Endocannabinoids in Liver Disease

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Endocannabinoids are lipid mediators of the same cannabinoid (CB) receptors that mediate the effects of marijuana. The endocannabinoid system (ECS) consists of CB receptors, endocannabinoids, and the enzymes involved in their biosynthesis and degradation, and it is present in both brain and peripheral tissues, including the liver. The hepatic ECS is activated in various liver diseases and contributes to the underlying pathologies. In patients with cirrhosis of various etiologies, the activation of vascular and cardiac CB1 receptors by macrophage-derived and platelet-derived endocannabinoids contributes to the vasodilated state and cardiomyopathy, which can be reversed by CB1 blockade. In mouse models of liver fibrosis, the activation of CB1 receptors on hepatic stellate cells is fibrogenic, and CB1 blockade slows the progression of fibrosis. Fatty liver induced by a high-fat diet or chronic alcohol feeding depends on the activation of peripheral receptors, including hepatic CB1 receptors, which also contribute to insulin resistance and dyslipidemias. Although the documented therapeutic potential of CB1 blockade is limited by neuropsychiatric side effects, these may be mitigated by using novel, peripherally restricted CB1 antagonists. (Hepatology 2011;53:346-355)

Marijuana has been used for its psychoactive and medicinal properties for millennia. Like other plant-derived substances, marijuana has been slow to yield its secrets, with insights into its mechanism of action beginning to emerge only during the last decades. The existence of specific cannabinoid (CB) receptors in mammalian tissues was first revealed by radioligand binding, and this was followed by the molecular cloning of two G protein–coupled CB receptors.1 CB1 receptors are the most abundant receptors in the mammalian brain, but they are also expressed in peripheral tissues, including various cell types of the liver, at much lower yet functionally relevant concentrations.2-8 CB2 receptors are expressed primarily in immune and hematopoietic cells and have also been detected in the liver in certain pathological states.9,10 Additional CB receptors may exist,11 but their potential role in liver biology is unknown.

The discovery of CB receptors triggered a search for endogenous ligands. Arachidonoyl ethanolamide (AEA), also known as anandamide, was the first such ligand discovered,12 with 2-arachidonoyl glycerol (2-AG) identified 3 years later.13,14 Additional endogenous ligands have since been identified1 but have received less attention. AEA and 2-AG are generated on demand via a high-affinity uptake mechanism17; this is followed by their enzymatic degradation. AEA is metabolized primarily by membrane-associated fatty acid amide hydrolase (FAAH),18 whereas 2-AG is preferentially degraded by monoglyceride lipase.19

The psychoactive properties of CBs and the abundance of CB1 receptors in the brain could suggest that
the endocannabinoid system (ECS) is primarily a neuronal signaling system; therefore, evidence for the presence and functional importance of the ECS in the liver was unexpected. Indeed, early studies of brain CB1 receptors used the liver as a negative control. However, recent reports have documented low-level CB1 expression in the whole liver, hepaticocytes, stellate cells, and hepatic vascular endothelial cells (see Fig. 1). CB1 receptors are present in human hepatocytes and in the whole human liver, with increased expression noted in patients with hepatocellular carcinoma (HCC) or primary biliary cirrhosis. CB2 receptors are undetectable in the normal liver but are induced in pathological conditions such as nonalcoholic fatty liver disease (NAFLD), the embryonic state, the regenerating liver, and HCC. Hepatic endocannabinoids levels are similar to those in the brain, whereas FAAH expression is higher in the liver versus the brain. Evidence implicating the ECS in the regulation of hepatic hemodynamics, fibrogenesis, and lipid metabolism and in the dysregulation of these functions in pathological states such as cirrhosis, NAFLD, alcoholic fatty liver, and ischemia/reperfusion (I/R) injury is discussed next.

