was allowed. The percentage of each cell population was, as follows: 15 ± 3% neurons, 75 ± 8% astrocytes, and 10 ± 2% microglia. In addition, the experiments were conducted at two weeks after seeding.

Oxygen and glucose deprivation (OGD)

The experiments were performed at nine or 10 days after sub-culturing. In order to simulate the ischemia *in vitro*, OGD was performed in a chamber (Forma Scientific) at 37°C, with the total absence of glucose, and an anaerobic nitrogen atmosphere (95% N_2/5% CO_2). Before commencing any treatment, the cells were washed twice with the "ischemia buffer" (130 mM of NaCl, 5.4 mM of KCl, 1.8 mM of $CaCl_2$, 0.8 mM of $MgCl_2$, 1 mM of NaH_2PO_4 H_2O, and 26 mM of $NaHCO_3$; pH = 7.2) used during OGD, in order to remove the growth medium. During the OGD, the pressure (0.5 psi) and temperature of the system were kept constant. The control group was maintained in an aerobic atmosphere with the glucose buffer (the same as the ischemia buffer, but contains 33 mM of glucose) for the same duration. At the end of the OGD period, the cells were washed twice with reperfusion medium (the same as the growth medium, but gentamicin was replaced with 0.15 ng/mL of penicillin), and fixed in this medium for a 24-hour reperfusion period. The OGD duration in the glutamate release experiment was 150 minutes. The time range of 140–160 minutes was chosen, because glutamate was observed to be released at this time, and there was no lactate dehydrogenase (LDH) release. Thus, experiments in which the LDH values of the control and OGD groups significantly differed were not considered. This ruled out the possibility of unwanted glutamate release due to cell rupture and its subsequent consequences (excitotoxicity), which would interfere with the study of the release mechanisms and the effect of Nosustrophine in this process. For the experiments, the OGD duration was 150 minutes. After the experiments, different cell viability parameters, such as LDH, were measured. Then, the medium was removed after 150 minutes, and the cells were washed with reperfusion buffer and kept in an incubator at 37°C. This allowed the LDH to be measured at 0, 3 and 24 hours after OGD. These experiments were performed to verify the occurrence of cell death after OGD, and determine whether Nosustrophine is capable of reducing this. Different concentrations of Nosustrophine (10, 50 and 100 µg/mL) were added at the start of the OGD period.

Preparation and treatment of mouse organotypic hippocampal slice cultures (OHSCs)

The OHSCs were prepared from postnatal day 4–6 mice, following an established protocol.[34] After decapitation, the brains were removed, and the hippocampi were dissected and transversely cut in 350 µm sections using a McIlwain tissue chopper. In the intact state, the hippocampal sections were selected and placed onto porous polyethylene (PTFE) membrane inserts (PICM0RG50, Merck Millipore), with three sections per insert. Then, the inserts were transferred to 6-well plates, with each well containing 1.2 mL of culture medium. Next, the culture medium comprised of MEM supplemented with 2 mM of GlutaMAX™ (Thermo Fisher Scientific), and this was adjusted to pH 7.3. In addition, the medium was supplemented with 20% heat-inactivated horse serum, 0.00125% ascorbic acid, 1 µg/mL of insulin, 1 mM of $CaCl_2$, 2 mM of $MgSO_4$, 13 mM of D-glucose, and 1 mM of GlutaMAX™. Then, the OHSCs were cultured at 37°C in a humidified CO_2-enriched atmosphere, and the medium was changed twice each week for the subsequent 2–3 weeks.

In order to prepare the Aβ1-42 peptide solution, 1 mg of human amyloid β-peptide (1-42) (Tocris, Bio-Techne, Wiesbaden, Germany) was dissolved in 1 mL of sterilized distilled water, and stored at −20°C. Then, the peptides were aggregated by incubation at 37°C for 72 hours.[35,36] In order to induce the Aβ1-42-induced neurotoxicity, the slices in the serum-free medium were exposed to a final concentration of Aβ1-42 (25 µM) on day 22 of the *in vitro* culture. The culture medium (300 µL) that contained the Aβ1-42 peptides were applied on top of the slices, and 700 µL was added underneath the slices. The control slices were only treated with serum-free medium. For slices that were exposed to both Aβ1-42 and Nosustrophine, the OHSCs were initially pretreated with 50 µg/mL or 100 µg/mL of Nosustrophine in serum-free media for 72 hours at 37°C. Then, these slices were exposed to 25 µM of Aβ1-42 in the presence of Nosustrophine (50 µg/mL or 100 µg/mL) for 48 hours. Finally, the slices were washed with serum-free media and harvested.

Immunofluorescence

A total of nine cultured hippocampal explants were fixed in 4% paraformaldehyde, and blocked with 5% bovine serum albumin (BSA; Sigma, Japan) in phosphate buffered saline (PBS), which contained 0.1% Triton X-100, for 1.5 hours. Then, these were permeabilized with 0.3% Triton-X 100 in 0.1 M of PBS for 30 minutes, and blocked again in 5% BSA in 0.1 M of PBS, which contained 0.1% Triton X-100, for 1.5 hours. Afterwards, the slices were incubated overnight with the primary antibody against the neuron-specific protein NeuN (1:1,000; MAB-377, Millipore), and detected using the Alexa Fluor-488-tagged secondary antibody (Thermo Fisher Scientific). The specificity of the fluorescent immunostaining for each antibody was confirmed by omission of the primary antibody. Then, the slices were counterstained with DAPI (Vector Laboratories). Several images of the three hippocampal explants from each treatment group were captured using the Leica DM6 B upright microscope and LAS X software. The mean density among the triplicates of immunofluorescence cell markers relative to the background in each explant image was quantified using the area/pixel analysis software (Pixcavator 4).

Determination of lactate dehydrogenase activity

LDH is a cytosolic enzyme released into the extracellular space as a consequence of cell lysis. The demand for ATP, when compared to aerobic ATP supply, causes the accumulation of ADP, AMP and pyruvate. This glycolytic flux leads to the production of pyruvate, which exceeds the metabolic capacity of pyruvate dehydrogenase and other shuttle enzymes that metabolize pyruvate. This mecha-

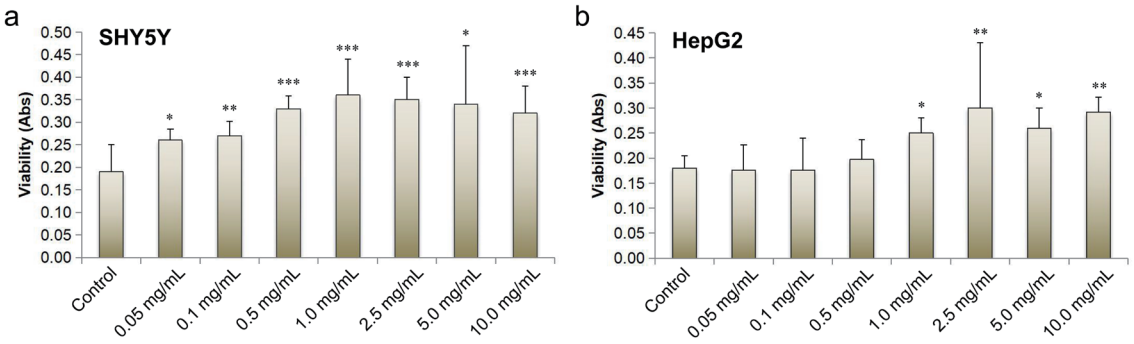

Fig. 1. Nosustrophine increases cell viability. (a) Viability assay in the neuroblastoma SH-SY5Y cell line; (b) Viability assay in hepatocarcinoma HepG2 cell line. Cells were treated with 0.05–10.00 mg/mL of Nosustrophine, and the cell viability was measured after 72 hours of incubation; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. HepG2, hepatocarcinoma cell line; SH-SY5Y, neuroblastoma cell line.

nism directs the flow of pyruvate and NAD+ via LDH, producing lactate and NADH in the process. In order to measure this, after OGD, a volume of medium was removed and mixed with the same amount of phosphate/NADH/pyruvate buffer (a final concentration of 350 µM of NADH, and 900 µM of sodium pyruvate; pH 7.4). Then, the kinetics of the different cell groups with specific ATP concentrations (0, 1 and 3 mM) were measured for 150 seconds in room temperature using a spectrophotometer at 340 nm. This wavelength was used to measure the increase in fluorescence, which is an indirect method to record the disappearance of NADH upon oxidation, due to the presence of LDH in the medium. Specifically, the reaction was, as follows:

$$\mathrm{NADH} + \mathrm{H}^{+} + \text{Sodium pyruvate} \xrightarrow{\text{LDH}} \mathrm{NAD}^{+} + \text{Lactate}$$

LDH was expressed as a percentage of the total LDH. In order to calculate this value, the cells were lysed with Triton X-100, and measured at 340 nm. Then, the value, together with the values previously obtained at 0, 3 and 24 hours, were recorded as the total value of LDH. Thus, the released LDH value was determined, as follows:

$$\text{LDH (\%)} = 100 \times \frac{\text{LDH (mean)}}{\text{LDH (mean)} + \text{LDH (cells)}}.$$

Statistical analysis

The data was tested for the normality and equality of variances using the Shapiro-Wilk test and Levene's test. The statistical significance was determined using one-way ANOVA with post-hoc Bonferroni correction: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. All values were expressed as the mean ± standard error of the mean (SEM) of the number of experiments indicated in each case. A p-value of < 0.05 was considered statistically significant (Newman-Keuls test).

Results

Effect of Nosustrophine on the viability of human cell lines

The impact of Nosustrophine on the viability of human cell lines was investigated by evaluating the cell viability rates of two distinct cell lines: hepatocarcinoma (HepG2) and neuroblastoma (SH-SY5Y) cells. In order to assess the cytotoxicity of Nosustrophine, the different increasing concentrations of the drug that was added to the SH-SY5Y cell culture medium were tested (Fig. 1a). The control group presented with the expected low viability rates, since Nosustrophine was not added to the medium. However, as the concentration of Nosustrophine increased in the medium, there was a corresponding significant effect on cell viability. Even the low concentration of Nosustrophine (0.05 mg/mL) resulted in a considerable difference in viability rate, when compared to the control group. The most substantial effect was detected at a concentration of 5 mg/mL of Nosustrophine. The present primary findings demonstrate that Nosustrophine has a significant effect on the viability of neuroblastoma SH-SY5Y cells at concentrations greater than 1 mg/mL (Fig. 1a).

Given its integral role in drug metabolism, the liver represents a suitable organ for assessing drug-induced cytotoxicity. The HepG2 cell line, which is derived from human liver carcinoma, was used as the experimental model system for analyzing the possible adverse effects of Nosustrophine. In order to evaluate the potential impact of this drug on cell viability, a standardized experimental protocol was used, and increasing concentrations of Nosustrophine were applied to the culture medium for HepG2 cells (Fig. 1b). The control group under OGD, in which no Nosustrophine was introduced, had low rates of viability, akin to those detected in the SH-SY5Y cell line (Fig. 1a). Conversely, a moderate, concentration-dependent impact on the viability of HepG2 cells upon the administration of Nosustrophine was observed (Fig. 1b). Furthermore, a modest increase in cell viability rates was observed with Nosustrophine concentrations of greater than 0.5 mg/mL, and the most significant effects were observed with Nosustrophine concentrations that exceeded 1 mg/mL (Fig. 1b). These results collectively suggest that Nosustrophine, even at high doses, may not confer any toxic effects on human cell lines, including both the SH-SY5Y and HepG2 cell lines (Fig. 1a, b).

Effect of Nosustrophine on mice neuronal and glial cell viability

As a model of oxidative stress, primary rat neurons were subjected to OGD (150 minutes). This induced neuronal death, as shown by the LDH viability marker in the control group. However, the administration of Nosustrophine to the culture medium sustained the neuronal survivability rate at all three studied time points. Compared to control cells, the amount of LDH released to the medium after OGD decreased at 0, 3 and 24 hours (Fig. 2). Neurons treated with Nosustrophine (10, 50 and 100 µg/mL) had similar levels of cell viability at zero and three hours after OGD. Nevertheless, at 24 hours after OGD, all Nosustrophine-treated groups presented with a small increase in viability levels (Fig. 2). The LDH marker indicated a significant loss in astroglial cell viability in the OGD-exposed control group. However, the astroglia that were treated with various concentrations of Nosustrophine (10, 50 and 100 µg/

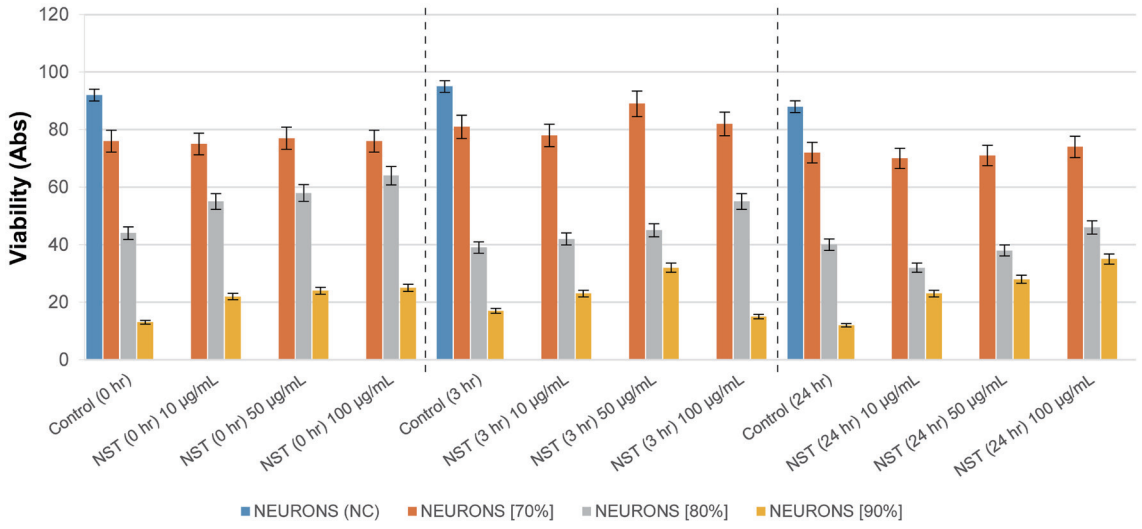

Fig. 2. Cellular screening of the effect of Nosustrophine in the primary culture of neurons. The 150-minute OGD process induced neuronal death in the model used through a cell viability marker (LDH). There was an increase in LDH levels released to the medium at three and 24 hours after OGD. When neurons in glutamate medium (10 µM) were incubated with Nosustrophin in the absence of magnesium for five minutes, this induced cell death. The incubation with Nosustrophin (10, 50 and 100 µg/mL) significantly modified the LDH values at all different confluence cell rates. LDH, lactate dehydrogenase; NST, Nosustrophine; OGD, oxygen and glucose deprivation.

mL) presented with significant improvements in viability levels, when compared to the control group (Fig. 3). This effect was positively associated with the concentration of Nosustrophine across all three time points. These data suggest that Nosustrophine significantly enhances astroglial cell survival.

In cultured microglial cells, the identical OGD methodology was used on two separate sets of microglia, depending on the type of major histocompatibility complex (MHC) molecule expressed. Both kinds of microglial cells had a similar rate of viability reduction at all three time points, with varying concentrations of Nosustrophine (10, 50 and 100 µg/mL). The protective effect of Nosustrophine on microglia was inversely correlated to the drug concentration in the culture medium (Fig. 4). OHSCs offer a sensitive model for investigating pathogenic responses. The treatment with both Nosustrophine (50 µg/mL) and Aβ1-42 (25 µM) resulted in pyknotic changes, and the loss of pyramidal neurons (Fig. 5b), mainly in the CA1-CA2 and dentate gyrus regions, when compared to the control slices (Fig. 5a). However, higher concentrations of Nosustrophine (100 µg/mL) protected against the Aβ1-42-induced neurodegeneration, preserved the neuronal structure, and led to fewer pathogenic manifestations (Fig. 5c), when compared to 50 µg/mL of Nosustrophine.

Discussion

Neurotrophic factors have potential as treatments for neurodegenerative diseases, but its clinical application remains limited by challenges related to its transport to the brain and suboptimal

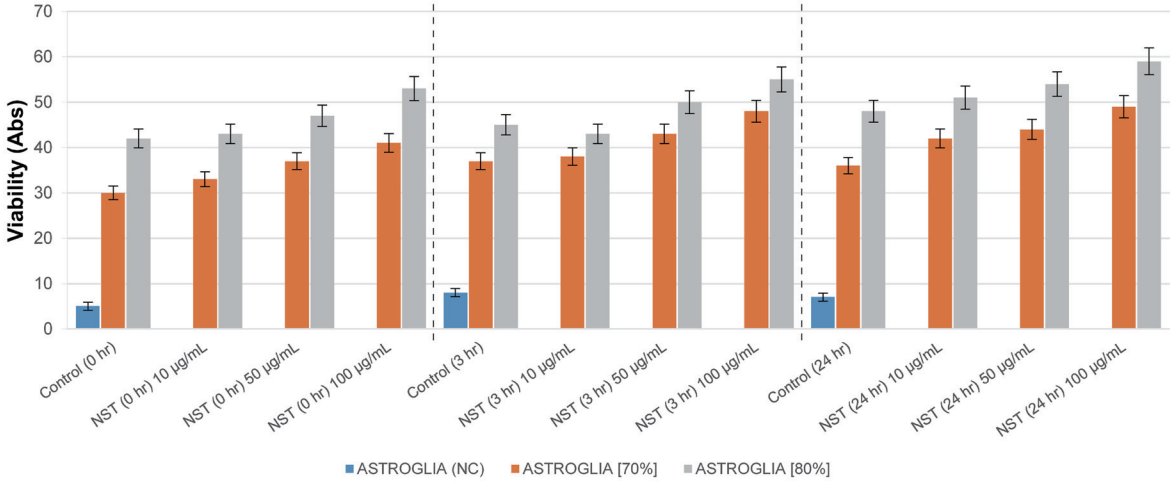

Fig. 3. Cellular screening of the effect of Nosustrophine in the astroglial cell culture. The results for the glutamate release were significantly modified by the presence of Nosustrophin in cultures of microglia exposed to OGD. The incubation with nosustrophin (10, 50, and 100 µg/mL) significantly modified the LDH values at all different confluence cell rates. LDH, lactate dehydrogenase; NST, Nosustrophine; OGD, oxygen and glucose deprivation.

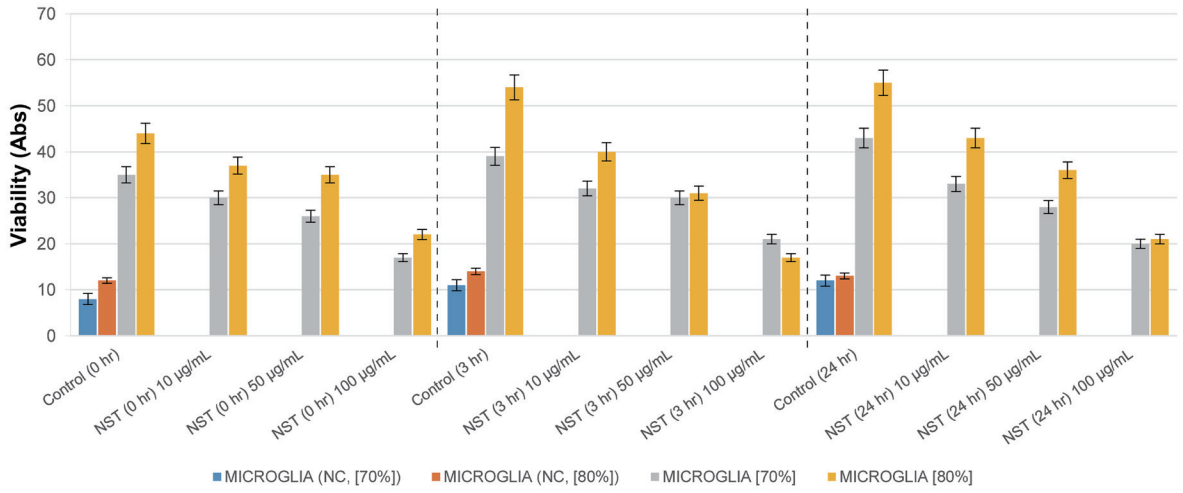

Fig. 4. Cellular screening of the effect of Nosustrophine in the microglia cell culture. The results for the glutamate release, although not significant, were modified by the presence of Nosustrophin in mixed-neuron-glia cultures exposed to OGD at all different confluence cell rates. NST, Nosustrophine; OGD, oxygen and glucose deprivation.

pharmacokinetic profiles. In order to address this, safe delivery methods and the investigation of the duration of its effects are necessary. The encapsulation of natural neuroprotective extracts is a promising approach for delivering neurotrophic factors to the brain during neurodegenerative disease phases. Combined with stem cell transplantation, this approach can enhance the neuroprotection, and promote tissue repair. The present study demonstrated the potential of Nosustrophine as a biological compound for harnessing the advantageous traits of PBE. Earlier studies have demonstrated the neuroprotective effects of PBE in hypoxia-induced diseased animal models, and the enhanced proliferation and differentiation of primary cells from the ovary, uterus and heart of rats, when PBE was added to the culture media.[9,37] However, the neuroprotective effects of PBE on mouse and human cell lines remain undocumented. The present study revealed that Nosustrophine, which is a pure PBE molecule, can promote high levels of cell viability and survival rates, in both animal and human cultured cells. The present findings suggest that Nosustrophine has neuroprotective effects, which increase glial density and promote neuronal survival. This effect may be beneficial for reducing common neuropathologies, since the cellular densities of the tested cell lines were higher than those of the control group. Furthermore, the present findings align with the findings of earlier reports that demonstrated the neuroprotective and neurotrophic properties of cerebrolysin, which is a commercially available porcine-derived brain extract.[38,39]

Growth factors regulate cell growth and proliferation *in vivo* and in culture. Growth factors are required for the proliferation of non-transformed cells in culture, and numerous factors are usually required. Since these deplete faster than other components of the culture media, these factors are rate-limiting for cell proliferation. Neoplastically altered cells may lack or require less growth factors, which may provide a growth advantage, and this is a distinguishing feature of cancer cells. At increasing concentrations, Nosustrophine exhibited a considerable impact on the viability of

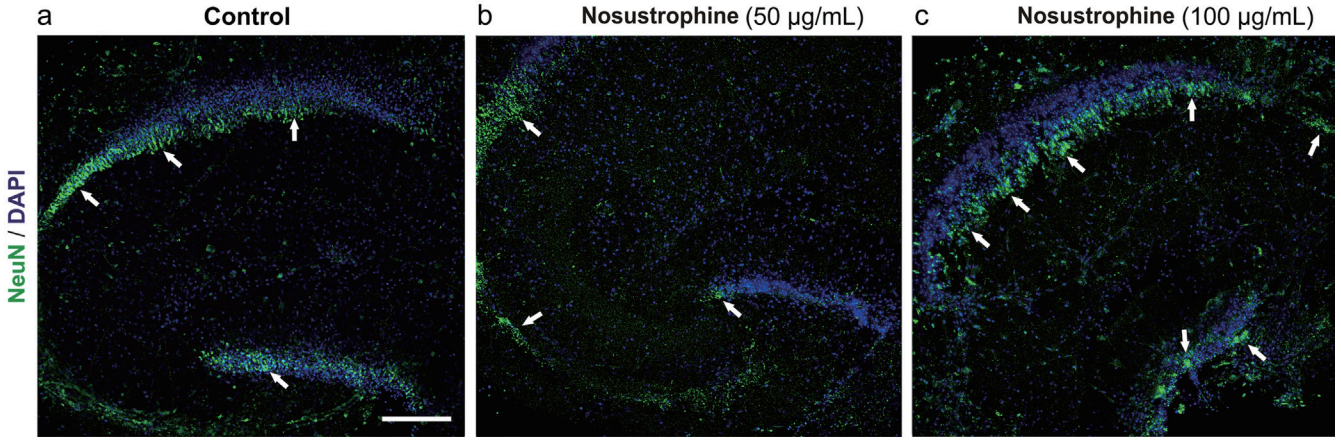

Fig. 5. Nosustrophine is protective against Aβ1-42-induced neurodegeneration in organotypic hippocampal slice cultures. Transverse slices of hippocampi obtained from neonatal mice pups were maintained on Millipore inserts for 22 days. The slices were exposed to (a) the vehicle (control), (b) Nosustrophine (50 µg/mL) with Aβ1-42 (25 µM), and (c) Nosustrophine (100 µg/mL)+Aβ1-42 (25 µM). Then, these were fixed and immunostained for NeuN (green). Afterwards, the nuclei were counterstained with DAPI (blue). The images were processed for maximal intensity projection. The white arrows point to the neurons. Scale bar: 80 µm. DAPI, neuronal nuclei; NeuN, marker of postmitotic neurons.

hepatocarcinoma HepG2 and neuroblastoma SH-5YS cells, possibly indicating the interaction with these protein factors. Furthermore, in the present study, the HepG2 cell line data indicated that the Nosustrophine treatment had no adverse effects on the liver. However, research into the molecular mechanisms related to the proteins in Nosustrophine remains at its early phases.

The organotypic hippocampal slice culture is a suitable model system for studying the mechanisms of neurodegeneration, since this preserves several key features of the hippocampal circuitry *in vitro*, including synapse maturation and intrinsic signaling pathways.[40] The investigators previously reported that in 8-9-month-old transgenic AD (APP/BIN1/COPS5) mice, Nosustrophine substantially reduced the Aβ1-42 immunoreactivity levels, when compared to saline-treated mice. The present study confirmed that finding, and revealed that the treatment with 100 µg/mL of Nosustrophine was protective by reducing cellular damage and neuronal loss after 48 hours of co-exposure to Aβ1-42. However, the treatment on hippocampal slices with 50 µg/mL of Nosustrophine did not prevent the Aβ1-42-induced neuronal death after 48 hours of exposure. Since Nosustrophine improves neurological injury outcomes through mechanisms other than reducing oxidative damage, gaining an understanding of the molecular basis of its protective effect can help to identify effective therapeutic targets against neurodegeneration.

Neurotrophins are an important group of chemicals that play a vital role in neuronal survival in vertebrates. These molecules are synthesized as large precursor forms, and undergo proteolytic processing to produce mature, and biologically functional ligands.[41,42] Brain-derived neurotrophic factor (BDNF) regulates synaptic plasticity, neuronal survival, and differentiation, and this has been considered a promising molecular target for the treatment of neurological disorders.[43] Furthermore, BDNF plays a significant role in neuronal growth and cell survival, and is essential for chemical processes that underlie synaptic plasticity. Thus, BDNF may provide new therapeutic options for neurodegenerative and neuropsychiatric disorders. In situations, such as epilepsy and persistent pain sensitization, the pathological levels of BDNF-dependent synaptic plasticity may be a contributing factor. Neurotrophic factors, including BDNF, are significant pharmacological targets of AD.[44,45] Low levels of BDNF are associated with synaptic loss and neurite atrophy in the brain of AD patients, while high levels of BDNF slow the AD progression and cognitive decline.[46,47] Furthermore, the injection of BDNF into the hippocampus reverses the learning deficits in the A1-42-induced AD rat model.[48] An *in vivo* experiment conducted using conditional knockout mice that targeted glial TrkB by crossing TrkBflox/flox mice with GFAP-Cre[49] revealed that the stimulation of the BDNF-TrkB signaling pathway in glial cells produces neuroprotective effects.[49–51] In addition, CNTF and bFGF levels are upregulated in BDNF-treated cultured Müller glia, conferring neuroprotective effects.[52] These findings suggest that glial BDNF-TrkB signaling induces an independent neuroprotective effect by upregulating several neurotrophic factors that promote prosurvival signaling in neurons and glia. Furthermore, earlier studies have revealed that several neurotrophic substances derived from PBE exhibit a positive effect on cultured brain cells. The neurotrophin nerve growth factor (NGF) enhances the development, differentiation, and survival of cholinergic neurons in the basal forebrain, making it an ideal cholinergic therapeutic agent.[53] Exogenously-applied NGF improves cognitive function in old, impaired, or cholinergic-depleted rats, and rescues cholinergic neurons in the basal forebrain.[53,54] These studies provide conclusive evidence of the neuroprotective effect of BDNF on brain cell culture models, which support the use of Nosustrophine as a reliable adjuvant medication for treating AD-related dementia. Free-radicals play a crucial role in the pathophysiology of brain damage following cerebral ischemia, and antioxidants reduce this impairment by boosting scavenger enzyme activity.[55] The positive effects of Nosustrophine were consistent with these findings, suggesting that its neuroprotective impact may be due to the increase in scavenger enzyme activity, which reduce oxidative stress or free-radicals in the various cell cultures examined. The pleiotropic effects of Nosustrophine may be attributed to the more precise targeting of its active components toward specific cellular target domains.

The present study aims to investigate the effects of Nosustrophine on the viability of astroglial and microglial cells, and the expression of MHC molecules, with the objective of assessing the neuroprotective potential of Nosustrophine. Inflammation is linked to microglia-mediated tissue damage, which underscores the importance of understanding the role of neurotrophic factors in maintaining tissue integrity and healing. The present data indicates that the Nosustrophine administration at various concentrations had a positive impact on the survival and proliferation of astroglial and microglial cells. This suggests that Nosustrophine may contain neurotrophic factors that regulate the apoptosis of enteric glial cells.[56] The disruption of this system may contribute to more severe inflammation. In addition, the availability of neurotrophic factors is essential for the survival and function of dopaminergic neurons, which are associated with neurodegenerative disorders, such as Parkinson's disease. Although the link between GDNF and degenerative diseases has not been conclusively proven, GDNF has the potential to improve the function of surviving dopaminergic neurons, and correct behavioral abnormalities that resulted from nigrostriatal degeneration.[57,58] Therefore, GDNF has emerged as a promising treatment option for Parkinson's disease.

Age is a significant risk factor for neurodegenerative disorders, and the neurotrophic factor expression decreases with age.[59] The present preliminary findings suggest that active porcine brain proteins and neurotrophic factors are neuroprotective against cognitive dysfunction in AD through the regulation of various intracellular processes. These findings support the potential use of neurotrophins as therapeutic agents for AD. However, more extensive research is required to evaluate the effectiveness of Nosustrophine across different stages of the disease. The present study was conducted on a limited number of cell lines, and further investigations are needed to establish the clinical efficacy of Nosustrophine. Nonetheless, the effectiveness of PBE in regulating scavenger enzymes and neurotrophic factors supports the potential of Nosustrophine as a neuroprotective agent. Two aspects should be given particular consideration for future research: First, PBEs are multi-component and multi-target drugs that can modulate neurotrophins in brain pathologies. However, the effective composition of Nosustrophine and its therapeutic effects require further investigations to optimize its clinical outcomes and prescription designs. Second, more comprehensive studies are required to evaluate the therapeutic effects of PBE. Scientific research in these areas would advance the development of PBE treatment for brain disorders, and provide a fair assessment of the clinical outcomes.

Future directions

The understanding of the molecular underpinnings of this nootropic substance may be enhanced by clarifying the mechanism of Nosustrophine neuroprotection in the human neuroblastoma SH-

SY5Y cell line. It may be possible to develop new therapies for the treatment of Parkinson's disease and other dopaminergic neurodegenerative processes by evaluating the molecular mechanisms underlying the neuroprotective effects of Nosustrophine, and other related neurotrophic factors derived from young porcine (*Sus scrofa domesticus*) brains. In order to preserve the high degree of compatibility and stability of the cell model, more effectively simulate the *in vivo* environment, and comprehend the mechanism of the disease state, it is crucial to choose the appropriate research object and preparation techniques. Future studies should concentrate in developing more precise *in vitro* models that might be utilized to create brand-new medications that target the affected areas in the central nervous system, as these would offer huge benefits to people.

Conclusions

The present study investigated the effect of Nosustrophine, which is an epigenetic bioproduct derived from the *Sus scrofa domesticus* brain using non-denaturing biotechnological processes, on the progression of neurodegeneration in the human neuroblastoma SH-SY5Y cell line. The present *in vitro* data indicated that Nosustrophine has therapeutic properties that prevent selective dopaminergic neuronal loss in the central nervous system, and reduce secondary degenerative effects caused by chronic neuroinflammation. Furthermore, Nosustrophine exhibited neuroprotective and anti-inflammatory effects, when this was administered before or after toxic neuroinduction. The present cell culture data highlights the potential of Nosustrophine as a preventive strategy against neuropathological damage. Nevertheless, further preclinical studies are required to validate these findings.

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Conflict of interest

Prof. Ramón Cacabelos has been an Editor-in-Chief of the *Journal of Exploratory Research in Pharmacology* since July 2015. The authors declare no other conflict of interests.

Author contributions

RC, IC, VL and LC conceived the experiments; IC, OMI, VN and VL conducted the experiments; RC, IC, OMI, LC and VL analyzed the results. All authors reviewed the manuscript, and accepted the final manuscript for publication.

Ethical statement

All experimental procedures were performed in accordance with the European Community Law (86/609/EEC), European Union Directive 2016/63/EU, and the Spanish Royal Decree (R.D. 1201/2005). Study procedures were reviewed and approved by the Ethics Committee of the International Center of Neuroscience and Genomic Medicine.

Data sharing statement

No additional data are available.

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Mini Review

A Narrative Review of Placebo and Nocebo Effects on Itch

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Abstract

There is limited information available on the evaluation of placebo or nocebo effects on itch. The present study developed a search strategy using PubMed to evaluate literature related to placebo and/or nocebo effects on itch. The search strategy identified 65 articles. After the independent review of each article, 10 studies were selected for inclusion. These studies varied, in terms of methods and outcome measures. Overall, verbal suggestion, conditioning, and/or placebo topical therapies led to placebo and/or nocebo effects on itch. Further understanding the mechanisms of placebo and nocebo effects on verbal suggestion and conditioning can open doors to the development of therapeutic strategies that could ameliorate or improve itch in patients.

Introduction

Have you ever had an itch you just can't scratch? Pruritus, or in layman's terms, itching, is essentially the miscommunication between sense of touch and the central nervous system. This can cause feelings of unease, irritation, or anxiety, often leading to an irresistible urge to quell this sensation. Recent studies have highlighted the importance of the emotional impact of itching, particularly in chronic cases. These studies demonstrated an association with higher rates of stress, anxiety, depression, and even suicidal ideation, leading to major deficits in quality of life.[1]

Although some itches may purely be due to physical or psychological causes, most pruritus occur due to the combination of these two. Thus, due to multifactorial causes, the treatment for pruritus may need to be tailored to target multiple causes. The use of the placebo effect to lessen itch has become a particular interest. This has become especially fascinating, because this has a potential for low risk of side effects and toxicities due to avoidance of pharmacologic therapy, and high rewards of effective itch relief for patients. This narrative review examined relevant studies for itching, especially for pruritus induced by physiological reasons, and treated using a psychological approach, placebo.

Keywords: Placebo; Nocebo; Itch; Pruritus.

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Methods

In order to identify relevant studies, the authors searched PubMed using a search strategy developed by the study team. The search strategy used a combination of keywords and controlled vocabulary words to capture the concepts of "itch", "pruritus", and "placebo" (refer to Table 1 for the details of the search strategy). The search was performed by S.W. in May 2022, and 65 articles were identified for review. Five reviewers independently checked the titles and abstracts. Then, the full articles of potential studies for inclusion were reviewed by the reviewers. For the inclusion of studies, the study was required to be specifically designed to evaluate the placebo or nocebo effect on itch. Among the reviewed studies, eight studies were identified for inclusion. Additional relevant studies were identified by checking the bibliographies of the relevant studies. Using this strategy, an additional two studies were identified for inclusion. Thus, a total of 10 studies were reviewed, and two additional studies were included, which provided additional statistical analysis related to the included studies.

Results

The results of the literature search resulted in a total of 10 studies (12 articles), and these were reviewed by the authors. One author (J.D.) reviewed the selected studies in detail, and compared the study methodology, size, interventions and findings. The details of the review are presented in Table 2. The study methodology varied among studies, and this often utilized verbal suggestion, conditioning, and/or the application of placebo topicals to elicit placebo responses. The sample sizes of all of the included studies

Table 1. Search strategy

Search strategy	Database	Article results	Excluded articles	Included articles
("placebos"[MeSH Terms] OR "placebo effect"[MeSH Terms] OR "placebo*"[Title/Abstract]) AND "open label"[Title/Abstract] AND ("pruritus"[MeSH Terms] OR "itch*"[All Fields])	PubMed	65	57	Eight studies were identified from the initial search for inclusion. Two additional studies were identified from the article references for inclusion. 10 studies in total (plus two additional articles that further analyzed the included studies).

