The endocannabinoid system and stroke: A focused review

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Abstract:

Stroke is an important cause of morbidity and mortality worldwide. Development of novel neuroprotectants is of paramount importance. This review seeks to summarize the recent evidence for the role of the endocannabinoid signaling system in stroke pathophysiology, as well as the evidence from preclinical studies regarding the efficacy of cannabinoids as neuroprotective therapies in the treatment of stroke. Recent evidence from rodent models implicating cannabinoid 1 receptor (CB1R), cannabinoid 2 receptor (CB2R), and CB1R and CB2R co-antagonism as neuroprotective strategies in stroke are reviewed. Rodent evidence for the therapeutic role of the endocannabinoid system in treating poststroke depression is reviewed. Finally, evidence for the role of cannabidiol, a publicly available cannabinoid that does not bind directly to known endocannabinoid receptors, as a stroke neuroprotectant is also reviewed. The review closes with a consideration of the role of human cannabinoid abuse in stroke and considers future directions for research on endocannabinoid-based stroke therapeutics.

Keywords:

Cannabidiol, endocannabinoid, neuroprotection, stroke

Introduction

Globally, overall stroke mortality has declined from 142/100,000 person-years in 1990–110/100,000 person-years in 2013, but the number of people affected or disabled from stroke has increased 1.4–1.8 folds over the same time period. In 2013, there were 25.7 million stroke survivors worldwide, with 6.5 million deaths and 10.3 million new strokes.[1] Recent advancements in acute stroke treatment such as mechanical thrombectomy have fundamentally reshaped the treatment paradigm for ischemic stroke (IS) caused by large-vessel occlusion.[2] On the other hand, novel neuroprotectants and rehabilitative facilitators for IS, although well-studied in preclinical settings, have yet to demonstrate major impact in the clinical setting.[3] Targeting of the endocannabinoid system for IS protection and rehabilitation is one such area that has received a significant recent attention in the basic science literature, with emerging applicability to human patients.

Cannabis is widely considered one of the first plants cultivated by man. The use of hemp fibers derived from cannabis for rope, textile, and the paper has been dated as far back as 4000 BC in China. Cannabis also has a long history of medicinal use in various cultures, including as a treatment for rheumatic pain, constipation, female reproductive problems, and even malaria.[4] The main psychoactive constituent of cannabis, delta-9-tetrahydrocannabinol (THC), was characterized in 1964. The endocannabinoid signaling system, consisting mainly of two cell-surface cannabinoid receptors, termed CB1R and CB2R, as well as endogenously produced ligands, known as the endocannabinoids, occurred in the late 1980s and early 1990s.[5-8] Therapeutic modulation of the endocannabinoid system to treat disease can occur in a variety of dimensions, either by agonism or antagonism of cannabinoid receptors themselves, or by

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targeted interaction with the various endocannabinoid pathway synthetic and degradative enzymes such as N-acylphosphatidylethanolamine-selective phospholipase D, fatty acid amide hydrolase, diacylglycerol lipase isozymes α and β, and monoacylglycerol lipase.

Alteration of the endocannabinoid signaling system has been implicated in a vast array of human diseases, including neurological disorders such as Parkinson's, Alzheimer's, and multiple sclerosis.[9] Recent evidence has shown that simple medicinal cannabis formulations can function as efficacious neuroprotectants. For instance, Sativex, an oral medicinal spray containing both THC as well as nonpsychoactive cannabidiol (CBD), has been found to decrease spasticity in multiple sclerosis.[10] A recent randomized controlled trial demonstrated that Epidiolex, a nonpsychoactive CBD formulation, is safe and effective in reducing seizures in Lennox–Gastaut syndrome.[11] Adverse events were reported in 86% of patients; however, most were mild. Medical cannabis has also been found in a small study to improve the Unified Parkinson's Disease Rating Scale scores in Parkinson's in addition to rigidity, tremor, and bradykinesia.[12]