**Endocannabinoids and Altered Hemodynamics in Cirrhosis**

The potent hypotensive action of Δ⁹-tetrahydrocannabinol (THC) has long been recognized, and attempts have even been made to exploit it for the treatment of hypertension. This and the similar effects of synthetic CB analogues and endocannabinoids are mediated by CB1 receptors located, in part, in the peripheral cardiovascular system, and they play a pathogenic role in various forms of shock, including endotoxic shock.

Advanced liver cirrhosis is associated with endotoxemia and hypotension, and this suggests endocannabinoid involvement. Indeed, cirrhosis in rats is accompanied by progressive hypotension reversible by CB1 blockade, which also reduces the elevated portal venous pressure and mesenteric blood flow. The likely source of endocannabinoids is activated macrophages, in which lipopolysaccharide induces the CD14-dependent synthesis of AEA. AEA levels are elevated in circulating macrophages of cirrhotic rats or patients, and such macrophages injected into normal rats elicit CB1-mediated hypotension. Cirrhosis increases CB1 expression in vascular endothelial cells and in mesenteric arteries and increases the vasodilator potency of AEA. In patients with cirrhosis, circulating AEA levels, but not 2-AG levels, are increased in peripheral blood but not in hepatic veins or liver tissue, and this suggests that the liver is not its source.

These findings implicate AEA as a mediator of the vasodilated state in cirrhosis. Although in one study of patients with cirrhosis the increase in circulating AEA did not correlate with the degree of hepatic and renal dysfunction, in another study of patients with...
primary biliary cirrhosis, the CB1 expression in hepatocytes and biliary epithelial cells and the CB2 expression in hepatocytes and cholangiocytes were positively correlated with the severity of the histological stage. Cirrhosis is associated with renal sodium retention, which has been attributed, in part, to portal hypertension secondary to liver parenchymal damage and fibrosis. In cirrhotic rats, rimonabant dose-dependently reduced ascites by ensuring a less positive sodium balance.

AEA induces CB1-mediated mesenteric vasodilation independently of nitric oxide. However, the effect of higher doses of AEA is resistant to CB1 blockade and may be mediated via putative AEA receptors implicated in the mesenteric vasorelaxant effect of AEA observed in CB1/CB2 double-knockout mice, which may also contribute to mesenteric vasodilation in cirrhosis.

The hyperdynamic circulation of advanced cirrhosis is associated with increased cardiac output and tachycardia. However, the cirrhotic heart has an underlying decrease in contractility and β-adrenergic hyposensitivity called cirrhotic cardiomyopathy, and this has been attributed to endocannabinoid activation of cardiac CB1 receptors on the basis of pharmacological studies using isolated myocardial preparations from bile duct-ligated rats. In vivo studies using an intraventricular pressure-volume microcatheter system revealed a profound decrease in baseline cardiac contractility, which was acutely normalized by CB1 blockade. The suppression of cardiac contractility by CB1 receptor activation may involve inhibition of L-type calcium channels and/or reductions in the myocardial cyclic adenosine monophosphate content. Of the 2 major endocannabinoids, AEA is more likely to be involved, as suggested by a cirrhosis-related increase in myocardial AEA levels but not 2-AG levels.

These findings raise the therapeutic potential of CB1 blockade in treating the hemodynamic abnormalities of patients with advanced liver cirrhosis. Because the increase in mesenteric blood flow may precipitate the rupture of varicosities and also contributes to ascites formation, CB1 blockade may avert these potentially fatal complications and thus keep patients alive until a liver transplant becomes available.