Table 2. Review of manuscripts: placebo and nocebo effects on itch

Data source/ Location	Study methodology	Study size	Interventions	Findings	Author's conclusions
(1) A. Strumpf *et al*.,[2] 2016/ GERMANY	Closed-label; Randomized	*N* = 100; Healthy volunteers	1) Nocebo-like control I: a) NaCl Application; b) Noted: "does not cause itch in most people". 2) Nocebo-like control II: a) Histamine Application; b) Noted: "causes some itch in most people". 3) Nocebo-like effect II: a) Histamine Application; b) Noted: "causes an enormous itch in most people". 4) Nocebo-like effect I: c) NaCl Application; d) Noted: "causes an enormous itch in most people".	Larger wheal developed, significantly higher itch intensities, and increased unpleasantness under the nocebo-like condition I, when compared to under nocebo-like control condition I (received NaCl; $p = 0.001$, $p = 0.003$, $p = 0.003$). Larger flare size and itch intensity ratings differed between the nocebo-like experimental condition and nocebo-like control condition II (received histamine; $p = 0.02$, $p = 0.007$). The differences were not significantly different between males and females.	Itch and even skin reactions can be induced and intensified by suggestions and instructions, but these are not significantly impacted by gender.
(2) A. Van Laarhoven *et al*.,[3] 2011/ NETHERLANDS	Part 1: Verbal suggestions regarding various somatosensory stimuli that can evoke itch/pain. Part 2: Suggestions of a decrease or neutral response related to the application of histamine. Groups related to placebo impact on pain were also part of the study - outside the scope of the review.	Part 1: *N* = 56. Part 2: *N* = 36 (only those in the high expectation group from Part 1).	Part 1: 1) Itch Nocebo Condition (high expectation); 2) Itch Nocebo Control Condition (low expectation). Measured the effect of different somatosensory stimuli (mechanical, electrical, and chemical stimuli). Part 2: 1) Itch placebo; 2) Itch placebo control condition.	Part 1: Itch levels were significantly higher in the high expectation group, when compared to controls ($p < 0.001$). This was true for each type of stimulus. Higher expectations of itch were associated with higher levels of experienced itch. Part 2: The decrease in itch was larger in the itch placebo group, when compared to the controls ($p < 0.05$).	Nocebo effects can be indicated on itch by manipulating expectations through verbal suggestions. Verbal suggestions designed to induce a placebo effect resulted in a decrease in itch.

(continued)

Table 2. *(continued)*

Data source/ Location	Study methodology	Study size	Interventions	Findings	Author's conclusions
(3) D. Bartels *et al.*,[4] 2014/ NETHERLANDS	Multi-arm parallel group; Single-blind; Randomized	*N* = 95	1) Verbal Suggestion - noted that the third electrode would impact itch intensity based on the color on the screen (an actual sham electrode - all medium intensity stimuli); 2) Conditioning – noted that the color changes represent the change in intensity (low, medium, and high intensity stimuli were used); 3) Conditioning and verbal suggestion; 4) Control – the colors were not associated with change in stimuli intensity, no verbal suggestions were given.	Significant nocebo effect in the conditioning with verbal suggestion group (3), when compared to controls ($p = 0.02$). Borderline significant nocebo effect in the verbal suggestion group (1), when compared to controls ($p = 0.063$). No significant difference between the conditioning group (2) and controls. Significant placebo effect in the conditioning group (3), when compared to controls ($p = 0.009$). This was not observed in the other groups.	The combination of conditioning and verbal suggestion can induce significant nocebo and placebo effects on itch.
(4) D. Bartels, *et al.*,[5] 2017/ NETHERLANDS	Multi-arm parallel group; Single-blind; Randomized	*N* = 129	Part 1: negative verbal suggestions "receive a series of electrical itch stimuli with and without the activation of the third electrode that influenced intensity" third electrode = sham electrode = placebo, screen turned different color when third electrode was "activated". Part 2: Group 1: Positive expectation group "the third electrode will now decrease itch intensity". Group 2: Same procedure as Part 1; Group 3: Extinction Group - no instructions were given. Part 3: Same groups as Part 2, histamine iontophoresis was used	Part 1: Significantly higher itch score for the conditioned trials, when compared to that for the neutral trials ($p < 0.001$). Part 2: Change in itch score: group 1: −0.4 ± 1; Group 2: 0.5 ± 0.8; Group 3: 0.3 ± 0.9; ($p < 0.001$, $p < 0.01$). Part 3: Significantly lower itch scores in the positive versus negative expectation groups ($p < 0.01$).	The study demonstrates that nocebo effects can be effectively minimized by positive expectation induction, and that these may even result in placebo effects.
(5) D. Bartels *et al.*,[6] 2018/ NETHERLANDS	Additional analysis of D. Bartels, *et al.*, 2017/NETHERLANDS	*N* = 129	Refer to D. Bartels, *et al.*, 2017/NETHERLANDS	Part 1: Localized scratching with greater frequency and duration in the conditioned trials, when compared to the neutral trials ($p < 0.001$), total body scratching with greater frequency ($p = 0.056$) and duration ($p < 0.001$) in the conditioned trials. Part 2: No significant change in scratching episodes in the positive expectation group versus controls.	No conclusive evidence was identified for the generalization of nocebo effects on itch to scratching - further research is needed.

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Table 2. *(continued)*

Data source/ Location	Study methodology	Study size	Interventions	Findings	Author's conclusions
(6) M. Darrah *et al*.,[7] 2013/ NEW ZEALAND	Single blinded; Randomized	*N* = 58: Healthy college student volunteers	Written information was provided along with verbal explanation of skin reaction and antihistamine effect. Baseline administration: Aqueous cream applied (placebo). Histamine administered. Second administration: 1) Control group: Same as the baseline protocol; 2) Expectancy group: Verbal instructions were provided on the effectiveness expected from the antihistamine (placebo cream).	Expected wheal area: Significant between group difference in the change in expected wheal area with the expectancy group, expecting a greater reduction ($p = 0.005$). Wheal Area: No significant difference ($p = 0.64$). Heart rate (HR): A greater reduction in HR from baseline to second administration in the expectancy group, when compared to the control group ($p = 0.45$).	The wheal area was not impacted by the participants' expectation based on verbal instructions - participants in the expectancy group did expect greater reduction in wheal size, and experienced a greater reduction in heart rate.
(7) M. Darragh *et al*.,[8] 2015/ NEW ZEALAND	Closed label; Cross-over; Randomized	*N* = 50: Healthy volunteers	1) Group 1: Session 1 – Control; Session 2 – Treatment. 2) Group 2: Session 1 – Treatment; Session 2 – Control. For the treatment session - watched video explaining that an anti-histamine treatment cream would be applied to reduce itchiness and the size of the weal. In the control session - informed the subjects that the purpose was to get an indication of skin reactivity without treatment. The placebo cream was applied in all treatment sessions, followed by histamine.	Reduction of itch in the treatment group was noted after one minute ($p = 0.009$), three minutes ($p < 0.001$), and five minutes ($p < 0.05$). There was no difference in itch at seven minutes ($p = 0.23$), and no difference in weal size ($p = 0.39$).	Demonstrates the placebo effect in the context of inflammatory skin reactions using verbal suggestions alone.
(8) S. Meeuwis *et al*.,[9] 2018/ NETHERLANDS: *Study 1*	Open label; Randomized	*N* = 92: Healthy volunteers	1) Open-label positive VS; 2) Control no VS. VS = verbal suggestion.	The positive verbal suggestion group had significantly lower itch expectations, when compared to the control group ($p < 0.001$). No statistically significant difference between groups in mean self-reported itch during iontophoresis. ($p = 0.24$). The self-reported skin condition scores were lower in the experimental group ($p = 0.059$). No difference in physical parameters ($p > 0.14$)	The proof-of-principle study demonstrated that the open-label positive verbal suggestions were successful in reducing the level of itch the participants were expected to experience, but not in reducing the itch that was actually experienced.

(continued)

Table 2. *(continued)*

Data source/ Location	Study methodology	Study size	Interventions	Findings	Author's conclusions
(9) S. Meeuwis *et al*.,[10] June 2019/ NETHERLANDS: *Study 2*	Placebo controlled Crossover study. Open and closed label groups. Randomized	*N* = 92: Healthy volunteers	1) Open-label positive VS; 2) Closed-label positive VS; 3) Open-label negative VS; 4) Closed-label negative VS. VS = verbal suggestion. Positive VS = "tonic has an itch reducing effect". Negative VS = "tonic has an itch increasing effect". Open label = "tonic is a placebo, but people still have a response in their brain, impacting itch even if they know they received a placebo". Closed label = no additional information given.	The area under the curve (AUC) for itch during histamine iontophoresis: the combined positive VS groups and negative VS groups revealed a small sized non-significant difference ($p = 0.19$). The open and closed label analysis revealed similar findings. Maximum itch during iontophoresis: the combined and separate groups had no significant difference ($p > 0.24$). AUC for itch during the follow-up iontophoresis: significant and medium sized difference in changes for scores during the four-minute follow-up for the positive VS groups ($p = 0.16$), but not the negative VS groups ($p = 0.98$). No differences in subjective or physical skin response among the groups.	Both open-label and closed-label verbal suggestions were able to influence itch expectations, with closed label suggestions having more effect in reducing itch during follow-up. However, experienced itch during histamine iontophoresis was not influenced by suggestions.
(10) S. Meeuwis *et al*.,[11] Nov/ Dec 2019/ NETHERLANDS	Placebo controlled Crossover study. Open and closed label groups. Randomized	*N* = 92: Healthy volunteers	Two-phase conditioning paradigm: 1) Open-Label Conditioned Group: a) CS + UCS with explanation of conditioning and expected effects; b) CS + placebo. 2) Closed-label conditioned group: a) CS + UCS no explanation given; b) CS + placebo. 3) Conditioned not evoked control group: a) CS + UCS; b) water + placebo. 4) Non-conditioned control group: a) CS + placebo; b) CS + placebo. CS = conditioned stimulus - flavored beverage; UCS = unconditioned stimulus - H1 antihistamine	No differences in expected itch, remembered itch, or expected medication efficacy were identified ($p > 0.11$). No significant differences were found for mean self-reported itch, clinical skin response to histamine iontophoresis, spirometry, heart rate, or skin conductance level. When the groups were combined for comparison, conditioning was identified to be marginally effective in reducing itch ($p = 0.076$).	The study provides preliminary support for the behavioral conditioning of antipruritic effects. The findings suggest that this conditioning may be effective when it is known that a learning paradigm is being used. Further investigation in the open-label setting may help facilitate the utilization of placebos in clinical practice.

(continued)

Table 2. *(continued)*

Data source/ Location	Study methodology	Study size	Interventions	Findings	Author's conclusions
(11) S. Meeuwis *et al.*,[12] Jan 2021/ NETHERLANDS: *Study 3*	Closed and open label arms; Randomized	*N* = 112: Healthy volunteers	1) Open-label positive VS; 2) Closed-label positive VS; 3) Open-label negative VS; 4) Closed-label negative VS. VS = verbal suggestion, Positive VS = "caffeine-containing patch to shoulder, influence both cognitive abilities and sensitivity to stimuli such as itch". Negative VS = "caffeine-containing patch made the itch worse". Open Label = the participants noted that the patch does not contain caffeine, test effects of positive suggestions, studies have shown that this reduces itch, even if it is known that this is a placebo.	Expected itch/Expected patch efficacy: the expected itch in the positive VS groups was significantly lower, when compared to the negative VS groups ($p < 0.001$), larger effect size in the open-label group, the differences in expected patch efficacy were small ($p = 0.059$). Self-rated mean itch: Significantly lower in the positive VS groups ($p < 0.001$). Self-rated skin response: Reported as less severe in the positive VS group ($p < 0.001$). Clinical skin response: No difference between groups	Both open and closed label positive suggestions related to a sham transdermal patch were able to influence the expectations for itch, mean itch experienced, and self-reported skin response in the experimental setting, when compared to negative suggestions.
(12) S. Meeuwis *et al.*,[13] Dec 2021/ NETHERLANDS	Data used from previous studies (refer to the study details above). *Study 1: S. Meeuwis et al. 2018; Study 2: S. Meeuwis et al. June 2019; Study 3: S. Meeuwis et al. January 2021.*	*N* = 295 in total from three studies	Data used from previous studies (refer to the study details above). *Study 1: S. Meeuwis et al. 2018; Study 2: S. Meeuwis et al. June 2019; Study 3: S. Meeuwis et al. January 2021.*	Effects of VS on mean itch as mediated by expectations: For open-label participants: Positive VS indirectly reduced post-VS mean itch through mediation of expectation ($p < 0.001$). The lower pre-VS expected itch was significantly associated with lower post-VS expected itch ($p < 0.001$, $p = 0.032$). Closed label participants: Positive VS reduced expected itch, when compared to negative VS ($p < 0.001$). Post-VS expected that the itch was not associated with the post-VS mean itch ($p = 0.9$), while positive VS was directly associated with lower post-VS mean itch ($p = 0.014$). Post VS expected that the itch did not mediate the effects of CS on mean itch in the closed label context. Interindividual differences in the relationship between verbal suggestions, expectations, and itch: BAS (behavioral activation system) and body ignorance may play a role in the effect of VS. VS = verbal suggestion.	Innovative statistical methods were used to obtain detailed mechanistic information on the influence of interindividual differences on how placebo effects are formed. The effects of open-label positive and negative VS on itch may be more dependent on mediation by expectations, while closed-label suggestions directly influence itch. Low BAS sensitivity (sensitivity to rewards) was associated with increased impact of expectation on itch response. High ignorance of bodily signals is associated with increased placebo response to VS.

(continued)

were relatively low, which ranged within 14–129 subjects, with an average of 108 subjects.

A. Strumpf *et al*. (Study 1, Table 2) used histamine or saline application as the physical intervention.[2] Then, the subjects were verbally inquired whether they expected the application to cause itch.[2] Verbal suggestion for itch to occur in the placebo saline group led to greater wheal development, and significantly higher reported itch intensities.[2] Similar results were observed in groups that received histamine, indicating that itch, and in this case, even skin reactions, can be induced by verbal suggestion.[2]

A. Van Laarhoven *et al*. (Study 2, Table 2) reported similar results using various itch-causing stimuli, including mechanical, electrical, and chemical stimuli.[3] For all stimuli, higher expectations for itch were associated with the reported increase in itch experience.[3] Verbal suggestion was also effective in inducing a response in these subjects, resulting in a decrease in itch response.[3]

D. Bartels *et al*. completed several trials using both active and sham electrodes, in order to stimulate itch (Studies 3, 4 and 5; Table 2).[4–6] The authors combined the techniques of verbal suggestion and conditioning to elicit placebo responses, and reported that the combination of these techniques was more effective than using verbal suggestion alone.[4] Furthermore, it was reported that electrodes can decrease an itch, even though the intensity produced by the electrode does not change, significantly lowering the itch score.[5] The additional analysis did not reveal significant changes in scratching episodes in the subjects, indicating that the nocebo effect does not conclusively extend from subjects that reported a feeling of itchiness to the act of physically scratching.[6] More research is likely needed in this area, since this was the only analysis that addressed scratching episodes.

The use of a placebo cream, which was represented to the study participants as an antihistamine cream, in combination with verbal suggestion, was examined in several studies conducted by M. Darrah et. al. (Studies 6 and 7, Table 2).[7,8] Both studies revealed that when the placebo cream and verbal suggestion were utilized, there was no significant difference in wheal area, but there were improvements in itch at various time periods.[7,8] In addition, a reduction in heart rate from baseline was realized, when subjects were provided instructions on the effectiveness of the antihistamine (actually placebo) cream.[7]

In the Netherlands, S. Meeuwis *et al*. (Studies 8–12, Table 2) completed a number of studies related to placebo response and its impact on itch.[9–13] Three separate studies were completed using histamine iontophoresis as the itch inducing stimuli.[9–11] The use of this process enabled histamine to be introduced through the skin using current, creating an itch response. This process is commonly used in clinical research related to itch response and treatment. The group that initially conducted a study in 2018 reported that positive verbal suggestions can lead to significantly lower itch expectations, but there was no significant difference in mean self-reported itch during iontophoresis.[9] This result was in contrast to previous trials, which revealed improvement in experienced itch with verbal suggestion. Another study completed by the group of S. Meeuwis revealed similar results for both positive and negative verbal suggestion, resulting in changes in itch expectations, but without significantly impacting the experienced itch when looking at the area under the curve for the itch experienced during histamine iontophoresis and maximum itch intensity.[10] The subsequent study completed by a group of investigators used a two-phase conditioning paradigm to invoke a placebo response.[11] Various research groups used the combination of a conditioned stimulus (flavored water), an unconditioned stimulus (antihistamine), and placebo comparators, along with verbal suggestion.[11] However, no significant differences were identified for mean self-reported itch, clinical skin response to histamine iontophoresis, heart rate, or skin conductance level.[11] When the results of these groups were combined for analysis conditioning, these were identified to be marginally effective in reducing itch.[11]

An alternate approach of using a placebo patch was utilized by the group of S. Meeuwis in a study published in 2021.[12] In that trial, all subjects were given a placebo patch, that is, the subjects were given patches that contained caffeine (closed label group) or placebo (open label group).[12] These groups were further divided into the positive suggestion group (patches that would improve the itch) and negative suggestion group (patches that would worsen the itch).[12] No difference in clinical skin response was realized among the groups. However, the expected itch, self-rated skin response, and self-rated mean itch were significantly lower in the positive suggestion groups.[12]

An additional analysis of previous trials (Studies 8, 9 and 11; Table 2) was conducted by S. Meeuwis *et al*., and published in 2021.[13] Innovative statistical methods were applied to determine the interindividual differences on how placebo effects are formed.[13] The researchers reported that the effects of open-label positive and negative verbal suggestions on itch may be more dependent on its expectation, while the closed label approach would directly influence the itch.[13] Furthermore, the researchers determined that low sensitivity to rewards and high ignorance of bodily signals were associated with increased placebo response to verbal suggestion.[13]

Summary of available research and limitations

A review of 10 trials on placebo and nocebo effects on the perception of itch and skin reactions in young healthy individuals by verbal suggestion, learning process, expectancy, or conditioning, or other means in open-label and closed label trials was completed. The results slightly varied among the studies. However, overall, verbal suggestion, conditioning, and/or placebo topicals appeared to produce a beneficial placebo effect on itch.

Itch, such as pain, is a subjective sensation of a person, and the intensity depends on various factors, including the frame of mind, psychological state, underlying medical or mental illnesses, and external cues, such as verbal suggestions, conditioning, etc. Therefore, it remains challenging to have an objective measure of itch. Merely four of the reviewed studies (Studies 1, 5, 6 and 7; Table 2) used certain objective measures, such as the size of wheals, intensity of scratching, and heart rate, to gauge the placebo or nocebo effects on itch. However, these are not well-established accurate surrogates to measure itch intensity, due to the psychological component of the patient's itch experience. The use of subjective measures to evaluate the improvement or worsening of an itch remains difficult to some degree, given the interpatient variability. Furthermore, it remains difficult to directly compare the presently available literature, since the research methods and reported outcome measures are not standardized. Moreover, the placebo response varied in the studies, in which some studies reporting statistically and clinically significant improvements in itch, while other studies reported more modest placebo effects. Combining visual or tactile (i.e. patch or topical cream) with verbal suggestion appeared to improve the placebo response. These trials indicate that there is a psychological component to itch that can be influenced by inducing a placebo response, but the exact

mechanisms remain unknown.

Subjects who were recruited for the studies were healthy individuals, who had no acute or chronic skin diseases, psychiatric disorders, or other underlying medical diseases. Further studies on patients with medical conditions that cause itch, especially chronic itch, are warranted. The challenges of applying placebo, regardless of whether these are open-label or closed label, to patients with itch in real-world clinical practice would include moral, ethical, and patient-clinician trust relationship issues.

Compared to the field of pain, studies conducted for the placebo and nocebo effects on itch have been relatively new and few, and far between. The limitations of the present study include the following: a small number of studies were identified, the reviewed studies had small sample sizes, and the heterogeneous design of these studies made the comparison across studies difficult; merely studies published in the English language from 2011 to 2021 were reviewed, which may have caused relevant publications in other languages and publications outside of the search period to be missed; since the present study was a narrative review, and not a systematic review or meta-analysis, the quality of the included literature was not assessed.

The use of placebos with verbal suggestion, conditioning, and/or physical placebos can conceivably lower the risk of systemic or local toxicities, when compared to systemic or topical medications. It would be beneficial to invest more resources in elucidating the mechanisms of placebo effects, thereby opening doors to the development of therapeutic strategies that could ameliorate or improve itch in patients who do not respond to conventional modalities of treatment for itch.

Future directions

Research in the area of placebo and nocebo effects on itch remains limited. In order to further investigate the use of these techniques in the therapeutic setting, additional research is needed. The present studies appeared to support the use of verbal suggestion, conditioning, and/or topical placebos to elicit improvements in itch response. Future studies should focus on replicating these results in larger study populations, and in subjects with chronic itch conditions. These data would be necessary to move forward in the development of techniques that can be used in clinical settings, that is, using the placebo effect to improve patient outcomes. Before these interventions can be therapeutically used in real world applications, additional discussions on its ethics and impact on patient-provider relationship are necessary.

Conclusions

Studies, including those that investigate placebo or nocebo effects, are difficult to conduct and evaluate, due to multiple variables, subjectivity, and moral/ethical/trust concerns for various disease states, including itch. As anticipated, the standardized review approach resulted in the limited inclusion of articles. Although there were multiple limitations, the present study contributes to the literature by gathering small study data to present the potential positive impact of the use of placebo to treat itch, and identify future areas of study in this field, with the hope of advancing treatment options.

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Author contributions

SW: research strategies; JD, AH, KH, NP and TL: review of articles for inclusion; JD, AH, KH, NP, TL and SW: drafting of the manuscript; JD: primary editing of the manuscript.

Disclaimer

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Mini Review

Efficacy of Ketamine Therapy in the Treatment of Refractory Major Depressive Disorder

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Abstract

Major depressive disorder (MDD) is a prevalent and highly debilitating illness that causes significant functional impairment in many patients. Conventional pharmacotherapy, such as monoaminergic antidepressant agents, usually takes several weeks to improve symptomatology and has some adverse side effects, and in many cases, patients show clinical non-response. This has resulted in a quest to identify novel means of targeting the illness. Ketamine, a glutamate N-methyl-D-aspartate receptor antagonist, has been widely researched as an alternative intervention. Originally developed as an anesthetic, ketamine has been shown to exert an antidepressant effect at subanesthetic doses. A single dose of ketamine has been shown to have a rapid effect in resolving serious depressive symptoms including suicidal ideation with antidepressant effects. However, further research is needed as, in longer-term use, ketamine has the potential to be abused and certain psychological side effects, including psychotomimetic or dissociative effects, must be considered. This review highlights some of the benefits and risks of the use of ketamine in the treatment of MDD.

Introduction

Major depressive disorder (MDD) is among the most disabling and potentially life-threatening illnesses globally and has been ranked by WHO as the third greatest cause of the burden of disease while being projected to rank first by 2030.[1,2] MDD is diagnosed when an individual exhibits persistent depressive episodes, anhedonia (a decrease in interest in pleasurable activities), feelings of worthlessness and/or guilt, low energy levels, impaired concentration, changes in appetite and sleep patterns, psychomotor retardation, agitation, or SI. According to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition,[3] to be diagnosed with MDD, a patient must experience at least five of these symptoms, of which one is depression or anhedonia and which results in interpersonal or occupational impairment.[2]

The etiology of MDD is believed to be multifactorial and includes biological, genetic, environmental, and psychosocial factors. Historically, MDD has been considered to be primarily influenced by anomalies in the functions of neurotransmitters, especially serotonin, norepinephrine, and dopamine. Conventional and widely-used antidepressants such as selective serotonin receptor inhibitors (SSRIs) and serotonin-norepinephrine receptor inhibitors (SNRIs) aim at modulating the monoaminergic system. However, an important limitation of SSRIs is the delayed onset of action, as they typically take about 14 days to begin exerting an effect and have the potential to worsen any pre-existing anxiety or suicidality during this time, especially in younger populations. Other possible side effects which limit the effectiveness and use of these conventional therapies may include insomnia, nausea, headaches, and sexual dysfunction, which negatively impact the patient's quality of life. Moreover, a significant proportion of patients fail to respond to treatment at all.[4,5]

Treatment of refractory MDD

Among those individuals who receive first-line treatment for MDD, up to 60% do not achieve remission[6] and a significant proportion of patients fail to achieve clinically notable benefits even with multiple antidepressant interventions. Patients with Treatment-Resistant Depression (TRD) continue to have residual depressive symptoms that affect both function and quality of life and increase the risk of suicide. Thus, alternative intervention for refractory cases is an important clinical need.[7]

TRD is a subset of MDD. While there is a lack of broad consensus on the definition of TRD, it may be described as depressive symptomatology that does not remit after two or more regimens of first-line antidepressant pharmacotherapy at optimal dose and

Keywords: Ketamine; Esketamine; Pharmacotherapy; Treatment-resistant depression; Non-conventional antidepressants.

Abbreviations: MDD, major depressive disorder; NMDA, N-methyl-D-aspartate; SI, suicidal ideation; TRD, treatment-resistant depression.

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duration in the course of a current depressive episode.[7] Treatment resistance includes persistent symptoms of a low mood, repeated depressive episodes, and poor response to medication or other therapeutic interventions, including brain stimulation such as electroconvulsive therapy, repetitive transcranial magnetic stimulation, magnetic seizure therapy, deep brain stimulation as well as psychotherapy.[1,6]

Recent studies have shown that patients with MDD having episodes of TRD have a significantly higher risk of self-harm, a greater than 20% increase in all-cause mortality, increased use of health resources, comorbidities such as anxiety disorders, obsessive-compulsive disorders, and fatigue when compared to patients without TRD episodes. Increased incidence of substance abuse was also found to be higher among these patients. One study also found that MDD episodes with TRD were of substantially longer duration than MDD without TRD.[7]

Research has suggested that the risk factors for the development of TRD include age, age at onset, psychiatric comorbidities, duration of, a history of abuse, and treatment-related factors.[7]

The efficacy of ketamine in TRD

In the quest for more rapid-acting treatments, newer theories have emerged that suggest MDD may be closely associated with more complex neuroregulatory systems, and the examination of novel molecular targets beyond the monoamine system has become marked in order to gain clinically groundbreaking advances in MDD therapeutics.[2,8]

Recent research involving in vivo brain imaging and studies of gene expression has implicated abnormalities in glutamatergic signaling in the pathophysiology of MDD and agents that modulate this system have significant therapeutic potential. In particular, ketamine, a non-competitive NMDA receptor antagonist, which has been in use primarily in veterinary and pediatric anesthesia and has well-established safety and efficacy qualities as an analgesic and anesthetic in these contexts, has been recently studied for use off-label as a treatment for psychiatric disorders.

A key factor in ketamine's efficacy is its role as an efficient glutamate receptor modulator. Ketamine works by blocking NMDA receptors in the brain, which increases levels of the neurotransmitter glutamate causing synaptogenesis or neurotransmission along new pathways.[4] As glutamate is the primary excitatory neurotransmitter in the central nervous system and any disruption in glutamate function or the operation of glutamatergic transmission may impair neural health, this has a significant effect on limiting the progression of many neurodegenerative and psychiatric diseases[4,9,10] and affects mood, thought patterns, and cognition[11] Moreover, recent studies have found that ketamine is better at crossing the blood-brain barrier than SSRIs, SNRIs and other widely used antidepressants.[12] This is the first non-monoaminergic agent that has demonstrated rapid-onset efficacy in the treatment of MDD and thus represents a pharmacologically novel therapeutic option for adults with TRD.[8,13]

Several studies have shown that antidepressant effects in patients with TRD were observed within approximately 2 hours of a single subanesthetic intravenous infusion of ketamine, after the acute, dissociative, and euphoric side effects subsided, with the effects gradually decreasing at seven days post-infusion—although twice weekly infusions have been demonstrated to prolong the antidepressant effect for up to 15 days.[4] This finding is especially significant for individuals needing immediate intervention such as those with concurrent SI or patients with personality disorders where high levels of suicidality render research on ketamine a priority.[9,10,14–17]

Moreover, ketamine has been demonstrated to be effective in other psychiatric contexts, including Bipolar Disorder, Social Anxiety, Generalized Anxiety Disorder, Obsessive Compulsive Disorder, Post-traumatic Stress Disorder, and eating disorders.[10]

Routes of administration

Although there is consensus regarding the therapeutic role of ketamine in depression, the comparative effects of different formulations of ketamine are less clear and the psychoactive and therapeutic effects have also been found to vary substantially by dose and route of administration.[10,18] Ketamine is a 1:1 racemate of two enantiomers, S-ketamine (esketamine) and R-ketamine. One of these enantiomers, S-ketamine (esketamine), binds more potently to the NMDA receptor than R-ketamine and thus has an anesthetic effect that is approximately 2 times higher but produces less lethargy and cognitive impairment.[9,19]

Ketamine and esketamine are similar as they have the same molecular makeup but esketamine has been shown to be not only more potent but also better tolerated than ketamine.[19] Recent research suggests that esketamine reduces the risk of relapse by between 50 and 70%.[16] The most researched formulations and routes of delivery of ketamine in TRD are intranasal (IN) esketamine and intravenous or parenteral (oral, sublingual, and IN) racemic ketamine.[13,20]

Route of administration is an important factor in the use of ketamine for disorders such as TRD, in which repeated dosing may be indicated. Intravenous delivery is widely used in clinical settings due to its superior bioavailability and dose control, while esketamine is usually given in the form of a nasal spray.[10,21]

Some studies suggest oral and intranasal formulations of ketamine are optimal for TRD, but there is still little data regarding the potential link between the rapidity of onset of action and the route of administration.[22]

Risks of ketamine use

While the clinical effectiveness of ketamine in TRD has been demonstrated, studies show that this varies considerably among patient populations, which has implications for general use.[23] Many important factors regarding ketamine use still need to be defined and relatively little is known about the overall risks of ketamine use as an antidepressant.[22,24] There is a dearth of research aimed at identifying optimal dosing strategies for ketamine use and common adverse side-effects of ketamine have been found.[25] These include transient and dose-dependent dizziness, headache, nausea, blurred vision, cardiovascular symptoms, neurotoxicity, cognitive dysfunction, and dissociative and psychotomimetic effects.[10] Such adverse effects tend to manifest in acute, low-dose treatments whereas extended exposure may put patients at risk of neurotoxicity and drug dependence. Since ketamine is associated with an increased risk of drug abuse, it cannot be recommended in routine clinical practice.[22,25] Ketamine abuse may lead to chronic cystitis, hepatotoxicity, and gall bladder pathology in addition to the psychiatric symptoms of impaired cognition and chronic dissociative effects.[25]

Experience with ketamine administration in patients with TRD indicates that higher doses of intravenous ketamine are associated with increased rates of treatment-related adverse events such as dissociation when compared with lower dosing. Thus, clinicians administering ketamine should be aware of the greater probability

of adverse events and potential safety issues when administering comparatively higher doses of intravenous ketamine.[13]

Other concerns

Ketamine use for TRD raises a complex set of ethical concerns. Given the rising popularity of off-label ketamine use for TRD, there is consensus that clinicians and professional bodies must ensure that guidelines for safe practice are administered, all experimental and trial data are made known through national registries, and that both the risks inherent in ketamine treatment and the patients themselves continue to be monitored.

In addition, ensuring equitable access to treatment resources is imperative for optimal treatment benefit. The associated cost and financial accessibility hold socioeconomic and ethical implications for practice and, where patients do access treatment facilities, they may experience different standards of care between treatment sites. These are issues that warrant further examination.[26]

Further directions

Scant research exists into the use of ketamine with other supportive interventions such as psychotherapy. It is thought that ketamine may assist in the creation of adaptive new neural pathways in the brain if treatment occurs within the context of a supportive environment and with the inclusion of concurrent psychotherapeutic interventions.[27] Some preliminary studies show that adjunct psychotherapy may prolong the antidepressant effect of ketamine, leading to a less frequent need for administration.[25,28]

Additionally, ketamine may improve treatment adherence and patient engagement, which makes it a valuable psychotherapeutic adjunct.[9] Ketamine's demonstrated antidepressant effect may be linked with the psychotherapeutic process, generating rapid change, increasing treatment engagement, and lowering the patient's defensiveness through relief from distressing symptomology.[29] As such, further research into this association is warranted.

Conclusions

Current neuroscience research has redefined the perception of TRD from a monoaminergic neurotransmitter system model where there is dysfunction of specific parts of the brain toward the finding that depression is a much more complex network disorder. The need for rapid-acting antidepressant therapies at the receptor level with targeted synaptogenesis and improved neural connectivity has led to studies of agents outside current models. Research on ketamine treatment for TRD is still in the relatively early stages but numerous studies have established that ketamine is a safe, effective, fast-acting, and sustained antidepressant that markedly reduces adverse symptoms associated with depression, even in patients who are resistant to conventional pharmacotherapy.

Further research into the risks of ketamine use is necessary. A lack of guidelines regarding the therapeutic monitoring of ketamine therapy for depression has implications for expanding the use of this treatment. Dose optimization, alternative routes of administration, and the role of concurrent pharmacotherapy in the antidepressant effects of ketamine are some of the issues that remain to be answered. Further work is needed to gain a more reliable understanding of ketamine's abuse liability and its side effects in the clinical setting. Moreover, the use of ketamine as an adjunct to other forms of anti-depressant therapy, such as concurrent psychotherapy, requires further investigation.

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Conflict of interest

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Review Article

Neurotoxic or Protective Cannabis Components: Delta-9-Tetrahydrocannabinol ($^{\Delta 9}$THC) and Cannabidiol (CBD)

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Abstract

Cannabis sativa contains phytocannabinoids that are psychoactive and neurotoxic (delta-9-tetrahydrocannabinol: $^{\Delta 9}$THC) or nonpsychoactive and presumptively neuroprotective (cannabidiol: CBD). Along with rising legalization, availability, and demand, the $^{\Delta 9}$THC:CBD ratio also has increased. Cannabis legalization means that use will likely increase in pregnant or breastfeeding women, affecting all stages of brain and neurodevelopment of their offspring. $^{\Delta 9}$THC exposure *in utero* or during development leads to lasting detrimental effects on behavior, cognition, locomotor activity, as well as epigenetic changes. Caution is urged with cannabis use. CBD is one of the most actively studied therapies for a broad spectrum of neurological, inflammatory, and mental diseases (e.g., Parkinson's disease, Huntington's disease, Alzheimer's disease, schizophrenia) because of its efficacy, low toxicity, and availability. While data indicate that the benefits of CBD may outweigh its risks, there are indications that it poses a risk for adverse effects on neurodevelopment from *in-utero* exposure as well as detrimental effects on male reproduction. Therefore, there is a clear need to continue researching the effects of $^{\Delta 9}$THC exposure as well as the optimal CBD treatment related to disease management while stressing the need to further characterize possible adverse effects.

Introduction

Medicinal and recreational cannabis use has increased globally, and continuation of this trend is anticipated as its use becomes legalized internationally.[1,2] *Cannabis sativa* is composed of over 100 "cannabinoids,"[3,4] but the psychoactive compound delta-9-tetrahydrocannabinol ($^{\Delta 9}$-THC), isolated in 1964, and the nonpsychoactive compound cannabidiol (CBD), isolated in 1940,[5] represent the most abundant components. Consumption of cannabis products occurs through diverse routes (inhaled smoke, vaping of liquid extracts, resins or waxes, lotions, edibles).[6,7] Inhaled cannabinoids are rapidly absorbed in the lungs[8] but less so by other routes (e.g., dermal, oral, rectal).[9] Due to their highly lipophilic properties, they are stored in adipose tissue for weeks or months and are concentrated in the breast milk of rodents and humans.[10,11] CBD products can have beneficial health effects and aid in various medical disorders (e.g., Parkinson's disease, anxiety, and epilepsy).[12,13] Accumulating evidence also indicates there are neurotoxic and reproductive effects from exposure.[14–18]

Due to increasing cannabis use, exposure to $^{\Delta 9}$-THC presents concerning health risks because use will likely also increase in pregnant or breastfeeding women, affecting all stages of brain and neurodevelopment of their offspring.[19–24] Along with increased legalization, social acceptance, and use, a change in the ratio of $^{\Delta 9}$-THC to CBD in cannabis has also occurred, leading to a change in potency (the $^{\Delta 9}$-THC:CBD ratio increased from 14:1 in 1995 to 80:1 in 2014).[25] Ultimately, the extent of cannabis neurotoxicity[26] is dependent on many variables, including the $^{\Delta 9}$-THC exposure level, purity,[25] route of administration,[7,9,27] developmental age at exposure,[23,28–30] health status,[31,32] pregnancy status,[21,33–36] lactational status,[37,38] and others.[39] Further, due to the lipophilic nature of these compounds, it has been shown that exposure at low, re-

Keywords: $^{\Delta 9}$THC, Delta-9-Tetrahydrocannabinol; Cannabidiol; Neurodevelopment; Endocannabinoid system; Cannabis; Neuroprotection; Neurotoxicity.