Current evidence for the neuroprotectant efficacy of cannabinoids in IS is predicated on preclinical work in animals. A 2015 meta-analysis examined 34 preclinical studies examining CBD for poststroke neuroprotection. The authors concluded that cannabinoids were able to statistically reduce infarct volume and improve functional outcomes in experimental stroke, with activity at both cannabinoid 1 (CB1) and cannabinoid 2 (CB2) receptors associated with positive outcomes.[13] For instance, one early study showed that CB1 knockout mice undergoing transient or permanent cerebral ischemia had larger infarct volumes, worse neurological deficits, decreased cerebral blood flow (CBF) to the penumbra on reperfusion, as well as increased N-methyl-D-aspartate (NMDA) excitotoxicity compared to CB1 wild-type mice, suggesting that the endocannabinoid system is intricately leaked with neuronal and endothelial behavior.[14] Building on these results, Zhang *et al.* noted that CB1 antagonists coupled with CB2 agonists reduced inflammation and leukocyte rolling while also increasing blood flow during occlusion, possibly through collaterals.[15] These and other studies have contributed to optimism regarding the possible applicability to cannabinoids in the treatment of stroke patients. To review the state of the literature, since the publication of the 2015 meta-analysis by England *et al.*, we searched the PubMed/Medline database using the Thomson Reuters Web of Science for articles published within the last 5 years using the search term "TITLE: ((stroke) OR ischemi*) AND (((((((cannabinoid) OR cannabidiol) OR

delta-9-tetrahydrocannabinoid) OR WIN 55212-2) OR "2-arachidonoylglycerol") OR endocannabinoid*) OR CB1) OR CB2))." In what follows, we provide a focused review of the results of this literature search, with special emphasis on clinically relevant findings [Table 1].

Cannabinoid Receptor Agonists and Antagonists as Stroke Neuroprotectants

Evidence that CB1R antagonism is neuroprotective

In a human autopsy study, Caruso *et al.* have shown an increase in CB1R expression in the ischemic regions of the brain tissue, using both neuronal and nonneuronal cell staining and CB1R antibodies.[16] It has also recently been shown that mice lacking CB1R expression on astrocytes demonstrate decreased neuronal death in a mouse model of stroke.[17] Knowles *et al.* administered a CB1 receptor antagonist to rats 30 min prior to induction of transient global cerebral ischemia and assessed the effects of CB1 blockage on hormone expression and neuronal damage, finding that CB1 antagonist pretreatment counteracted ischemia-associated changes in corticotropin-releasing hormone, vesicular glutamate transporter 2, tyrosine hydroxylase, and dopamine receptor 1 expression throughout the rat brain.[18] Reichenbach *et al.* have also found that CB1R antagonism used as both a pretreatment and a posttreatment is neuroprotective in a mouse model of permanent ischemia through photoinjury.[19] However, there is at least one recent study that suggests that CB1R agonism can also play a neuroprotective role in rodent models of stroke. Caltana *et al.* have demonstrated that CB1 agonists administered poststroke in mice reduces deleterious effects on astrocytes, neurons, and dendrites, while also counteracting stroke-associated deterioration in motor activity, suggesting that agonism at CB1R poststroke in mice is neuroprotective.[20] Further work is needed to fully determine the therapeutic possibilities of modulating activity at CB1R to treat stroke. For a summary of recent evidence related to the neuroprotective effects of CB1R antagonism see [Table 2].

