

Review Article

Endocannabinoids and liver disease – review

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Abstract: *Aims:* Endocannabinoids are endogenous compounds that bind to the same receptors as tetrahydrocannabinol, the active component in marijuana and hashish. They have been found to have many physiological and patho-physiological functions, including mood alteration, control of feeding and appetite, motor and co-ordination activities, analgesia, immune modulation and gut motility. In this review we aim to elucidate current knowledge as to their role in liver physiology and disease. *Methods:* The major findings published to date concerning endocannabinoids and liver disease are described, and their implications with regard to understanding disease mechanisms, and the development of new treatments is considered.

Results: Recently, endocannabinoids have been implicated in the hemodynamic alterations occurring in cirrhosis. These changes appear to be mediated via specific cannabinoid receptors (CB1) on splanchnic and hepatic vascular endothelium. Plasma levels of endocannabinoids also seem to be elevated in hepatitis, and are involved in apoptosis of hepatocytes by a membrane mechanism not related to a specific receptor. Other studies suggest a beneficial role for cannabinoids in reducing the inflammation of experimental hepatitis. In an animal model of acute hepatic failure, both endocannabinoids and the antagonist to the CB1 receptor have been found to have a beneficial effect on neurological and cognitive function.

Conclusions: Endocannabinoids appear to be involved in several aspects of acute and chronic liver disease, including vascular changes, modulation of inflammatory process and neurological function. Further research may provide new insights into the pathophysiology of liver disease, as well as a basis for novel treatment modalities.

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Cannabis has been used for psychoactive and recreational purposes as well as in traditional medicine, long before the advent of modern medicine and scientific research (1, 2). The active component of cannabis, tetra-hydro-cannabinol (THC) was discovered in 1964 (3). This finding led to the discovery of two specific receptors to cannabinoids. The cannabinoid receptor antagonist (CB1) receptor was found initially in the brain (4) and subsequently in the gut and vascular endothelium (5–7). The CB2 receptor was isolated primarily in the immune system (8). Both receptors are coupled to G-proteins and act via adenylate cyclase and calcium channels (4). The

first endogenous ligand for these receptors was found in 1992, and designated as Anandamide (from the Sanskrit ‘Ananda’-bliss) (9). This compound is an amide of arachidonic acid and ethanolamine, and is a member of the fatty acid amide (FAA) family. Following this breakthrough, several other ligands were reported, e.g. 2-arachidonyl-glycerol (2-AG), noladine and oleamide (10–13). Specific antagonists to each subtype of cannabinoid receptors were also found: SR141716A for the CB1 receptor and SR 14452 for the CB2 receptor (14, 15).

Cannabinoids have been studied extensively in recent years and have been found to have important functions in many physiological and pathophysiological processes. They have been found throughout the CNS, and effect many neurological and psychological phenomena such as mood, appetite, emesis control, memory, spatial coordination muscle tone and analgesia (5, 16–29). These effects are mediated primarily through the CB1 receptor. Outside the CNS, cannabinoids exert immunomodula-

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Abbreviations: HE, hepatic encephalopathy; CNS, central nervous system; FHF, fulminant hepatic failure; TAA, thioacetamide; SR141716A, CB1, cannabinoid receptor antagonist; 2-AG, 2-arachidonyl glycerol; GABA-ergic, gamma amino butyric acid; SR144528, CB2, cannabinoid receptor antagonist.

tory and anti-inflammatory effects via CB2 receptors found in lymphocytes, monocytes and neutrophils (30–34). Other effects include vasodilation of splanchnic vessels via receptors on the surface of endothelial cells (7, 35), and inhibition of gut motility by anandamide released from nerve ends (10, 16). In this review, we examine the implications of basic-science and clinical research of cannabinoids' role in hepatic physiology and disease.

Endocannabinoids and liver disease

Portal hypertension, a complication of cirrhosis, is caused by both increased resistance to flow in the portal vein, and, at a later stage, by increased flow in the mesenteric vasculature. Systemic vasodilation further complicates this condition, causing a decrease in effective blood volume, hypotension, fluid and salt retention, worsening ascites and deterioration of renal function (36).