Abbreviations: ACh, acetylcholine; 2-AG, 2-arachidonoylglycerol; 5-HT, serotonin; AEA, anandamide; ARfD, acute reference dose; βA, beta-amyloid; BDNF, brain-derived neurotropic factor; Ca^{+2}, calcium; CB1R, cannabinoid 1 receptor; CBD, cannabidiol; CNS, central nervous system; COX2, cyclooxygenase-2; D1 or D2, dopamine receptors; DA, dopamine; DAGL, diacylglycerol lipase; DRN, dorsal raphe nucleus; DS, Dravet syndrome; eCB, endocannabinoid; eCBS, endocannabinoid system; FAAH, fatty acid amide hydrolase; GABA, gamma-aminobutyric acid; GD, gestation day; GPR55, G-coupled protein receptor 55; i.p., intraperitoneal; i.v., intravenous; IL, interleukin; iNOS, inducible nitric oxide synthase; K^+, potassium; LOAEL, lowest-observed-adverse-effect level; LOEL, lowest-observed-effect level; MAGL, monoacylglycerol lipase; MOA, mode of action; NAc, nucleus accumbens; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMDA, *N*-methyl-D-aspartate; NOAEL, no-observed-adverse-effect level; PFC, prefrontal cortex; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; s.c., subcutaneous; SNc, substantia nigra; TNF, tumor necrosis factor; TRPV1, transient receptor potential cation channel subfamily V member 1, or vanilloid receptor 1; VTA, ventral tegmental area; $^{\Delta 9}$THC, delta-9-tetrahydrocannabinol.

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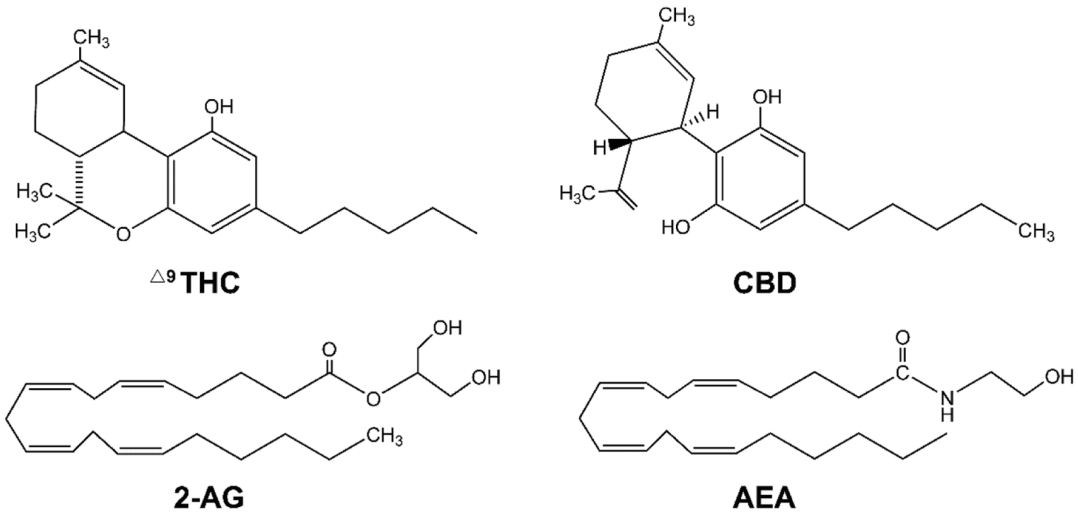

Fig. 1. Lipophilic structures for delta-9-tetrahydrocannabinol ($^{\Delta 9}$THC) and cannabidiol (CBD) as well as the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA). Each compound acts at the G protein-coupled receptors cannabinoid 1 and 2 receptors, which affect neurotransmitter release.

alistically achievable *in-vivo* concentrations causes specific molecular targets to be affected, resulting in behavioral or cognitive deficits in those with $^{\Delta 9}$THC exposure,[39–41] or potential benefits that greatly improve the health of those with neurodegenerative diseases.[42–44]

In this review, both the risks and benefits of exposure associated with $^{\Delta 9}$THC and CBD were investigated. Notably, the risks from CBD exposure, which is usually considered to be safe, are associated with reproductive and developmental health effects.[45] Recently, concerns have been raised about CBD use, since it is available in numerous over-the-counter products, with little data supporting its safety or efficacy.[46] The side effects and adverse health effects, along with questions regarding the ingredients, are often unknown. On the other hand, $^{\Delta 9}$THC exposure has been associated with adverse effects, depending on the dose, yet the benefits of this drug need to be emphasized. These phytocannabinoids were selected because they are the dominant compounds in cannabis, and they are often used as treatments for physical ailments as well as for recreational use. There is a vast amount of literature characterizing these compounds and their effects during development and throughout life in both animal and human studies, but it is important to present the risks as well as the benefits.

The endocannabinoid system (eCBS)

The eCBS was discovered in the 1990s while investigating the mode of action (MOA) of $^{\Delta 9}$THC. It is innate and multifaceted, affecting metabolic pathways throughout the body [e.g., muscle, adipose tissue, gastrointestinal tract, liver, and central nervous system (CNS)].[47] It helps to shape neuronal connectivity in the brain throughout development and into adulthood,[48] affecting the gamma-aminobutyric acid (GABA)ergic, glutamatergic, opioid, and dopaminergic systems.[49] Cell membrane-bound cannabinoid-1 receptors (CB1Rs) are the most abundant in the brain, while CB2Rs are mainly expressed on immune cells (T-cells, macrophages) in the periphery or glia/microglia in the brain.[47,50] Some researchers have suggested that the transient receptor potential cation channel subfamily V member 1 (TRPV1 or vanilloid receptor 1) could be classified as CB3R, as it is activated by CBD.[51] Each receptor type can act independently; however, depending on their location, CB1Rs and CB2Rs (possibly also CB3Rs) can act together, competitively, or in opposite directions, potentially through dimerization to regulate physiological effects.

Normally, neurotransmitters [e.g., glutamate, GABA, serotonin (5-HT), dopamine (DA), acetylcholine (ACh), or norepinephrine] in the CNS are released presynaptically via neuronal stimulation, or by G protein-coupled receptors and voltage-gated ion channel calcium (Ca^{+2}) and potassium (K^{+}) influx.[50,52] However, the elevation in postsynaptic Ca^{+2} affected by neurotransmitters/receptors through the ion channels [e.g., ionotropic glutamate receptors, *N*-methyl-D-aspartate (NMDA), or GABA],[53] stimulates endocannabinoid (eCB) postsynaptic biosynthesis.[50,52,54]

There are two principal eCB ligands [2-arachidonoylglycerol (2-AG) and anandamide (AEA)], which are synthesized postsynaptically from arachidonic acid by *N*-acyl phosphatidylethanolamine phospholipase D and diacylglycerol lipase alpha/beta (DAGLα/β), respectively.[55–57] These eCBs are produced, as needed,[47] postsynaptically by Ca^{+2}-dependent transacyclase and other enzymes, then they migrate from postsynaptic neurons to the presynaptic CBR.[53,58] Signaling then occurs as CBR couples to the guanosine-5′-triphosphate ($G_{i/o}$)/α-protein subunit dimer[58,59] and binds adenyl cyclase to generate cyclic adenosine monophosphate. The cascade decreases presynaptic Ca^{+2} influx by blocking the activity of voltage-dependent N-, P/Q- and L-type Ca^{+2} channels[60,61] and activation of some K^{+} channels.[53,62] The retrograde eCB (AEA and 2-AG) transmitters in the brain presynaptically inhibit the release of the neurotransmitters GABA,[63,64] glutamate,[63,65,66] DA,[65,67,68] norepinephrine,[69] 5-HT[67,70] and ACh,[71,72] thereby decreasing the probability of neurotransmitter release. eCBs are then degraded by the serine hydrolase monoacylglycerol lipase (MAGL) in the presynaptic cell and fatty acid amide hydrolase (FAAH) located in the postsynaptic cell.[49,57,73]

Figure 1 compares the lipophilic structures of the eCBs (2-AG and AEA) with cannabinoids (e.g., $^{\Delta 9}$THC and CBD). $^{\Delta 9}$THC and CBD toxicity or neuroprotection depends on factors such as potency, exposure, duration/frequency, vehicle, route of administration, and species-specific differences. Pharmacokinetic and pharmacodynamic parameters determine the extent of P450 (CYP1A, 3A4, 2C9, and 2C19) metabolic activation and glucuronidation elimina-

Table 1. Brain regions and pathways affected by endocannabinoids, $^{\Delta 9}$-THC and/or CBD

Neurotransmitter/Pathway	Brain region associations	Behavior/processes involving eCBS	Reference
Dopamine: DA			
Mesolimbic	DA from ventral tegmental area (VTA; midbrain) → ventral striatum (amygdala, pyriform cortex, lateral septal nuclei, nucleus accumbens)	Reward-related cognition (e.g., incentive: wanting; pleasure: liking; positive reinforcement, associative learning) & emotion	[78,80,81,88–91]
Mesocortical	DA from VTA (midbrain) → prefrontal cortex + hippocampus	Cognition: executive function (e.g., planning, attention, working memory, planning, self-control, etc.), emotion	
Nigrostriatal	DA from substantia nigra (pars compacta; substantia nigra SNc: midbrain) → dorsal striatum (i.e., caudate nucleus + putamen)	Neuromotor function, reward-related cognition, associative learning	
Tuberoinfundibular	DA from the hypothalamic arcuate (infundibular) + paraventricular nucleus → pituitary gland median eminence	Inhibits the release of prolactin.	
Glutamate			
Glutamatergic	Hippocampus, neocortex and over 90% of synapses in human brain.	Excitatory effects on VTA & SNc neurons, memory, learning, neural communication	[53,90,92,93]
γ-Aminobutyric Acid: GABA			
GABAergic	Hippocampus, thalamus, basal ganglia, hypothalamus, brainstem[a]	Inhibitory effects on VTA and SNc neurons	[90,94–96]
Serotonin: 5HT			
Serotonergic	Dorsal raphe nuclei, cortex, hippocampus	Modulator of receptors with effects depending on subtype (i.e., biphasic effect on VTA neurons)	[80,84,85,97,98]

[a]GABAergic transmission includes inhibitory median spiny neurons in the striatum/basal ganglia affected by the glutamatergic (AMPAR) and dopaminergic (D1 and D2) receptor inputs from the VTA, SNc, and PFC.[87]

tion of $^{\Delta 9}$THC and CBD.[9,74] A tipping point leading to an adverse health effect would depend on an individual's ability to handle various exposure loads based on age, genetic makeup, health status, and diet, among other influences.[75,76] These risk factors are often difficult to characterize in humans, since hepatic metabolism studies are, by necessity, generally performed *in vitro*.[75]

$^{\Delta 9}$THC-associated mechanisms and neurotoxicity

To understand the effects of $^{\Delta 9}$THC on the brain, it is helpful to know which areas are affected. The eCBS/CBRs throughout the brain[77] help to regulate glutamatergic (excitatory), GABAergic (inhibitory),[78,79] dopaminergic, and serotonergic neurotransmitter release at presynaptic terminals.[80,81] The interactions among these systems are complex, occurring via direct and indirect stimulation, which may or may not be overseen by the eCBS to regulate neuroplasticity and excitability toward locomotor activity, cognition (learning and memory), executive functions, reward, motivation, and neuroendocrine control, among other functions.[78,80,82–86] The striatum in the basal ganglia contains inhibitory GABAergic medium spiny neurons that are affected by the glutamatergic (AMPAR) and dopaminergic (i.e., D1 and D2) receptor inputs from the ventral tegmental area (VTA), substantia nigra (SNc), and prefrontal cortex (PFC).[87]

Table 1 summarizes some of the main brain regions, pathways, and neurotransmitters involving the neuronal connections in the eCBS and affected by $^{\Delta 9}$THC.[53,78,80,81,84,85,87–98]

Cannabinoid signaling can be disrupted through agonistic activity of $^{\Delta 9}$THC at the CB1Rs throughout areas of the brain. This process leads to inhibition of accumulation of 2-AG and AEA in the brain.[73,99,100] While there are many other neuronal circuits associated with the eCBS, the ones mentioned above are most frequently associated with cannabis.

$^{\Delta 9}$THC-associated neurotoxicity in rodent and nonhuman primate models

$^{\Delta 9}$THC exposure throughout all life stages is associated with effects on behavior, cognition, locomotor activity, birth weight, learning, and other adverse effects.[101–104] Cannabis smoke was listed as a reproductive toxicant on 3 January 2020, under California's Proposition 65.[104] However, to control for the dose intake and other technical issues, many neurodevelopmental studies performed in animals used intravenous (i.v.) $^{\Delta 9}$THC administration. Although this is not a likely exposure scenario for humans, the immediate absorption by i.v. could be compared to pulmonary exposure by inhalation.[105,106] Subcutaneous (s.c.), oral (i.e., gavage), and intraperitoneal (i.p.) administration are more slowly absorbed and are subject to local metabolic processes prior to entering the blood stream.[107,108] Other considerations contributing to potential variabilities in evaluating the study results are as follows: 1) often only a single exposure dose was used, limiting potential observations of a dose–response relationship; 2) $^{\Delta 9}$THC dosing vehicles varied among studies; 3) different species/strains of rodent were

used; 4) different exposure scenarios were used; and 5) many different laboratories contributed to the list of studies.

Gestational exposure to $^{\Delta 9}$THC

The eCBS is involved in the earliest developmental stages, including fertilization, implantation, and neuronal progenitors in the brain, leading to migration, morphogenesis, and axonal guidance.[94,109,110] The effects of $^{\Delta 9}$THC on these processes can be seen in rodents' pulmonary exposure by inhalation.[105,106] Administration via a s.c., oral, or i.p. route is more slowly absorbed and is subject to local metabolic processes prior to entering the blood stream.[107,111] $^{\Delta 9}$THC has profound effects on CB1Rs in areas of the brain regulating GABA, 5-HT, glutamate neurotransmitters, and DA release, influencing, for example, the development of locomotor activity, cognition, learning, memory, and emotional regulation (Table 2).[11,29,34,112–141] Notably, the lowest doses of $^{\Delta 9}$THC (0.15 mg/kg/day) in the offspring of Long-Evans rats treated *in utero* affected preproenkephalin, an endogenous opioid precursor in the nucleus accumbens, amygdala, and striatum, in addition to showing evidence of decreased cognition and other behavioral effects.[112–114] Treatment *in utero* or from paternal exposure during a full cycle of sperm development, even at low $^{\Delta 9}$THC doses (0.15 mg/kg/day), resulted in developmental deficits and epigenetic transmission.[112,113,115–117] Male Wistar adult rats treated throughout sperm development (gavage, 2.0 mg/kg/day) had offspring with affected locomotor activity, feeding behavior, and visual operant signaling.[118] Moreover, epidemiological evidence supported findings that cannabis exposure during gestation or during male sperm development results in children with cognitive, motor, and behavioral (including severe psychoses) effects.[33,142–145] Infants with gestational exposure to cannabis may show an exaggerated startle response or an inability to adapt to novel stimuli.[146,147] Furthermore, women who used cannabis during pregnancy had an increase in fetal deaths, premature births, heart rhythm disorders, and fetal intrauterine growth restrictions.[36]

In support of the gestational exposure findings, a meta-analysis was performed on the behavioral effects in animal offspring exposed to $^{\Delta 9}$THC during gestation and lactation.[148] A compilation and meta-analysis of behavior in offspring from 15 selected studies in Long-Evans, Sprague-Dawley, or Wistar female and/or male rats exposed from mothers exposed via oral, i.v., or s.c. administration indicated significant effects on cognitive, locomotor, and emotional behavior.

Postnatal exposure to $^{\Delta 9}$THC

Postnatal exposures to young C57BL/6J male mouse pups resulted in behavioral effects from $^{\Delta 9}$THC treatment at 1.0 mg/kg/day administered s.c.[119] This and other studies performed in male and female Wistar rats at 2.0 mg/kg/day (s.c.)[11] or 10 mg/kg/day (gavage)[149] included effects on anxiety and neurodevelopmental deficits similar to those seen in autism, epilepsy, and schizophrenia.[11,119,149] Perinatal exposure in children would likely be from nursing, secondhand smoke, or accidental ingestion, causing long-term effects.[37,150] The transfer of cannabis in the milk to nursing babies was shown to affect DA receptors, resulting in hyperactivity, poor coordination, and cognitive function and leading to an increased risk of future drug abuse.[37,150] For example, GABA is primarily excitatory in early development and then it switches to inhibitory postnatally. Disruption of this process in humans may result in neurodevelopmental patterns affecting chronic pain, neuroplasticity, and psychiatric diseases (e.g., autism, epilepsy, and schizophrenia).[11,151] Data indicate that perinatal cannabis exposure increases the risk of future drug use.[152]

Adolescent exposure to $^{\Delta 9}$THC

Exposure to $^{\Delta 9}$THC i.p. in Long-Evans and Wistar male rats throughout adolescence at low doses (1 or 1.5 mg/kg/day) showed disrupted neural development in the PFC and hippocampus resulting in effects on neuroplasticity, cognition, social interactions, memory and others.[29,120] Similar effects (i.e., increased: CB1R density, anxiety, learning deficits, anhedonia) were observed at higher doses (2.5–10 mg/kg/day) in Long-Evans females, male and female Sprague-Dawley rats and male CD1 mice receiving various dosing regimens (Table 2).[120–123,153] $^{\Delta 9}$THC treatment in adolescents disrupted development of brain areas (e.g., PFC) associated with adverse behaviors like schizophrenia in humans, which often occurs in adolescence.[155] Adolescence is a stage of peak eCB (2AG and AEA) and CB1R expression.[156] The brain is still developing and is at heightened risk for disruption of normal neurodevelopmental processes.[157,158] Where pre- or postnatal exposures may be involuntary in developing young, adolescence is where preteens and teens may begin to experiment with cannabis on their own.[159] Vaping cannabis has become one of the most preferred methods of consumption, that will not only increase the concentration of $^{\Delta 9}$THC but also potentially increase exposure to residues of pesticides used on cannabis crops.[99,160–162] Cannabis use in adolescents greatly increases the risk of psychosis by 3–4-fold and has been shown to lower the age of schizophrenia onset.[163,164] Further, adolescent cannabis use will increase the probability of future drug use,[165] as shown by evidence from animal and epidemiological studies.[152,166,167]

Adult exposure to $^{\Delta 9}$THC

Acute adult effects in Long-Evans male rats as well as C57BL/6Arc and CD1 male mice showed behavioral effects (attention and learning, decreased anxiety and locomotor activity) at low $^{\Delta 9}$THC doses (i.p.: 0.25, 0.8, or 1.0 mg/kg/day; Table 2).[124–127] Notably, these studies used 2–8 treatment levels and could therefore establish a dose–response relationship. C57BL/6J male mice treated at 10 mg/kg/day also experienced a decreased thermic response and increased catalepsy and analgesia.[126] This study demonstrated the "cannabinoid tetrad": increased catalepsy, hypomobility, hypothermia, and antinociception.[128] At the low acute doses, the animals showed decreased anxiety; but at higher doses graduating from 1 to 3 to 10 mg/kg/day at 7-day intervals, the animals had increased anxiety measures with both acute and chronic exposures in male Wistar rats.[129] Human studies also showed that cannabis use versus nonuse was associated with an earlier onset of psychoses, death by suicide, depression, mania, anhedonia, cognitive deficits, and anxiety/paranoia as well as brain effects (decreases in glutamine, affected DA, and decreased hippocampal volume (systematic review).[168] This review also reported associated harmful effects of exposure on driving, stroke, pulmonary function, vision, and negative drug-drug interactions. With cannabis legalization, it is likely that there will be more health-related deficits and an increased need for public and clinical policy changes. Table 2 lists the lowest-observed-effect levels (LOELs) reported from each *in-vivo* study (mg/kg/day).

Nonhuman primate exposure to $^{\Delta 9}$THC

Studies in nonhuman primates have been performed in pregnant animals. Rhesus macaques were fed $^{\Delta 9}$THC in a cookie at 2.5 g/7 kg at gestation day (GD) 0–155.[169] There were decreases in the

Table 2. Neurotoxic and behavioral effects from $^{\Delta 9}$THC treatment during development in animal studies

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
Gestational treatment				
Long-Evans Dam: GD 5-PND 2; F1 fostered PND 2–21. Dose: i.v. 0.15 mg/kg/day. Vehicle: Tween 80/saline	F1 M/F Pups: PND 2 or PND 62, Adult	NAc: ↓striatal DRD2 mRNA expression; ↓DR2 receptor & binding sites; epigenetic regulation of DRD2 mRNA expression disrupted; affected DA receptor gene regulation. Significance: Increase in sensitivity to opiate reward in adulthood	0.15*	112
Long-Evans Dam: GD 5-PND 2 fostered PND 2-21. Dose: i.v. 0.15 mg/kg/day. Vehicle: Tween 80/saline	F1 M Pups PND 55, Adult	↓PENK mRNA expression NAc (pup), ↑PENK in NAc & amygdala (adults); ↑Self-administer heroin; ↓latency between active lever press; ↑active lever press; ↑responses on stress test; ↑total responses on active lever on 1st & last extinction days; ↓distance traveled during acquisition & maintenance. Significance: Increased opioid seeking behavior (motivation/reward) & stress response in adulthood	0.15*	114
Long-Evans Dam every 3rd day; PND 28–49; mated PND 64–68; F1 fostered. Dose: i.p. 1.5 mg/kg/day. Vehicle: saline/Tween 80	F1 M/F Pups: PND 35 (Adolescence) or PND 62 Adult	Striatal dysregulation of CB1R gene expression, affecting striatal plasticity; ventral to dorsal striatum disruptions between adolescence & adulthood; F ↓novelty seeking. Significance: Supports relevance to age-dependent vulnerability for neuropsychiatric disorders	1.5*	130
Long-Evans Dam every 3rd day PND 28–49; mated PND 64–68; F1 fostered. Dose: i.p. 1.5 mg/kg/day. Vehicle: saline/Tween 80	F1 M/F Pups: PND 35 (Adolescence) or PND 62, Adult	Epigenetic effects & altered CB1R mRNA expressions in NAc associated with glutamatergic system regulation; F ↓ locomotor activity. Significance: Cross-generational epigenetic vulnerability to drug abuse	1.5*	117
Wistar Dam: GD 15–PND 9. Dose: Gavage 3.0 mg/kg/day. Vehicle: sesame oil	F1 M Pup: PND 90, Adult	Disrupted hippocampal GABAergic system; ↓GABA outflow & uptake in hippocampus; ↓ CB1 binding; cognitive impairments. Significance: Long term cognitive deficiency & disrupted GABA neuronal development	5.0*	115
Wistar Dam: GD 5–14, 16, 18, 21 & PND 1 & 5. Dose: Gavage 5.0 mg/kg/day. Vehicle sesame oil	F1 M/F GD 14, 16, 18, 21 + PND 1 & 5 Neonate	Disrupted tyrosine hydroxylase gene activation (rate limiting in DA production); ↑ DOPACL DA metabolite forebrain. Significance: Tyrosine hydroxylase plays a large part in neurodevelopment through DA production	5.0*	131
Wistar Dam: GD 7–22. Dose: i.p. 3 mg/kg/day. Vehicle: Not stated	F1 M/F Behavior PND 70–100	M: ↓Time on light side of test box (↑anxiety); ↑transition to light; ↓Time in open arm of EPM; ↑VTA spike activity; ↓DA & NMDAR2B PND 21; ↑GAD87 PND 21; F: ↑GAD67, vGLUT1-2; PPARα & PPARY1-2 & NMDAR2B in the mesolimbic system (VTA-NAc); M/F: ↑Altered fatty acid concentrations in the nucleus accumbens core & shell up to PND 120 (M) or PND 21 (F). Significance: Sex difference with M more affected than F; Fatty acid deficits disrupt the DA/GLUT/GABAergic neurotransmissions affecting neurodevelopment	3.0*	132
SD Dam: GD 5–PND 2 foster-nursed PND 2–21. Dose: i.v. 0.15 mg/kg/day. Vehicle: Tween80/saline	F1 M/F Pups: PND 22, 45 & 60 Weaning, adolescent, adult	Pup: ↓anxiety: ↓active place avoidance acquisition; ↑active place avoidance reversal phase entries; Adult: ↓attention (acquisition, reversal & distraction) & cognition. Significance: Decreased anxiety, attention & cognitive function	0.15*	113

(continued)

Table 2. *(continued)*

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
SD Dam: Group 1: GD 5–20. Group 2: GD 5–20 + PND 15. Dose: s.c. 2.0 mg/kg/day; PND 15 2.5 mg/kg/day. Vehicle: Tween80/saline	F F1 Pups: Groups 1 & 2: PND 15–28 Juvenile	Group 1 & 2: Male behaviors affected: ↑distance traveled; ↓stretch-attend postures; Group 2: ↓ latency in passive avoidance training; ↑AMPA from DA cells; ↓stretch-attend postures; ↓DA 240 min postacute dose. Significance: Behavioral effects from mesolimbic (NAc) dopaminergic disruptions are greater in males & greater after $^{\Delta 9}$THC challenge	2.0*	1331
SD Dam: GD 5–GD 20. Dose: s.c. 2.0 mg/kg/day. Vehicle Tween 80/saline	F1 M/F Pups: Tests done PND 24–28, Juvenile	VTA DA neuron effects: ↑ firing rate; ↓ cells/track; ↓ spikes/burst, burst rate; ↓after hyperpolarization period; ↑DRD2 sensitivity & acute stress vulnerability; ↑ activity, ↓ PPI average in acute restraint & forced swim test. Significance: Sensorimotor gating deficits leading to an increase in susceptivity to stimuli triggering psychotic-like behaviors	2.0*	134
SD M Adult 28 days; mated 2 days post dose. Dose: s.c. 2.0, 4.0 mg/kg/day. Vehicle: Tween 80/saline	F1 M Pups: PND 30, 60, 100 & 150 Adolescent, adult	↓ACh activity; ↑ ChAT: ACh biomarker for number of ACh terminals in striatum; ↓ ChAT hippocampus; ↓HC3/ChAT (ACh activity index) n frontal/parietal cortex & striatum. Significance: Paternal $^{\Delta 9}$THC leads to disruptions in developmental trajectory of ACh potentially affecting attention	2.0	116
Wild-type Mouse Dam: GD 12.5–16.5. Dose: i.p. 3.0 mg/kg/day. Vehicle: saline/DMSO/Tween 80	F1 M/F Pups: PND 20; 2 months; Juvenile, adult	CB1R →affected cortical neuron synaptic signaling development →affected connectivity in cortical GABAergic & glutamatergic systems → ↓fine motor skills; ↓ skilled motor function; 2 months: ↓ success in pellet retrieved in skilled steps test; ↑seizure. Significance: Disrupted CB1 signaling leading to disrupted glutamate & GABA signaling leads to increased susceptibility to seizures and cortico-spinal function.in adulthood	3.0*	135
C57Bl/6 Mouse Dam: GD 14.5–18.5. Dose: i.p. 3.0 mg/kg/day. Vehicle: DMSO	F1 M/F: GD 18.5; PND 10 & 120, Fetal, pup, adult	↓ CB1R & misrouted hippocampal CB1R afferents, ↑CB1R density in striatum; Impaired LTD in pyramidal cell synapsis; ↓ synaptic plasticity in the cortical circuitry; Impaired cortical axonal development; ↓2-AG signaling, ↓ CB1R & ↑ MAGL expression, ↓DAGL; abnormal growth cones & cytoskeleton in axonal region. Significance: Abnormal axonal development in growth cone disrupts neuronal circuitry, memory encoding, cognition & executive skills	3.0*	136
Postnatal Treatment				
Wistar Dam: PND 1–10. Dose: s.c.2.0 mg/kg/day. Vehicle: DMSO/cremophore/saline	F1 M/F Pups: PND 10, 15, 20; 9–21 Preweaning, juvenile	↓Bodyweight gain; GABA excitatory to inhibitory switch in PFC (eCB disruption); ↓upregulation & expression of KCC2 (K+ transporter), Vocalizations ↑in frequency (kHZ). Significance: Delayed development of GABA switch leads to sensorimotor gating deficits, potential autism, epilepsies, schizophrenia-like behavior.	2*	11
Wistar M Adult: 12 days mated to untreated F. Dose: Gavage 2.0 mg/kg/day. Vehicle: EtOH/TritonX100/saline	F1 M/F Pups: PND 28–140, Adolescent, adult	↑Habituation of locomotor activity, Novelty suppressed feeding: ↓latency to begin eating; ↓Visual operant signal. Significance: Impaired operant attention into adulthood	2*	118
SD Juvenile M/F: PND 10–16. Dose: Gavage 10 mg/kg/day. Vehicle: corn oil	F1 M/F Pups: PND 29 & 38, Adolescent	↓Bodyweight gain; High Illumination: ↑entries & time in open arm; Low Illumination: ↑stretch attend posture; ↑head dips; ↓ exploration, ↑frequency of nape attacks; ↑time & frequency play fighting. Significance: Altered social behavior in adolescence.	10*	137

(continued)

Table 2. *(continued)*

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
C57BL/6J Mice M Pup: PND 5–16 & 5–35. Dose: s.c. 1.0, 5.0 mg/kg/day. Vehicle not stated	F1 M Pup: PND 16 or PND 35 Preweaning, adolescent	Hippocampal cell rearranged CB1R; changes key molecular constituents of mitochondrial respiratory chain; Thinning of pyramidal cell layer; Neurochemical deficits Significance: Developmental deficits from neuronal disorganization, misrouted differentiation & associated pathologies.	1.0	119
Adolescent Treatment				
Long-Evans M PND 28 each 3rd day to PND 50. Dose: i.p. 1.5 mg/kg/day. Vehicle: saline/H_2O/Tween80	M: PND 50 or PND 63, Adolescent, adult	Adolescent: Disrupted development of dendritic arbors PFC (pyramidal neurons); Adult: prolonged atrophy in distal apical arbors of PFC neurons; Prematurely pruned dendritic spines attenuated neuroplasticity. Significance: Disrupted PFC neural networks lead to decreased cognitive & emotional dysregulation & affected decision making similar to pathology in human schizophrenia	1.5*	29
Long-Evans F PND 35–75. Dose: i.p. 5.6 mg/kg/day. Vehicle: saline	F: PND 75–160 & 159 to 200 Adult	Adult: ↑CB1R density; Persistent impairment of working memory & task performance. Significance: Long term effects on operant learning	5.6	123
Long-Evans M/F "Puberty Onset" for 14 days. Dose: i.p. 5 mg/kg/day. Vehicle: EtOH/Cremophor/saline	M/F: Day 14 treatment	M/F combined: ↓Total attacks, total pins, percent defense & complete rotation.	5.0 (only dose)	138
Wistar M i.p. 1.0 mg/kg/day PND 28–30 →5.0 mg/kg/day alternate days PND 34–52 or PND 60–62 →5.0 mg/kg/day alternate days PND 66-84 or Acute: 5 mg/kg/day: PND 52. Vehicle: Tween80/saline	M: PND 52, 55, 67, 70, 71, 72, 84, 87, 99, 102, 103, 104, Adolescent, adult	Adolescent: ↑Latency to emerge; ↓time in open areas; ↓rearings; ↓novel object preference; ↑memory deficits; alterations in hippocampal structure/function remaining to adulthood. Significance: Hippocampal alterations lead to persistent memory deficits that developed in adolescence	1.0	120
SD M/F PND 35–37; 5; 38–41; 10; 42–45. Dose: i.p. 2.5 mg/kg/day, twice/day. Vehicle: EtOH/cremophor/saline	M/F: PND 75: Adult	Adult: ↓ Bodyweight & food intake; ↓CB1R binding & stimulation (NAc, amygdala, VTA, hippocampus); ↓sucrose preference (anhedonia); ↓CREB activation in prefrontal cortex, NAc, hippocampus; ↑ dynorphin (indicates depression). Significance: Disruption of neural circuitry related to emotion and depression during adolescence	5.0	34
SD M PND 35–37; 5; 38–41; 10; 42–45. Dose: i.p. 2.5 mg/kg/day, twice/day. Vehicle: EtOH/cremophor/saline	M: PND 75 Adult	Adult: ↓Radial maze learning; ↓dendritic length in hippocampal dentate gyrus; ↓spine density; ↓NMDA receptors & biomarkers indicating ↓neuroplasticity. Significance: Spatial memory & cognitive deficits	5.0	122
CD1 Mice M PND 28–48; 69–89. Dose: i.p. 3.0 mg/kg/day. Vehicle EtOH/chermophor/saline	PND 49–53 & PND 90–94 Adolescent. PND 90–94 & PND 131–135 Adult	Adolescent: Impaired object recognition/working memory (novel object recognition & discrimination); repetitive/compulsive behaviors (↑percent shredded in nestlet; ↑marble burying); ↓delayed anxiety to move out of the dark; Adult: ↓novel object recognition performance; elevated plus maze ↓ anxiety to venture out. Significance: Behaviors common to those seen in animal schizophrenia models & humans	3.0*	121
Adult treatment				

(continued)

Table 2. *(continued)*

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
Long-Evans M. Dose: Acute i.p. 1.0, 1.5, 2.0 mg/kg. Vehicle: detergent/EtOH/saline	~15 min time increments postdose	↓Attention; ↓hippocampal functional cell types. Significance: Information not likely to be encoded correctly & unlikely to be accurately retrieved or recalled	0.5	127
Long-Evans M. Dose: Acute i.p. 0.01, 1.0 mg/kg. Vehicle: Tween80/saline	30 min postdose	↑Trials to achieving reversal task between stimulus & reward; affects c-fos expression associated with negative behavioral effects (orbital limbic & striatal regions in brain). Significance: Effects in orbitofrontal cortex & striatum (potential inelasticity) leading to an inability to perform reversal discriminations	1.0	124
Wistar M: 5 days. Dose: i.p. 2.0, 4.0 mg/kg/day. Vehicle: Tween 80/saline	30 min postdose	↓Short-term memory & discrimination affected by eCB increase at the CB1R. Significance: Disrupted CB1Rs is detrimental to memory & cognition	2.0	139
Wistar M: i.p. 7 days per dose. Dose: i.p. 1.0, 3.0, 10 mg/kg/day. Vehicle: EtOH/Tween 80/saline	20 min postdose	↓ Body weight; Anxiety measures: ↓time spent in emergence test; ↑ hide time; ↓ open field time; ↓percent open arm time; ↓active time; ↓total social interaction time & distance traveled; Place conditioning: ↓preference for the conditioned side; ↓CB1 R binding in hippocampus, substantia nigra, caudate putamen, cigulate gyrus. Significance: Affected anxiety, learning, memory & social interaction due to disruptions in CB1R binding in critical brain regions	1.0, 3.0, 10	129
SD M 2 times/day for 14 days. Dose: i.p. 5.0 mg/kg twice per day. Vehicle: Tween 80/saline	Post terminal dose	↓Performance attention, executive functions, memory, cognition associated with ↓DA in PFC. Significance: Disruption of the cortical dopaminergic pathways lead to cognitive & attention dysfunction	20	140
SD M: Acute (1 treatment). Dose: i.p. 5.0 mg/kg. Vehicle: OH-β-cyclodextrin/saline	30 min postdose	↑Working memory impairments; ↑DA turnover (DOPAC/DA); ↑NE turnover PFC. Significance: Cognitive impairment	5.0*	141
C57BL/6JArc mice M: 1 or 21 days. Acute & Chronic Doses: i.p. 0.3, 1.0, 3.0, 10 mg/kg. Vehicle: EtOH/Tween 80/saline	Acute & chronic 60 min postdose	Acute & chronic: ↑analgesia & catalepsy; ↓ thermic response & locomotor activity; Anxiety: ↓ distance traveled light/dark; ↓ frequency of entries in elevated + maze; ↓ vertical activity, rearing & head dipping; ↓startle response; ↓passive avoidance/anogenital sniffing, social interaction; ↑latency passive avoidance; ↑prepulse inhibition. Significance: Effect on neurotoxicity (anxiety) occurs after both acute & chronic exposure	1.0	126
CD1 mice M. Dose: Acute i.p. 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12, 48 mg/kg/day. Vehicle: EtOH/CremophorEL/saline	30 min postdose	↑Percent time in the open arm in the elevated plus maze; ↓anxiety; ↑percent swim time; ↑closed arm entries. Significance: ↓Anxiety & depression behaviors	0.8	125

ACh, acetylcholine; 2-AG, 2-arachidonoylglycerol; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CB1, cannabinoid 1 receptor; CHaT, choline acetyltransferase; CREB, cyclic adenosine monophosphate response element-binding; DA, dopamine; DAGL, diacylglycerol lipase; DOPAC, L-3,4-dihydroxyphenylacetic acid; DRD, dopamine receptor; eCB, endocannabinoid; F, female; GABA, gamma-amino butyric acid; GD, gestation day; GAD67, glutamic acid decarboxylase 67; GLUT, glutamate; H3C, hemicholinium-3; i.p., intraperitoneal; i.v., intravenous; LOEL, lowest-observed-effect level; LTD, long-term depression; M, male; MAGL, monoacylglycerol lipase; NAc, nucleus accumbens; NE, norepinephrine; PENK, preproenkephalin; PFC, prefrontal cortex; PND, postnatal day; PPI, prepulse interval; s.c., subcutaneous; SD, Sprague-Dawley; VTA, ventral tegmental area; ↓, decrease; ↑, increase; →, leads to; *, only one dose was used in the study.

amniotic fluid volume throughout pregnancy and decreased placental perfusion (oxygen availability decreases) accompanied by increased placental microinfarctions. In addition, there were significant changes in the RNA signature sequences in the placental transcriptome. These data indicate that disruptions in vascular development and angiogenesis affect the offspring through decreased testes weights and relative heart weights. Adult male rhesus macaques were treated with $^{\Delta 9}$THC in a cookie at 0.5 mg/7 kg/day (1–70 days), 1.0 mg/7 kg/day (71–140 days), and 2.5 mg/7 kg/day (141–210 days). At 210 days, there were dose-related decreases in testicular and epididymal weights.[170] Follicle-stimulating hormone, luteinizing hormone, and prolactin were increased, and total testosterone and estradiol were decreased. These effects indicate potential disruption of the hypothalamus–pituitary–gonadotropin axis, impacting testicular function.[171] In another study, adult female rhesus macaques were treated with $^{\Delta 9}$THC in a cookie at 0.5 mg/7 kg/day (1–3 weeks), 1.0 mg/7 kg/day (4–6 weeks), 2.0 mg/7 kg/day (7–9 weeks), and 2.5 mg/7 kg/day (10–12 weeks). At 12 weeks, the animals showed increases in menstrual cycle length and increased follicle-stimulating hormone concentrations, another indication of hypothalamus–pituitary–gonadotropin axis disruption.[171] The disruptions in hormonal balance, menstrual cycle, and ovulatory function would likely affect fecundity.[172]

$^{\Delta 9}$THC-associated effects in humans

A review by Frau and Melis[173] provides evidence showing that *in utero*, transplacental $^{\Delta 9}$THC exposure deregulates the mesolimbic dopaminergic system in males, potentially predisposing them to schizophrenia. Prenatal exposure in humans can act to prime the sensorimotor gating development in the brain, primarily in the VTA region associated with the dopaminergic system. Subsequent environmental exposures such as $^{\Delta 9}$THC or other stressors can lower the threshold to initiation of psychotic-like effects.[134] In addition, $^{\Delta 9}$THC exposure to infants during breastfeeding can continue more than 6 weeks after the last maternal consumption, potentially affecting brain development.[9,38,142,174] Monfort, Ferreira, Leclair, and Lodygensky[22] have described the pharmacokinetics of cannabinoid exposures during pregnancy, in infants, and during breastfeeding. While consumption may be due to depression, anxiety, nausea, or pain, data indicate that there are significant irreversible risks to neuronal development in fetuses, neonates, and the developing young.[22] Data also support the increased risks of dysregulated glucose-insulin measurements as well as obesity in children after maternal use of cannabis during pregnancy.[175]

Although $^{\Delta 9}$THC (cannabis) is not federally legal in the United States, acute and repeated human exposure to $^{\Delta 9}$THC is regulated by the European Food Safety Authority.[176] Human data were used by this agency to establish a lowest-observed-adverse-effect level (LOAEL) for an administered $^{\Delta 9}$THC exposure of 2.5 mg/kg/day (corresponding to an internal dose of 0.036 mg/kg/day). Applying an uncertainty factor of 3 to extrapolate from a LOAEL to a no-observed-adverse-effect level (NOAEL) and 10 for intraspecies differences produced 1 µg/kg/day (acute reference dose: ARfD = [0.036 mg/kg/day ÷ 30] = 1 µg/kg/day). However, it is evident from gestational treatment in Table 2 that offspring experienced neurodevelopmental effects related to motivation/reward, stress response, and increased sensitivity to opiate reward in adulthood at 0.15 mg/kg/day.[112,114] Establishing an ARfD would require the same uncertainty factors in addition to an interspecies default of 10 ([LOAEL 0.15 mg/kg/day ÷ 3 = NOAEL 0.05 mg/kg/day] ÷ [10 interspecies × 10 intraspecies]) = 0.5 µg/kg/day.[177–179] Gestational exposure to $^{\Delta 9}$THC may need a different ARfD than that of adults, since effects occur at very low doses. This is especially critical to re-evaluate because the low-dose animal studies used only one dose, and there were no doses below 0.15 mg/kg/day in which effects might also be seen in developing fetuses.