Evidence that CB2R agonism is neuroprotective

Recent work has examined the effects of CB2R agonism in a rat model of stroke when used as a pretreatment versus as a poststroke treatment.[21,22] When used as a pretreatment, CB2R agonists were able to suppress neurodegeneration in rat model of large-vessel IS created by occlusion of the right middle cerebral artery (MCA), whereas this effect was lost when the CB2R agonist was used in the 2–5-day period immediately following ischemic insult.[22] Ronca *et al.* were able to demonstrate significantly smaller infarct volumes in a mouse model of IS in which mice received pre- and posttreatment with a selective cannabinoid CB2R agonist.[23] Importantly, this animal model, rather than using transient middle cerebral occlusion to model ischemia/reperfusion

Table 1: Key publications on the endocannabinoid system and stroke published in the past 5 years

Title	Year published	Significance
Detrimental effects of 2-AG on whole-blood platelet aggregation and on cerebral blood flow after a focal ischemic insult in rats[31]	2018	Using blinded evaluators, demonstrated significant reductions in cerebral blood flow following administration of 2-AG, the main endocannabinoid receptor ligand in a rat model of stroke. Suggests needed caution in future human trials using CB1R or CB2R ligands therapeutically
Surprising outcomes in cannabinoid CB1/CB2 receptor double-knockout mice in two models of ischemia[32]	2018	Previous work suggested that knockout of CB1R or CB2R alone was deleterious in rodent models of stroke, but this study presents evidence that simultaneous knockout of both genes is neuroprotective in stroke models. This suggests previously unappreciated complexity in the relationship between the endocannabinoid system and stroke
Nonselective cannabinoid receptor antagonists and hinokiresinols reduce infiltration of microglia/macrophages into ischemic brain lesions in rat through modulating 2-AG-induced migration and mitochondrial activity[30]	2015	Provided data demonstrating the direct anti-inflammatory actions of nonspecific cannabinoid receptor antagonism in a rat model of stroke
The cannabinoid CB2 receptor agonist GW405833 does not ameliorate brain damage induced by hypoxia-ischemia in rats[26]	2014	Provided data contradicting previous studies suggesting that CB2 agonism is neuroprotective in models of cerebral ischemia by measuring outcomes over longer time periods; demonstrates the need for caution in extrapolating results from one study over longer time periods
Cannabidiol enhances morphine antinociception, diminishes NMDA-mediated seizures, and reduces stroke damage through the sigma-1 receptor[39]	2018	Provides a possible mechanism for the actions of CBD in stroke neuroprotection
Short-term effects of cannabidiol after global hypoxia-ischemia in newborn piglets[42]	2016	Contradicted previous studies finding neuroprotective effects from CBD administered after hypoxia-ischemia in a piglet model of global hypoxia-ischemia; demonstrates the need for further basic research before such therapies are extended to the clinic

2-AG: 2-Arachidonoylglycerol, CBD: Cannabidiol, CB1R: Cannabinoid 1 receptor, CB2R: Cannabinoid 2 receptor, CB1: Cannabinoid 1, CB2: Cannabinoid 2

Table 2: Evidence that altered activity at CB1R is neuroprotective in rodent models of stroke

Study	Intervention	Effect
Wang *et al*.[17]	Gene knockout (mice)	Neuroprotective
Knowles *et al*.[18]	Pharmacologic antagonist (rats)	Neuroprotective
Reichenbach *et al*.[19]	Pharmacologic antagonist (mice)	Neuroprotective
Caltana *et al*.[20]	Pharmacologic agonist (mice)	Neuroprotective

CB1R: Cannabinoid 1 receptor

injury, used a photoinjury method to model permanent ischemia. These results build on previously published data from the same group, demonstrating a similar effect in mice experiencing transient MCA occlusion rather than permanent photoinjury.[24] In addition to animal model data suggesting that CB2R agonism is neuroprotective, there is also data that suggests that CB2R antagonism is neuroharmful. For example, administration of a CB2R antagonist in a mouse model of chronic IS resulted in decreased neuroblast migration toward the infarct boundary, while both mice treated with a CB2R antagonist and mice with deletion of the CB2R gene demonstrated decreased numbers of new neurons and worse sensorimotor performance 28 days poststroke, when compared to controls.[25] The authors also demonstrated that CB2R agonists, but not CB2R antagonists, increased neural progenitor cell migration *in vitro*. Taken together, these results suggest that signaling interactions at the CB2R receptor play a critical role in poststroke neurogenesis, but further experiments are needed to determine whether this observation can be therapeutically leveraged, whether by pretreatment or poststroke treatment with CB2R agonists.

