Basic Science Advances for Clinicians

Section Editors: Anna M. Planas, PhD, and Midori A. Yenari, MD

Cannabis and Cannabinoid Biology in Stroke
Controversies, Risks, and Promises

Sang-Ho Choi, PhD; Yongshan Mou, MD; Afonso C. Silva, PhD

Stroke is the fifth leading cause of death and affects ≈0.8 million Americans annually.[1] The causes, duration, localization, and injury severity, as well as the presence of comorbidity factors, impact the overall outcome and likelihood of survival. The current therapeutical approach for acute ischemic stroke includes thrombolysis and mechanical thrombectomy. Reperfusion with intravenous alteplase (recombinant tissue-type plasminogen activator; activase and actilyse) remains the mainstay treatment for ischemic stroke, and a recent pooled analysis of 9 clinical trials showed that this therapy is beneficial regardless of patient age and stroke severity, as long as it can be administered within 4.5 hours of onset.[2] Although alteplase increases the risk of early hemorrhage, the number of patients with a good outcome exceeds the ones with a fatal intracranial hemorrhage.[2]

Nevertheless, immediate reperfusion therapy does not directly address the secondary neurological sequelae that lead to continued brain injury after stroke. Depending on injury severity, complex cascades of biochemical events are activated, generating deleterious responses, such as excitotoxicity, oxidative stress, and inflammation that affect both the central nervous system and the overall stroke outcome. Homeostatic cellular functions governing ATP-dependent ion gradients, calcium influx, endoplasmic reticulum, and mitochondrial function, membrane stability, redox balance, and blood-brain barrier permeability all become dysfunctional after ischemic stroke.[3] Pharmacological interventions targeting ≥1 steps of this cascade have led to the development of many neuroprotective drugs over the last 2 decades.[4] While some of the drugs produced encouraging results, particularly when combined with alteplase therapy, many proved ineffective in clinical trials.[5] Consequently, no neuroprotective treatment options currently exist to improve neurological outcome after ischemic stroke, making it imperative to conduct new research for novel therapies.

Endocannabinoid System

In recent years, the endocannabinoid system (ECS) has become the subject of great interest in neurobiology and neuropharmacology, mainly because of its predominant distribution in the central nervous system.[6] The ECS is composed of endocannabinoids, endogenous lipid-based retrograde neurotransmitters that bind to the cannabinoid receptors CB1 and CB2, as well as cannabinoid receptor proteins expressed throughout the central nervous system and peripheral nervous system (Figure). Importantly, modulating the activity of the ECS turned out to hold therapeutic promise in a wide range of pathological conditions and neurological disorders.[6]

The ECS has emerged as a new therapeutic target in a variety of neurological disorders with a robust neuroinflammatory component that leads to brain tissue injury.[7] There is convincing evidence that the components of the ECS are altered during ischemic stroke in both animals and humans, indicating that this system may contribute to the consequences of ischemic stroke.[8] Although numerous studies have examined the effects of cannabinoids and the role of the ECS in experimental models of ischemic stroke, results have been somewhat conflicting in support of either a beneficial or detrimental role. Nevertheless, a recent systematic review and meta-analysis of the currently available preclinical studies reported neuroprotective effects from a range of approaches to use of cannabinoids.[9] The main advantage of cannabinoids in neuroprotection is their broad-spectrum activity at multiple cellular and molecular mechanisms that involve not only the ECS itself but also the immune system.[10] Cannabinoids can limit excitotoxicity, oxidative stress, and neuroinflammation, and to enhance the trophic and metabolic support of neurons by acting through either specific cannabinoid receptor–mediated signaling pathways or via direct interactions with transcription factors. There are no previous or current clinical trials using cannabinoids in stroke, but their pleiotropic effects on the ischemic penumbra and cerebral vasculature after stroke, combined with their excellent tolerability, make them promising candidates for future treatment development.