CBD-associated mechanisms

While it can make up as much as 40% of cannabis extract,[180] CBD has been purified in products for use by people and even their pets. CBD is one of the most actively studied therapies for a broad spectrum of neurological, inflammatory, and mental diseases because of its efficacy, low toxicity, and availability (e.g., over the counter and online order). The exact mechanism for the therapeutic effects is still under investigation,[42,181] but the proposed MOA for CBD indicates several targets associated with neuroprotection (Fig. 2[182] and Table 3).[86,183,184] Like $^{\Delta 9}$THC, CBD has effects on many interacting targets, and there is evidence for direct and indirect CBD actions on inflammatory and neurological parameters.[180]

Glial cells

CNS connective tissue (e.g., macroglia: astrocytes and microglia) consists of nonneuronal cells that link neuronal cells to the blood supply (blood–brain barrier), regulate blood flow to the brain, and regulate neurotransmission (macroglia) or serve as macrophages to mount immune responses in the brain (microglia).[185] When neuronal injury occurs, astrocytes can signal microglia to initiate an immune response; however, when the immune response becomes unbalanced, neuronal injury will occur.[181] CBD can decrease the microglial immune response to injured dopaminergic neurons in diseases like Parkinson's disease, and it increases the recruitment of astrocytes to promote neuronal regeneration through brain-derived neurotrophic factor (BDNF) (Table 3).

Adenosine receptor 2A ($A_{2A}R$)

Adenosine acts at a G protein-coupled receptor ($A_{2A}R$) on neuronal membranes to suppress immune responses due to inflammation or cell stress. CBD serves as an agonist to decrease adenosine reuptake, thereby increasing adenosine signaling and decreasing neuroinflammation.[186,187] CBD exposure decreases proinflammatory cytokine interleukin (IL)1β, microglial activity, tumor necrosis factor-alpha (TNFα), cyclooxygenase-2 (COX2), and inducible nitric oxide synthase (iNOS) activity in the brain (Table 3). These pathways have been shown to improve the effects of multiple sclerosis, hypoxic-ischemic brain damage, Alzheimer's disease, and hepatic encephalopathy.[183]

5-HT receptors

The dorsal raphe nucleus (DRN) is the primary serotonergic center (5-HT) in the brain where GCPR 5-HT_{1A} receptors are expressed. Receptor stimulation inhibits voltage-gated Ca^{2+} channels, activates K^+ channels, and inhibits neurotransmission in the DRN.[44,188] CBD has an anxiolytic effect by acting through 5-HT_{1A} receptor in male Wistar rats, previously stressed by foot shocks or restraint, but it can also induce anxiogenic behaviors in rats experiencing contextual fear conditioning,[182–190] perhaps by serving as an agonist at the 5-HT_{1A} receptor.[188] Acting on the serotonergic system, CBD is associated with improved locomotor activity (after striatal damage), cognition, cerebral ischemia, seizure disorders, and hepatic encephalopathy (Table 3).[183] Through the 5-HT1A receptor, CBD is associated with antiepileptic, anticataleptic, neu-

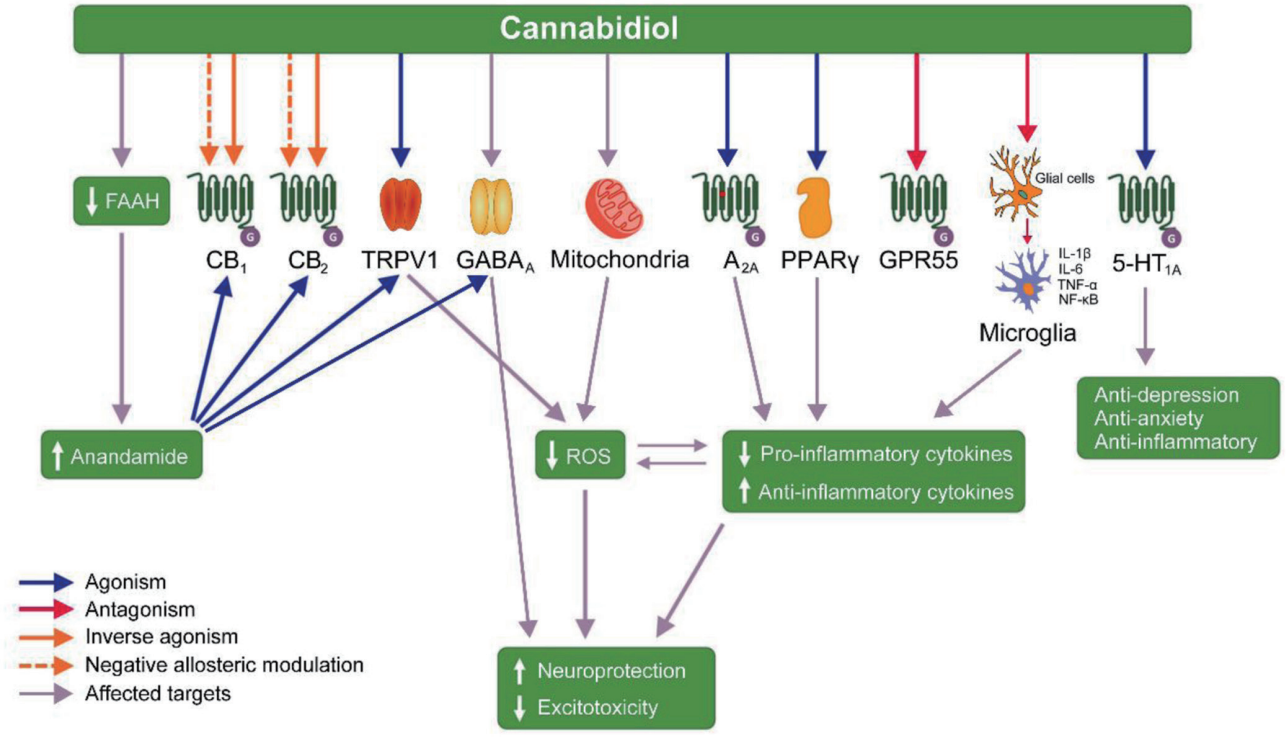

Fig. 2. The cannabidiol (CBD) mechanism of action includes: (1) agonistic activity toward the transient receptor potential vanilloid type 1 (TRPV1), the peroxisome proliferator activated receptor γ (PPARγ), and the serotonin$_{1A}$ (5-HT$_{1A}$) receptor; (2) antagonist activity at the G-protein coupled receptor GPR55; (3) antagonist to CB1 and CB2Rs in addition to acting as a reverse agonist and negative allosteric modulator; (4) antagonist of FAAH leading to increased anandamide (AEA), which goes on to activate the CB1, CB2, and TRPV1 receptors.; (5) direct action on the GABA$_A$ receptor (also influenced by AEA), leading to neuroprotection; (6) increased mitochondrial activity leading to antioxidant and anti-inflammatory action. Overall CBD has anti-depressant, anti-anxiety, and anti-inflammatory effects. Figure adapted with permission: Copyright © 2018.[182] FAAH, fatty acid amide hydrolase; GABA, gamma-aminobutyric acid; GPR55, G protein-coupled receptor 55; ROS, reactive oxygen species.

roprotective, antiemetic, anxiolytic, antidepressant, antipsychotic, and analgesic effects.[86,191–195] Others have also indicated that CBD acts via a negative allosteric mechanism in DRN somatodendritic 5-HT$_{1A}$ receptors that does not require CB1, 5-HT$_{2A}$, or GABA$_A$ receptors.[86,186]

CB1Rs and CB2Rs

CBD at the CB1Rs regulate excitotoxicity by inhibiting glutamate release to the NMDA receptors and normalizing glutamatergic activity. CBD acts to increase the blood supply to areas after ischemic incidents by decreasing endothelial-derived endothelin-1 or nitric oxide to increase vasoconstriction.[197] Neurodegeneration occurs with activation of microglial cells (immune cells in the brain); however, CB1R activation by CBD leads to a decrease of TNFα and IL12 and an increase of IL10. Activation of CB2 then decreases the proliferation and migration of microglial cells while decreasing TNFα by inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB; Table 3).[198,199] The anti-inflammatory action of CBD has been shown to improve neuronal damage from ischemic stroke, Tardive dyskinesia, and Parkinson's disease.

FAAH

CBD can act indirectly at the CB1R through inhibition of FAAH and the AEA transporter, leading to increased AEA and activation of CB1R.[200,201] Increased CB1R agonism leads to decreased eCB degradation and transport (Table 3).

TRPV1

TRPV consists of a vanilloid channel on the plasma membrane, considered by some to be a CB3R,[51] that induces neuropeptide release associated with pain perception, neuroinflammation, and body temperature regulation.[200] CBD at TRPV-1 channels leads to increased Ca^{2+} levels, resulting in desensitization and subsequent decreased pain. TRPV1 binding decreases microglial activation and migration as well as oxidative stress (Table 3). In addition, CBD can increase AEA levels by inhibition of FAAH.[202] However, AEA and CBD are both TRPV1 channel agonists. TRPV1 channel activation by CBD presynaptically increases glutamate release in the brain, which may serve to counteract/antagonize the inhibitory action of CB1R binding by CBD on colocalized glutamatergic neurons. TRPV1 activation by CBD agonism can increase the PI3K/Akt pathway signaling to decrease the incidence of hallmarks of Alzheimer's disease.

G-coupled protein receptor 55 (GPR55)

GPR55 binding protects against excitotoxicity potentially through GABA$_A$ receptor. CBD, as an antagonist, decreases GPR55 activation in the CNS to regulate such processes as neuropathic pain and antiepileptic activity.[203] CBD has a high affinity for GPR55, resulting in a decreased glutamate release in the hippocampus, thus causing anti-convulsive effects, also seen in human subjects.[180] Moreover, the use of CBD has been shown to result in improved Parkinson's disease and Dravet syndrome (DS) symptoms (Table 3).[183,204]

Table 3. ***In-vivo*** **and** ***in-vitro*** **examples of neuroprotective effects of CBD in different neurological diseases[183]**

Model	CBD dose	Treatment	Biological/pharmacological effect	Neurological disease
Neuroprotection through activation of A_{2A}Rs				
SJL/J mice: F	5.0 mg/kg, i.p.	Days 1–7 post infection	Microglia activation attenuated, downregulating the expression of VCAM1, CCL2 and CCL5 & proinflammatory cytokine IL1β. CBD improved motor deficits in the chronic phase of the disease	Multiple sclerosis
Newborn C57BL6 mice: M/F	0.1–1,000 µM	15 min pre-incubation	↓Acute brain damage & apoptosis; ↓glutamate concentration, IL6 & expression of TNFα, COX2, and iNOS	Hypoxic-ischemic brain damage
Primary rat microglial & N13 microglial cells & C57Bl/6 mice: M/F	20 mg/kg, i.v.	1/day for 7 days; 3 days/ week for 2 weeks	Inhibited ATP-induced intracellular Ca^{2+} increase in cultured N13 & primary microglial cells and A_{2A} receptors may be involved in this mechanism. *In vivo:* ↓gene expression of proinflammatory cytokine IL6 & prevented cognitive impairment induced by βA	Alzheimer's disease
Sabra mice: F	5.0 mg/kg, i.p.	28 days	↓Hippocampal TNFα-R 1 gene expression but ↑expression of the BDNF gene. Indirect activation of A_{2A}R, ↑cognitive & motor function in rats with hepatic encephalopathy.	Hepatic encephalopathy
Neuroprotection through the activation of the 5-HT_{1A}				
MCA occlusion mice: M	3.0 or 10 mg/kg, i.p.	Before & 3 h after damage	CBD significantly ↓infarct volume induced by MCA occlusion through 5-HT_{1A} receptor	Cerebral ischemia
Swiss mice: M	5.0, 15, 30, or 60 mg/kg, i.p.	30 min before receiving drugs to induce catalepsy	CBD pretreatment ↓catalepsy in a dose-dependent manner, through the 5-HT_{1A}R	Striatal disorders
Swiss mice: M	15–60 mg/kg or 60 nmol, i.p.	30 min before or 2.5 h after receiving the drugs to induce catalepsy	CBD pretreatment ↓catalepsy in a dose-dependent manner, through the 5-HT_{1A}R	Striatal disorders
Wistar Kyoto rats: M	100 mg/kg	60 min before seizure induction	CBD significantly mitigated PTZ-induced seizure	Seizure disorders
Adult Wistar rats: M	0.1–1.0 mg/kg & 5.0 mg/kg, i.p.	Acute treatment + cumulative injections every 5 min & repeated at 5 mg/kg/day for 7 days	CBD protected nerve injury-induced deficits in dorsal raphe nucleus 5-HT neuronal activity & exerted antiallodynic effects by TRPV1 activation & anxiolytic properties through 5-HT_{1A} receptor activation	Allodynia & anxiety
Sabra mice: F	5.0 mg/kg, i.p.	28 days	CBD, by 5-HT_{1A}R activation, ↑cognition & motor function, impaired by bile-duct ligation. CBD ↓neuroinflammation, ↑BDNF gene expression & ↓TNFαR 1 gene expression in hepatic encephalopathy model	Hepatic Encephalopathy
Sabra mice: F	5.0 mg/kg, i.p.	Single acute dose	CBD ameliorated cognitive deficits & locomotor activity; restored brain 5-HT levels & improved liver function	Hepatic Encephalopathy
C57BL/6J mice: M	30 mg/kg/day, i.p.	7 days	↑Time spent interacting; ↓psychotic-like behaviors acting through 5-HT 1A receptors	Schizophrenia
Neuroprotection by antagonistic activation of GPR55				
Scn1a mutant mice (DS model): M/F	10, 20, 100, or 200 mg/kg/day	Twice/day for 7 days	Acute CBD ↓thermally induced seizures & ↓spontaneous seizure rate. Low doses ameliorated autism-type social interaction deficits in genetically induced DS model, ↑GABA inhibitory transmission impaired in DS mediated by GPR55	DS

(continued)

Table 3. *(continued)*

Model	CBD dose	Treatment	Biological/pharmacological effect	Neurological disease
Adult C57BL/6 mice: M	5.0 mg/kg	5 days/week, 5 weeks	↓Density of microglial cells in the cell body. In the haloperidol-induced catalepsy model, through GPR55-activation.	Parkinson's disease
C67BL/6 mice M/F	5.0–10 & 50 mg/kg	Increasing doses from 5.0 to 10 mg/kg 3 times/ week, or daily, at 50 mg/kg, for 23 days	EAE disease ameliorated (all doses), ↓encephalitogenic cell vitality, ↓levels of IL6, production of ROS, ↓apoptosis & GPR55R in CNS	EAE disease
Neuroprotection through activation of the TRPV receptors				
Wistar rat: M	10 mg/kg, i.p.	2 h after the induction of model	CBD inhibited carrageenan-induced hyperalgesia by desensitization of TRPV1R	Hyperalgesia
hPBMECs & hCMEC/ D3 Cells[a]	0.1, 0.3, 1.0, 3.0, 10, 15 μM	7 or 24 h of incubation	Dose-related ↑in intracellular Ca^{2+} through activation of TRPV2 enhanced cell proliferation, cell migration & tubulogenesis in human brain endothelial cells.	—
U87MG Human glioblastoma cell line	10 μM	Cells treated with different CBD doses 1 day or co-treated with CBD 10 μM & chemo drugs 6 h	TRPV2 activation & ↑Ca^{2+} improved chemotherapy drug action by enhancing absorption & ameliorating cytotoxic activity in human glioma cells	—
Human gingival mesenchymal stem cells	5 μM	24-h incubation	TRPV1 desensitization promoted the PI3K/Akt pathway[b] signaling, which can reduce Alzheimer hallmarks	Alzheimer's disease
Neuroprotection through the activation of the PPARγ				
SH-SY5Y^{APP+} cells	10^{-9}–10^{-6} M	24 h	↓Expression of amyloid precursor protein & its ubiquitination, leading to ↓Aβ & neuronal apoptosis. Effects mediated by PPARγ activation	Alzheimer's disease
Primary rat astrocytes & SD rat: M	10^{-9}–10^{-7} M: *in vitro;* 10 mg/ kg: *in vivo*, i.p.	15 days	*In vitro:* Dose response ↓ in Aβ mediated through inhibition of NF-κB; Aβ-induced neuronal damage led to ↓gliosis & glial fibrillary acidic protein. Effects exerted through PPARγ activation	Alzheimer's disease
Hippocampal slices from C57Bl/6 mice	10 μM	30 min before addition of Aβ	Improved synaptic transmission & long-term potentiation in the hippocampus slice of C57BL/6 mice, protecting it from cognitive deficits induced by Aβ 1–42. CBD effects exerted through interaction with PPARγ	Alzheimer's disease
Newborn C57/ BL6 & Swiss mice primary microglial cultures: M/F	60 mg/kg: *in vivo,* i.p.; 10 μM: *in vitro*	2 injections/day 30 min prior to haloperidol: 21 days	Dyskinesia prevented after induction haloperidol. ↓Oxidative stress in corpus striatum, ↓activation of microglial, inflammatory cytokine (e.g., IL1β and TNFα), ↑anti-inflammatory cytokine IL10. CBD affects PPARγ actions on lipopolysaccharide-stimulated microglial cells	Tardive dyskinesia
Adult C57/BL6 mice: M	15, 30, or 60 mg/kg, i.p.	15 min before L-DOPA administration for 3 days	CBD did not prevent L-DOPA-induced dyskinesia. Cotreatment of CBD + capsazepine, acting through CB1R & PPARγ, ameliorated dyskinesia.	Parkinson's disease

(continued)

Table 3. *(continued)*

Model	CBD dose	Treatment	Biological/pharmacological effect	Neurological disease
Human brain microvascular endothelial cell/human astrocyte co-cultures	100 nM, 1.0 & 10 µM	Before or directly after induction of ischemic damage	10 µM prevented enhanced BBB permeability after ischemic damage induced by oxygen-glucose deprivation, through by activating PPARɣ & $5HT_{1A}R$.	Ischemic stroke
Neuroprotection through positive allosteric modulation of $GABA_A$ receptors				
Surgical human DS & TSC cortical tissue in Xenopus oocytes	5.0 µM	Pre-incubation of cells 10 s before co-application of GABA & CBD	Positive modulation of $GABA_AR$, ↑amplitude of GABA-evoked current in brain tissues of patients with DS &TSC	DS & TSC
Scn1a+/- mice (M/F) & Xenopus oocytes expressing $GABA_A$ receptors	*in vivo* 12 or 100 mg/kg, i.p.; *in vitro* 10 µM	*In vivo*: CBD administered i.p. 45 min before CLB. *In vitro*: CBD (10 µM) co-applied with GABA, for 60 s	↑CLB concentration & active metabolite N-CLB in plasma & brain. Co-administration ↑anticonvulsant effect by enhancing the activity of the $GABA_A$ receptor	DS

[a]hCMEC/D3 cells: Human hematopoietic stem-cell-derived cells (HBLECs) and human primary brain microvascular endothelial cells (hPBMECs) used in BBB models. [b]PI3K/Akt pathway: Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt kinase phosphorylation involved in the cell cycle is decreased in the Alzheimer's brain (associated with amyloid-β and tau pathologies).[184] βA, β-amyloid; A2AR, adenosine 2A receptors; ATP, adenosine triphosphate; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CBD, cannabidiol; CB2R, cannabinoid receptors type 2; CCL-2, chemokine ligand 2; CCL-5, chemokine ligand 5; CLB, clobazam; N-CLB, N-desmethylclobazam; CNS, central nervous system; COX2, cyclooxygenase-2; DS, Dravet syndrome; F, Female; GABA, ɣ-aminobutyric acid; GPR55, G-coupled protein receptor 55; hPBMECs, human primary brain microvascular endothelial cells; IL1β, interleukin 1β; IL6, interleukin-6; IL17, interleukin-17; iNOS, inducible nitric oxide synthase; L-DOPA, l-3,4-dihydroxyphenylalanine; M, male; MCA, middle cerebral artery; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PPARɣ, peroxisome proliferator activated receptor-ɣ; PTZ, pentylenetetrazole; ROS, reactive oxygen species; Scn1a+/-, heterozygous loss of function SCN1A; SD, Sprague-Dawley rat; SH-SY5Y^{APP+}, SH-SY5Y cells transfected with the amyloid precursor protein; TNFα, tumor necrosis factor α; TSC, tuberous sclerosis complex; TRV1 or 2, transient receptor potential vanilloid type 1 or 2; VCAM1, vascular cell adhesion molecule-1. Table adapted with permissions: Creative Commons — Attribution 4.0 International — CC BY 4.0.[86,183]

Peroxisome proliferator-activated receptor gamma (PPARɣ) receptors

CBD is an agonist of PPARɣ, a nuclear receptor and ligand-inducible transcription factor that produces anti-inflammatory and antioxidative effects.[199] PPARɣ modulates inflammation by inducing ubiquitin-proteasomal degradation of p65, resulting in inhibition of proinflammatory gene expression of cyclooxygenase (COX2) and proinflammatory mediators (e.g., TNFα, IL1β, and IL6) in addition to inhibition of NFκB-mediated inflammatory signaling. CBD agonist activity with PPARɣ also contributes to the inhibition of TNFα, IL1β, and IL6 transcription to prevent NFκB signaling, and it also produces antioxidant properties.[198,199] It increases eCBs by antagonist activity at CB2Rs, and the eCBs then act as PPARɣ agonists to promote anti-inflammatory and antioxidant actions. Furthermore, Alzheimer's disease has been demonstrated to be improved via the PPARɣ-mediated protective effects of CBD (Table 3).

$GABA_A$ receptors

As the main inhibitory neurotransmitter in the CNS, GABA disruption is associated with neurological diseases, including cognitive deficits, drug addiction, chronic stress and anxiety, epileptic disorders, and Huntington's disease.[180,205] CBD stimulates GABAergic neurotransmission, meaning that the inhibitory neurotransmission and frequency are increased.[206] Seizure frequency, duration, and severity were reduced in addition to increased social behaviors in a mouse model of DS and other diseases after CBD treatment. In addition, overexcitation in the dentate gyrus of the hippocampus was decreased through CBD effects on $GABA_A$ receptors.[206] Therefore, with CBD bound to the $GABA_A$ receptor, anticonvulsant and anxiolytic actions are seen in the CNS. Moreover, since CBD does not bind competitively with the benzodiazepine receptor, it is potentially useful in patients resistant to benzodiazepines, which is the standard antiseizure treatment (Table 3).[207]

CBD-associated neuroprotection in animal studies

CBD has shown neuroprotective effects in animal models with several neural-associated disease states (Table 3).[86,181,183,208] The areas studied have focused mainly on neuroprotection and treatment of brain-related diseases (e.g., multiple sclerosis, Alzheimer's disease, and schizophrenia), rather than effects on other areas of the body (e.g., local pain). CBD at doses from 5.0 mg/kg/day in rodents has many beneficial effects (Table 3). Note that doses administered *in vivo* were by i.p.; therefore, CBD is more slowly absorbed and subject to local metabolic processes prior to entering the blood stream, as would occur with oral exposure.[107,111] Table 3 indicates pathways specifically shown to be associated with CBD exposure.

CBD neuroprotection in human studies

The neuroprotective effects of CBD observed in animal studies are supported by observations in human subjects. CBD is well tolerated in children and adults and has a broad spectrum of therapeutic benefits to help with significant neurological disease states,[209] including neurological damage and disorders, brain tumors, Parkinson's disease, Huntington's disease, Alzheimer's disease, multiple sclerosis, neuropathic pain, and childhood seizures (e.g., Lennox-Gastaut syndrome and DS).[180,210] Additionally, synthetic forms of CBD have been used to treat drug-resistant epilepsies in children

Table 4. Neuroprotection for Parkinson's disease initiated with cannabidiol treatment

CBD target	Biological effect
CBD neuroprotection in Parkinson's disease (review)[42]	
CB1 activation	↓Microglial activation and microglial NADPH oxidase expression; ↓Production of proinflammatory agents (IL1β, TNFα, iNOs, COX2); ↓Dopaminergic neuronal damage; ↓Excitotoxicity (↓glutamate release); ↓ROS and lipid peroxidation
CB1 antagonism	↑Astrocyte activation in substantia nigra pars compacta
CB2 activation	↓Microglia number and production of proinflammatory agents (IL1β, TNFα, iNOs, nitric oxide); ↓Dopamine depletion; ↓Myeloperoxidase-positive astrocytes; ↑Antioxidant enzyme activity and antioxidant agents
MAGL inhibition	↓Microglia and astrocyte number; ↑CB2 activation; ↑GDNF
FAAH inhibition	↑Motor activity; prevents excitotoxicity by inhibiting glutamate release due to neuroinflammation; ↓Protein carbonylation; ↓ROS and lipid peroxidation
PPARɣ activation	↓ROS
CBD neuroprotection in Huntington's disease (review)[42]	
CB1 activation	↓Excitotoxicity (↓glutamate release)
CB2 activation	↓Reactive microglial cell number; ↓Production of proinflammatory agents (TNFα); ↓ROS and nitric oxide; ↑Production of neurotrophins & anti-inflammatory mediators (IL10, IL1 antagonist)
Phytocannabinoid structure	↓ROS (phenolic structure acts as an ROS scavenger)
PPARɣ activation	Interference with the NFκB signaling pathway; Induction of antioxidant enzymes
CBD neuroprotection in Alzheimer's disease (review)[42]	
PPARɣ activation	↓Apoptosis during neurodegeneration; ↓Astrocyte activation; ↓Expression of proinflammatory cytokine IL1β and iNOS (↓neuroinflammation); ↓Amyloid plaque and inflammation
CB1 activation	↓Amyloid β-induced memory impairment
CB2 activation	↓Proinflammatory mediators from microglial cells and astrocytes; ↓Neuroinflammation

CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; COX2, cyclooxygenase 2; FAAH, fatty acid amide hydrolase; GDNF, glial cell-derived neurotrophic factor; IL, interleukin; iNOS, inducible nitric oxide synthase; MAGL, monoacylglycerol lipase; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; TGF, transforming growth factor; TNF, tumor necrosis factor.

(age ≥2 and older) (Lennox-Gastaut syndrome or DS).[210] Epidiolex/Epidyolex (>99% CBD) is approved by the United States Food and Drug Administration and the European Medicines Agency to treat these diseases.[211] The benefits of CBD also have been shown in human subjects to treat anxiety, depression, post-traumatic stress disorder, and obsessive-compulsive disorders;[212,213] furthermore, it has demonstrated antipsychotic properties in those with schizophrenia.[214] A few examples of CBD affecting neurological diseases are listed in Table 4 (review).[42]

Parkinson's disease

The hallmark of Parkinson's disease is the accumulation of α-synuclein and the degeneration of dopaminergic neurons in the SNa in addition to motor alterations (bradykinesia, resting tremors, rigidity, and postural instability), depression, and dementia (review).[42] Improvement in the disease by CBD occurs via numerous pathways acting through the eCBS (e.g., CB1Rs, CB2Rs, FAAH, and MAGL) to modulate excitotoxicity, dopaminergic neuronal degeneration through inflammation, and microglial inhibition (Table 4).[43,202,215–217] Importantly, CBD has been used to improve the effects of Parkinson's disease in human subjects (review).[218]

Huntington's disease

Huntington's disease is an autosomal-dominant neurodegenerative disease that is progressive, leading to degeneration of striatal GABA and dopaminergic neuronal destruction in the globus pallidus.[43] CB1R activation by CBD in the striatum can inhibit glutamatergic transmission to protect damaged neurons and serve as an antioxidant (Table 4).[43,217,219,220]

Alzheimer's disease

CBD has been shown to decrease or block hyperphosphorylation of tau protein, acetylcholinesterase activity, oxidative stress, apoptosis, neuroinflammation, gliosis, and deposition and expression of beta-amyloid (βA).[210] The mechanism is associated with selective activation of PPARɣ, resulting in increased clearance of βA peptides through autophagy in the hippocampus, ubiquitination of amyloid precursor proteins, and decreased βA deposition (Table 4).[43,210]

CBD-associated toxicity

Since it is not considered to be intoxicating, compared to $^{\Delta 9}$THC, CBD has been widely used for medicinal purposes and is of great interest to medical communities.[17] While CBD use has increased in humans for a plethora of conditions, little is known about the potential for risks from consumption during pregnancy or in children using CBD to treat epilepsy.[17,221] The effects of CBD on brain development *in utero* are not well understood; however, C57BL6/J dams treated with 3.0 mg/kg s.c. GD 5-18 had pups with sex-specific behavioral effects (Table 5).[15–17,23,24,183,222,223,228], The male

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Table 5. Neurotoxic, behavioral, and reproductive effects from CBD treatment during development in animal studies

Animal strain sex/duration/dose/vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
Gestational treatment				
In-vitro C57Bl/6J mouse whole embryos. 6 somite embryos for 24–30 h of culture. Dose: 0, 15, 30 µM CBD. Vehicle: EtOH	24–30 h	No effects on embryo growth. ↓cranial neural tube closure 15, 30 µM. Significance: Adverse effects on brain development *in vitro*	15 µM	23
C57Bl/6J mouse M/F: GD 5–18 S.C. Dose: 0, 3 mg/kg/day. Vehicle: Cremophor EL, EtOH, saline	PND 10 and 13	10d: Mean USV duration ↓ (M) and frequency ↑ (F); PND 10, 13, 16, 19, 22: ↓body weight (M); Syllabic repertoire of sound communication sex specific; ↓Homing behavior: Distance moved, velocity, movement distance moved from nest (F). Significance: Adverse neuronal development *in vivo*	3 mg/kg/day (only dose tested)	24
Postnatal treatment				
In Vitro Wistar primary neonatal (PND 2) rat cerebral cortices (astrocytes + neurons) 1–24 h. Dose: 0, 0.5, 1, 5 µM CBD. Vehicle: EtOH	24 h	Neuron: All doses tested: Viability ↓ LDH ↑ at ≥0.1 µM; Only 0.1 µM tested: Change in mitochondrial membrane potential, ↑ATP depletion & caspase 4/7 activation, ↑apoptosis & chromatin condensation; ↓dendrite length; Astrocytes: All doses tested: Viability ↓ LDH ↑ at ≥0.5 µM; Only 0.5 µM tested: dysregulated mitochondrial membrane potential, ↑ATP depletion & caspase 8, 9, 4/7 activation, ↑apoptosis & necrosis. Significance: Cytotoxic to neurons and astrocytes *in vitro*	Neurons: 0.1 µM; Astrocytes: 0.5 µM	17
In vitro 18-week-old human M: Sertoli cells mouse sertoli cell line. Dose: Human: 7, 8, 9, 10 µM. Mouse: 10, 12.5, 15, 17.5, 20 µM. Vehicle: DMSO	24 h	Human & Mouse: ↑Cytotoxicity & cell senescence; ↓DNA replication & DNA repair; disruptions in cell-cycle related genes; ↓Cell viability; inhibition of G1/S phase cell cycle transition; ↓mRNA for Wilms' tumor 1 biomarker. Significance: Adverse effects on human Sertoli cells *in vitro*	Human: 7.0 µM; Mouse: 10 µM	228
Adolescent treatment				
Swiss mice M: PND 21–55 (4 spermatogenic cycles), gavage. Dose: 0, 15 & 30 mg/kg/day. Vehicle: Sunflower oil	PND 90	↓Testosterone (30 mg/kg/day); ↓spermatogenesis (≥15 mg/kg/day); ↑sperm with head abnormalities & cytoplasmic droplets (≥15 mg/kg/day); affected seminiferous tubule morphology (≥15 mg/kg/day). Significance: Disrupted sperm development likely affected fertility	15 mg/kg/day	223
Swiss Mice M: PND 21–55 (4 spermatogenic cycles), gavage. Dose: 0, 15 & 30 mg/kg/day. Vehicle: Sunflower oil	PND 90	Germinal epithelium stages disrupted & seminiferous tubule dysmorphology during spermatogenesis (≥15 mg/kg/day); ↑malonaldehyde & ↓sperm motility, super oxide dismutase & catalase at 30 mg/kg/day; ↑abnormal acrosome reaction & sperm velocity (≥15 mg/kg/day). Significance: Potentially affected fertility & ↑oxidative stress	15 mg/kg/day	15
Adult CBD treatment				
Wistar rat: 1 treatment (M/F) or 4 days (F). Dose: 0, 0.3, 3, 30 mg/kg. Vehicle: Not stated	F: pro- and late diestrus 1 h/4 d; M: 1 h	Acute: Late diestrus ↑entries into & time spent in open arms EPM (0.3 mg/kg/day F; 3.0 mg/kg/day M); 4-day F: Late diestrus ↑entries & time spent into open arms EPM. Significance: Disrupted behavior, indicating neuronal damage in both sexes.	F: 0.3 mg/kg/day; M; 3.0 mg/kg/day	16

CNS, central nervous system; EPM, elevated plus maze; F, female; GABA, gamma-aminobutyric acid; GPR55, G-coupled protein receptor 55; hPBMECs, human primary brain microvascular endothelial cells; IL1β, interleukin 1β; IL6, interleukin-6; IL17, interleukin-17; iNOS, inducible nitric oxide synthase; L-DOPA, l-3,4-dihydroxyphenylalanine; LOEL, lowest-observed-effect level; M, male; MCA, middle cerebral artery; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PPARγ, peroxisome proliferator-activated receptor gamma; PTZ, pentylenetetrazole; ROS, reactive oxygen species; Scn1a+/-, heterozygous loss of function SCN1A; SD, Sprague-Dawley rat; SH-SY5Y^{APP+}, SH-SY5Y cells transfected with the amyloid precursor protein; TNFα, tumor necrosis factor alpha; TSC, tuberous sclerosis complex; TRV1 or 2, transient receptor potential vanilloid type 1 or 2; USV, ultrasound vocalizations; VCAM1, vascular cell adhesion molecule-1. Table adapted from the review by Silvestro, Schepici, Bramanti, and Mazzon,[183] with permissions: Creative Commons — Attribution 4.0 International — CC BY 4.0.

pups showed higher body weights, and there were effects on ultrasonic vocalizations (both sexes), homing behavior, and decreased motor and discriminatory abilities (females). These findings indicate that CBD has effects on psychopathology after *in-utero* exposure at 3.0 mg/kg/day and may not be as safe as previously considered when consumed during pregnancy.