Regarding CB2R agonism is a stroke therapeutic, an important question to consider is how durable any observed positive effects are over time. Rivers-Auty *et al*. have shown that a CB2 agonist failed to show positive histological and behavioral effects at 15 days after injury in an animal model of cerebral hypoxia-ischemia.[26] This important article suggests that many of the neuroprotective properties associated with CB2 agonists or other endocannabinoid signaling molecules may be early and transient and that further rigorous experimentation is needed to assess the exact properties of CB2 agonists with respect to stroke neuroprotection in animal models, let alone in humans.

Another important question for researchers to answer is the exact mechanism by which CB2R agonism provides a neuroprotective effect. One possible mechanism by which the putative neuroprotective effects of cannabinoids are achieved is through the endocannabinoid-mediated modulation of the poststroke inflammatory response. Using CB2R tracers and positron emission tomography, Hosoya *et al*. have demonstrated increased levels of the CB2 receptor in the cerebral cortex surrounding ischemic

lesions in rats undergoing photothrombotic stroke surgery.[27] The authors were also able to demonstrate using immunohistochemistry that there was an elevation in CB2R expression within the microglia around the peri-infarct area, suggesting that the CB2R receptor may mediate microglia recruitment and activity in the postischemic inflammatory response. However, there are also data that suggest that decreased activity at CB2R also promotes inflammation. For instance, Kossatz *et al.* have shown that CB2R knockout mice demonstrate increased levels of HIF-1-alpha and TIM-3 expression by infiltrating microglia.[28] Further work is needed to determine the exact role of CB2R signaling in poststroke inflammation. For a summary of recent evidence regarding the neuroprotective effects of CB2R agonism and antagonism see [Table 3].

Evidence that co-antagonism at CB1R and CB2R is neuroprotective

The primary CB1 and CB2 endogenous agonist 2-arachidonoylglycerol (2-AG) is difficult to measure *in vivo*, and Brose *et al.* have recently shown that brain 2-AG levels rise dramatically following global ischemia, which both demonstrate the intimate connection between endocannabinoid signaling and brain ischemia as well as the importance of developing novel laboratory techniques to prevent ischemia-associated surges when measuring endocannabinoid levels in experimental animal models.[29] Jalin *et al.* have shown that postischemic treatment with the nonselective CB1R and CB2R antagonist hinokiresinols reduced infarct volume as well as infiltration by inflammatory cells into ischemic lesions, while co-administration of 2-AG abolished these positive effects, elegantly demonstrating the important role the endocannabinoid system plays in poststroke inflammation.[30] After administration of 2-AG to rats receiving permanent MCA occlusion surgery, Shearer *et.al.* performed blinded measurements of CBF.[31] They found that CBF was severely reduced in the 2-AG group when compared to vehicle controls for up to 4 h following the ischemic insult. These results further suggest that blocking the effects of 2-AG, the main endogenous ligand for CB1R and CB2R could play a neuroprotective role in stroke. Finally, in a recent study published by Ward *et al.*, it was shown that CB1R/CB2R double-knockout mice showed improved poststroke outcomes in both permanent and transient MCA occlusions, further suggesting that agonist activity at CB1R and CB2R may be driving deleterious physiological processes in the poststroke brain microenvironment.[32] Future research is needed to explore the full therapeutic possibilities of counteracting 2-AG signaling in the poststroke microenvironment. For a summary of recent evidence that co-antagonism CB1R and CB2R is neuroprotective in rodent models of stroke [Table 4].