Neurological Benefits of Cannabis and Cannabinoid Use

Cannabis (*Cannabis sativa*), also known as marijuana, has been used for centuries as a medicinal and recreational drug. It contains >120 different cannabinoids that have been identified, but the role and importance of most of them are yet to be fully understood.[11] Broadly, cannabinoids are defined as phytocannabinoids, synthetic cannabinoids, and endogenous cannabinoids. The 2 most notable and thoroughly investigated phytocannabinoids are the Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Studies of Δ^9-THC and CBD led
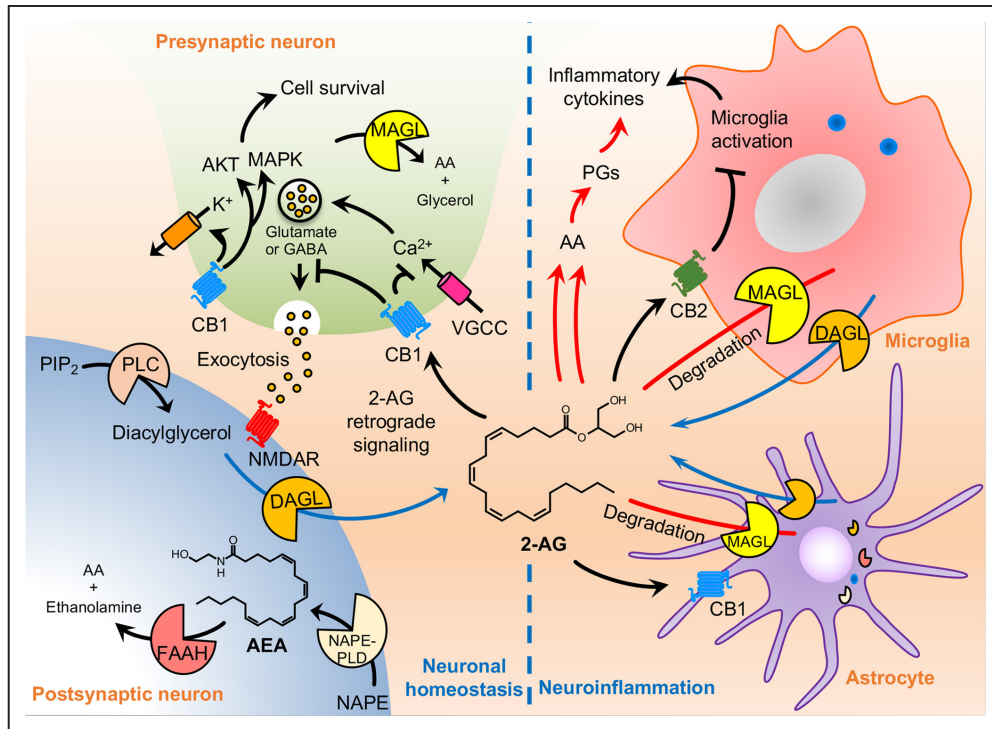
Figure. Overview of biosynthesis and degradation of endocannabinoids and its multiple cellular actions in the brain. The endocannabinoid system is composed of cannabinoid receptors (CB1 [cannabinoid receptor type 1] and CB2 [cannabinoid receptor type 2]), endocannabinoids (2-arachidonoylglycerol [2-AG] and *N*-arachidonoylethanolamine [AEA]), and its synthesizing (DAGL [diacylglycerol lipase] and NAPE-PLD [*N*-arachidonoyl phosphatidyl ethanol-preferring phospholipase D]) and degrading enzymes (MAGL [monoacylglycerol lipase] and FAAH [fatty acid amide hydrolase]). CB1 receptors are abundant in the central nervous system, particularly in the cortex, basal ganglia, hippocampus, hypothalamus, and cerebellum. CB2 receptors are primarily present in microglia and immune system and are highly inducible after tissue injury or during neuroinflammation. As a lipid signaling molecule, 2-AG readily cross the membrane and activate CB1 receptors located in the presynaptic neurons. Activated CB1 receptor inhibits neurotransmitter release through the suppression of calcium influx and activates potassium (K^+) channels, as well as induces the activation of AKT (protein kinase B) and MAPK (mitogen-activated protein kinase) survival pathway. Although 2-AG mainly mediates retrograde endocannabinoid signaling, AEA also activates presynaptic CB1 receptors and intracellular CB1 receptors as well. 2-AG functions as the primary cannabinoid receptor signaling molecule. 2-AG is also a significant precursor for arachidonic acid (AA) and, therefore, plays a substantial role in proinflammatory pathways. 2-AG is synthesized from diacylglycerol by DAGL, whereas AEA is synthesized from NAPE by NAPE-PLD. MAGL is the rate-limiting enzyme in the degradation of 2-AG. 2-AG also modulates the activity of microglia and astrocytes. These glial cells also are a source for brain endocannabinoids. Increased number of microglia and astrocytes are typically found in the ischemic brain and result in increased production of proinflammatory cytokines. This process leads to a change toward a more proinflammatory milieu in the brain. GABA indicates gamma aminobutyric acid; NMDAR, *N*-methyl-D-aspartate receptor; PGs, prostaglandins; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; and VGCC, voltage-gated calcium channel.