Ascites is associated with increased plasma levels of the bacterial endotoxin lipopolysaccharide (LPS). In a study designed to assess the prognostic value of plasma endotoxin levels, it was found that plasma endotoxin levels increased progressively as liver function deteriorated. In short-term survival analysis, plasma endotoxin levels were significantly higher in non-survivors than in survivors. In long-term survival analysis, plasma endotoxin levels did not differ significantly between survivors and non-survivors, and was not an independent predictor of long-term survival. The authors concluded that in patients with cirrhosis, plasma endotoxin levels progressively increase as liver function deteriorates and may be useful in predicting short-term survival (37). LPS appears to contribute to the hemodynamic changes observed in cirrhosis, possibly mediated via enhanced nitric oxide (NO) production (38). LPS acts on macrophages, in a complex mechanism involving the CD14 protein expressed on their membranes, resulting in increased production of cytokines, including interleukin (IL) 1- β and tumor necrosis factor- α (39, 40). Anandamide is found in macrophages (41). The finding that activation of peripheral CB1 receptors in rats by macrophage- and platelet-derived substances contributes to the hypotension in hemorrhagic shock (42) prompted the investigation of the possible role of endocannabinoids in endotoxin-induced shock. In that study, rat platelets were found to contain 2-AG. *In vitro* exposure of platelets to LPS markedly increases 2-AG levels. Prolonged hypotension and tachycardia were elicited in rats treated with LPS, as well as by macrophages plus platelets isolated

from LPS-treated donor rats; rat macrophages or platelets preincubated *in vitro* with LPS had a similar effect. In all four cases, the hypotension but *not* the tachycardia was prevented by pretreatment of the recipient rat with the CB1 receptor antagonist SR141716A (3 mg/kg i.v.), which also inhibits the hypotensive response to anandamide or 2-AG. The hypotension elicited by LPS-treated macrophages or platelets remains unchanged in the absence of sympathetic tone or after blockade of NO synthetase. These findings indicate that platelets and macrophages generate different endogenous cannabinoids, and that both 2-AG and anandamide may be paracrine mediators of endotoxin-induced hypotension via activation of vascular CB1 receptors (43).

These results led to further studies designed to explore the possible role of endocannabinoids in the hemodynamic changes in chronic liver disease.

Hypotension in rats with biliary cirrhosis was improved by the CB1 receptor antagonist SR141716A. Similar results were also observed in rats with CCl₄-induced cirrhosis, wherein SR141716A also reduced the elevated mesenteric blood flow and portal pressure. Monocytes from cirrhotic humans and rats elicited SR141716A-sensitive hypotension in normal recipient rats. Significantly elevated levels of anandamide were found in these cells. Compared with non-cirrhotic controls, in cirrhotic human livers there was a three-fold increase in CB1 receptors on isolated vascular endothelial cells indicating up-regulation of these receptors in chronic liver disease (44).

In a similar study, arterial pressure, cardiac output, and total peripheral resistance were measured before and after the administration of SR141716A to cirrhotic rats with ascites, and to control rats. CB1 receptor blockade increased arterial pressure and peripheral resistance in cirrhotic animals, but not in healthy ones. A suspension of blood cells from cirrhotic rats induced hypotension in recipient rats. Monocyte levels of anandamide were significantly elevated in cirrhotic animals as compared to those in healthy controls. Here too, the conclusion was that in cirrhosis, monocytes increase their production of anandamide, and that this process contributes to hypotension and hemodynamic deterioration (45).

It would thus seem that endocannabinoids are involved in the hemodynamic alterations of cirrhosis by acting as mediators between endotoxin/LPS and blood vessels. LPS acts on monocytes and platelets, thereby increasing production of anandamide and 2-AG, respectively. The endocannabinoids produced in response to LPS may then act on systemic and mesenteric vasculature, decreasing blood pressure and effective blood

volume, increasing fluid and solute retention, thereby worsening ascites and contributing to renal dysfunction. The mechanism of vasodilation is at least partially mediated by the CB1 receptor, as demonstrated by the effects of SR141716A. However, a non-CB1 receptor mechanism cannot be ruled out as a contributor to these processes. One such mechanism may be via the vanilloid receptor and possibly by the subsequent release of CGRP that requires NO production, as demonstrated in endothelium-denuded aortic rings in rabbits (46). Given the central role of NO in the hemodynamics of cirrhosis, such a mechanism would seem plausible.