Table 3: Evidence that altered activity at CB2R is neuroprotective, neuroharmful, or has no effect in rodent models of stroke

Study	Intervention	Effect
Yu *et al.*[22]	Pharmacologic agonist (rats)	Neuroprotective
Ronca *et al.*[23]	Pharmacologic agonist (mice)	Neuroprotective
Bravo-Ferrer *et al.*[25]	Pharmacologic antagonist and gene knockout (mice)	Neuroharmful
Bravo-Ferrer *et al.*[25]	Pharmacologic agonist (mice)	No effect
Rivers-Auty *et al.*[26]	Pharmacologic agonist	No effect

CB2R: Cannabinoid 2 receptor

Table 4: Evidence that altered activity at CB1R and CB2R is neuroprotective or neuroharmful in rodent models of stroke

Study	Intervention	Effect
Jalin *et al.*[30]	Pharmacologic antagonist (mice)	Neuroprotective
Shearer *et al.*[31]	Pharmacologic agonist (rats)	Neuroharmful
Ward *et al.*[32]	Gene knockout (mice)	Neuroprotective

CB1R: Cannabinoid 1 receptor, CB2R: Cannabinoid 2 receptor

The Endocannabinoid System and Poststroke Depression

In a rat model of poststroke depression, Wang *et al.* found that CB1 receptor expression was downregulated in the hypothalamus of rats subjected to MCA occlusion following by chronic unpredictable mild stress (CUMS), while also showing that intraperitoneal injections of CB1 and CB2 receptor agonists during the administration of CUMS were able to attenuate poststroke depression behavior in rats.[33] These data suggest a possible role for cannabinoid receptor agonists in the treatment of poststroke depression in humans. Zhang *et al.* have also investigated the role of the endocannabinoid system in poststroke depression, showing that interaction between sevoflurane and the CB1R resulted in a reduction in depressive-like behavior in rats following transient occlusion of bilateral common carotid arteries.[34]

Cannabidiol as a Stroke Neuroprotectant

Unlike experimental CB1R agonists and antagonists, CBD is a widely available cannabinoid that does not bind directly to CB1R or CB2R, and its specific target has yet to be established. It has also received attention for its possible neuroprotective properties. Khaksar and Bigdeli have shown that infusion of CBD into the lateral ventricle through a surgically implanted cannula for 5 days resulted in reduction in neurological deficit, infarction, edema, and blood–brain barrier permeability at 24 h following 60 min of MCA occlusion.[35,36] Recent work in a neonatal rat model of IS has demonstrated that CBD administration following MCA occlusion for 3 h increased neurobehavioral function at 1 week and 1 month without reducing the volume of infarct.[37] Using a mouse model of cerebral ischemia induced by

bilateral common carotid artery occlusion, Mori *et al*. have demonstrated that CBD aids in global function recovery following ischemic insult while also resulting in reduced hippocampal neurodegeneration, reduced white matter injury, reduced glial response, while also demonstrating increased levels of the brain-derived neurotrophic factor.[38] Rodríguez-Muñoz *et al*. have recently shown that the poststroke neuroprotective effects of CBD may be mediated by an antagonist-like activity at the sigma-1 receptor, which itself is known to inhibit NMDA receptor activity, suggesting that the neuroprotective effects of CBD may result from counteracting the effects of NMDA receptor overactivity in the brain.[39] Building on the above results, Lafuente *et al*. have shown that co-therapy with CBD and hypothermia in neonatal piglets subjected to hypoxic-ischemic insult reduced excitotoxicity, inflammation, oxidative stress, and cell damage more than either alone.[40] Similar results have been reported in newborn mice as well, with the therapeutic window for CBD administration stretching to 18 h posthypoxia-ischemia.[41] In conflict with these results, Garberg *et al*. demonstrated in newborn piglets that the administration of CBD alone did not have significant effects on postischemia neuropathology scores, S100B levels in the CSF, hippocampal proton magnetic resonance spectroscopy biomarks, plasma troponin-T, or urinary neutrophil gelatinase-associated lipocalin, whereas the administration of CBD plus hypothermia reduced urinary neutrophil gelatinase-associated lipocalin compared to hypothermia alone.[42] These conflicting results suggest that further research is needed to understand the role of CBD signaling in ischemic brain injury, both in the context of adult stroke and neonatal hypoxia-ischemia.