to the discovery of the 2 endogenous cannabinoid receptors CB1R (cannabinoid receptor type 1) and CB2R (cannabinoid receptor type 2).[12,13] THC exerts its well-known psychoactive effects, including relaxation, euphoria, dreaminess, feelings of anxiety, and paranoia, through the CB1R. THC has also been demonstrated to impair cognition and psychomotor performance.[14,15] However, recent studies have shown that THC promotes hippocampal neurogenesis and restores memory and cognitive function in aged animals.[16,17] Despite the beneficial effects of THC, its psychoactive effects have limited the medical use of cannabis.

On the contrary, CBD is known as the main nonpsychoactive component of cannabis and has shown anti-inflammatory, immunosuppressive, analgesic, and anxiolytic effects. In particular, CBD has a low affinity in the micromolar range for the cannabinoid receptors and was found to be an anticonvulsant in animal models and humans with epilepsy.[18] Although cannabis is still listed by the US Drug Enforcement Administration as a schedule I substance of the Controlled Substances Act, the US Food and Drug Administration has approved on June 25, 2018, the first prescription drug

derived from cannabis for treating 2 rare and severe forms of epilepsy.[19] The Epidiolex oral solution contains highly purified plant-derived CBD. In September 2018, the Drug Enforcement Administration classified Epidiolex as a schedule V substance, clearing the final hurdle for its legal prescription in the United States. Although Epidiolex is the first Food and Drug Administration–approved drug directly derived from the cannabis plant, it is not the first cannabinoid-based drug approved in the United States. The Food and Drug Administration approved 3 synthetic cannabinoid-based drugs: Marinol, Syndros, and Cesamet. Marinol and Syndros include the active component dronabinol—a synthetic form of THC. Cesamet includes the active ingredient nabilone, which is synthetically derived and has a chemical structure similar to THC. All 3 synthetic compounds are prescribed to adults for the treatment of nausea and vomiting associated with chemotherapy, while Marinol and Syndros are also indicated for the treatment of anorexia associated with weight loss in AIDS patients. Although Epidiolex is currently only approved for 2 rare forms of epilepsy, this sets a precedent that may benefit the world of cannabinoid research.

Accumulating preclinical studies suggest that cannabinoids have significant therapeutic value in stroke. A recent systemic review and meta-analysis by England et al[9] demonstrated that all subclasses of cannabinoids, cannabis-derived, synthetic, specific CB1R, and CB2R agonists, significantly reduced infarct volume in transient and permanent ischemia and improve both early and late functional outcome in experimental stroke when given after stroke onset. CBD showed trends to infarct reduction with delayed administration ≤6 hours after stroke onset.[9] Repeated treatment with CBD from day 1 or day 3 to day 14 improved functional outcome and survival rates, which suggest that CBD may have neuroprotective effects not only at the early phase but also at the late time point.[20] Multiple targets have been proposed to mediate the neuroprotective effects of CBD such as a combination of a potent antioxidant, immunosuppression, and anti-inflammatory actions.[21] CBD also counteracts the cerebral hemodynamic impairment and produces beneficial cardiac effects. Sultan et al[22] conducted a systemic review and meta-analysis with 25 studies and concluded that CBD is associated with changes in hemodynamics in vivo. Acute and chronic administration of CBD did not affect blood pressure or heart rate under control conditions but reduced blood pressure and heart rate in stressful conditions. Jadoon et al[23] found that acute administration of CBD reduced resting systolic blood pressure and blunted the blood pressure response to stress in humans. In mouse and piglet models of stroke, CBD significantly increased cerebral blood flow.[24,25]