In adults, aspects of CBD neurotoxicity are related to sex and strain in rodent studies.[208] For example, male and female Swiss and C57BL/6 mice were treated with a single dose of CBD at 0 (saline/Tween 80), 10, and 20 mg/kg/day, and Flanders-sensitive line rats and Flanders- resistant line rats were treated with CBD at 0, 10, 30, and 60 mg/kg/day i.p. The mice were tested in the elevated plus maze, which measures anxiety behavior, and in the tail suspension test, which measures immobility and antidepressant behavior) 30 min after treatment. There were no effects from treatment with either strain of females in the tests, but male Swiss mice showed increased immobility in the tail suspension test at all doses (antidepressant). In the elevated plus maze test, the female Swiss mice showed decreased entries into the enclosed arm, indicating decreased exploratory behavior (antidepressant-like effect). Meanwhile, male and female C57BL/6 mice did not show effects in the elevated plus maze test. Rats were also tested 50 min after treatment in the forced swim and open field tests. The Flanders-sensitive line rats showed decreases at all doses in the forced swim test (measure of immobility), with no effects on distance traveled in the open field test and no effects in these tests with Flanders-resistant line rats. When the interval between treatment and testing was increased to 2 h, there was a slight increase in immobility in the Flanders-sensitive line rats at 30 mg/kg CBD. Therefore, it is significant to note that the exposure time, sex, strain, and species differences with CBD treatment were related to anxiety/depressive behaviors. The doses used in this study and those shown in Table 5 are within the range of those showing neuroprotection in Table 3, also administered i.p. *In-vitro* studies with mouse embryos also support the toxic effects of CBD during development.[16]

Animal studies have shown that doses of CBD that are neuroprotective (Table 3), can be toxic to the male reproductive tract.[14,15,223–225] CBD treatment at 15 mg/kg/day (gavage) for three sperm development cycles in mice can lead to disrupted sperm development, abnormal seminiferous epithelium, decreased testes weights, and other effects that would impact fertility.[14] Studies also have demonstrated reduced testosterone, inhibition of sperm maturation, and thinning, atrophied cells, pyknosis in seminiferous tubules, and other pathologies.[14] The presumptive MOA involves CBD inhibition of 17α-hydroxylase in Leydig cells, leading to decreased testosterone production. However, in humans, the effects on sperm and other reproductive parameters in males have been mainly attributed to the $^{\Delta 9}$THC content in cannabis, rather than CBD.[226,227] But based on animal studies, CBD in cannabis could contribute to the negative effects in males; hence, this area needs more research. *In-vitro* studies performed on human and mouse Sertoli cells obtained postnatally support the toxic effects of CBD observed in animal studies.[18] Dose exposure, route, species, sex, frequency of consumption, and susceptibility to the effects from exposure contribute to health outcomes.

Future directions

With increasing use of cannabis with higher concentrations of $^{\Delta 9}$THC, there are concomitant risks to safety in the general population from intoxication while driving or in the workplace. Methods have been developed to measure impairment from cannabis in a timely manner on site (e.g., in a car or workplace) through brain imaging to provide assessments of intoxication.[39] Functional near-infrared spectroscopy provides a measurable signature of neural impairment of the PFC, and the results are supported by blood and urine assessments to indicate whether participants were exposed but not impaired or exposed and impaired. Such measures acknowledge the growing need for detection and mitigating safety measures due to cognitive impairment from cannabis use.

Neurotoxicity of CBD is also in need of more study. For example, CBD injured neonatal rat cortical neurons and astrocytes *in vitro* at low therapeutic levels that could affect patients treated with CBD.[17,221] CBD is known to be neuroprotective in Parkinson's disease, where dopaminergic neurons of the substantia nigra pars compacta are shown to degenerate.[78,194,229] Conversely, in animal models, dopaminergic pathways are attenuated by CBD, resulting in decreased motor functions.[24] While data indicate that for some, the benefits of CBD may outweigh the risks, it is clearly necessary to continue researching optimal treatment levels related to disease improvement. Persons exposed to higher doses of CBD for severe illnesses, such as DS to control seizures (Epidiolex®, Epidyolex® in Europe), may need to weigh the risk versus benefit and exert caution for use in pregnant women and children.

Finally, one of the biggest challenges in characterizing the effects of cannabis during developmental life stages is knowing the exposure and individual health risk factors. In laboratory experiments, the exact dose, purity of cannabinoids, animal strain/sex/pregnancy status, duration of exposure, and other parameters are controlled; however, with human subjects, it is difficult to characterize exposure. Nevertheless, knowledge of the dose and product components being consumed as well as the life stage of exposure, route of exposure (i.e., inhalation, s.c., i.v., oral, or i.p.), body fat composition, age, health status, frequency of use, and other factors will determine the absorption, distribution, and metabolism of cannabinoids entering the blood stream.[107,111] Many of these parameters are not consistent among studies performed in animals (e.g., different animal species/strains, dosing regimens, vehicles), and data may be difficult to obtain in epidemiological studies with human subjects. Thus, there is a need for further study to protect fetuses, infants, and children from harmful exposures during development. There is also a need for further research related to risks for male reproductive toxicity.

Conclusions

This review focused on neurotoxicity and neuroprotection of the most thoroughly characterized phytocannabinoids in cannabis—$^{\Delta 9}$THC and CBD. Most cannabis exposure is not a pure form of either compound, but it contains a combination of those and over 100 others. Due to the increasing use of cannabis or CBD, not just recreationally, but for the treatment of diseases (e.g., depression, anxiety, inflammation, pain, and seizures) and a plethora of other conditions, it is critical for the industry to thoroughly characterize expected exposures. The extent of the risk versus beneficial effects of compounds in cannabis is dependent on many factors, but, as indicated by studies with $^{\Delta 9}$THC, there is a high risk for long-lasting neurodevelopmental effects from exposure to fetuses, infants, children, and adolescents, including severe mental dysfunction (e.g., depression, anxiety, and schizophrenia), decreased cognition, drug dependency tendencies, and decreased motor function. Adolescent use can present unique challenges because adolescence is a developmental stage of increased independence and potential for experimentation with cannabis. In addition, brain development as

well as major dynamic changes in the eCBS continue for the first 25, or more, years of life; hence, cannabis exposure during adolescence can still attenuate brain development. Adolescent exposure has been shown to lead to persistent adverse neurodevelopmental changes, increasing the risks for major depressive disorder, drug addiction, and severe psychotic disorders.

On the other hand, CBD is nonpsychotropic and has positive therapeutic applications to treat childhood epilepsy, multiple sclerosis, stroke, Alzheimer's disease, Parkinson's disease, and other severe disorders. The focus has been mainly on the health benefits; however, the reported developmental effects from exposure *in utero*, effects on male reproduction, and associations with human genotoxicity have not been well studied, and a significant data gap remains.

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Conflict of interest

I have no competing interests (financial/personal) to declare.

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MHS is the sole author of this work.

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Review Article

Ustekinumab in Dermatology: Approved Indications and Off-label Uses

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Abstract

Ustekinumab is a human antibody that interacts with the p40 chain shared by both interleukin (IL)-12 and IL-23. Treatment with ustekinumab can effectively inactivate the biological functions of IL-12 and IL-23 to control aberrant Th1 and Th17 immunological responses. Ustekinumab is the first unique IL-12/IL-23 blocker approved by the Food and Drug Administration for the treatment of patients with moderate or severe psoriasis. Subsequently, its application has extended as a therapeutic option for psoriatic arthritis and inflammatory bowel diseases. Given its therapeutic mechanism, usterkinumab may be used as a potential alternative for treatment of a variety of inflammatory skin conditions. More importantly, usterkinumab is relatively safe, as the associated adverse reactions are generally non-serious and rare; although continuous monitoring of its adverse events is warranted. Here, we discuss the therapeutic effects of ustekinumab and its clinical applications specifically in dermatology.

Introduction

Ustekinumab (CNTO-1275, Stelara) is a unique fully humanized monoclonal antibody (mAb) that interacts with the p40 chain shared by interleukin (IL)12/23 and functionally attenuates Type 1 T helper (Th1) and Type 17 (Th17) responses.[1] Ustekinumab was approved for treatment of adult patients with moderate to severe psoriasis by both the European Medicine Agency (EMA) and the U.S. Food and Drug Administration (FDA) in January 2009 and September 2009, respectively. Subsequently, the FDA has expanded the approval for treatment of adolescents and children (≥6 years old) (Fig. 1). Although it was first approved for treatment of patients with psoriasis,[2–5] ustekinumab has proven effective for treatment of other immune-mediated disorders (IMD), including active psoriatic arthritis (PsA) and active forms of major inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease. Aside from these labeled indications, ustekinumab has been used "off-label" for other inflammatory diseases.[6] However, while multiple publications, mainly case reports and a small number of case series, have shown excellent results of ustekinumab when prescribed off-label for treatment of various skin conditions, there is a lack of systemic reviews in the literature. Hence, in this review we discuss ustekinumab's pharmacological effects, efficacy, and safety in the treatment of psoriasis. More importantly, this review offers a special emphasis on the potential applications of ustekinumab in dermatology, based on its specific mechanism of action to inactivate both IL-12 and IL-23, and extend its therapeutic applications to a variety of skin disorders.

Keywords: Ustekinumab; IL12; IL23; IL 12/IL 23 inhibitor; Biologic therapy.

Abbreviations: ACH, acrodermatitis continua of Hallopeau; AD, Atopic dermatitis; AE, adverse event; BD, Behçet disease; BP, bullous pemphigoid; DBT, dual biological therapy; EMA, European Medicine Agency; EP, erythrodermic psoriasis; FDA, Food and Drug Administration; GP, guttate psoriasis; GPP, generalized pustular psoriasis; GWAS, genome-wide association studies; HS, hidradenitis suppurativa; IFN, interferon; IL, interleukin; IMD, immune-mediated disorders; JAK2, Janus kinase 2; LE, lupus erythematosus; LP, lichen planus; mAb, monoclonal antibody; MCVE, major adverse cardiovascular event; MTX, methotrexate; ND, neutrophilic dermatoses; PG, Pyoderma gangrenosum; PP, pustular psoriasis; PPP, palmoplantar pustulosis; PRP, pityriasis rubra pilaris; PsA, psoriatic arthritis; QoL, quality of life; RCT, randomized placebo-controlled trials; SAPHO, Synovitis, Acne, Pustulosis, Hyperostosis and Osteitis; SS, Sweet syndrome; STAT, signal transduction activation of transcription; TNF, tumor necrosis factor; TYK2, tyrosine kinase.

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Pharmacological mechanisms of ustekinumab

Successful mAb therapy began with the generation of chimeric, humanized, and, most recently fully human mAbs. Most mAbs that have been approved and are in the pipeline are indicated for the treatment of cancer, but there have also been other breakthroughs in the field of IMD.[1] Presently, one of the largest classes of mAb therapy includes mAbs that bind and neutralize tumor necrosis factor α (TNFα), a potent inflammatory mediator associated with various IMD, such as rheumatologic, dermatologic, and gastroen-

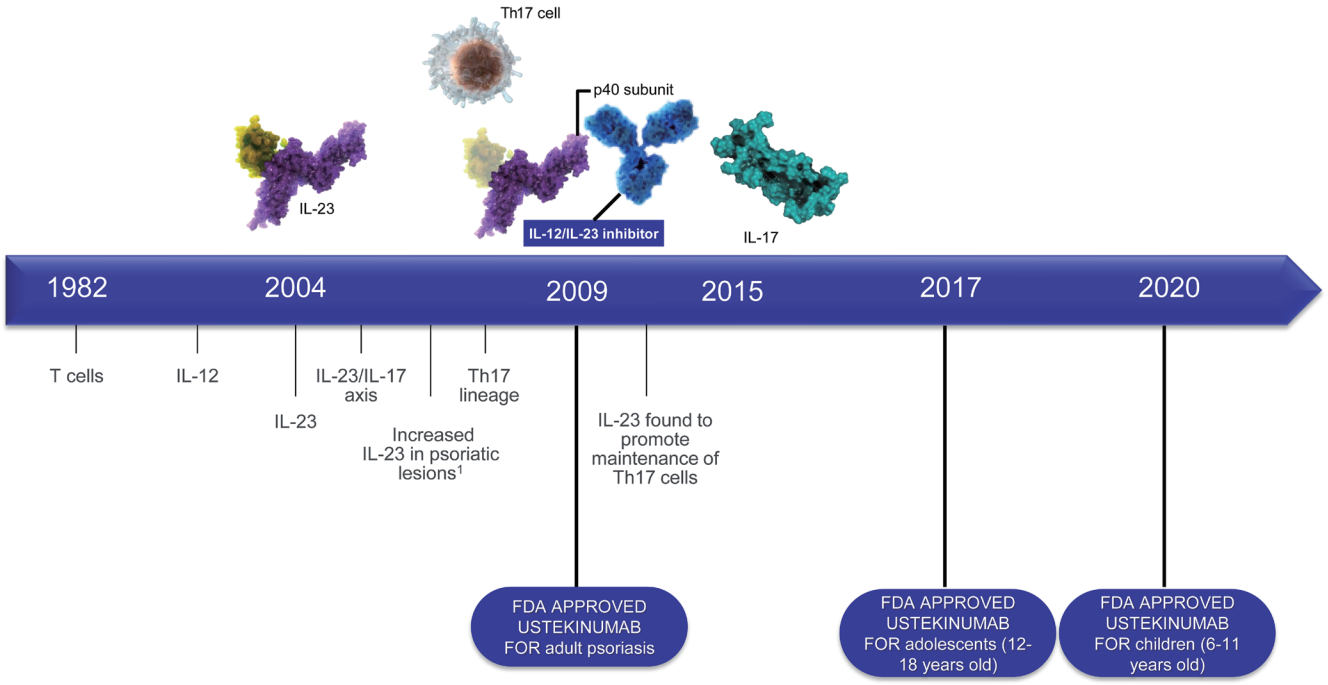

Fig. 1. Timeline of the development and approval of ustekinumab for psoriasis. FDA, Food and Drug Administration; IL, Interleukin; Th, T-helper.

terological diseases.[7] IL-12 and IL-23 are significant contributors to the pathogenesis of IMD.[8] IL-12 is a pro-inflammatory cytokine that consists of two different chain units designated by their average molecular weight as p40 and p35. The binding of IL-12 to its specific receptor (IL12Rβ1/IL12Rβ2), which is usually upregulated on pro-inflammatory T cells, stimulates tyrosine kinase 2 (TYK2) and Janus kinase 2 (JAK2) to activate signal transduction activation of transcription (STAT) 4. Once phosphorylated, STAT4 translocates to the nucleus where it modulates transcription of numerous genes, primarily interferon (IFN)-γ.[9] Thus, IL-12 promotes the differentiation of activated CD4+ T cells into Th1 cells, a subset of CD4+ T cells involved in the pathogenesis of several IMD.[10] IL-23 is also a heterodimeric cytokine formed by two chains of p19 and p40 (similar to IL-12). Engagement of IL-23 receptors (IL12Rβ1/IL23R) by IL-23 can activate STAT3 to induce IL-17, IL-22, and other cytokine production, leading to Th17 responses that contribute to the pathogenesis of various IMD and tissue damage.[1]

Within the skin, IL-17 can promote keratinocyte proliferation and the production of different chemoattractant molecules, such as CXCL1, CXCL8, and CCL20.[11–13] According to animal and human studies, there is a strong link between Th1/Th17 signaling dysregulation and certain IMD, like psoriasis, PsA, rheumatoid arthritis, and inflammatory bowel disease. Furthermore, genome-wide association studies (GWAS) have identified a strong association between genetic alterations that affect the Th1/Th17 axis and chronic inflammation.[14] Thus, in genetically susceptible individuals, over-activated IL-12 and IL-23 trigger aberrant Th1/Th17 responses, subsequently leading to IMD. Ustekinumab is a unique fully human IgG1 kappa mAb against that interacts with the p40 chain of IL-12 and IL-23 and blocks the binding of these two cytokines to their common receptor, IL12Rβ1. Importantly, ustekinumab preferably binds to soluble but not membrane-associated IL-12/IL-23 and does not usually induce complement activation or cell lysis through its immunoglobulin Fc domain.[1]

Figure 2 is an illustration of the mechanisms underlying the action of the drug in inflammatory skin diseases.

Ustekinumab current approved indications in dermatology

Plaque psoriasis is the only validated indication for ustekinumab in dermatology. Psoriasis is a frequent, chronic skin IMD marked by sharply well-circumscribed erythematous-squamous lesions and is significantly associated with systemic comorbidities.[15] The World Health Organization defines psoriasis as a serious, chronic, disfiguring, disabling, and non-communicable disease. Psoriasis affects approximately 2% to 3% of people worldwide, and 30% of cases are moderate to severe forms.[16] Psoriasis and its related comorbidities may substantially lower a patient's quality of life (QoL) and lead to a high degree of cumulative life course impairment.[17] Clinically, psoriasis can manifest with a multitude of phenotypes; 90% of cases display chronic plaque psoriasis (also named psoriasis vulgaris).[18] Therefore, all currently available treatments are approved for psoriasis vulgaris. Psoriasis can also exhibit other less common variants, including guttate, erythrodermic, and pustular psoriasis. Currently, the management of these variants relies on empiric therapies.[16]

During the past twenty years, the understanding of psoriasis pathogenesis has progressed considerably. The TNFα-IL23-Th17 axis has been recognized as a major inflammatory pathway for the pathogenesis of plaque-type psoriasis,[19] which is supported by immunological and genetic studies. While GWAS have shown a link between psoriasis pathogenesis and genetic alterations in the IL-23/IL-17 axis,[20] immunological researchers have stressed the important roles of IL-23 in the development and progression of psoriasis by enhancing Th17 responses. IL-17 is a key orchestrator of chronic inflammation in psoriasis. IL-17 induces the secretion of many other cytokines and chemokines, which promote the chemotaxis of immune cells to the site of inflammation and sustain the positive inflammatory loop and epidermal hyperplasia.[21]

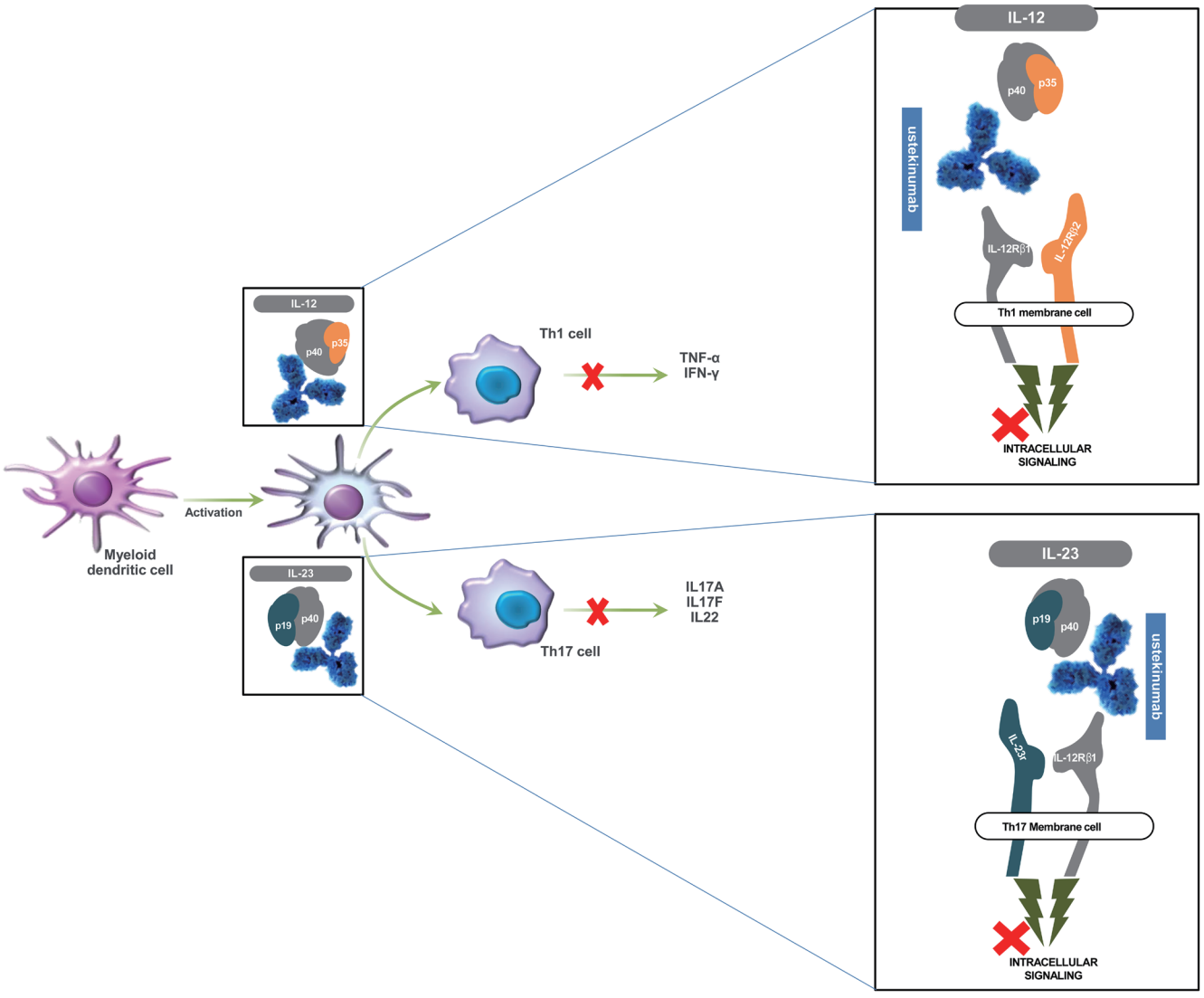

Fig. 2. The mechanisms of actions of ustekinumab in inflammatory skin diseases. IFN: Interferon; IL: Interleukin; Th: T-helper; TNF: Tumor necrosis factor.

However, until late last century, topical therapies and/or ultraviolet light therapies were the mainstay of psoriasis treatment. Subsequently, the first major therapeutic advancements have been conventional systemic drugs (methotrexate, cyclosporine, fumarates, and acitretin). Besides these older agents, the novel small molecule apremilast has recently expanded into the psoriasis armamentarium.[22] Although these treatments may benefit some patients, they have lower therapeutic efficacy and higher adverse events (AEs) owing to a non-specific modulation of the immune system. For example, the PASI 75 (a decrease in PASI score by 75%, the current benchmark of psoriasis treatment) of methotrexate is typically 35.5–41%.[22] The need for alternative and/or small molecule therapeutics and a better understanding of the immunopathogenesis of psoriasis have prompted the discovery of biological drugs directed against the aberrant immune response. Three main groups of biological agents, including blockers for TNFα, IL-23, and IL-17, have been approved for the treatment of psoriasis.

Ustekinumab is the first approved biological drug for treatment of chronic plaque psoriasis, based on its anti-IL-23 effect.[23] Ustekinumab, manufactured by Johnson & Johnson Pharmaceutical, is created by immunizing human mAb-producing mice with recombinant human IL-12. Currently, this biological drug is approved by the FDA for treatment of patients aged ≥6 years who have moderate to severe psoriasis and who are eligible for systemic treatment or phototherapy.[23] The efficacy-safety profiles of ustekinumab have been demonstrated through four large phase III studies: three placebo-controlled trials namely PHOENIX 1/2 and PEARL, and one active comparator-controlled trial (ACCEPT).[2–5] The results from these trials indicate that ustekinumab has a more favorable efficacy-safety profile compared to anti-TNFα drugs. A total of 2,000 psoriatic patients with moderate to severe disease participated in the PHOENIX 1/2 trials. After the initial induction doses of the drug administered subcutaneously every 4 weeks, followed by a maintenance dosage every 12 weeks, 66.4% to 75.7% of participants achieved PASI 75, which was significantly greater than in the placebo groups (3–4%). Moreover, while the improvement was maintained during the 3-month interval between doses, the incidence of AEs (52% and 49%, respectively) and serious AEs

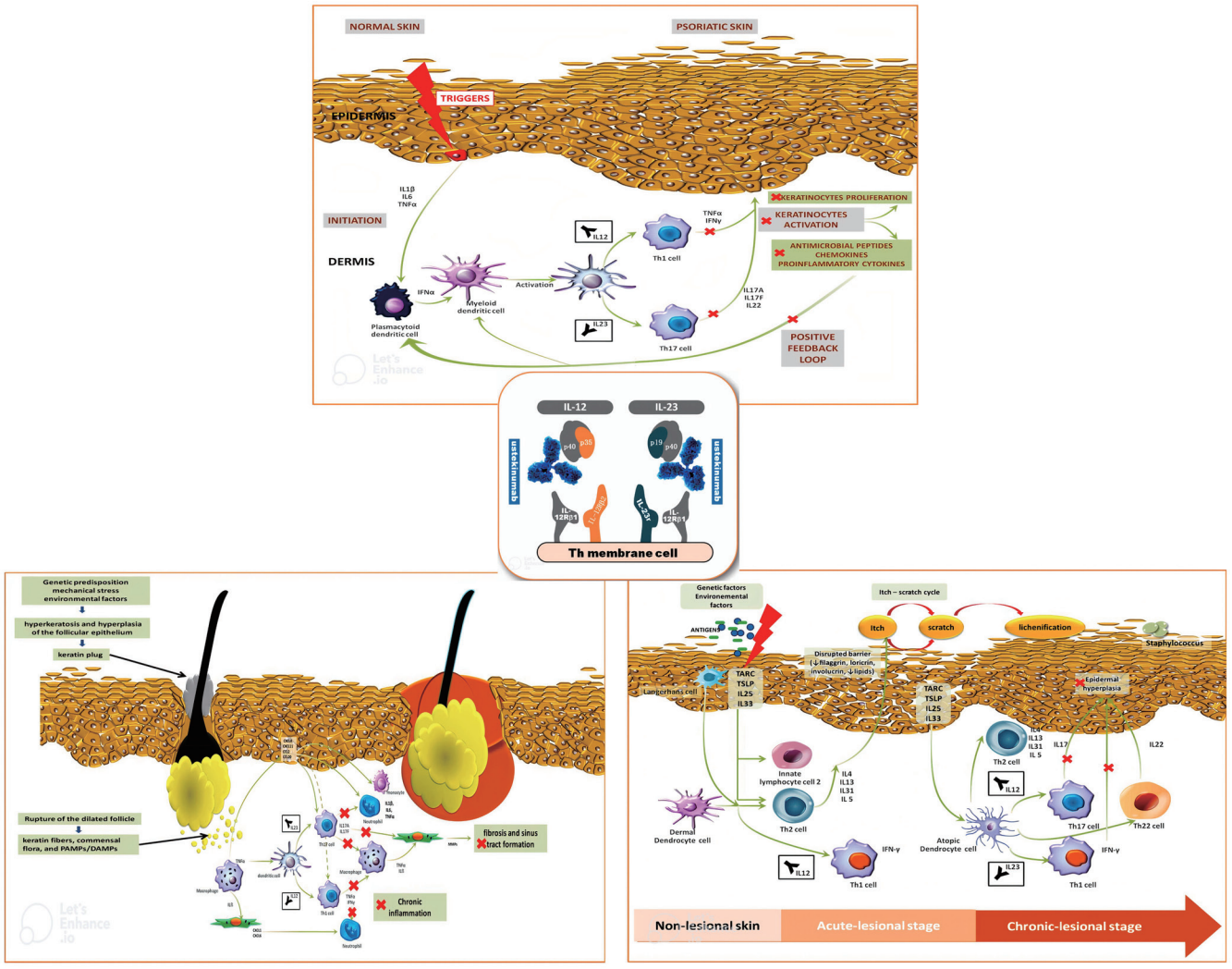

Fig. 3. Therapeutic effect of ustekinumab on psoriasis (top figure), atopic dermatitis (lower right figure), and hidradenitis suppurativa (lower left figure). IFN, interferon; IL, Interleukin; Th, T-helper; TNF, Tumor necrosis factor; TARC, thymus and activation regulated chemokine; TSLP, thymic stromal lymphopoietin.

(1.4% and 1.5%, respectively) were not significant between these two groups. In the ACCEPT trial, similar results were obtained in the ustekinumab group (67.5% to 73.8%) with a higher efficacy compared to etanercept (56.8%) and a comparable safety profile. Consistent data regarding the efficacy of ustekinumab and its safety profile were observed through 5 years of follow-up.[24] However, dose escalation (90 mg every 8 weeks) resulted in better improvement in psoriatic patients who failed to respond to the initial regimen. In addition, since most patients with psoriasis experienced a flare-up after stopping ustekinumab therapy, there is no available data to support the long-term use of this biological drug.[25]

Figure 3 is an illustration of the therapeutic effect of ustekinumab on psoriasis and two other major skin diseases, atopic dermatitis and hidradenitis suppurativa.

Ustekinumab off-label uses in dermatology

Ustekinumab has been used to treat many skin diseases given its distinct and targeted mechanism of action. However, robust evidence from well-designed studies addressing uncommon and life-threatening diseases is rare, and the scientific data available in this field are often restricted to small clinical reports. Thus, such limited evidence cannot support the use of ustekinumab as an initial therapy. On the other hand, there are a few low-level quality studies comparing IL-12/IL-23 blockers versus standard treatments. For this reason, ustekinumab should be reserved to treat cases that have failed or did not tolerate the first-line therapy and where other therapeutic alternatives are lacking. Table 1 summarizes the studies concerning the off-label use of ustekinumab.[26–122]

Ustekinumab for other subtypes of psoriasis

Psoriasis encompasses other infrequent variants namely guttate (GP), erythrodermic (EP), and pustular psoriasis (PP). GP accounts for nearly 2% of psoriatic patients and appears as red, scaly, small, raindrops-shaped papules that often erupt suddenly throughout the entire body.[123] Although there is no consensus on the treatment of GP, severe forms of GP are commonly treated with topical corticosteroids, phototherapy, immunomodulatory drugs, or even bio-

Table 1. Cases of diseases treated off-label with ustekinumab

Cutaneous disorders	Study	Study design	No of patients	Patient (s) Age (y)/sex	Prior systemic treatment	Ustekinumab dose (mg)	Reported efficacy/ outcomes	Serious AEs
GP	Brummer GC[26]	cs	6	29–42/3M–3F	CsA, Apr, Phototherapy	90 once/45Q4W/45Q4W, 90Q4W/90Q8W	Success 6/6	None
	Amarnani[52]	CR	1	56/1F	Phototherapy Aci	SD	Clearance	None
EP	Santos-Juanes[28]	CS	2	32 and 41/2F	CsA, MTX, SCS, PUVA, Efa, anti-TNFα	SD	Success 2/2; PASI 90	None
	Viguier[53]	CS	3		Anti-TNFα	SD	33% PASI 50	-1 sudden death; -Widespread skin Staphylococcus
	Wang[29]	CS	8	28–55/7M–1F	CsA , MTX, PUVA, Efa, alefacept, Aci, anti-TNFα	-7 patients: 45Q4W two doses, -1 patient: 45Q4W two doses + W16 + W 32	50% PASI 75; 50% PASI 90	None
	Pescitelli[27]	CS	22	NA/14M–8F	CsA, MTX, SCS, UVB Ret, anti-TNFα	SD	68.2%; PASI 90	None
GPP	Storan[30]	CR	1	90/1F	CsA, MTX, Ret, Ada	SD	Success 1/1	None
	Arakawa[32]	CS	4	20–50/4F	CsA, MTX, Ana, Aci , anti-TNFα	SD	Success 4/4	None
	Dauden[31]	CR	1	47/1M	None	SD	Success 1/1	None
	Matsumoto[54]	CR	1	70/1F	CsA, etretinate, IFX	Started at 45 then 90	Fail 1/1	None
PPP	Morales-Munera[55]	CS	5	30–50/2M–3F	CsA, MTX, PUVA, Aci, Efa, leflunomide, anti-TNFα	SD	Success 5/5	None
	Au[56]	open-label study	20	18- 85/9M–11F	Systemic therapy, anti-TNFα	SD	Improvement 12/20, Clearance 7/20	None
	Bulai Livideanu[57]	CS	2	29 and 42/1F and 1M	CsA, MTX, Ret, phototherapy, anti-TNFα	45 or 90, not regularly	Success 2/2	None
	Bissonnette[58]	RCT	15	NA/14F–1M		45Q4W two doses or placebo	No difference compared to placebo	1 leg cellulitis
	Husson[35]	CS (PPP + ACH)	30	NA	NA	NA	Success 21/30	2 PPP worsening, 1 Paradoxical psoriasis, 1 Pneumonia
ACH			4				Success 4/4	
	Adisen[59]	CR	1	50/1M	Dap, CsA, MTX, Ret, phototherapy,anti-TNFα	Start 90 then SD 45	Success 1/1	None
	Palacios-Alvarez[33]	CR	1	67/1M	MTX, phototherapy, Aci, anti-TNFα	SD 45	Success 1/1	None
	Saunier[60]	CR	1	53/1M	Aci , MTX, phototherapy, CsA, anti-TNFα, Ana	SD 45	Success 1/1	None

(continued)

Table 1. *(continued)*

Cutaneous disorders	Study	Study design	No of patients	Patient (s) Age (y)/sex	Prior systemic treatment	Ustekinumab dose (mg)	Reported efficacy/ outcomes	Serious AEs
	Cymerman[34]	CR	1	20/1F	CsA	SD 45	Success 1/1	None
	Adas[61]	CR	1	61/1M	Eta	45Q4W two doses then 90Q8W	Success 1/1	None
PRP	Ruiz Villaverde[62]	CR	1	45/1M	None	SD 45	Success 1/1	None
	Byekova[63]	CR	1	75/1M	IFX, Aci	SD 45	Success 1/1	None
	Chowdhary[64]	CR	1	52/1F	Aci, UVB, minocycline, SCS, MTX	SD 90	Success 1/1	None
	Di Stefani[65]	CR	1	31/1M	CsA, Aci, MTX	SD 45	Success 1/1	None
	Eytan[66]	CR	1	57/1M	MTX, Ret, PUVA, Efa, anti-TNFα	90Q8W	Success 1/1	None
	Humme[67]	CR	1	50/1M	Aci, PUVA, CsA, IFX	45–90	NA	CD30(+) anaplastic large cell lymphoma
	Lernia[68]	CR	1	29/1F	CsA, MTX, anti-TNFα, PUVA,	SD 45	Fail 1/1	None
	Lwin[69]	CS	2	20 and 49/1M and 1F	Ret, phototherapy	-1 patient: SD 45; -1 patient: Started at SD 45 then 90	Success 2/2	None
	Paganelli[70]	CR	1	78/1F	Ret, phototherapy, MTX, CsA, IFX	SD 45	Success 1/1	None
	Wohlrab[71]	CR	1	28/1M	Aci, Bath-PUVA,	SD 45	Success 1/1	None
	Aragón-Miguel[72]	CR	1	30/1M	Aci, PUVA	SD 45	Success 1/1	None
	Feldmeyer[73]	CR	1	40/M	None	SD 45	Success 1/1	None
	Kalogeropoulos[74]	CR	1	60/1M	Aci	SD 45	NA	Meningococcal and HSV-2 Meningitis
	Napolitano[75]	CS	5	28–62/3M–2F	CsA, SCS, Aci, MTX	SD 45	Success 4/5, 1partial improvement	None
	Craiglow[76]	CS	6	NA	NB-UVB, PUVA, Ret, MTX, CsA, anti-TNFα,	-5 patients: 0,7 mg/kg-1,1 mg/kg Q12W; -1 patient 1,2 mg/kg Q8W	Success 5/6, 1partial improvement	NA
	Matsuda[77]	CR	1	72/1M	SCS, Secu, IFX, etretinate, CsA, MTX, Apr	SD 45	Success 1/1	None
	Ponholzer[78]	CS	5	NA/3M–2F	NB-UVB, PUVA, Aci	-3 patients: SD 45; -1 patient: SD 90; -1 patient: Started at SD 45 then 90	Success 5/5	NA
HS	Gulliver[79]	CS	3	30–32/1M–2F	ATBs, Ret, anti-TNFα, SCS, Efa	SD 45	- HS-PGA: 2/3; - DLQI: 2/3; - Pain: 2/3	None