The development of new chromatographic techniques has allowed for more accurate measurements of serum cannabinoids. Using this method, four-fold and three-fold increased levels of ANA and 2-AG, respectively, were found in the sera of patients with endotoxic shock compared to healthy subjects (47). In a subsequent study, the investigators demonstrated that compared to serum anandamide levels in healthy humans (4.0 ± 0.79 nM), a significant increase was seen in acute non-severe hepatitis (8.8 ± 0.98 nM), with a further rise in severe acute hepatitis (15.8 ± 10.25 nM). In chronic cirrhosis, anandamide levels were also elevated at 11.64 ± 1.93 nM. In the acute hepatitis group, serum anandamide levels were found to correlate with the extent of tissue damage as indicated by serum ALT levels. Furthermore, tissue samples from the cirrhotic patients showed massive hepatocellular apoptosis and liver fibrosis. Since anandamide has been known to induce apoptosis, particularly of lymphocytes (48), the effect of anandamide on hepatocellular apoptosis was therefore assessed. Anandamide induced apoptosis of human hepatoma cells (HepG2) in a dose-dependent manner. This was preceded by G0/G1 cell cycle arrest, and the activation of pro-apoptotic signaling pathways such as p38 mitogen-activated protein kinase. The mechanism of apoptosis was thought to be either CB receptor-mediated as in thymic lymphocytes (48) or vanilloid receptor (VR1) mediated, as seen in human neuroblastoma CHP100 and lymphoma U937 cells (49). As neither SR141716A nor SR144528 produced significant inhibition of apoptosis in VR1 knock-out mice, it was concluded that the principal mechanism of apoptosis was non-receptor mediated. Depletion of membrane cholesterol by MCD or treatment with HMG-coA-reductase inhibitors, did result in suppression of apoptosis, as did treatment with the antioxidant *N*-acetyl-cystein. Polymixin binding studies indicated that anandamide interacts with membrane cholesterol. Similar results were observed in nor-

mal rat hepatocytes. This indicated that the apoptotic effect of anandamide on liver cells is mediated through a direct effect on membrane cholesterol designated as lipid-lipid plasma membrane interaction and not through specific receptors. The effect seems to involve enhanced susceptibility to oxidative stress (50).

The effects of endocannabinoids on neurological and cognitive aspects of hepatic encephalopathy (HE) were studied in an animal model of acute hepatic failure. The pathogenesis of HE is a complex process involving several mechanisms including functional changes in neurotransmitter systems such as the opiodergic (51, 52) and gamma amino butyric acid (GABA-ergic) (53–58) systems that are known to interact with the endocannabinergic system (16, 59).

Fulminant hepatic failure (FHF) was induced by thioacetamide (TAA). Neurological performance (assessed by a fourteen point scale based on reflexes and task performance), activity (evaluated in an infra red maze), and cognitive function (performance in an eight arm maze) were measured after administration of the 2-AG SR141716A or both. Encephalopathic mice treated with SR141716A or 2-AG or both, showed improved neurological function, activity and cognitive function compared to untreated animals. SR141716A showed a dose-response pattern in the improvement of neurological function. CNS levels of 2-AG were found to be elevated in mice with TAA induced liver failure when compared to healthy mice. Administration of exogenous 2-AG resulted in decreased brain levels of endogenous 2-AG. The endocannabinoid system may therefore have a role in the pathogenesis of hepatic encephalopathy. The similar effect of 2-AG and SR141716A could be explained by two hypotheses: end-product inhibition of 2-AG and exertion of the effect through a non-CB1-receptor target (60).

Other publications have shown that certain cannabinoid compounds decrease the extent of experimental hepatitis. The synthetic, non-psycho-tropic cannabinoid (PRS-211,092) decreased concanavalin A-induced liver injury in mice that was accompanied by promotion of early gene expression of IL-6 and IL-10, induction of early gene expression of the suppressors of cytokine signaling (SOCS-1 and 3), and inhibition of several pro-inflammatory mediators, including IL-2, monocyte chemoattractant protein-1 (MCP-1), IL-1 β , interferon- γ , and tumor necrosis factor α . PRS-211,092 inhibited IL-2 production and nuclear factor of activated T cells activity in cultured T cells (61).