Moreover, CBD reduced brain edema and blood-brain barrier permeability associated with ischemic condition.[26] CBD has negligible activity on cannabinoid receptors but may interfere with the ECS and directly or indirectly stimulate 5-hydroxytryptamine 1A receptors, adenosine receptors, transient receptor potential vanilloid subtype 1, and nuclear receptors of the peroxisome proliferator-activated receptor family.[21] THC also showed trends to infarct reduction with delayed administration ≤4 hours.[9] The neuroprotective effect of THC is related to the CB1R-mediated inhibition of voltage-sensitive calcium channels, which reduces calcium influx, excessive glutamate release, hypothermia, and enhances cerebral blood flow.[27,28] Also, THC, when acting on the CB2R found in the immune system, decreased the severity of stroke. Oral treatment with a low dose of THC inhibited atherosclerosis progression in a murine model of established atherosclerosis, through pleiotropic immunomodulatory effects on lymphoid and myeloid cells. CBD was also effective in diabetic complications and related atherosclerosis.[29] Administration of the CB1R agonist HU-210 significantly reduced motor disability and infarct volume in a dose-dependent manner and was useful 4 hours after stroke onset.[30] This neuroprotection is associated with the indirect protective effects of hypothermia.

Despite a large number of preclinical studies, there are no previous clinical trials using cannabinoids in stroke, although relevant clinical trials using cannabinoids in other neurological disorders already exist.[31] THC/CBD oromucosal spray (Sativex) is the first cannabis-based medicine to be licensed in the United Kingdom and currently available in numerous countries worldwide for the treatment of multiple sclerosis-related spasticities not responded adequately to other medication.[32] Spasticity is common after stroke and other neurological conditions and causes significant limitations on activities of daily living.[33] Importantly, a clinical trial investigating THC:CBD oromucosal spray efficacy and safety for poststroke spasticity has been registered.[34] Also, another relevant clinical trial was efficacy and safety evaluation of a single intravenous dose of dexanabinol (HU-211)—a synthetic and nonpsychoactive cannabinoid derivate—in patients experiencing severe traumatic brain injury.[35] This phase III trial concluded that dexanabinol was safe but was not efficacious for the treatment of traumatic brain injury.

Neurological Complications of Cannabis and Cannabinoid Use

Cannabis is the most commonly produced and consumed illicit substance in the world, and the contents of THC and CBD varied widely in street cannabis. Because THC is the primary psychoactive component cannabinoid, cannabis users prefer the strains of the plant with higher THC content. Potter et al[36] carried out the potency of THC and CBD seized by police in England in 2005 and found that the median content of THC was significantly higher than the one recorded 10 years before. In contrast, CBD content was found to be extremely low in more recent cannabis. Cascini et al[37] also performed a meta-analysis to assess the potency of THC from 1970 to 2009 and reported a temporal trend of increasing potency worldwide. These findings indicate that trends for preferring higher THC content variants carry significant health risks, particularly to those who are susceptible to its harmful effects.

The growing popularity of recreational consumption of cannabis, especially among the young population, raises immediate concerns regarding its safety. Recent case-control studies and systemic reviews have shown that cannabis use can significantly affect physical and mental health and lead to substance dependence.[38] Mainly, ischemic stroke is the most commonly reported adverse neurovascular effect of cannabis use in the young population. Kalla et al[39] conducted a study in patients aged 18 to 55 years and found that cannabis use was independently associated with a 26% increase in the risk of stroke after corrected for known risk factors, such as obesity, hypertension, smoking, and alcohol use. This study drew data from the Nationwide Inpatient Sample, which includes the health records of patients admitted over 1000 hospitals comprising about 20% of US medical centers.