(continued)

Table 1. *(continued)*

Cutaneous disorders	Study	Study design	No of patients	Patient (s) Age (y)/sex	Prior systemic treatment	Ustekinumab dose (mg)	Reported efficacy/ outcomes	Serious AEs
	Sharon[80]	CR	1	55/1M	ISO, ATBs, SCS, MTX, ada, MMF	SD 45	Improvement 1/1	None
	Baerveldt[81]	CR	1	39/1F	Diclofenac/misoprostol, colchicine, CsA	SD 45	Improvement 1/1	None
	Santos-Pérez[82]	CR	1	50/1F	ISO, anti-TNFα, SCS	SD 45	Improvement 1/1	
	Blok[36]	open-label study	17	20–53/4M–13F	ATBs, ISO, anti-TNFα, SCS	SD	- HiSCR: 8/17; - DLQI: 07/17	urticaria
	Romani[83]	CS	12	19–60/9M–3F	ATBs, CsA, MTX, AZA, anti-TNFα, Ana	IV loading dose adjusted by weight then SC 90 Q8W	- HiSCR: 8/12; - DLQI: 11/12; - Pain: 11/12	None
	Scholl[84]	CS	3	25–31/2M–1F	ATBs, Ada, dapsone, Secu	IV loading dose adjusted by weight then SC 90 Q8 or 12W	- SAHS: 3/3; - DLQI: 3/3	None
	Takeda[85]	CR	1	29/1M	IFX, SCS	360 IV loading dose then SC 90 Q8W	Improvement 1/1	Non
	Montero-Vichez[86]	CS	10	14–52/6M–4F	ATBs, Ret, CsA, anti-TNFα, SCS, interferon, phototherapy,	SD	-HSPGA: 7/10; - Pain: 8/10	None
	Sánchez-Martínez[87]	CS	6	31–59/3M–3F	ATBs, Ret, MTX, finasteride, AZA, Ada	IV loading dose adjusted by weight then SC 90 Q8W	HiSCR: 3/6	None
	Hollywood[88]	CS	16	22–77/4M–12F	ATBs, Ana, metformine, liragultide, Dap, sironolactone, anti-TNFα.	NA	Improvement 8/16	Recurrent infection
	Smith[89]	CR	1	49/1F	Anti-TNFα.	SD 90	Primary Improvement	Multifocal myositis
	Provini[90]	CR	1	17/1F	ATBs, sironolactone, SCS, Ada	90Q8W then 90Q4W	Improvement 1/1	None
	Valenzuela-Ubiña[91]	CS	10	26–58/4M–6F	ATBs, Ret, MTX, finasteride, AZA, SCS, metformin, Dap, Ana, CsA, Anti-TNFα.	- 9 patients: 90Q8W; -1 patient: SD 45	-HSPGA: 9/10	None
Neutrophilic diseases								
PG	Goldminz[92]	CR	1	47/1M	SCS, Dap, MTX, CsA, AZA, Anti-TNFα.	90Q4W then 90Q8W	Complete healing	None
	Cosgarea[93]	CR	1	71/1M	SCS, CsA	NA	Complete healing	None
	Benzaquen[94]	CR	1	56/1F	Ada	SD 45	Complete healing	None
	Guenova[37]	CR	1	37/1F	SCS	Two doses 45Q4W	Complete healing	None

(continued)

Table 1. *(continued)*

Cutaneous disorders	Study	Study design	No of patients	Patient (s) Age (y)/sex	Prior systemic treatment	Ustekinumab dose (mg)	Reported efficacy/ outcomes	Serious AEs
	Nunes[95]	CR	1	45/1M	SCS, IFX, AZA, CsA	520 IV loading dose then SC 90 Q8W	Complete healing	None
	Piqueras-García[39]	CR	1	33/1F	SCS, 6-Mercaptopurine, CsA, Anti-TNFα, vedolizumab, tacrolimus	90 week 0, 4, 10 then Q8W	Complete healing	None
	Petty[40]	CR	1	50/1F	SCS, CsA, IFX	90 week 0, 4 then Q8W	Complete healing	None
	Low[96]	CS	3	36 –57/3F	SCS, MMF, Dap, IVIG	-1 patient: 90 week 0, 4 then Q6W then 45 Q3W; -2 patient: 90 week 0, 4 then Q8W then 45 Q4W	Complete healing 3/3	None
	Greb[97]	CR	1	50/1M	SCS, CsA, Anti-TNFα, Dap	90 Q8W then 90 Q6W then 135 Q6W	Significant improvement	None
	Fahmy[98]	CR	1	34/1F	Tacrolimus, AZA	90 week 0, 2 then Q8W	Complete healing	None
	López González[99]	CR	1	29/1F	ATB, SCS, Ada	260 IV loading dose then SC 90 Q8W	Complete healing	None
	Vallerand[100]	CR	1	47/1M	SCS, IVIG, MMP	520 IV loading dose then SC 90 Q8W	Significant improvement	None
	Nieto[101]	CR	1	62/1M	Ada, Azacytidine, CsA, IFX, IVIG, Thalidomide, SCS	90 Q8W	Complete healing	None
	Westerdahl[102]	CS	8	24–88/4M–4F	SCS, Anti-TNFα, colchicine, Ixe, ATB,	-3 patients: 90 Q8W; -1 patient: 90 Q12W; -2patients: 45 Q12W; -1 patient: 45 Q8W; -1 patient: 180 Q8W	Complete healing 7/8, improvement 1/8	None
	de Risi-Pugliese[38]	CS	4	30–44/1F	AZA, IFX, MTX, Ada, AZA, aminosalicylates, Mercaptopurine	-2 patients: 90 Q8W; -1 patient: 90 Q2W then Q8W; -1 patient: 90 Q4W then Q8W	Complete healing ¾; Significant improvement 1/4	None
APF			2	32 and 41/2F	SCS, Dap, ISO, CsA, colchicine, Ana, ATB, Ada	-1 patient: 90 Q8W; -1 patient: 90 week 0, 2 then Q8W	Significant improvement 2/2	None
SS			1	28/1M	Colchicine, MTX, SCS, Ada, Dap	90 Q8W	Fail 1/1	None
BD	Baerveldt[81]	CR	1	39/1F	diclofenac/misoprostol, colchicine, CsA	SD 45	Remission	None
	Lopalco[103]	CR	1	36/1F	SCS, colchicine, MTX, CsA, AZA, Anti-TNFα, Ana	SD 45	Remission	None

(continued)

Table 1. *(continued)*

Cutaneous disorders	Study	Study design	No of patients	Patient (s) Age (y)/sex	Prior systemic treatment	Ustekinumab dose (mg)	Reported efficacy/ outcomes	Serious AEs
	Mirouse[41]	Prospect-ive study	14	34–41/10M–4F	Colchicine, AZA, SCS, MMF, tocilizumab, tacrolimus	-11 patients: SD 90; -3 patients: SD 45	-Complete response: 9/14; -Partial response: 3/14; -Fail: 2/14	None
	Mirouse[104]	open-label study	30	33–45/16M–14F	Colchicine, SCS, thalidomide, hydroxyl-chloroquine, MTX, cyclophosphamide, AZA, MMF, everolimus.	SD 90	-Complete response: 18/30; -Partial response: 9/30; -Fail: 3/30	None
	London[105]	open-label study	15	NA/9M–6F	Colchicine, SCS	SD 90	-Complete response: 9/15; -Partial response: 2/15; -Fail: 4/15	None
AD	Puya[106]	CR	1	21/1F	SCS, UVB, CsA, Efa	SD 45	Complete response	None
	Agusti-Mejias[107]	CR	1	16/1F	SCS, phototherapy, AZA, CsA	SD 45	Complete response: SCORAD 0	None
	Shroff[108]	CR	1	70/1F	UVB, CsA, MMF	45 week 0, 3, 11 and 19	Complete response: SCORAD 0	None
	Fernández-Antón Martínez[42]	CS	4	23–29/4M	SCS, phototherapy, AZA, CsA, MTX, MMF	SD 45	Significant improvements	None
	Lis-Święty[109]	CR	1	21/1M	None	SD 45	Exacerbation	None
	Ishiuji[110]	CS	2	59 and 39/1M and 1F	None	SD 45	Exacerbation	None
	Samorano[111]	CS	2	47 and 20/1M and 1F	phototherapy, SCS, CsA, MTX, MMF, Eta	-1 patient: SD 45; -1 patient: 45 week 0, 6, 12	Fail 2/2	None
	Nic Dhonncha[112]	CS	10	20–50/8M–2F	phototherapy, AZA, CsA, MMF, Eta, Efa	SD 45 or 90	Significant improvements: 4/10; Fail: 6/10	None
	Saeki[113]	RCT phase 2	52 vs 27 placebo	20–57/36M–16F	phototherapy, SCS, CsA	SD 45 or 90	No significant improvement	None
	Wlodek[114]	CR	1	13/1F	SCS, AZA, CsA, MTX, MMF	SD 45	Partial improvement	None
	Khattri[115]	RCT phase 2	16 vs 15 placebo	NA/10M–16F	NA	SD 45 or 90	No significant improvement	None
	Weiss[116]	CS	3	27–55/2M–1F	SCS, phototherapy, CsA, MMF	45 week 0, 4, 12 then Q8W	Improvement 3/3	None
AA	Guttman-Yassky[43]	CS	3	NA/2M–1F	NA	SD 90 (3 doses)	Improvement 3/3	None
	Aleisa[44]	CS	3	9–16/3F	SCS,	1 patient: 90 week 0, 12, 24; 2 patients: one dose 90	Significant hair regrowth 3/3	None

(continued)

Table 1. *(continued)*

Cutaneous disorders	Study	Study design	No of patients	Patient (s) Age (y)/sex	Prior systemic treatment	Ustekinumab dose (mg)	Reported efficacy/ outcomes	Serious AEs
	Ortolan[117]	CS	4	8–44/2M–2F	SCS, CsA, MTX, ruxolitinib, tofacitinib	1 patient: 45 week 0, 60 week 90 week 12, 20; 1 patient: 90 Q8W; 2patients: 90 week 0, 4, 12	Fail 4/4	None
	Elkady[34]	CR	1	39/1F	Vitamines B complex	90 week 0, 4 then Q8W	Hair regrowth	None
Vitiligo					None		Repigmentation	
SAPHO	Wendling[46]	CS	3	32–61/3F	Phototherapy, MTX, CsA, Anti-TNFα	SD 90	Remission 1/3	Paradoxical psoriasis
	Firinu[117]	CR	1	NA/1F	anti-TNF-α, Ana	SD 90	Significant improvement	None
LP	Solimani[47]	CS	1	72/1F	CsA, SCS, Aci,AZA	SD 45	Significant improvement	None
	Webster[48]	CR	1	70/1F	Hydroxychloroquine	SD 45	Fail	None
LP pemphigoides	Knisley[118]	CR	1	71/1F	Cycline, nicotinamide, SCS, Dap, AZA, MMF Hydroxychloroquine	SD 45	Significant improvement	None
BP	Loget[49]	CR	1	88/1F	None	SD 45	Remission	None
antilaminin-γ1 pemphigoid	Majima[119]	CR	1	69/1M	SCS, Ada	NA	Remission	None
Cutaneous lupus	De Souza[120]	CR	1	58/1F	NA	SD 45	Remission	None
	Varada[122]	CS	1	68/1F	NA	NA	Remission	None
	Dahl[51]	CR	1	79/1F	SCS, hydroxychloroquine, AZA, thalidomide, MTX, IV IG	45 week 0, 4, 16, 34	Improvement	None
	Winchester[50]	CR	1	41/1M	MTX, hydroxychloroquine, CsA	SD 45 then 90	Significant improvement	None
	Mazgaj[122]	CR	1	65/1F	Chloroquine, Aci, MTX, CsA, thalidomide, lenalidomide, alitretinoin.	SD 45 then 90 Q8W	Remission	None

AA, alopecia areata; ACH, Acrodermatitis continua of Hallopeau; Aci, acitretine; AD, atopic dermatitis; Ada, adalimumab; AEs, adverse events; Ana, anakinra; anti-TNFα, tumor necrosis factor α inhibitor; APF, Amicrobial pustulosis of the folds; Apr, apremilaste; ATB, antibiotic; AZA, azathioprine; BD, Behçet disease; BP, bullous pemphigoid; CR, case report; CS, case series; CsA, cyclosporine; Dap, dapsone; Efa, efalusumab; EP, erythrodermic psoriasis; Eta, etanercept; GP, generalized psoriasis; GPP, generalized pustular psoriasis; HS, Hidradenitis suppurativa; IFX, infleximab; ISO, isotrétinoïne; IV IG, intravenous immunogolobulines; IV, intravenous; Ixe, ixekizumab; LP, lichen planus; MMF, mycophenolate mofetil; MTX, methotrexate; PG, pyoderma gangrenosum; PPP, palmoplantar pustuloses; PRP, Pityriasis rubra pilaris; Q(number)W, every (number) week; Ret, retinoid; SAPHO, synovitis, acne, pustulosis, hyperostosis, osteitis; SC, subcutaneous; SCS, systemic corticosteroids; SD, ustekinumab at weeks 0, 4, and then every 12 weeks. Weight <100 kg, 45 mg/weight >100 kg, 90 mg; secu, secukinumab; SS, Sweet syndrome.

logical therapy.[124] Successful use of ustekinumab in recalcitrant GP has been reported in only a few of cases. For example, Brummer *et al*[26] reported that treatment with ustekinumab successfully cleared lesions in six patients with resistant GP.

EP accounts for 1–2.25% of all cases and represents one of the most severe and potentially life-threatening subtypes of psoriasis. It manifests as erythema covering >75% of the entire skin surface.[123] There is little scientific evidence that supports biological therapy in EP owing in part to the paucity of high-quality studies. However, the efficacy of ustekinumab in this rare form of psoriasis was highlighted in multiple clinical studies[27–29,125,126] with impressive response despite the failure of a first-line anti-TNFα therapy. A multicenter retrospective study in Italy showed that 80% of EP patients achieved PASI 75 following treatment for seven months.[27]

PP is a rare form of psoriasis characterized by non-follicular small pustules on erythematous and edematous skin. There are three clinical forms of PP: generalized form (GPP), palmoplantar pustulosis form (PPP), and acrodermatitis continua of Hallopeau (ACH). It was postulated that the formation of pustules is caused by elevated levels of certain immune mediators, including IL-17F and IL-8, which can be targeted specifically by IL-23/IL-17 blockers. GPP (also known as von Zumbusch disease) can cause serious complications and can be life-threatening, especially if not diagnosed early and treated appropriately.[123] There are only a few case reports[30,31] and one case series[32] of GPP that have been successfully treated with ustekinumab. Arakawa *et al*[123] reported that ustekinumab therapy led to remission in four GPP cases for 17 months. PPP is a chronic, debilitating form of PP and is usually resistant to treatment. PPP manifests pustules as an erythematous base, hyperkeratosis, and scales, affecting both palms and soles.[123] Despite the lack of sufficient evidence supporting ustekinumab use for PPP, there have been some reports of a satisfactory response among patients with PPP, including significant improvement in the QoL.[127,128] ACH is an uncommon disease presenting with long-lasting sterile pustules specifically affecting the extremities of the digits.[123] This form of PP has been found invariably recalcitrant to available antipsoriatic therapies. There are some case reports and one retrospective study on the efficacy of ustekinumab in the treatment of ACH.[33–35] Treatment with ustekinumab effectively improved clinical symptoms in seven patients with ACH and cleared skin lesions in 75% of patients, similar to that of anti-TNFα therapy.

Ustekinumab for pityriasis rubra pilaris (PRP)

PRP is a rare disease characterized by erythematous and papulosquamous eruption and is classified into six major types depending on clinical aspects, age of disease onset, and outcome.[129] The etiology of PRP is still not completely understood, and its management relies heavily on small clinical studies. There are diverse treatments for PRP with varying outcomes, including topical corticosteroids, phototherapy, systemic retinoids, and immunosuppressive drugs. In recalcitrant cases, anti-TNFα therapy can significantly improve clinical symptoms, supporting the immunological pathogenesis theory. In refractory PRP cases, ustekinumab has been reported to be valuable. A review of the PubMed database and the Cochrane Library until September 2017 by Kromer *et al*,[130] included all studies that evaluated the risks and benefits of systemic treatments for PRP. There were about 182 studies (including 475 patients) on systemic treatment of PRP. Ustekinumab was successful in 62.5% of patients compared to adalimumab (46.4%), etanercept (53.3%), and infliximab (57.1%). The comparison between ustekinumab and acitretin (which is commonly considered as a reference treatment in PRP) showed a substantially elevated rate of excellent response in patients treated with ustekinumab (p = 0.001). The general AE reporting rate was 26.4%, but this was significantly elevated with retinoids (34.1%) then MTX (16.5%) and the lowest proportion was reported with biological agents (8.8%).

Ustekinumab for hidradenitis suppurativa (HS)

HS is a long-term inflammatory dermatosis that often causes serious morbidity and manifests mostly after puberty with inflamed nodules and painful deep-seated abscesses with sinus tracts mainly localized in body zones rich in apocrine glands including axillary and anogenital areas.[131] Although some drugs have been proven to be successful in managing HS symptoms, there is a lack of solid evidence supporting them. The pathogenesis of HS is complex, but TNFα and IL-17 are recognized as central players in HS pathogenesis.[132] One study suggests that gene polymorphisms in IL12Rβ1 may be linked to some severe forms of HS.[133] Currently, adalimumab, a type of anti-TNFα antibody, is the only biological agent available for the treatment of HS.[134] However, failure of treatment was common and consideration of second-line biological drugs, like ustekinumab, may be valuable for inhibiting Th17 responses. In this perspective, an open-label, uncontrolled trial was conducted in 17 patients with HS to determine the benefit of ustekinumab therapy. At week 40 when the clinical trial ended, 47% of patients achieved HiSCR-50 (50% improvement in HS inflammatory lesions), and >82% of cases obtained moderate or remarkable relief of their modified Sartorius score.[36]

Ustekinumab for neutrophilic diseases

Pyoderma gangrenosum (PG), Sweet syndrome (SS), subcorneal pustular dermatosis, and erythema elevatum diutinum are heterogeneous diseases that may be grouped as neutrophilic dermatoses (ND) hallmarked by a sterile, neutrophil-rich infiltrate on the skin.[135] Clinical management of ND is challenging due to the lack of universally accepted and validated guidelines. The standard treatment for idiopathic PG and SS is systemic corticosteroids, whereas dapsone is the first line of treatment for subcorneal pustular dermatoses.[136] However, the use of biological therapy, primarily TNFα blockers, anti-IL-1, anti-IL-17, and anti-IL-23, is rapidly expanding for the management of widespread and aggressive PG.[137] Although detailed knowledge of how biological drugs work for ND is lacking, the expression of several cytokines, including TNFα, IL-8, IL-17, and IL-23, is up-regulated in PG, which may explain the favorable clinical results obtained with biological agents such as ustekinumab.[37] A literature search found 21 out of 23 ND patients had responded positively to ustekinumab (17 PG, 4 amicrobial pustulosis of the folds, 1 Bowel-associated dermatosis-arthritis syndrome, and 1 SS), 16 (70%) were complete responders and 5 (21%) were partial responders, whereas no responses were seen in one PG and one chronic recurring Sweet syndrome.[38–40,138]

Ustekinumab for muco-cutaneous manifestations of Behçet disease (BD)

BD is a primary vasculitis that manifests specifically as repetitive attacks of oral-genital ulcers, cutaneous inflamed lesions, and uveitis with multiple organ system involvement, including the gastrointestinal, cardiopulmonary and nervous systems.[139] Whereas systemic vasculitis may lead to worse outcomes, repetitive aphthous ulcers often lead to substantial QoL impairment. These mucocutaneous lesions could be managed with colchicine, azathioprine, thalidomide, and more recently apremilast, although with varying success and potential serious AEs. However, increased knowledge of the immune mechanisms responsible for BD has prompted the

use of biological drugs to manage the more intractable mucocutaneous lesions. One randomized controlled, 4-week trial and several observational studies and case series have showed that TNF-α inhibitors are promising treatment options for recalcitrant mucocutaneous disease.[140] Additionally, emerging evidence suggests that Th1 and Th17 responses may contribute to the pathogenesis and progression of BD. This, together with higher IL23 levels and Th17/Th1 ratios in BD patients, suggest that ustekinumab may be reasonable and effective for the management of BD. In support of this, ustekinumab has been demonstrated to be effective in ameliorating recalcitrant oral aphthous ulcers in BD patients in clinical trials.[41,140]

Ustekinumab for atopic dermatitis (AD)

AD is a frequent pruritic inflammatory dermatosis, which commonly follows a remitting-relapsing chronic course and commonly develops in a patient with atopic diathesis.[141] The pathogenesis of AD is thought to be both skin barrier alteration and immune system dysfunction. The conventional treatments rely on topical anti-inflammatory drug medication and adequate skin hydration. However, systemic immunosuppressant medications are required for moderate to severe forms of the disease; although they are discouraged, owing to their transient efficacy and a poor AE profile.[142] The identification of new immune targets involved in the process of AD has prompted the development of innovative therapeutics, including biological therapy and small molecules. Studies have showed that IL17 and IL22 expression are upregulated in AD lesions and represent therapeutic targets for ustekinumab treatment.[42] Indeed, many studies have investigated the efficacy of IL-12/IL-23 inhibitors for patients with recalcitrant AD. Pan Y *et al*[143] conducted a systematic review on the current scientific literature up to September 2017 concerning the benefits of ustekinumab in AD. They found that this biological drug has been administered in 8 case reports and 2 randomized placebo-controlled trials (RCTs) of 107 cases. In general, the observational studies have shown more clinically relevant effects, whereas RCTs have not shown a significant advantage of ustekinumab over the placebo.

Ustekinumab for Alopecia Areata (AA) and Vitiligo

AA is a common IMD that causes temporary and permanent non-scarring alopecia.[144] Treatment of AA by conventional systemic therapy is hampered by its AEs and limited efficacy. Nevertheless, the discovery of the role of various immunological mediators, including Th1, Th2, and IL-23, in the pathological process of AA has opened a door to test the efficacy of ustekinumab for AA.[145] There are some reports on the therapeutic efficacy of ustekinumab for new onset AA and some cases with hair regrowth.[146] Guttman-Yassky *et al*[43] demonstrated that treatment with ustekinumab for 20 weeks improved clinical symptoms in three moderate-to-severe AA patients without AEs. Likewise, ustekinumab has safely ameliorated clinical symptoms in three pediatric patients with mild, moderate, and severe AA.[44]

Vitiligo is also a long-lasting IMD and consists of depigmented skin macules. Like AA, Th17 cells are the major immune players in vitiligo pathogenesis.[147] Therefore, it is reasonable to test the therapeutic efficacy and safety of ustekinumab for vitiligo. However, there is only one report on the use of ustekinumab for repigmentation in a patient with both psoriasis and vitiligo, and these findings contrast other observations.[45,148,149]

Miscellaneous

Ustekinumab has been reported for the treatment of miscellaneous cutaneous disorders, like SAPHO syndrome (Synovitis, Acne, Pustulosis, Hyperostosis and Osteitis), lichen planus (LP), bullous pemphigoid (BP), and lupus erythematosus (LE). Some data have demonstrated an aberrant Th17 response in SAPHO patients, which suggests that ustekinumab may be promising for SAPHO syndrome.[46] However, only 5 SAPHO cases have been treated with IL-12/IL-23 blockers with mixed results on cutaneous symptoms, as less than half of the patients had improved symptoms.[150]

There is little evidence on the efficacy of IL12/IL23 blocker for LP. Although treatment with ustekinumab was reported to remarkably improve extensive erosive oral LP in one report,[47] ustekinumab treatment failed to show any efficacy in another report with concomitant psoriasis and erosive LP.[48] There are controversial reports on the therapeutic effect and deteriorative outcomes of ustekinumab in BP patients.[151,49]

Regarding systemic LE, a phase II RCT conducted by Ronald van Vollenhoven *et al*[152] to test the therapeutic effect of ustekinumab in 102 active systemic LE patients has revealed that addition of ustekinumab to standard therapy enhances therapeutic efficacy. Although peculiar cases of ustekinumab-induced lupus-like cutaneous reactions have been reported, many successful cases have been widely reported on the therapeutic efficacy of ustekinumab for cutaneous and discoid LE.[50,51,153]

AEs observed with ustekinumab

Most AEs associated with ustekinumab use are non-serious, occasional, and usually do not lead to drug discontinuation.[154] The most commonly encountered AEs are headaches, asthenia, abdominal pain, and upper respiratory infections. Local injection site reactions are also usually mild in severity and infrequent, probably due to a minimal injection regimen.[3] Moreover, there have been no reported differences in the frequency of AEs or abnormal laboratory tests between ustekinumab- and placebo-treated patients in clinical trials.[154]

IL-17 is a pro-inflammatory cytokine that can participate in immune responses against bacterial and fungus infections. Therefore, treatment with ustekinumab to block the IL-12/IL-17-related signaling may increase susceptibility to infections.[155] However, the infectious risk due to ustekinumab was low in clinical trials. Furthermore, analysis of published register-based data did not show higher rates of severe infections when comparing ustekinumab to either anti-TNF agents or conventional systemic therapies.[156,157] In particular, the potential risk of active tuberculosis infection due to ustekinumab seems to be reduced when compared to TNFα inhibitors.[158] In addition to infectious hazards, the most reported AEs of ustekinumab treatment are the risk of major adverse cardiovascular events (MCCEs); a meta-analysis of RCTs in 2011 reported an increase in MCCEs during the first months of drug exposure, although there was no significant increase in the frequency of MACEs when compared to placebo.[159] This report is correlated with experimental studies, in which the IL23/IL17 pathway negatively affects atherosclerotic plaques stability. Nonetheless, a case-control study of ustekinumab from the French National Health Insurance database involving more than 9,000 subjects during the period of 2010–2016, revealed a significant link between ustekinumab therapy and the onset of acute coronary syndrome, while stroke was identified only with high cardiovascular risk patients.[160] Another important concern regarding ustekinumab use is its potential oncogenic effect. Animal-based studies have revealed that blockade of IL12/IL23 signaling may increase

Table 2. Skin adverse events associated with ustekinumab

Skin lesions related to the administration of treatment	Bruising, pruritus, pain, erythema, swelling, skin rash
Skin infections	Bacterial infections: cellulitis, mycobacterium abscessus, secondary syphilis, staphylococcal skin colonization; Viral infections: disseminated verrucae, condyloma acuminate, herpes zoster; Fungal infection: cutaneous candidiasis, Nocardia infection, disseminated sporotrichosis; Parasitic infections: plurifocal cutaneous leishmaniasis, cutaneous protothecosis
Skin neoplasia	Non melanoma skin tumors: basocellular carcinoma, spinocellular carcinoma; Malignant melanoma; Skin lymphomas/Lymphoproliferative disorders: Jessner-Kanof type, anaplastic large T cell lymphoma, mycosis fungoides; Multiple dermatofibromas
Immune mediated diseases	«de novo» psoriasis and exacerbation of prior psoriasis or psoriasis subtypes; Atopic-dermatitis and its exacerbation; Lupus-like paradoxical reaction; Alopecia areata; Skin vasculitis; Vitiligo; Dermatomyositis; Localized scleroderma (morphea); Lichen or lichenoid reaction; Frontal fibrosing alopecia; Linear IgA bullous dermatosis; bullous pemphigoid; Erythema multiforme; Erythroderma, exfoliative dermatitis and hypersensitivity reaction; Erythematous annular eruptions; Fixed drug eruption; Urticaria
Other skin events	Hidradenitis suppurativa; Seborrhoeic keratosis; Thrombotic thrombocytopenic purpura; Sarcoidosis-like paradoxical reaction; Wells syndrome; Erythema annulare centrifugum; Cutaneous focal mucinosis; Lentigines; Spiny follicular hyperkeratosis

IgA, Immunoglobulin A.

the risk of malignancies.[161] However, despite scarce reports of malignant tumors, cancer incidence itself was low in clinical trials and in register-based data.[157] There are neither observational studies, nor case reports on any increase of adverse outcomes in pregnant women.

Furthermore, ustekinumab treatment-related uncommon cutaneous and systemic AEs have been reported, including immune-mediated dermatological disorders. The most reported skin and systemic AEs associated with ustekinumab use are summarized in Tables 2 and 3, respectively.

Despite the reassuring data, the real-life, long-term safety of ustekinumab application still requires investigation across international, multicentric registry-based cohorts and from long-term outcome trials. Hence, greater vigilance should be applied when starting treatment: a thorough history, a holistic clinical examination with careful assessment for active infections (screening for possible tuberculosis, checking cardiovascular and neurological functions and ruling out any malignancy), along with laboratory workup (complete blood count and metabolic profile) should be considered before the initiation of ustekinumab. Subsequent laboratory tests and follow-up monitoring are recommended.

Future prospects

Currently, a myriad of biological drugs (either approved or used off label) are available for the treatment of skin diseases. For psoriasis, physicians have a plethora of biological drugs with different immunological mechanisms that could be used. Nonetheless, some patients can be resistant or show a declining response to biological agents over time.

Combination therapies with biological and conventional systemic drugs are well documented and have become a routine practice for many clinicians. However, for patients with a severe, debilitating skin disease, who do not respond to biological monotherapy and combination with conventional systemic agents, dual biological therapy (DBT) could be considered. However, uncertainty in the real safety profile of such a combination still exists, particularly for the high risk of opportunistic infection and MACEs, and data on the safety of such DBT in dermatology remain anecdotal. The limited number of case reports and case series mostly originated from gastroenterology and rheumatology-based studies and/or registries, but DBT has been used in many cases with PsA/psoriasis or inflammatory bowel diseases/psoriasis simultaneously. Available studies have shown that DBT ustekinumab/anti-TNFα blockers have better efficacy than each drug alone, although there are different safety profiles, without serious AEs. In dermatology, only one case of DBT of ustekinumab/adalimumab has been reported in a patient with a long-lasting, resistant PPP for a quasi-complete clearance over 4 months with a good overall tolerance. Thus, a window of opportunity does exist for the use of DBT with ustekinumab and other biological drugs for the treatment of psoriasis or other skin diseases, paving the way to a tailored, personalized treatment regimen. Despite the paucity of data, dermatologists can be inspired from the use of DBT in other fields, like gastroenterology and rheumatology. The main future challenges are to determine the optimal treatment dosing regimen and the best timing for DBT to result in the most effective and safest outcomes for patients.

Conclusions

Continual progression in psoriasis research has revealed the crucial role of Th17 responses in its pathogenesis. The successful treatment with IL-12/IL-23 blockers for moderate-to-severe psoriasis is considered a major scientific breakthrough, being the first non-TNFα targeted biological drug in the treatment of psoriasis and heralded as a new era of more precise biological therapy with higher efficacy and favorable safety profiles. Additionally, the recommended dosage regimen of ustekinumab is appropriate for most patients because its initial efficacy seems to be sustained fairly well over a 5-year treatment duration. The potential risk of infection or other AEs in patients with ustekinumab are mild, similar to that in placebo-treated patients, and there is no evidence of any overall increased risk in post-marketing reports. However, like other new biological drugs, high cost and unknown long-term effects limit the approval of this drug as a first-line treatment for moderate-to-severe psoriasis. Emerging data suggest that ustekinumab may be well tolerated and efficient for HS, PRP, and BD, as well as several

Table 3. Adverse systemic reactions associated with ustekinumab

Systemic allergic reactions to the administration of treatment	**Flushing, anaphylactoid reaction, nausea, vomiting, blurred vision and/or confusion, dizziness, difficulty in breathing**
Whole body (general disorders)	Asthenia, Flu-like symptoms, myalgia, anorexia, depression, sleep disturbance
Infections	*Bacterial infections*: latent tuberculosis reactivation, miliary tuberculosis, meningococcal meningitis, pneumonia, Clostridium difficile infection, Mycobacterium fortuitum ventriculoperitoneal shunt infection, perianal abscess, dental abcess, urinary tract infection, Staphylococcus aureus bacteremia with iliac artery endarteritis, Streptococcal sepsis; *Viral infections*: HSV-2 meningitis, Varicella zoster virus meningitis, acute hepatitis B, HBV reactivation, HCV reactivation, herpes simplex virus encephalitis, nasopharyngitis , Respiratory tract infections; *Fungal infection*: mycotic oesophagitis; *Parasitic infections*: Amoebic liver abscess, Ocular toxoplasmosis, severe acute toxoplasmosis
Neoplasia	Anal adenocarcinoma, cancer of anal fistula, endometrial cancer, esophageal cancer, hepatocellular carcinoma, pancreatic adenocarcinoma Malignant peritoneal mesothelioma, Gastric Mucosa-Associated Lymphoid Tissue Lymphoma, Exacerbation of Hodgkin's lymphoma, chronic lymphocytic leukaemia, multiple myeloma, papillary thyroid cancer, breast cancer
Cardiovascular events	Hypertension, congestive heart failure, dilated cardiomyopathy, unstable angina, Vasculitis, central retinal vein and artery occlusion
Gastrointestinal events/ Hepatobiliary events	Acute hepatitis, elevated alanine transferase levels, fatty liver infiltration, diverticulitis, retroperitoneal fibrosis, pancreatitis
Musculoskeletal events	Paradoxical psoriatic arthritis, arthralgia, multifocal myositis, polymyositis, myasthenia gravis
Renal adverse events	Lupus nephritis, new-onset autoantibody-mediated nephritis, nephrotic syndrome, IgA nephropathy, focal segmental glomerulosclerosis
Nervous system events	Headache, neuropathic pain, memory loss, parkinsonism, benign intracranial hypertension, posterior reversible encephalopathy syndrome, demyelination, limbic encephalitis, Facial palsy, reversible cerebral vasoconstriction syndrome, ischaemic stroke, Guillain-Barré syndrome, peripheral neuropathy
Respiratory adverse events	Noninfectious pneumonia, bronchospasm crisis, pneumothorax, sarcoidosis
Urogenital and obstetric events	Urolithiasis; Epididymo-orchitis, erectile dysfunction; Foetal death and miscarriage;
Other systemic events	-Monoclonal gammopathy of undetermined significance; Autoimmune thyroiditis

HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; HSV-2, Herpes Simplex Virus-2.

other dermatological conditions, but there are few clinical trials to evaluate the therapeutic efficacy and safety of ustekinumab for these disorders.

This review highlights the significant progression during the past decade on the optimal use of ustekinumab for skin diseases beyond its labeled indications. However, there are some limitations, like the lack of RCTs and the limited amount of available data, especially regarding the off-label use of the biological drug. Further studies with larger cohorts of patients and robust designs are warranted to investigate ustekinumab's efficacy, safety, and long-term effects in off-label uses for other skin diseases.

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Conflict of interest

Dr. Hakim Rahmoune has been an editorial board member of *Journal of Exploratory Research in Pharmacology* since June 2017. The authors have no other conflict of interests to declare.

Author contributions

Contributed to study concept and design (ASC, NB, and HR), acquisition of the data (ASC and HR), data analysis (ASC), drafting of the manuscript (ASC and HR), critical revision of the manuscript (NB and HR), and supervision (ASC).

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Review Article

Utilization of Ketamine for Major Depression

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Abstract

Major depressive disorder (MDD) continues to be a prevalent disease worldwide. While selective serotonin reuptake inhibitors and other medications continue to be prescribed, further research has been conducted toward other treatment modalities. Within the past decade, ketamine, an N-methyl-d-aspartate receptor antagonist, has been extensively studied as a new treatment for MDD. Recent studies show that ketamine at subanesthetic doses provides antidepressant effects. An extensive overview of the latest statistics of MDD and treatment plans are emphasized, with a review of current medications and their subsequent side effects. However, an important factor to consider with ketamine is dissociation, and given ketamine's psychotomimetic side effects, it must be reviewed further. Despite such side effects of hallucinations and depersonalization, studies have shown administration achieves rapid and lasting antidepressant effects. The synergistic effects of ketamine are also analyzed with recent studies to summarize the effects of different treatment modalities. A summary of the latest research studies of ketamine as a possible treatment for MDD is provided. By focusing on the evolution of ketamine as a treatment for MDD, physicians can now utilize newer techniques for depression, with better short-term and long-term outcomes for the patients.