Human Studies of Cannabinoid Use in Stroke

Despite considerable number of animal and preclinical studies, there are only a limited number of clinical studies assessing cannabinoids in human ischemic cerebral injury. Keles *et al*. noted significant increases in oxygenated hemoglobin in the prefrontal cortex after THC use, as well as increased prefrontal blood flow on near-infrared spectroscopy.[43] These physiological effects associated with THC use could be therapeutically leveraged in the appropriate setting. In addition to its effects on brain oxygenation, cannabinoids have also received recent attention as a treatment for various types of spasticity. Along these lines, a double-blind, placebo-controlled, crossover trial is currently recruiting patients to investigate the efficacy of cannabinoids in reducing poststroke spasticity.[44]

Cannabinoid Abuse and the Risk of Stroke

Prior studies have shown a complex and varied interaction between cerebrovascular system regulatory pathways and cannabinoids. In a recent detailed review of literature from animal models, Richter *et al*. demonstrated that administration of cannabinoids can lead to vasodilation or vasoconstriction in the animal depending on the experiment.[45] Of a total of nine studies addressing the effect of cannabinoids on cerebral vasculature, three indicated vasoconstriction in response to the drug, while others showed vasodilatory effects. Depending on the timepoint, at which vasodilation is activated, this protection may be beneficial in early stages of ischemia. If it occurs at a later stage, it might accelerate the recuperation of cerebral function.

These data from animal models are important when putting in the context of recent concern over an apparent clinical connection between cannabinoid use and IS. A recent study reviewed possible stroke-related complications in the young and reported a temporal relationship between cannabinoids' use, whether natural or synthetic, and the occurrence of stroke.[46] Reversible cerebral vasoconstriction triggered by cannabinoid use has been reported as a possible underlying factor predisposing these patients to ischemic complications. Generation of reactive oxygen species leading to an oxidative stress and mitochondrial damage is other postulated underlying mechanisms that have been reported as a possible predisposing factor for cerebrovascular complications following chronic cannabinoid use. However, despite the widespread use of cannabinoids, the low number of their ischemic complications has raised the possibility for a genetic predisposition for cerebrovascular complications in these patients. Future randomized or well-designed population-based epidemiologic studies are required to assess the association of cannabinoids with the risk of IS in general population.

Conclusion

A great deal of supporting evidence exists for the involvement of the endocannabinoid system in IS pathophysiology. While no definitive human studies demonstrating poststroke benefit yet exist, the available preclinical data are promising. However, recent work has also demonstrated that the role of the endocannabinoid system in stroke is more complex than initially thought. First, the 2018 study by Shearer *et al*. has demonstrated an intimate connection between poststroke CBF and 2-AG signaling, and further work should be done to unpack this relationship in order to better predict the full effects of endocannabinoid therapeutics in human.[31] Second, Rivers-Auty *et al*. have cast doubt on the idea that CB2 agonism can reduce brain damage associated with hypoxia-ischemia, and further work is needed to examine the true potential of CB2 agonism as a promising poststroke neuroprotectant.[26] Finally, the

endogenous ligands for CBD have yet to be determined, and conflicting evidence for the neuroprotective effects of CBD in animal models of stroke have recently been published.[39,42] In our opinion, further work is required to establish satisfactory answers to these CBD-related questions before human trials. In sum, more studies will be needed to determine more precisely which cannabinoid receptors are most beneficial to neural recovery, the optimum timing of administration, and whether THC, CBD, or another synthetic agonist or antagonist will be the most efficacious agent with the least amount of adverse effects for stroke treatment.

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