Similarly, Jouanjus et al[38] conducted a systemic review with 116 cases published between January 2011 to March 2016 in patients aged 18 to 44 years and concluded that although cannabis use is linked to several adverse cardiovascular events, the evidence is the most persuasive for ischemic stroke. Similar results were also reported from the US nationwide inpatient sample, showing that recreational use of cannabis was independently associated with a 2.25-fold increase in the risk of acute ischemic stroke among people aged 25 to 34 years.[40] On the contrary, a recent population-based cohort and long-term follow-up study of 45 000 Swedish men performed by Falkstedt et al[41] analyzed the effects of cannabis, tobacco, and alcohol use on the risk of early stroke. They

found no apparent association between cannabis use in young adults and stroke, including strokes occurring before 45 years of age. However, tobacco smoking showed a clear association with stroke. Because most studies were based on hospital discharge records, the findings may not be reflective of the general population regarding cannabis use and early stroke. Furthermore, epidemiological studies have not performed an individual analysis of patients without other cardiovascular risk factors, which may limit the estimation of the risk of stroke associated with cannabis use alone. Nevertheless, there are currently limited data, inadequate assessment of cannabis use, and few population-based studies to confirm or reject the hypothesis of the effects of cannabis use and early stroke.

It is critically important to identify all factors that may play a role in the recent increase in the incidence of stroke among the young population. One striking element reported in the majority of the case studies was a temporal relationship between cannabis use and the occurrence of stroke. However, a temporal correlation does not mean causation, and other factors may be involved. Currently, reversible cerebral vasoconstriction triggered by cannabis use may be a possible mechanism of stroke. Chronic cannabis use leads to impairment in cerebrovascular function, which has been associated with an increased risk for stroke.[42] Cannabis-related angiopathy has also been linked with ischemic stroke in heavy users.[43] Another possible mechanism of acute ischemic stroke due to cannabis use could be an increase in procoagulant effects, as THC increases the expression of glycoprotein IIb-IIIa and P-selectin on human platelets in a concentration-dependent manner.[44] Platelet aggregation is a significant risk factor for acute ischemic stroke,[45] and it is relatively more important in younger than in older stroke patients.[46] Earlier research has indicated that THC induces tachycardia.[47] It has been suggested that CBD reduces THC-induced adverse cardiovascular effects.[48] Jamil et al[49] reported a case of possible concurrent use of cannabis and other drugs on the development of stroke. Cannabis often precedes or is used along with other substances.

Besides this vascular role of cannabis in the occurrence of stroke, a cellular effect of cannabis on brain mitochondrial respiratory chain dysfunction and oxidative stress was recently suggested as a potential mechanism involved in cannabis-related stroke.[50] Indeed, despite the widespread use of cannabis, the low frequency of neurovascular complications after their use may be due to a genetic predisposition to neurovascular toxicity in some individuals.

There is still debate about the possible behavioral and pathological consequences of cannabis use. Because several questions remain unsolved, further research is still needed to assess the pathophysiological mechanisms involved in young cannabis users with stroke.

New Approaches to Targeting the ECS With Selective Inhibitors of Monoacylglycerol Lipase

In recent decades, multiple lines of research have provided insights into the biochemical regulation, pathophysiological roles, and therapeutic potential of the endogenous cannabinoid 2-arachidonoylglycerol (2-AG).[51] 2-AG is synthesized on demand and acts as a full agonist of cannabinoid receptors, but its rapid degradation by MAGL (monoacylglycerol lipase) results in short-lived actions. Many physiological and pathological processes involve 2-AG, which behaves as a retrograde signaling lipid that inhibits neurotransmitter release at both excitatory and inhibitory synapses. Several neurological disorders with an inflammatory involvement, including ischemic stroke, show elevated levels of 2-AG.[52] Although the exact role of 2-AG is not clear, the neuroprotection exerted by exogenous 2-AG suggests that 2-AG contributes to neuroprotection not only by reducing neuronal excitotoxicity and inhibiting the production of proinflammatory cytokines and reactive oxygen species but also by lowering cerebral vasoconstriction.[53]