A recently published study, examined the potential hepatotoxic effects of marijuana use in humans. A transversal study was conducted among

123 patients over 2 years in Sao-Paolo, Brazil. The patients were divided into three groups: 26 (21%) using only marijuana, 83 (67.5%) using marijuana and crack, and 14 (11.4%) consuming marijuana and alcohol. Among patients who reported using marijuana alone, hepatomegaly was observed in 57.7% and splenomegaly in 73.1%. These patients were found to have slightly elevated AST (42.3%), ALT (34.6%) and AP (53.8%). The prevalence of hepatomegaly, splenomegaly and hepatosplenomegaly was not different in the three groups. Patients who consumed both marijuana and alcohol had the highest levels of aminotransferases. These results suggest that chronic marijuana use, alone or in combination with alcohol or other drugs, may have hepatotoxic effects (62). The methodological issues involved, particularly the isolation of cannabis use from the use of other substances warrants caution as to the interpretation of these results.

What are the therapeutic implications of these data for patients with liver disease? Very little clinical data are available as to the efficacy of endocannabinoids or their receptor antagonists in humans with hepatic disease.

THC treatment for intractable cholestatic-related pruritus (ICRP) was evaluated in a report of three patients who had previously been treated with standard therapies for ICRP including: diphenhydramine, chlorpheniramine, cholestyramine, rifampicin, phenobarbital, doxepin, naltrexone, UV therapy, topical lotions and plasmapheresis. Patients were given 5 mg of delta-9-THC (Marinol) at bedtime. All three patients reported a decrease in pruritus, marked improvement in sleep, and were eventually able to return to work. Resolution of depression occurred in two of three. Side effects related to the drug included coordination disturbance in one patient. Marinol dosage was decreased to 2.5 mg in this patient with resolution of symptoms. The duration of antipruritic effect was approximately 4–6 h in all three patients suggesting that frequent dosing may be needed (63). One possible mechanism for the beneficial effect on cholestasis may be related to an increase in the threshold for nociception. This hypothesis was tested in rats with bile-duct-resection induced cholestasis and in sham-resected controls. Administration of the cannabinoid agonist WIN 55, 212–2 resulted in an increase in the tail-flick latency – an index of the nociception threshold – in both groups as compared to baseline. These results support the notion that the analgesic effect of cannabinoids may be of use in treating pruritus, a nociceptive stimulus, in patients with liver disease (64).

Despite the scarcity of information, certain conclusions may be drawn from clinical experi-

ence in other fields. Cannabinoids such as THC, cannabis and nabilone have been tried in patients with multiple sclerosis with some success (28). The safety profile of these agents may also prove problematic. A review of cannabinoids in clinical practice found that some patients, particularly women and the elderly had panic or anxiety attacks. Psychosis is also a possible consequence of cannabis use that must be considered. The slow elimination time of cannabinoids from the body may impair task performance and driving (65). The development of new, non-psychotropic cannabinoids such as HU-320 (66) may enable the development of new therapeutic agents, while avoiding the legal and social issues that may be associated with the use of compounds derived from illicit drugs.

SR141716A, the CB1 receptor antagonist is currently under investigation for treatment of obesity and for smoking cessation, given the cannabinoids' known effect in appetite stimulation and reward (1, 5, 16, 67). Preliminary results of the RIO–Europe trial, now in phase III show that this agent (labeled Rimonabant) was safe and effective in achieving weight reduction, improved lipid profile and amelioration of the metabolic syndrome compared to placebo (68, 69).

In summary, endocannabinoids are relatively recently discovered compounds, that have many physiological and pathophysiological functions. Though much has been learned about their effects in the CNS, immune system and gut, relatively little is known about their role in liver physiology and disease. There is convincing evidence for their role in the hemodynamic compromise as seen in cirrhosis. The mechanism underlying this phenomenon appears to involve an increase in production of 2-AG and anandamide in platelets and macrophages, respectively, and a subsequent vasodilation, at least partially mediated by CB1 receptors on endothelial cells. Studies have revealed an increase in serum anandamide concentration in both acute and chronic liver disease in humans, as well as an apoptotic effect of anandamide on liver cells, via a direct interaction with membrane cholesterol. The endocannabinoid system may also be involved in the pathogenesis of HE, as are several other neurotransmitter systems. Some epidemiological data supports the notion of a hepatotoxic effect for marijuana; however, methodological problems preclude conclusion in this context. Certain cannabinoids may improve hepatic inflammation and pruritus secondary to liver disease, but more data are needed to substantiate these propositions. The development of therapeutic modalities based on cannabinoids or their antagonists depends on their safety as well as efficacy. Syn-

thetic, non-psychotropic cannabinoids may be better tolerated and reduce social and legal tensions that may impede the pharmacological use of substances derived from illicit drugs.

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