Introduction

Major depressive disorder (MDD) is a common psychiatric diagnosis affecting millions worldwide. While monoaminergic drugs have long been the preferred method of treatment for MDD, they can often take weeks or months to provide a therapeutic effect, with many patients failing to find any relief in their depressive symptoms. Patients who have failed after two oral therapies are considered to have treatment-resistant depression (TRD). Given the high prevalence of MDD and potentially subsequent TRD, there has been a surge of interest in finding new short-acting therapies, novel to the standard methods of treatment. Ketamine was first discovered in the 1970s as an anesthetic medication but has since been extensively studied in multiple research projects. Through antagonism of the N-methyl-D-aspartate (NMDA) receptor, ketamine works to decrease hyperalgesia but has also been found to affect descending inhibitory serotonergic pathways. Research has shown that Ketamine, given regularly in a controlled manner, could be a promising potential treatment for patients with TRD and other depressive disorders, including post-traumatic stress disorder (PTSD), as it has been shown to rapidly reduce suicidality and depression in patients who have failed other therapies.

MDD and other depressive disorders have become increasingly prevalent within the United States, especially since the beginning of the COVID-19 pandemic in 2020.[1,2] Data from the National Survey of Drug Use and Health (NSDUH) reported that around 21.0 million US adults (8.4% of all US adults) experienced a major depressive episode in 2020, an increase from 16.2 million (6.4% of all US adults) in 2016.[3] Despite increased public health efforts and advancements in the field of psychiatry, there is still an increasing amount of depression without a compensatory increase in treatment response.[4] A recent study showed that this phenomenon, otherwise known as the "treatment-prevalence paradox" (TPP), was prevalent in that despite increased efforts to address MDD, the incidence of MDD diagnosis continues to rise.[4,5] The TPP strongly suggests an overdiagnosis of MDD or lack of response in treatment for MDD as the causes for this discrepancy.[6,7]

Many patients who suffer from MDD are hesitant to seek proper treatment, and those who do start taking common antidepressant medications tend to experience high treatment failure rates or minimal improvement in their symptoms.[8] Some randomized control trials (RCTs) have shown that serotonin selective reuptake inhibitors (SSRI), the current standard of treatment, were only 20–30% more effective than that of a placebo.[9] Other medications such as oral monoaminergic drugs carry a dangerous warning of increasing risks of suicidal ideation and behaviors. Patients must

Keywords: Depression; Ketamine; Anesthesiology; Psychiatry.

Abbreviations: AMPAR, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF, brain-derived neurotrophic factor; CBT, cognitive behavioral therapy; ECT, electroconvulsive therapy; mTOR, mechanistic target of rapamycin; MDD, major depressive disorder; NMDA, N-methyl-D-aspartate; SSRI, serotonin selective reuptake inhibitor; TRD, treatment-resistant depression; TPP, treatment-prevalence paradox.

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wait weeks to months to experience any form of symptomatic relief.[10] When patients are unable to respond to over two treatment regimens for their depressive symptoms, they are considered to have treatment-resistant depression (TRD) which is associated with greater levels of suicidality and higher risks of repeated hospitalization.[11–13]

Traditional pharmacological methods of treatment

The standard of care for pharmacological treatment of depression has gone largely unchanged for many years.[13] The basis of using these monoaminergic drugs ties into the pathophysiology of MDD, where levels of monoamines such as dopamine, norepinephrine, serotonin, epinephrine, and histamine are seen to be reduced. As such, the traditional pharmacological approach was to address this monoamine shortage by using pharmacological methods to increase their levels.[14] Despite initial success with this approach, there has been a large number of patients reporting no significant improvement in their symptoms, thus necessitating newer treatment methods to be considered.[15–17] With each subsequent generation, monoaminergic medications have become more tolerable, however, the targets and basis behind treatment have remained largely unchanged.[18] There is interest in research beyond the scope of the monoamine hypothesis—such as looking at other signaling cascades associated with stress responses and depressive symptoms.[18,19]

Mechanisms of action of current antidepressant treatments

Monoamine oxidase inhibitors (MAOIs)

MAOIs inhibit the activity of monoamine oxidase, which functions to break down serotonin, norepinephrine, and dopamine in the brain. By inhibiting monoamine oxidase, MAOIs increase the levels of these neurotransmitters in the brain, thus enhancing their neurotransmission. While MAOIs have been shown to have greater efficacy than subsequent generations of antidepressants, they are also much less tolerable. Major side effects of MAOIs include an increased risk of hypertensive crisis, dizziness, sexual dysfunction, and GI upset.

Tricyclic antidepressants (TCAs)

TCAs work by blocking the reuptake of both serotonin and norepinephrine but also affect other neurotransmitter systems, such as acetylcholine and histamine, thus precipitating antimuscarinic effects, including potential cardiovascular complications. Some examples of TCAs include amitriptyline, nortriptyline, and imipramine.

Serotonin-noradrenaline reuptake inhibitors (SNRIs)

SNRIs work by inhibiting the reuptake of both serotonin and norepinephrine, which increases the availability of both neurotransmitters in the synaptic cleft, thus enhancing their neurotransmission. SNRIs include venlafaxine, duloxetine, and desvenlafaxine.

Selective serotonin reuptake inhibitors (SSRIs)

SSRIs increase the availability of serotonin in the synaptic cleft by inhibiting the reuptake of serotonin in the brain, leading to subsequent increased serotonin neurotransmission. With both SSRIs and SNRIs, patients require at least 4–6 weeks to acquire any optimal effect.

Atypical antidepressants

These miscellaneous medications have different mechanisms of action that have been proven to act as an antidepressant. Some examples include bupropion, which works by increasing the levels of dopamine and norepinephrine in the brain, and mirtazapine, which works by enhancing the release of both serotonin and norepinephrine and also blocks certain serotonin receptors.

Ketamine as a treatment for depression

Mechanism of action

Discovered first in the 1960s as an anesthetic, ketamine has been extensively utilized for intraoperative anesthesiology and in acute trauma settings. Ketamine is a phencyclidine derivative, which is a compound primarily classified as a hallucinogen. Through competitive antagonism of the *N*-methyl-d-aspartate (NMDA) receptor, ketamine antagonizes the amplification of pain signals and modulates the central sensory processing of pain for analgesic effects. Higher doses of ketamine work on other receptors, including monoamine transporters, dopamine D2 receptors, and voltage-gated sodium channels. By activating other receptors, major side effects of ketamine start to take effect; primarily, hallucinations, euphoria/dysphoria, agitation, and anxiety. By inhibiting the NMDA receptors on the GABAergic interneuron, ketamine causes a large surge of glutamate activity that works to depolarize the postsynaptic neuron in releasing sodium and calcium. The subsequent ions cause the release of vesicle-filled brain-derived neurotrophic factor (BDNF) into the synaptic cleft.[20] Please see Figure 1 for further analysis of ketamine's molecular mechanism of action as an antidepressant.

Ketamine inhibits NMDA receptors primarily present on inhibitory GABAergic interneurons at lower subanesthetic doses. By inhibiting an inhibitory neurotransmitter, there is a surge of glutamate release from the increased depolarization of the presynaptic neuron, which then binds to and activates postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR). The subsequent Na+ and Ca2+ ions entering the postsynaptic cell activate the voltage-gated calcium channels, which release vesicles filled with brain-derived neurotrophic factor (BDNF) into the synaptic space. BDNF acts upon tropomyosin receptor kinase B (TrkB) to activate the MEK–ERK and PI3K-Akt signaling pathways to produce the mechanistic target of rapamycin (mTOR). mTOR acts as a facilitator of protein synthesis and is believed to play a role in neuroplasticity. The downstream protein synthesis resulting from this cascade ultimately gives rise to increased synapse generation, thought to play a role in ketamine's antidepressant effects.

Current studies showcasing antidepressant effects of ketamine

Once studies found that lower/subanesthetic doses of ketamine could provide antidepressant effects, a significant surge of interest in studying ketamine took place and has continued over the past decade or so. Ketamine can be administered by a variety of routes including the oral, sublingual, transmucosal, intranasal, intravenous, intramuscular, and subcutaneous routes. The relationship between the route of admission, dosing, timing of doses, and resulting effect is complex, given that ketamine has major effects on multiple organ systems, especially, and significantly, the cardiovascular system. Further studies should be conducted towards deciding optimal routes of administration, given that these factors are limiting for ketamine to be prescribed in an outpatient setting.[21] There is some evidence that the mechanism of Ketamine's antidepressant effects comes from downstream signaling as a result of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) activation. Studies have shown that applying an antago-

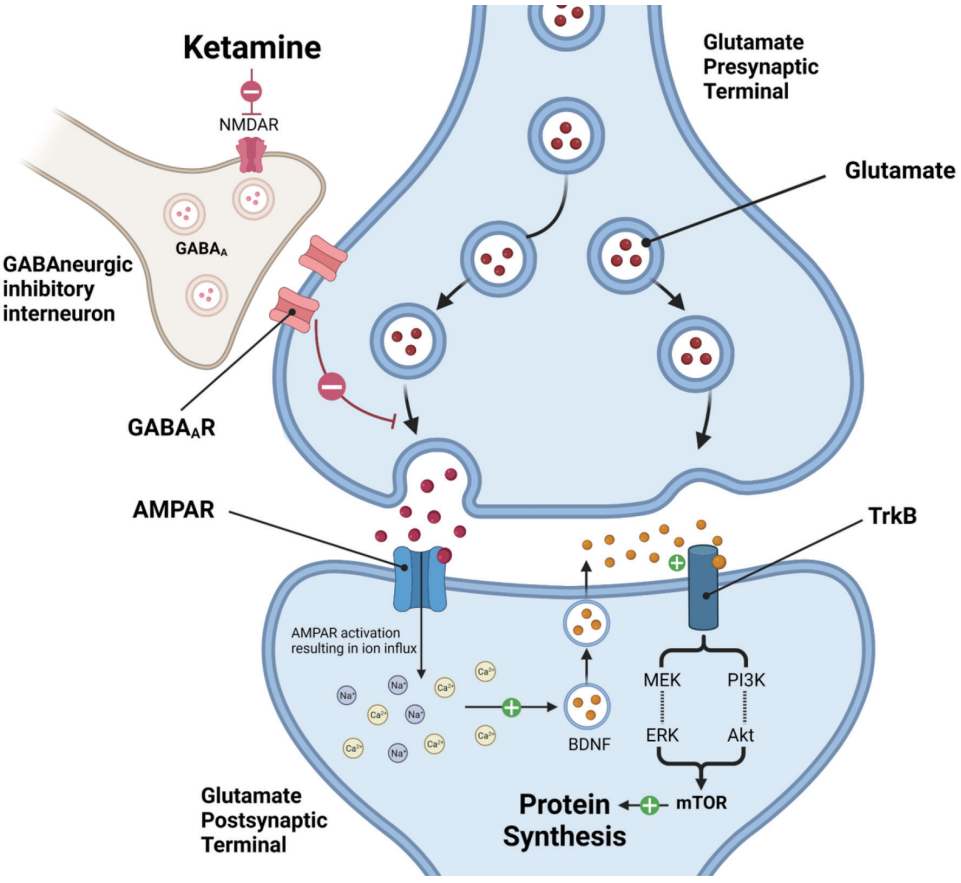

Fig. 1. Mechanism of ketamine as an antidepressant.

nist to AMPAR greatly diminishes the resulting antidepressant effect, which suggests that much of ketamine's ability to function as an antidepressant comes from the downstream effects of AMPAR activation, notably the activation of tropomyosin receptor kinase B (TrkB) resulting in the production of the mechanistic target of rapamycin (mTOR).[22]

A recent study conducted in 2011 focused on the administration of ketamine to induce antidepressant-like effects in rats. Intraperitoneal ketamine of 10 mg/kg was administered 30 minutes prior to inducing escape failures for learned helplessness and subsequent antidepressant effects. One of the controls was a specific inhibitor of the AMPA receptor to measure learned helplessness and depression by utilizing the tail suspension test. Through multiple reviews, this study concluded that the anti-depressant effects of ketamine were partially abolished in the cohort of subjects that had direct AMPA receptor antagonism, suggesting that AMPA receptor activations play a crucial role in the antidepressant-like effects of ketamine.[23]

Studies aimed at understanding ketamine's use as an antidepressant have suggested that its psychotomimetic side effects play some role in facilitating its antidepressant effects. However, because of its addictive potential and side effect profile, many providers are concerned about its long-term administration, thus necessitating further research into mitigating its psychotomimetic side effects and addictive potential. These studies suggest that the R-ketamine enantiomer is able to facilitate antidepressant effects with limited psychotomimetic side effects and reduced addictive properties, which suggests enantiomerically pure R-ketamine as a potential novel treatment with reduced side effects.[24–26] While S-ketamine seems to produce psychotomimetic effects and with greater addictive potential, these side effects could play some role in producing its antidepressant effect.[26–28] Current ketamine infusion treatment involves a racemic mixture of both R- and S-ketamine. A ketamine intranasal spray (esketamine/Spravato) of enantiomerically pure S-ketamine was approved by the FDA in 2019 as a treatment option for patients who have TRD. Early case reports showed acute resolution of depression and suicidality in patients in patients who had taken the spray.[29] suggesting a potential role for ketamine in the treatment of depression. More recent clinical trials following FDA approval have shown that treatment of esketamine and an oral antidepressant together can even lead to longer-lasting effects and can prevent relapse of symptoms.[30,31]

Unique qualities of ketamine treatments

As discussed previously about ketamine's mechanism of action, ketamine is unique in that it affects multiple receptors at higher doses, similar to other select medications. With a half-life of 45 minutes, ketamine can work on cholinergic, aminergic, opioid, and voltage-gated sodium channel receptors with increasing doses, having a modulatory role in sedation and analgesia.[32] It also allows for spontaneous respiration by not affecting the pharyngeal and laryngeal reflexes, especially important during extubation for anesthesia. As a cyclohexanone derivative, ketamine creates a dissociative effect on the patient that has been actively analyzed for

analgesia and sedation. From realizing its dissociative properties, ketamine was further studied as a synergistic medication, given its effect on multiple receptors. In 2004, a research study focused on the synergistic effect between ketamine and morphine for analgesic purposes. They found that humans who received both ketamine and morphine as opposed to morphine alone experienced a greater amount of pain relief in their burns, opening the door for central sensitization.[33] Along with affecting the other receptors, ketamine at higher doses blocks high-affinity dopamine D2 receptors. With its increasing popularity as a "club drug" in the 1990s, the addicting ketamine attracted younger generations for its role in producing delirium, slowing down the perception of time, and altering states of consciousness.[34] A study analyzed the effects of chronic ketamine use in the said population, which revealed atrophy of the frontal, parietal, occipital cortices, prefrontal lobes, brain stem, and corpus striatum. If administered in a controlled environment to limit its potential abuse, ketamine's psychotomimetic effects could have a strong potential role in the treatment of analgesia and even depression.

Dissociative effects and their role in ketamine treatments

While ketamine has been shown to be a promising treatment option for TRD, it is associated with robust psychotomimetic side effects. These side effects limit its potential as a treatment choice, as there are concerns over treatment safety and potential substance abuse. Discussion on the topic generally takes place over whether these side effects are necessary for ketamine treatment efficacy or if they are merely an unintended off-target effect.[35] Studies performed on ketamine's full psychotomimetic effect profile show that dissociation is the most closely correlated psychotomimetic side effect to treatment effectiveness though it is still unclear whether dissociation is an unintended off-target effect of ketamine or if the subjective experience itself plays a key role in facilitating its antidepressant effects.[36] Some studies suggest that dissociation should be viewed as a facilitator of depression treatment, rather than an unintended side effect.[37] This association is typically compared in parallel to that of other dissociatives that can produce similar antidepressant effects, albeit through different mechanisms of action with the resolution of depression thought to be associated with subjective dissociative experiences produced by these substances.[38] Studies regarding dissociatives suggest that the subjective experience during dissociation has a psychological role, rather than a physiological one, in producing positive mood changes and treating depression.[39] Clinical studies have shown that other NMDA receptor antagonists with little to no dissociative properties are unable to produce a comparable response to that of ketamine. While these NMDA receptor antagonists were able to produce antidepressant effects, they are shorter-lived and less profound.[40,41] However, some clinical trials have suggested that there is no real correlation between the response to ketamine as an antidepressant and its acute dissociative effects.[42,43] These studies have suggested that dissociation, rather than being a feature of ketamine's antidepressant effect, is an unintended side effect associated with its mechanism of action, unrelated to its antidepressant effect.[44] Research continues to be carried out to evaluate the safety of ketamine as an antidepressant due to its dissociative properties. As mentioned before, the R-ketamine enantiomer seems to have a reduced side effect profile while retaining its antidepressant effectiveness, making it a promising safer treatment option than that of current ketamine infusions and S-ketamine.[25] An analysis of literature has been conducted to assess if studies are able to correlate dissociation with treatment outcome but concluded that there is too much variation between studies to truly make a conclusion about the relationship.[45] The variation between studies suggests that there is a need for further research to determine whether or not dissociation truly plays a role in ketamine treatment efficacy.

Synergistic use of ketamine with traditional methods of treatment

Ketamine has been analyzed for its potential to be used synergistically with other traditional methods of treatment. Oral antidepressants were studied with concurrent use of ketamine, with results showing that the antidepressant effects were prolonged in patients with TRD.[30] This finding has opened up discussion and the potential to use sub-dissociative doses of ketamine alongside other treatments to enhance their effects. A study from 2017 showed that the coadministration of ketamine and fluoxetine had significant antidepressant effects on rats; however, the combination of ketamine and quetiapine did not produce similar results. The aforementioned ketamine/fluoxetine combination concurrently showed an increase in the antioxidant activity of superoxide dismutase, leading to decreased oxidative damage, and opening future studies towards anti-inflammatory properties for neuroprotection.[46] Alongside oral antidepressants, studies have shown that the administration of ketamine in patients undergoing electroconvulsive therapy (ECT) results in increased cognitive functioning compared to patients who receive ECT alone. Patients who receive ECT are typically those who have TRD and have failed psychotherapy and medication management. The possibility of harmful behaviors between the start of treatment and the response to ECT is also a major challenge given that ECT takes some time to achieve its optimal effect. A study was conducted in 2015 to analyze the relationship between ketamine and ECT for its synergistic effects on the recovery of patients. Given the strong concern for ketamine's potential for cognitive impairments, especially at certain doses, further studies will continue to be conducted for proper research. This research analyzed 22 patients with MDD who underwent ECT and received ketamine and propofol vs. only propofol. While the results were not statistically significant in showing a reduction in depression severity between the two groups, this study does point towards a better recovery time for cognitive performance in the group who received ketamine compared to the control group.[47]

Cognitive behavioral therapy (CBT) and other conservative forms of treatment, including psychotherapy, have been shown to sustain and enhance the effects of ketamine as an antidepressant, further supporting ketamine's synergistic theoretical effects. There are very limited studies that evaluate ketamine-assisted psychotherapy for TRD. One study from 2022 concluded that adjunct psychotherapy may play a role in the treatment of TRD, but patients were found to have temporary neural changes.[45] A separate clinical trial from 2021 assigned patients to receive CBT alongside intravenous ketamine vs. the control group of only ketamine for TRD. Subjects in the CBT group showed clinically and statistically significant improvement towards the end of the study, which further supports this treatment approach.[48] The synergistic properties that ketamine has been shown to demonstrate alongside other methods of treatment suggest clinical use as a potentiating agent. Further research on using ketamine as a potentiating or synergistic agent alongside well-established traditional forms of treatment is needed before making any conclusions; this proposed use of ketamine at sub-dissociative doses to potentiate the effects of more well-established treatment options could be a theoretically safer option for patients with TRD.

Future directions

Because of its success in treating previously treatment-resistant patients, using ketamine as a glutamate modulator has sparked interest in using drugs with similar mechanisms of action as new potential treatments for depression. Like ketamine, propofol is also able to induce antidepressant effects, and xenon, which also has theoretical use as an anesthetic, has been shown to be successful in the rapid reduction of depressive symptoms and anxiety symptoms in animal models.[49,50] However, because of its potential for abuse and addiction, there are valid concerns about the long-term repeated use of ketamine**.** Despite many case studies and short-term studies reporting treatment success, there is still limited data on the long-term repeated administration of ketamine and its longitudinal risks and complications in patients with TRD and other psychiatric illnesses. Future large-scale longitudinal studies regarding treatment efficacy and safety are strongly encouraged before ketamine can be widely administered safely in an outpatient setting for TRD.

Conclusion

FDA approval of ketamine as a potential treatment for TRD has undoubtedly already affected how the treatment of MDD, alongside other psychiatric disorders, will be approached. In this review, we presented the efficacy of ketamine as a treatment for depression and identified major targets of its mechanism of action. While previous treatment centered on monoaminergic modulation, ketamine treatment relies upon NMDA receptor antagonism leading to downstream AMPAR blockade, which may explain its considerable efficacy for those who have failed treatment with first-line monoaminergic drugs such as SSRIs. The success of ketamine treatment has revealed the complex pathogenesis of MDD; there is hope that future studies on ketamine may lead to the exploration of new pharmacological targets and future drug development.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Manuscript planning (RD and AA), manuscript drafting (RD), manuscript writing (RD and AA), critical revision of article (RD and AA). All authors have made a significant contribution to this study and have approved the final manuscript.

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Review Article

Role of TRPV1 in Health and Disease

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Abstract

Transient receptor potential vanilloid 1 (TRPV1) channel is a non-selective cation channel that plays a pivotal role in pain transduction. However, more than a pain sensor, it is involved in an array of vital processes in different body systems. The findings of several studies illustrated that many disorders are associated with alterations in the function and/or expression of the TRPV1 channel. Accordingly, the TRPV1 channel has become an important target in numerous therapeutic interventions. Several TRPV1 antagonists are already in the market, however, there is a need for new drugs with fewer or no side effects. This review highlights the involvement of the TRPV1 channel in a plethora of physiological and pathological conditions and points to its importance as a therapeutic target.

Introduction

Transient receptor potential vanilloid 1 (TRPV1)

In 1997, TRPV1 receptor was cloned from the dorsal root ganglia (DRGs) neurons of rats.[1] Since then, multiple studies have been conducted to elucidate the structure, mechanisms and roles of the TRPV1 channel in health and disease. The TRPV1 channel is a non-selective cation channel characterized by cation influx when activated[1] with a very high calcium (Ca^{2+}) permeability ($P_{Ca}/P_{Na} \sim 10$).[1] Previous research highlights that several endogenous and exogenous stimuli activate the TRPV1 channel. More specifically, the channel is activated by noxious heat (>43 °C), anandamide, low extracellular pH, redox state, prostaglandins (PGs), nerve growth factor (NGF), substance P (SP), oxytocin, lysophosphatidic acid, 9, 13 and 20-hydroxyoctadecadienoic acid, linoleic acid as well as the highly selective agonists capsaicin and resiniferatoxin (RTX).[1–3]

TRPV1 structure

Figure 1 depicts TRPV1 structure. TRPV1 channel possesses a tetrameric structure with 6 transmembrane domains and pore-forming hydrophobic stretch linking segment 5 (S5) and S6.[4] The channel has an unusual characteristic in which it has cytosolic intracellular C and N termini.[5] Notably, a considerable amount of literature showed that the TRPV1 channel contains multiple phosphorylation sites whereby its activity can be regulated by various kinases, including protein kinase A (PKA), PKC, Ca^{2+}/calmodulin dependent kinase II (CaMKII), sarcoma (Src) kinase, and the Ca^{2+}-dependent phosphatase, calcineurin.[6]

TRPV1 activation

There are several mechanisms for TRPV1 activation. In more detail, TRPV1 agonists (e.g. capsaicin and anandamide) activate the channel by direct binding while the non-agonist activators can induce sensitization for the channel through post-translational modifications, changing one or more of the following parameters: membrane potential, pH, temperature threshold, or trafficking to the plasma membrane.[7,8] Overall, when the TRPV1 channel is activated, sodium (Na^+) and Ca^{2+} channels open leading to ion influx, initiation of depolarization, additional Ca^{2+} entry through voltage-gated Ca^{2+} channels, propagation of action potential into the central nervous system (CNS) and finally, different sensations such as stinging, burning, itching or a feeling of warmth.[9,10] Xin *et al*. (2005) reported the involvement of the TRPV1 channel in Ca^{2+} release from intracellular stores due to its expression in the endoplasmic reticulum (ER), sarcoplasmic reticulum and membrane.[11] Accordingly, the TRPV1 channel contributes to the increase in Ca^{2+} concentration through four sources including the TRPV1 channel in the plasma membrane and ER; Ca^{2+}-induced

Keywords: TRPV1; Expression; Function; Health; Disease; Target.

Abbreviations: ATP, adenosine triphosphate; BAT, black adipose tissue; BoNTA, botulinum toxin a; Ca^{2+}, calcium; CaMKII, calmodulin dependent kinase II; CBD, cannabidiol; CGRP, calcitonin gene-related peptide; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; dlPAG, dorsolateral periaqueductal gray; DRGs, dorsal root ganglia; ER, endoplasmic reticulum; GABA, gamma- amino butyric acid; GI, gastrointestinal; IBS, irritable bowel syndrome; ICV, intracerebroventricular; Na^+, sodium; NGF, nerve growth factor; NSAIDs, non-steroidal anti-inflammatory drugs; PAG, periaqueductal gray; PAR2, protease-activated receptor 2; PGs, prostaglandins; PKA, protein kinase A; PNS, peripheral nervous system; ROS, reactive oxygen species; RTX, resiniferatoxin; S5, segment 5; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNPs, single nucleotide polymorphisms; SP, substance P; Src, sarcoma; TGs, trigeminal ganglia; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1.

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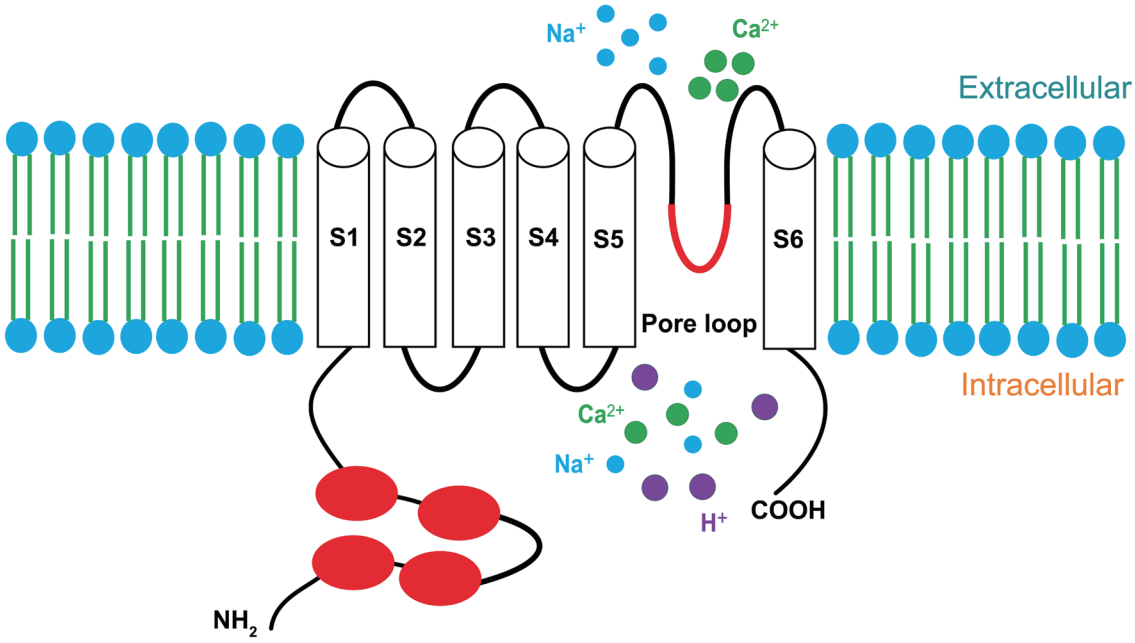

Fig. 1. Transient receptor potential vanilloid 1 (TRPV1) structure. The TRPV1 channel possesses a tetrameric structure with 6 transmembrane domains and a pore-forming hydrophobic stretch linking segment 5 (S5) and S6. The channel has an unusual characteristic in which it has cytosolic intracellular C and N termini. When the TRPV1 channel is activated, sodium (Na^+) and calcium (Ca^{2+}) channels open leading to ion influx, initiation of depolarization, additional Ca^{2+} entry through voltage-gated Ca^{2+} channels, propagation of action potential into the central nervous system (CNS) and finally, different sensations. H^+ refers to protons.

Ca^{2+} release and store-operated Ca^{2+} entry.[12] On the other hand, Ferrini *et al*. (2007) reported that the administration of capsaicin to the spinal lamina II neurons causes SP release that excites inhibitory neurons in laminae I, III and IV, leading to an increase in the release of inhibitory neurotransmitters (e.g. gamma-amino butyric acid (GABA)/glycine) in mice.[13] Thus, capsaicin enhances the inhibitory neurotransmission as a parallel alternative pathway to glutamate in the transfer of nociceptive signals.[13]

TRPV1 expression

It is well documented that the TRPV1 channel is highly expressed in DRGs, trigeminal ganglia (TGs) and the spinal cord.[1] Also, it is found in the striatum, amygdala, thalamus, microglia, astrocytes and other regions in the CNS as well as non-neuronal tissues such as hair follicles, mast cells, smooth muscles, keratinocytes, liver, tongue, oral cavity, bladder, kidneys, lungs, spleen and cochlea.[10,14] Related research shows that low levels of the TRPV1 channel are expressed in the entorhinal cortex, olfactory bulb, hippocampus, periaqueductal gray (PAG) and other regions.[15] Moreover, the TRPV1 channel is widely present in multiple peripheral tissues/systems including the vasculature, gastrointestinal (GI) tract, urinary bladder, and immune system.[16–18]

TRPV1 in health

Appealing evidence shows that the TRPV1 channel plays key roles in thermosensation, oral sensation, proteasome activity, modulation of autophagy, energy homeostasis, muscle physiology, GI motility, and the release of inflammatory mediators as well as crosstalk between the immune system and sensory nervous system.[1,18–24] In addition, the TRPV1 channel is involved in the modulation of synaptic transmission through pre- and post-synaptic mechanisms and microglia-to-neuron communication.[10] To elaborate, the TRPV1 channel modulates glutamatergic and GABAergic transmission and causes changes in neuronal firing.[25,26] Thus, it has a role in brain plasticity and development.[10,27] Moreover, numerous studies have shown that the TRPV1 channel is implicated in the regulation of long-term potentiation of excitatory postsynaptic potentials in the hippocampus which is responsible for learning and memory.[28]

In the urinary bladder, the TRPV1 channel is involved in the micturition reflex, regulation of the contractility in muscle cells, blood flow and nerve excitability.[17,29] In addition, the TRPV1 channel is involved in the regulation of vascular tone and blood pressure due to its wide expression in smooth muscle cells, perivascular nerves, and endothelial cells of the cardiac system.[30] Moreover, previous studies point to the vasodilatory effect of the TRPV1 channel and its role in the stimulation of mucus secretion in the gut.[31] In the stomach and duodenum, the TRPV1 channel takes part in the maintenance of tissue integrity in addition to its protective role against aggressive compounds.[32] Also, the TRPV1 channel plays a role in the control of motor function in the GI tract.[10] Also, the TRPV1 channel is a key component in the fertility outcome in men.[33] In other contexts, it is increasingly recognized that the channel is a fundamental contributor to the healing of different wounds as reviewed by Bagood and Isseroff (2021) and other researchers.[34] The TRPV1 channel acts as a mechanosensor in the lens and contributes to the regulation of water and ion transport to restore lens volume and maintain internal lens hydrostatic pressure gradient.[35]

Figure 2 shows body systems that have TRPV1 expression.

TRPV1 in disease

As the TRPV1 channel is implicated in several physiological processes, many disorders have been associated with alterations in the function and/or expression of the TRPV1 channel. Close attention

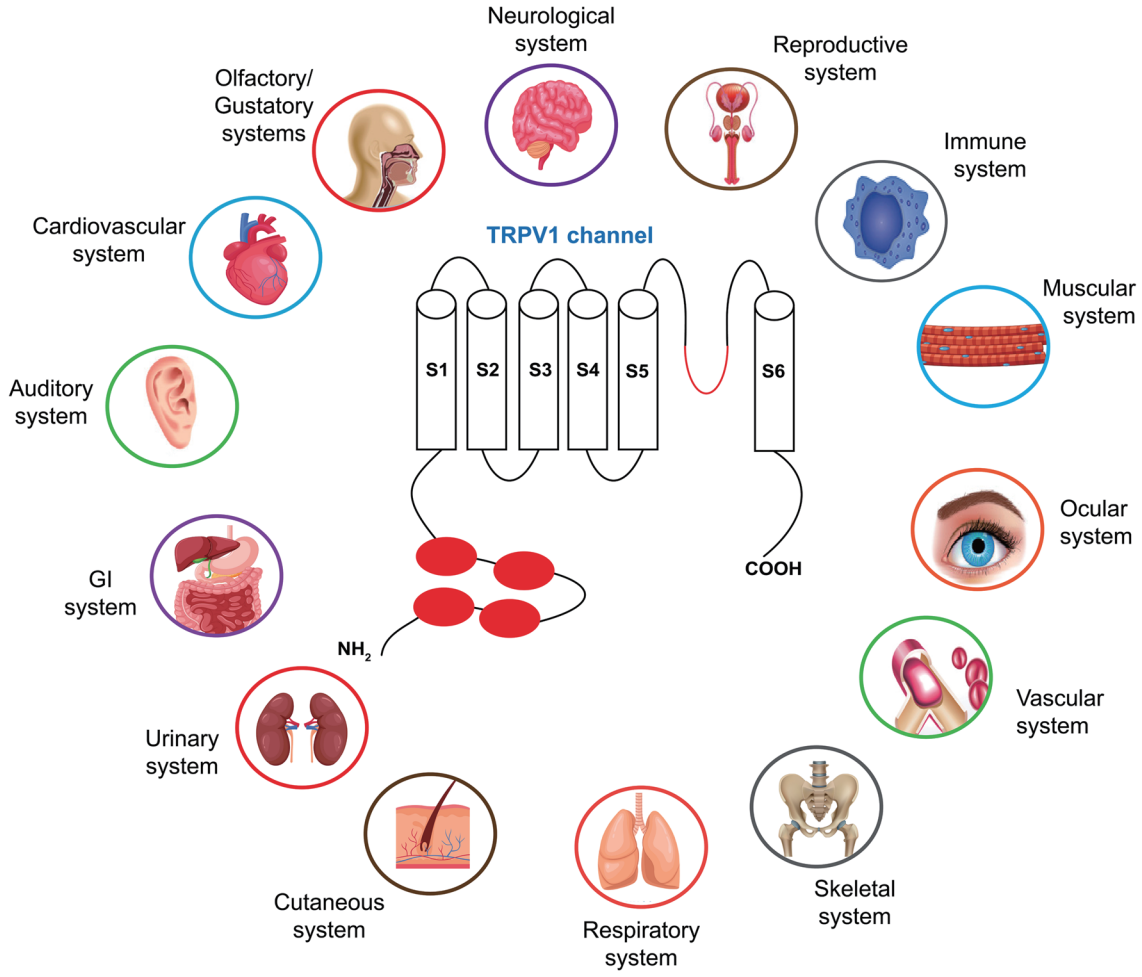

Fig. 2. Body systems that have transient receptor potential vanilloid 1 (TRPV1) expression. GI, gastrointestinal; S, segment.

is currently paid to the involvement of the TRPV1 channel in diseases, pointing to its importance as a promising therapeutic target. This review highlights up to date findings regarding the involvement of the TRPV1 channel in diseases.