Inactivation of MAGL leads to the elevation of the level of 2-AG, thus resulting in an enhancement of the endocannabinoid signaling. Beyond this critical role, the study by Nomura et al[54] provides compelling evidence linking both the endocannabinoid and eicosanoid signaling pathways through MAGL, which hydrolyzes 2-AG to arachidonic acid. Arachidonic acid is of particular interest as the precursor of the eicosanoid family that includes proinflammatory prostaglandins and leukotrienes. It is possible that pharmacological inhibition of MAGL might be a promising therapeutic target not only by enhancing anti-inflammatory and neuroprotective 2-AG signaling through cannabinoid receptor–dependent mechanisms but also by reducing proinflammatory eicosanoid production. Although early MAGL inhibitors had poor selectivity and low potency, they contributed significantly to advancing the understanding of the pathophysiological roles of MAGL.[55] Inhibition of MAGL activity with JZL184 reduced 2-AG hydrolysis by ≈85% in the mouse brain and led to dramatic elevations in brain 2-AG levels. A single dose of JZL184 was capable of inhibiting MAGL for ≤24 hours, with a maximal 8-fold elevation of brain 2-AG levels for at least 8 hours.[55]

Most importantly, acute MAGL blockade with JZL184 has been shown to exhibit a wide range of beneficial effects in neurodegenerative diseases.[54] Recent studies demonstrated that the MAGL inhibitor JZL184 significantly reduces infarct volume in both transient and permanent models of ischemic stroke and improves the functional outcome when administered it after stroke onset.[56] CPD-4645—a newly characterized MAGL inhibitor—modified the transcription profile of brain vasculature and restored its functional homeostasis in the photothrombotic model of ischemic stroke.[57] These results highlight a bidirectional mechanism of action—simultaneous enhancement of cannabinoid signaling through elevation of 2-AG and reduction of arachidonic acid and downstream eicosanoids—that can achieve therapeutic efficacy through either cannabinoid receptor–dependent or independent mechanisms.

Over the past decades, considerable efforts were made for developing novel MAGL inhibitors with improved selectivity and cross-species activity compared with JZL184. KML29—an analog of JZL184—was the most selective for MAGL over other serine hydrolase family of enzymes.[58] MJN110—another recently developed selective MAGL inhibitor—showed markedly increased potency and no significant

cross-reactivity with fatty acid amide hydrolase.[59] Interestingly, those MAGL inhibitors do not cause cannabimimetic activity such as catalepsy and hypothermia. Clinical research in the field of MAGL inhibitors is still at early stages. During the past 5 years, several academic groups and pharmaceutical companies have patented about 20 MAGL inhibitors for a large number of therapeutic uses, such as pain, inflammation, metabolic, and neurodegenerative diseases, as well as the treatment of cancer, anxiety, and epilepsy.[60] Recently, a potent human MAGL inhibitor ABX-1431 (Abide Therapeutics) has completed a placebo-controlled phase I for safety and tolerability.[61] ABX-1431 is currently subjected to phase II trials in patients with Tourette Syndrome and neuropathic pain. Although the translational potential of MAGL inhibitors still needs basic and preclinical studies, the results of ongoing and future clinical trials will hopefully unveil the MAGL inhibitors as a new drug class for the treatment of human diseases, including stroke.

Conclusions

Neurovascular complications, such as reversible cerebral vasoconstriction syndrome, intracranial hemorrhages, and ischemic strokes and their association with cannabis and cannabinoids are an emerging area of research as more states legalize cannabis for medical and recreational use. However, several questions remain unanswered about the pathophysiological role of cannabis and cannabinoids in such complications. Epidemiological studies must provide detailed information concerning not only the quantity and the frequency of cannabis use but also the type of cannabis used. Longitudinal studies are needed to clarify the impact of cannabis on the severe consequences associated with their use. Despite the controversy of cannabis use, the first cannabis-derived Epidiolex received the US Food and Drug Administration approval as a treatment for some types of epilepsy and opened new horizons for medical use of cannabis. This approval highlights the importance of critical benefit-risk analysis and careful evaluation in the drug development process.

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Disclosures

None.

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