TRPV1 and dysregulation of temperature

It is widely accepted that TRPV1 knockout mice show altered responses to heat.[36,37] The animals exhibited little thermal hypersensitivity during inflammation and impairment in painful heat detection.[37] In another study, it was revealed that the sensitivity to noxious heat was attenuated after silencing the TRPV1 gene by short hairpin ribonucleic acid.[38] Other research implicated that the expression of the TRPV1 channel accounted for the activity of hypothalamus in thermoregulation.[39] Importantly, the use of several TRPV1 antagonists was associated with side effects such as hyperthermia and accidental burns (e.g. AMG0347) or hypothermia (e.g. 1165901) as a further indication to the link between TRPV1 and thermoregulation.[40,41]

TRPV1 and pain

Many studies have depicted that the TRPV1 channel is expressed in sensory neurons.[1] In more detail, the TRPV1 channel is expressed in the unmyelinated C-fibers and the myelinated Aδ-fibers.[1] Thus, the TRPV1 channel is involved in the nociception of mechanical, thermal, and chemical stimuli during pain.[42] In detail, it has been long recognized that the TRPV1 channel plays a fundamental role in inflammatory and neuropathic types of pain.[43] By virtue of this fact, mice that lack the TRPV1 channel display a significant decrease in pain sensation.[37] Additionally, emerging evidence shows that TRPV1 expression changes after nerve injury.[44] In addition, it was revealed that the alterations in TRPV1 expression and function were major contributors to diabetes-induced variations in thermal pain.[45] Furthermore, cumulative evidence confirms that the TRPV1 channel is implicated in inflammatory pain through the activation of kinases (e.g. PKA and PKC) and an increase in TRPV1 activity by many inflammatory mediators.[46] Additionally, the TRPV1 channel is a major contributor to cases of neuropathic pain such as chemotherapy-induced peripheral neuropathy.[47] In this regard, one study has shown that paclitaxel causes TRPV1 sensitization through the release of mast cell tryptase that causes activation for the protease-activated receptor 2 (PAR2) and other kinases.[48] On the other hand, abundant evidence shows that the TRPV1 channel contributes to fibromyalgia which is a chronic pain disorder characterized by fatigue, widespread body pain, and mental health problems.[49,50] Importantly, the TRPV1 channel, among other pain receptors, has been implicated in different types of pain during coronavirus disease 2019 (COVID-19) and after recovery (post-COVID-19).[51,52]

Since the TRPV1 channel is involved in the nociception of different stimuli, it is widely considered a promising target for pain control.[42] Notably, despite the fact that the first exposure to TRPV1 activators causes pain, repeated exposure to these activators inhibits pain perception due to TRPV1 desensitization, thus representing a unique form of analgesia.[9]

TRPV1 and inflammation

It is well known that tissue injury is associated with inflammation and the release of multiple inflammatory mediators such as PGE_2 NGF, and bradykinin as well as protons that are responsible for tissue acidosis indicating that there is interplay between the TRPV1 channel and inflammation.[53] Many inflammatory mediators sensitize the TRPV1 channel by lowering its threshold leading to its activation at body temperature by several mechanisms that differ according to the types of nociceptors and inflammatory mediators.[43,54] These mediators have significant effects on the TRPV1 channel. Also, growing evidence demonstrates that inflammation promotes the sensitized state of the TRPV1 channel through increased activity of PKC and PKA. Thereby, the TRPV1 channel is considered a key detector for brain inflammation and autoimmune encephalitis.[27,55] Besides, the literature supports the fact that inflammation causes TRPV1 anterograde transport from the cell body to the periphery via the sciatic nerve.[56] Evidently, inflammation-induced reactive oxygen species (ROS) increased the translation of TRPV1 mRNA and caused anterograde transport of the TRPV1 protein to the periphery.[57] In this context, it has been found that the trafficking and expression of the TRPV1 channel change at the transcriptional, translational, and post-translational levels during nerve injury and inflammation.[58] Moreover, there is growing evidence indicating that the recruitment of vesicular TRPV1 pools to the membrane and the surface insertion of the TRPV1 channel onto the surface of DRGs are complementary mechanisms required for the enhancement of TRPV1 functionality by some inflammatory mediators such as NGF, insulin-like growth factor 1 and adenosine triphosphate (ATP).[54] Supporting this contention, earlier reports showed that numerous inflammatory mediators lower the threshold of TRPV1 activation via phosphorylation.[4] Likewise, there is substantial evidence revealing that NGF produced after inflammation and/or tissue injury has an impact on a regulatory region located upstream of the TRPV1 gene and hence evokes TRPV1 expression in nociceptors, partly through transcription.[59] Additionally, it was demonstrated that the administration of TRPV1 antagonists inhibits ovalbumin-induced coughing in guinea pigs, indicating that the TRPV1 channel plays a crucial role in inflammatory coughing.[60] Additionally, Orliac *et al*. (2007) proposed that the effect of anandamide during endotoxic shock (a case of severe inflammatory response) was enhanced by TRPV1 overexpression in rats.[61]

TRPV1 and cancer

Research evidence has proved the involvement of the TRPV1 channel in tumorigenesis (cell proliferation, death, and metastasis) as the channel contributes to cell division.[62,63] The effects and mechanisms of using various TRPV1 agonists/antagonists on different cancer cells were reviewed by Li *et al*. (2021).[63] Accumulating knowledge shows that the anti-tumor potential of capsaicin is demonstrated in different cancer cell lines via one or more of the following mechanisms: suppressing angiogenesis, increasing apoptosis, changing different signaling pathways or inhibiting proliferation and motility of cells.[63,64] The fact that TRPV1 activation leads to Ca^{2+} influx indicates that there is interplay between the TRPV1 channel and intracellular Ca^{2+} concentration, which is needed in many processes such as cell migration, cytotoxicity and ultimately cell death.[65,66] In this regard, one study demonstrated that the administration of the TRPV1 agonist, RTX, induced cell death in pancreatic cancer cells.[66] More precisely, it was revealed that the TRPV1 channel contributes to the proliferation of different human cancer cell lines and tumors such as osteosarcoma, colorectal cancer cells, dermal cancer cells, pancreatic cancer cells, urothelial cancer cells, renal cancer cells, hepatocellular carcinoma, nasopharyngeal carcinoma, breast carcinoma, neuroblastoma, and melanoma.[63] Meanwhile, the channel has an impact on the apoptosis/necrosis of breast carcinoma, osteosarcoma, lung cancer cells, gastric cells, oral squamous cell carcinoma, nasopharyngeal carcinoma, uterine cervix cancer, endometrial cancer, cutaneous melanoma, cervical carcinoma and bladder cancer cells.[63] Additionally, evidence suggests that the TRPV1 channel has a role, via different mechanisms, in cancer cell metastasis and invasiveness in different cells such as colorectal cancer cells, pancreatic cancer cells, urothelial cells, papillary thyroid carcinoma, dermal cancer cells, lung cancer cells, cervix adenocarcinoma, hepatoblastoma, nasopharyngeal carcinoma, neuroblastoma and melanoma.[63] In addition, the TRPV1 channel plays a role in bone cancer due to its activation by tissue acidosis mediated by osteoclasts.[67] In the oral cavity, TRPV1 expression was detected in the cell carcinoma of the human tongue.[68] Also, in cultured DRGs, it was found that treating the animals with the anticancer drugs oxaliplatin and cisplatin caused upregulation for TRPV1 mRNA.[69] Besides, a considerable body of work shows that the TRPV1 channel is implicated in several hematological malignancies due to its expression in macrophages, monocytes, and dendritic cells.[70] Moreover, previous research has shown that there is a link between TRPV1 expression and the efficiency of chemotherapy as well as radiotherapy.[63] Notably, caution has been raised in some studies regarding the association between the long term use of capsaicin and the emergence of cancer in animals.[71]

TRPV1 and psychiatric/neurological disorders

It is widely recognized that the TRPV1 channel is involved in several psychiatric and neurological disorders such as anxiety, conditioned fear, depression, drug-addiction disorders, epilepsy and Alzheimer's disease.[10,35,65,72] In more detail, earlier reports revealed that the TRPV1 channel was expressed in the hippocampus and cortex of patients who had epilepsy.[73] Additionally, it was found that the administration of the TRPV1 antagonist capsazepine suppressed seizures in genetically epilepsy-prone animals.[74] Remarkably, multiple studies have demonstrated that the TRPV1 channel promotes the migration of astrocytes and release of pro-inflammatory cytokines from astrocytes into the nearby neurons to maintain epileptogenesis.[75] In the substantia nigra, it is evident that the activation of astrocytic TRPV1 prevents the degeneration of dopaminergic neurons in a model of Parkinson's disease in rats.[76] Furthermore, You *et al*. (2012) reported that TRPV1 knockout mice exhibited antidepressant behavior.[77] Also, TRPV1 activation reversed memory impairment and hippocampal damage caused by the cytotoxic effects of Amyloid-β peptide.[65] Additional lines of evidence documented the potential role for the TRPV1 channel in schizophrenia.[78] Importantly, it merits consideration that the TRPV1 channel has been detected in brain areas that are involved in the control of stress such as the hippocampus, locus coeruleus, medial prefrontal cortex, hypothalamus, and dorsolateral periaqueductal gray (dlPAG).[79] In this regard, the TRPV1 channel in dlPAG has been implicated in the attenuation of cannabidiol (CBD)-me-

diated anxiolysis.[79]

TRPV1 and disorders of the auditory system

In the study of Takumida *et al*. (2005), the authors documented that the TRPV1 channel was detected in the inner ear of guinea pigs; more specifically, in hair cells and supporting cells of the organ of Corti; spiral ganglia of the cochlea; and the vestibular end organs.[80] Further, multiple studies showed that the cochlear expression of the TRPV1 channel was involved in drug-induced cochleotoxicity (hearing loss) during systemic inflammation.[81] Additionally, TRPV1 expression was up-regulated in the vestibular and spiral ganglia in the inner ear of mice after kanamycin challenge.[82] Besides, earlier studies shed new light on the role of the TRPV1 channel in cisplatin ototoxicity as its absence provided protection against hearing loss.[83] In addition, a significant amount of research has shown that several cochlear stressors (e.g. noise and ototoxic drugs) affect the TRPV1 channel indicating the role of this channel in the regulation of cytoprotection and/or cell death pathways.[83] Consistent with these findings, it was found that inhibiting inflammation or oxidative stress decreased TRPV1 expression, modulated the apoptotic and inflammatory signals and provided protection against cochlear damage and hearing loss.[83]

TRPV1 and disorders of the ocular system

It is well documented that the TRPV1 channel is expressed in different regions of the lens including the epithelium, outer cortex and inner cortex.[35,84] *In vivo*, TRPV1 absence was associated with impairment in the healing of the epithelium in debrided corneal defects in rodents.[84] Furthermore, a considerable body of work has revealed that TRPV1 activation by mechanical injury causes cytoskeletal rearrangement, an increase in Ca^{2+} concentration, and enhances the migration of isolated retinal astrocytes.[85] In ganglion cells, it has been published that the increase in intraocular pressure augments TRPV1 expression, which is involved in protecting ganglion cells from apoptosis.[86] Additionally, the application of capsaicin to the corneal epithelium causes TRPV1 activation, an increase in intracellular Ca^{2+} concentration, the release of inflammatory mediators, and protection against infection by microorganisms.[87]

TRPV1 and anosmia/ageusia

People experience a burning sensation on their tongues when eating chili peppers. Thus, multiple studies have highlighted the involvement of the TRPV1 channel in taste perception.

Remarkably, the TRPV1 channel is expressed in neurons innervating the oral cavity.[88,89] There are several pieces of evidence indicating that the TRPV1 channel responds to a number of substances (e.g. allicin, capsaicin, alcohol and gingerol) and modifies salt stimuli.[90] Also, appealing evidence shows that a TRPV1 channel variant is expressed in the epithelial cells and taste buds of the tongue.[89] Besides, it has been reported that TRPV1 polymorphisms are linked to alterations in the sensitivity to the taste of salts.[91] Notably, earlier research mentions that capsaicin can decrease sucrose preference and inhibit voltage-dependent Na^{+} channels in taste cells in TRPV1 knockout mice.[91] In this context, Hu *et al*. (2016) reported that the TRPV1 channel was involved in rimonabant-induced olfactory discrimination deficit and that the impaired olfactory discrimination was rescued by the TRPV1 antagonist capsazepine.[92] Further, the TRPV1 channel seems to be linked to the anosmia/ageusia symptoms in COVID-19 patients.[51,52]

TRPV1 and infections

Several reports demonstrated that the TRPV1 channel plays important roles in bacterial, fungal and viral infections.[51,52,93–95] In more detail, Maruyama *et al*. (2017) reported that the topical *Candida albicans* skin infection stimulated the release of calcitonin gene-related peptide (CGRP) in a TRPV1 dependent manner during bone infection.[93] Another study showed the beneficial effects of TRPV1 ablation on inducing immunosuppression against *Streptococcus pyogenes* in the skin.[94] Likewise, the TRPV1 channel has been implicated in the anti-inflammatory and immunosuppressive responses in animals infected with *Staphyloccocus aureus* in the skin and lung.[95,96] In a model of sepsis (cecal ligation and puncture), it was revealed that the animals that are deficient in the TRPV1 channel suffered from severe symptoms such as decreased phagocytosis in macrophages, increased apoptosis of peritoneal mononuclear cells, increased levels of inflammatory mediators, decreased levels of ROS, and reduced bacterial clearance.[21] In fact, the link between the TRPV1 channel, Ca^{2+} concentration and ROS provides evidence for the involvement of the TRPV1 channel in viral infections.[97] More precisely, an accumulation of knowledge showed that Ca^{2+} entry into the cells is of key importance to the viral lifecycle at several steps including its entry, replication, assembly, and release.[98] Further, it has been reported that there is interplay between the increase in intracellular Ca^{2+} and ROS levels in mitochondria, which is crucial for the lifecycle of many viruses.[99]

As shown from previous studies, the TRPV1 channel is one of the receptors that provide favorable environments for viruses including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[51,52] The wide expression of the TRPV1 channel in tissues that were frequently infected by SARS-CoV-2 suggests that the channel plays a crucial role in COVID-19, one of the world's worst pandemics in the current century. In the review of Jaffal and Abbas (2021), the authors summarized the studies that demonstrated a correlation between the TRPV1 channel and several symptoms of COVID-19 including fever, pain, myalgia, inflammation, cough, headache, pulmonary edema, anosmia, ageusia, as well as problems of the GI and cardiovascular systems.[51] Also, the TRPV1 channel can be implicated in other manifestations of COVID-19 disease such as anxiety as well as visual, renal, and hepatic problems.[51] Figure 3 shows a representation of a SARS-CoV-2-induced cytokine storm,[52] which is considered the leading cause of death in COVID-19 patients. The activation of the TRPV1 channel in the peripheral nervous system (PNS) and CNS contributes to Ca^{2+} influx and the release of neuropeptides that induce liberation of more inflammatory mediators. These mediators cause sensitization of more TRPV1 channels, among others leading to excessive stimulation and providing a favorable environment for SARS-Cov-2. In summary, the inflammatory cytokine storm produces a loop of amplified release of mediators at different levels leading to more adverse outcomes.

TRPV1 and disorders of the reproductive system

It is well known that the TRPV1 channel is expressed in the head, midpiece, and tail of sperm and is involved in the regulation of acrosomal reaction and sperm capacitation.[33,100] As such, there is correlation between TRPV1 expression and the fecundity potential of sperm.[33] In this context, earlier reports have shown that the TRPV1 channel is downregulated in the spermatozoa of idiopathic infertile men, subfertile men, and normozoospermic infertile males.[33] Further, in TRPV1 knockout mice, it was found that the testes of mice were more susceptible to oxidative stress, testicular damage, and dysfunctional sperm development.[101] It was

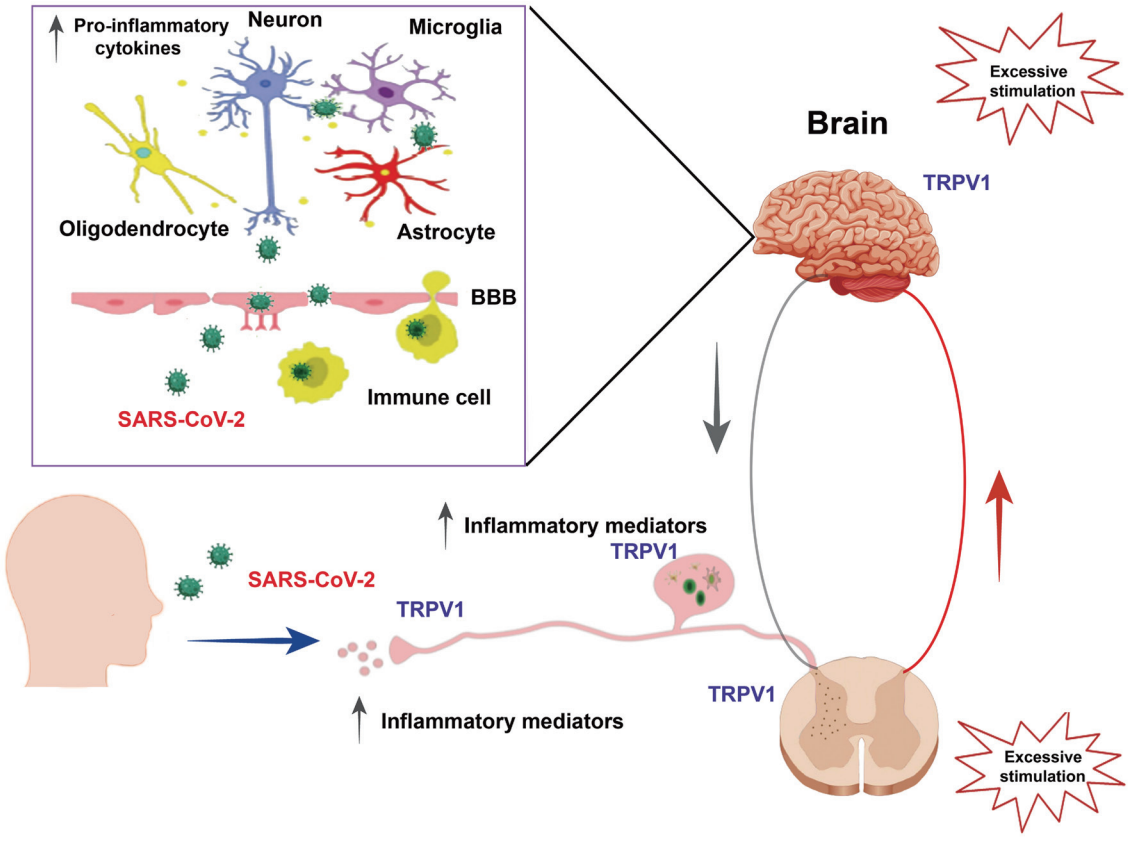

Fig. 3. Representative sketch for the cytokine storm in Coronavirus disease 2019 (COVID-19) and the involvement of the transient receptor potential vanilloid 1 (TRPV1) channel (Modified from Jaffal, 2021).[52] Severe acute respiratory syndrome coronavirus (SARS-CoV-2) can cross the blood brain barrier (BBB) and cause more devastating effects. During COVID-19, the activation of the TRPV1 channel in the peripheral nervous system (PNS) and central nervous system (CNS) contributes to calcium (Ca^{2+}) influx and the release of neuropeptides that induce the liberation of more inflammatory mediators. These mediators cause sensitization of more TRPV1 channels, among others leading to excessive stimulation and providing a favorable environment for SARS-Cov-2. DRGs, dorsal root ganglia.

also found that vulvodynia (a condition of pain in the opening of vagina) is linked to more epithelial innervation when accompanied by more TRPV1 expression in vulva.[102] Besides this, the TRPV1 channel contributes to the sensory symptoms experienced by patients who suffer from hyperalgesia, allodynia, and a burning sensation in the vulvar vestibulus region.[102]

TRPV1 and disorders of the respiratory system

A remarkable amount of literature demonstrates that the TRPV1 channel is expressed in several regions in the upper and lower respiratory tracts such as the vascular endothelial cells, submucosal gland cells, smooth muscle cells, cholinergic neurons, inflammatory cells, laryngeal epithelial cells, blood vessels, fibroblast cells, T cells, and the airway epithelium.[103,104] Also, the TRPV1 channel is expressed in neurons of the vagal nerve that innervate the airways.[38] Moreover, previous studies highlighted that TRPV1 antagonism decreased airway hyperresponsiveness in guinea pigs and exerted anti-tussive effects in a capsaicin-induced cough model of guinea pigs.[105,106] In line with the involvement of the TRPV1 channel in respiratory disorders, it was found that TRPV1 expression increased in patients suffering from chronic obstructive pulmonary disease (COPD) and chronic cough.[107,108] In addition, the TRPV1 channel was critical for the effect of NGF (when administered via inhalation or intracerebroventricular (ICV) injection) in enhancing cough and airway obstruction in guinea pigs.[109,110] Moreover, accumulated data suggest that the activation of the TRPV1 channel on respiratory effector cells can lead to tracheal mucosal edema, bronchoconstriction, protein secretion and inflammatory cell chemotaxis.[109,111] Interestingly, earlier reports have shown a relationship between TRPV1 single nucleotide polymorphisms (SNPs) and protective effects against wheezing in patients who suffer from asthma.[112] Additionally, a recent study documented the increase in TRPV1 expression in rhinovirus that contributes to asthma exacerbations.[113] In this regard, several studies have shown that capsaicin nasal spray is useful in the treatment of idiopathic rhinitis.[114]

TRPV1 and obesity

Previous reports have demonstrated that the TRPV1 channel is expressed in adipocytes and plays a key role in the regulation of metabolic processes that are related to obesity.[115,116] Capsaicin promotes weight loss by increasing the sympathetic nervous system activity, decreasing appetite as well as increasing energy expenditure, fat oxidation, insulin and leptin resistance.[117,118] Furthermore, capsaicin improves endurance capacity and energy metabolism in skeletal muscles.[119]

The findings of a recent meta-analysis of clinical trials showed that the daily consumption of capsiate (a non-pungent vanilloid) or capsaicin increased thermogenesis and decreased appetite, and

can thus be useful in weight management.[120] Further, it has been published that dietary capsaicin and capsinoids increase energy expenditure and thermogenesis mediated by an increase in brown adipose cells and a decrease in white adipogenesis.[115] It is evident that the administration of low-dose dietary capsaicin improved insulin sensitivity, increased fat oxidation, decreased body fat and improved the functions of liver.[116] Despite that, there are conflicting results about the role of the TRPV1 channel in weight management due to the risk of developing myocardial infarction.[121] Of note, the effects of capsaicin depend on the administered dose and duration of its application. Further research is needed in this regard.

TRPV1 and disorders of the GI tract

In the GI tract, the TRPV1 channel is expressed in the afferent neurons (vagal and spinal) in the esophagus, jejunum, stomach, rectum, colon as well as the small intestine.[16,119,122] In fact, accumulated evidence supports the findings that TRPV1-labeled nerve fibers are distributed in each layer of the GI tract including submucosa, mucosa, muscle, and myenteric plexus.[123] Thus, the TRPV1 channel is implicated in the cases of irritable bowel syndrome (IBS), neurogenic pancreatitis, and ileus.[123] It is well established that CGRP released after TRPV1 activation in primary nociceptive nerves leads to a strong inhibitory effect on gastric acid induced irritation.[124] Additionally, many substances (e.g. tachykinins) are released when the TRPV1 channel is activated causing gastric motility and acceleration for gastric emptying.[125] Furthermore, it has been illustrated that ulcer formation in rats is suppressed by the injection of low dose capsaicin and that the perfusion of capsaicin into the stomach of rats can inhibit gastric mucosal injury.[126,127] Evidently, several studies have been published about the effects of capsaicin on reducing the symptoms of functional dyspepsia caused by duodenal and gastric dysfunction, reducing upper abdominal symptoms as well as increasing GI dysfunction, leading to IBS-related symptoms.[19] Interestingly, TRPV1 expression increased in a rat model of chronic pancreatitis and in patients of ulcerative colitis and Crohn's disease.[128,129] Further, it is increasingly apparent that the channel is involved in gastric pain hypersensitivity and gastroesophageal reflux disease.[123] Moreover, it was revealed that capsaicin could improve liver function in a mouse model of hepatic failure.[130] The fact that TRPV1 expression has been found to increase in oesophagitis, colonic inflammation, acute haemorrhoidal disease, and distal colitis is further evidence of the involvement of the TRPV1 channel in the disorders of the GI tract.[8]

TRPV1 and disorders of the cardiovascular system

Previous studies have confirmed that the TRPV1 channel is densely expressed in the sensory neurons that innervate the ventricles, endothelial cells, epicardial surface of the heart, myocardium, cardiomyocytes, the adventitia of the ascending aorta, aortic arch, and the vascular smooth muscle cells.[131] Moreover, TRPV1 expression is detected in large arteries, aorta and carotid arteries.[132] Following this, other studies have shown that the TRPV1 channel plays a role in sensing blood pressure fluctuations.[133] Furthermore, it has been found that TRPV1 activation mediates the hypotensive action and is implicated in myogenic vasoconstriction in the Bayliss reflex in the resistance arteries.[10,134] In this regard, previous studies showed that the administration of capsaicin increased coronary flow and decreased left ventricular end diastolic pressure and infarct size in wild type mice.[135] In addition, it has been found that TRPV1 activation can alleviate atherosclerosis induced by a high-fat diet in mice through cellular cholesterol cleavage.[136] Specifically, dietary capsaicin decreased atherosclerosis by regulating lipid metabolism and decreasing endothelial dysfunction.[136,137] According to Harper *et al*. (2010), TRPV1 receptors that exist on platelets can promote inflammatory mediators leading to platelet activation and the formation of atherosclerosis.[138] The TRPV1 channel, being expressed in the perivascular nerves, also plays a crucial role in cardioprotection by stimulating the release of potent neuropeptides such as CGRP and SP that cause vasodilation or vasoconstriction.[138–140] Moreover, it has been documented that there is association between decreased expression of the TRPV1 channel in metabolic syndrome and increased ischemic reperfusion injury in isolated mice hearts.[141] Further, emerging evidence indicates that the TRPV1 channel mediates relaxation of smooth muscle cells in the endothelium.[142] However, previous studies have implicated that high consumption of capsaicin can cause myocardial infarction and vasospasm.[143] In this regard, Song *et al*. (2017) documented that TRPV1 activation is responsible for the contraction of smooth muscle cells in pulmonary artery, vasoconstriction and the pathogenesis of idiopathic pulmonary arterial hypertension.[144]

TRPV1 and diabetes

A considerable body of work shows that nerve fibers that express the TRPV1 channel innervate Langerhans islets in the pancreas.[145] Also, previous research has confirmed an alteration in the activity and/or expression of the TRPV1 channel in insulin resistance.[118] In the long-term diabetic microenvironment, earlier studies demonstrated that TRPV1 desensitization in DRGs decreased TRPV1 activity and contributed to peripheral diabetic neuropathy.[146] Furthermore, the injection of capsaicin attenuated hyperglycaemia in Zucker diabetic fatty animals which is a model of human type 2 diabetes mellitus.[145] In this sense, TRPV1 knockout mice exhibited impairment in glucose metabolism manifested by a decrease in glucose-induced insulin secretion.[147] Importantly, it has been found that the TRPV1 channel is a modulator for clock gene oscillations in black adipose tissue (BAT) and is involved in the regulation of hepatic functions and glucose metabolism.[148,149] Besides, earlier studies revealed that hepatic glycogen storage was compromised in TRPV1 knockout mice due to impairment in glucose homeostasis.[149] Further, it was shown that the livers of TRPV1 knockout mice exhibited changes in proteomics and a decrease in glycogen storage in addition to an enhancement in glycogenolysis, gluconeogenesis, and the levels of inflammatory parameters.[149]

TRPV1 and disorders of the cutaneous system

The burning feeling of capsaicin in the skin was discovered by Hogyes in 1878 before the discovery of the TRPV1 channel.[33] Since then, several studies have been conducted to unravel the effects and mechanisms of the TRPV1 channel on different systems including the cutaneous system. In the skin, it is evident that the TRPV1 channel presents in epidermal keratinocytes, mast cells, epithelial cells of hair follicles, blood vessels, eccrine sweat glands, keratinocytes, nociceptors, immune cells, sebocytes, fibroblasts, and melanocytes.[33,150] Interestingly, it has been documented that TRPV1 positive nociceptors in hair follicles play a role in the proliferation and migration of stem cells to improve healing.[151] Many people have used capsaicin to treat psoriasis, atopic dermatitis, and allergic contact dermatitis.[152–154] Also, the channel plays an important role in the healing of wounds in different models such as incision wounds, tape striping, burn wounds, corneal wounds and ultraviolet B wounds.[33] Therapeutically, it has been found that honokiol (a natural compound extracted from magnolia plants) is effective in treating third degree burns by decreasing the mRNA and protein

expression of TRPV1.[155] Moreover, in one study, mice lacking the TRPV1 gene showed reduction in histamine-induced scratching and itching sensation compared to wild-type mice.[156] Regarding hair growth, Bodo *et al*. (2005) suggested that the TRPV1 channel can influence human hair growth and that TRPV1-based therapy can be used for the treatment of hirsutism (unwanted hair growth), effluvium, and alopecia (hair loss).[157]

TRPV1 and headache

Several studies have unraveled the role of the TRPV1 channel in migraines. It is well known that one of the factors that contribute to migraines is the release of neuropeptides through the activation of trigeminal afferents in the cranial vasculature (trigeminovascular system).[158] Due to the expression of the TRPV1 channel in TGs and dural nerves, it is well documented that this channel is implicated in headache and migraine mechanisms.[159] In this regard, previous studies have shown that the anti-migraine drug sumatriptan alleviates headache in a TRPV1 dependent manner.[159] Other pieces of research elucidated the mechanisms of botulinum toxin A (BoNTA) in treating chronic migraine. The studies shed new light on the inhibition of TRPV1 trafficking to the plasma membrane in TGs and the decrease in capsaicin-induced pain after BoNTA treatment.[160,161] Moreover, many studies used TRPV1 agonists and antagonists to probe meningeal afferents and reported the effectiveness of TRPV1 agonists, rather than antagonists, in treating migraines.[162,163] In this regard, the repeated administration of intranasal capsaicin to chronic migraine patients resulted in 50–80% amelioration of migraine attack due to TRPV1 desensitization.[163] Likewise, it was found that the use of an intranasal TRPV1 agonist (civamide) decreased the frequency of headache attacks in 72.7% of patients and caused absence of pain in 33% of patients.[164] Importantly, it has been revealed that neurogenic vascular effects of the TRPV1 channel are implicated in migraine pathophysiology through CGRP release and dural vasodilation.[158] Widely popular, pro-inflammatory mediators stimulate trigeminal nociceptors possibly via the TRPV1 channel highlighting the role of the TRPV1 channel in migraines and the role of non-steroidal anti-inflammatory drugs (NSAIDs) in treating them.[165,166] Of relevance, it was found that the transient receptor potential ankyrin 1 (TRPA1) channel requires co-activation of the TRPV1 channel to initiate afferent signaling from the meninges and that ethanol triggers migraine attacks through release of CGRP in a TRPV1-dependent manner.[167–169]

TRPV1 and disorders of the urinary system

In the urinary tract, the TRPV1 channel is expressed in sensory nerve fibers, smooth muscles and the urothelium.[169] Importantly, the expression of the TRPV1 channel has been correlated with the severity of inflammation in interstitial cystitis or bladder pain syndrome.[170] According to clinical studies, capsaicin is recommended for the treatment of neurogenic bladder hyperreflexia as it causes a decrease in bladder capacity, pressure threshold for micturition and the patients' desire to void.[17] Also, the TRPV1 channel is expressed in the renal pelvis and contributes to the maintenance of diuresis, natriuresis, water and Na^+ homeostasis.[171] Additionally, previous findings have shown that the TRPV1 channel responds to many chemicals (e.g. allicin, alcohol, capsaicin, and gingerol) that are known to modify salt stimuli.[172] In this context, capsaicin has been effective in treating incontinence in people suffering from dysfunctional micturition reflex.[40] Additionally, recent preclinical data revealed that TRPV1 activators improved the outcome of ischemic acute kidney injury.[173]

TRPV1 and disorders of the muscular system

It is well established that the TRPV1 channel is expressed in muscle afferents and is involved in muscle nociception and muscle pain conditions.[8] Moreover, TRPV1 mutations are associated with muscle disorders such as exertional heat stroke and malignant hyperthermia.[24] Additionally, several studies have shown that TRPV1 activation leads to Ca^{2+} release, membrane excitability, neurotransmitter release, and muscle contraction.[174] Supporting this contention, it has been revealed that the upregulation of nitric oxide and peroxynitrite in overloaded muscle activates the TRPV1 channel.[175] Also, TRPV1 knockout mice exhibited stronger muscles with improvement in neuromuscular function compared to wildtype counterparts.[24] In frogs, it was documented that TRPV1 activation decreased the tension of fast skeletal muscle fibers causing a change in muscle activity.[176]

TRPV1 and disorders of the skeletal system

It has been long recognized that capsaicin attenuates key parameters that are responsible for symptoms of adjuvant arthritis.[177] Also, there is mounting evidence that the TRPV1 channel is involved in bone remodeling and bone diseases such as osteoporosis which is characterized by a decrease in bone density, increase in bone resorption, and fragile bones.[178,179] In this context, Alexander *et al*. (2013) reported the up-regulation of the TRPV1 channel in osteoclasts obtained from osteoporotic patients.[178] In addition, it was found that TRPV1 genetic deletion, inhibition, or desensitization in mice decreased the activity of osteoclasts *in vitro* and inhibited ovariectomy-induced bone loss as well as osteoporosis *in vivo*.[179] Moreover, previous studies documented that capsazepine inhibited the differentiation of osteoclasts and osteoblasts *in vitro* as well as ovariectomy-induced bone loss *in vivo*.[180] Accordingly, it is strongly suggested that the TRPV1 channel is involved in several bone problems.

Pharmacological agents that interact with the TRPV1 channel

As the TRPV1 channel is involved in multiple biological and pathological processes, several pharmacological agents that target this channel have been synthesized and it is increasingly recognized that there are multiple endogenous and exogenous agonists for the TRPV1 channel.[181] Capsaicin is an exogenous TRPV1 agonist extracted from the plant *Capsicum annuum* L.[182] The agonistic action of capsaicin has been exploited therapeutically by synthesizing patches that include high doses of capsaicin, leading to TRPV1 desensitization.[143] Remarkably, accumulating knowledge illustrates that capsaicin creams and patches attenuate pain due to TRPV1 desensitization on local cutaneous nociceptors and a loss of responsiveness to many sensory stimuli.[9] Accordingly, capsaicin (8% patch; Qutenza™) was approved by the United States Food and Drug Administration in 2009 for the treatment of postherpetic neuralgia-induced neuropathic pain.[143] Also, it has been revealed that capsaicin, formulated as a topical cream or a transdermal patch, is effective for the management of pain in minor muscle strains or cramps and joint pain.[143] On the other hand, many endogenous agonists (also called endovanilloids) for the TRPV1 channel have been identified including anandamide, *N*-oleoylethanolamine, *N*-Arachidonoyl-dopamine, *N*-oleoyl dopamine, lysophosphatidic acid, 20-hydroxyeicosatetraenoic acid, AM-404, hydroperoxyeicosatetraenoic acids [5-(*S*), 8-(*S*), 12-(*S*) and 15-(*S*)], hepoxilins A3, ATP, ammonia, polyamines (e.g. spermine, spermidine, putrescine), linoleic acid, in addition to 9, 13 and 20-hydroxyoctadecadienoic acid.[181] TRPV1 antagonists are classified into competitive or non-competitive antagonists according to their binding sites.[181,182]

Capsazepine is the first reported competitive TRPV1 antagonist that blocks capsaicin-or RTX-induced channel activation. Other examples include JYL-1421, A-425619, BCTC, JNJ-1720, SB-705498, SB-366791, AMG-9810, MK2295 and AMG-2674.[181,182] Examples of non-competitive antagonists are ruthenium red, RRRRWW-NH_2, methoctramine, AG-489, AG-505, DD-161515, and DD-191515.[181] In another context, TRPV1 antagonists can be classified according to their effects on body temperature. In more detail, the antagonists can increase, decrease, or un-change body temperature. Some antagonists (e.g. AMG-0347 and AMG-517) can cause hyperthermia, which is a drawback, while hypothermia can be caused by other antagonists such as A-1165901. Meanwhile, one group of antagonists do not change body temperature (thermoneutral antagonists).[182]

Future directions

There is no doubt that the TRPV1 channel is an important therapeutic target and that the pharmacological modulators of the TRPV1 channel can be potential drug targets for several disorders. The fact that there are drawbacks for several TRPV1 antagonists that are available in the market strengthens the need to discover novel TRPV1 modulators.[181,182]

TRPV1 modulation has been implicated in the anti-nociceptive effect of several medicinal plants, a finding that was proved by molecular docking studies.[183–185] In accordance with this idea, Abbas, (2020) reviewed 137 natural ingredients that affect TRPV1 activity in different *in vivo* and *in vitro* assays.[186] On the other hand, it has been long recognized that several toxins or venoms extracted from snakes, frogs, bees, spiders, scorpions, and marine organisms can act as TRPV1 modulators.[1,7,51] Continuing the search for novel compounds that can be exploited therapeutically and target the TRPV1 channel without adverse effects is of vital importance.

Conclusions

Since its cloning in 1997, research on the TRPV1 channel has grown rapidly. Several reports have documented the role of the TRPV1 channel in many biological and pathological conditions. Accordingly, attention has been directed towards the development of effective drugs that target the TRPV1 channel to treat different diseases. This review provides knowledge on the functions of the TRPV1 channel in health and diseases and highlights its importance as a target in pharmaceutical industries.

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