Endocannabinoids and Liver Fibrosis

CB2 receptors, which are normally undetectable in the liver, are prominently expressed in the cirrhotic human liver and are also detectable in nonparenchymal liver cells in the fibrotic mouse liver. THC suppresses the proliferation and induces the apoptosis of human hepatic myofibroblasts and stellate cells via CB2 receptors and thus may be antifibrotic and hepatoprotective. Accordingly, CB2−/− mice had an enhanced response to fibrogenic stimuli. CB2 receptor activation by AEA also inhibits the hyperplastic proliferation of cholangiocytes, which is a frequent result of extrahepatic biliary obstruction, cholestasis, and toxic liver injury. This has been associated with the increased production of reactive oxygen species and cell death via the induction of the activator protein 1 complex and thioredoxin. In cirrhotic rats, chronic treatment with the CB2-selective agonist JWH-133 attenuated cellular markers of fibrosis and enhanced the regenerative response to acute liver injury. Accordingly, CB2−/− mice had delayed liver regeneration in response to CCl4-induced injury, whereas JWH-133 treatment reduced the injury and accelerated liver regeneration. These findings signal the therapeutic potential of nonpsychoactive CB2 agonists in the treatment of liver fibrosis.

Paradoxically, in patients with hepatitis C virus infection, daily cannabis use increased fibrosis progression instead of protecting patients against it. Thus, endocannabinoids also exert a profibrotic effect that is possibly mediated by CB1 receptors. This is compatible with the finding of increased CB1 expression in stellate cells and hepatic myofibroblasts in the cirrhotic human liver and in the livers of mice with three different models of fibrosis. Genetic or pharmacological ablation of CB1 receptors protected mice against liver injury; this was reflected by the reduced expression of smooth muscle z-actin and transforming growth factor β. 2-AG is the likely fibrogenic mediator because its hepatic level is preferentially increased by the CCl4 treatment of mice and rats. Higher doses of 2-AG induce apoptosis in activated hepatic stellate cells (HSCs) in vitro through a receptor-independent, membrane cholesterol–dependent mechanism; this means that 2-AG also has antifibrotic activity. AEA showed a similar effect, although the eventual cell death was by necrosis rather than apoptosis. For both endocannabinoids, these effects occur in the 2 to 50 μM range. The hepatic concentration of AEA is orders of magnitude below such levels, whereas 2-AG may reach low micromolar concentrations. Because the proapoptotic effect of 2-AG is independent of CB receptors, it could contribute to the reduction of fibrotic activity observed after CB1 blockade. The profibrotic and adverse hemodynamic effects of CB1 activation could provide a rationale for the use of CB1 antagonists in the medical management of advanced liver cirrhosis.
Endocannabinoids and Metabolic Syndrome

The CB₁-mediated, appetite-promoting effect of endocannabinoids was the primary impetus for the development of brain-penetrating CB₁ receptor antagonists for the treatment of obesity. The first-in-class compound rimonabant caused weight reduction and improved the associated cardiometabolic risk factors, but neuropsychiatric side effects, including depression and anxiety, have prevented its approval in the United States and have led to its withdrawal from the market in other countries (reviewed by Rosenson).

Accumulating evidence indicates, however, that the metabolic effects of endocannabinoids are mediated, at least in part, by peripheral CB₁ receptors, as discussed in some detail later. Indeed, a non–brain-penetrating CB₁ antagonist was recently reported to retain the beneficial metabolic effects of rimonabant in obese mice without producing the behavioral effects that predict neuropsychiatric side effects in humans; this may revive interest in the therapeutic potential of CB₁ antagonism.

Reduced food intake is not the primary mechanism of weight reduction by CB₁ blockade in obesity. In mice with diet-induced obesity (DIO), chronic use of rimonabant caused a transient reduction in food intake and sustained weight loss, and this indicated food intake–independent effects on energy balance. Increased de novo hepatic lipogenesis has been documented in DIO mice and in people with NAFLD, and may be mediated by endocannabinoids. Indeed, lipogenic gene expression and the rate of de novo hepatic lipogenesis were increased by CB₁ agonists and decreased by CB₁ antagonists in rodents. A high-fat diet increases hepatic CB₁ expression and the hepatic levels of AEA. Thus, endogenous AEA acting via hepatic CB₁ receptors contributes to increased de novo lipogenesis in mouse models of obesity. CB₁-mediated hepatic lipogenesis may explain the finding that in patients with chronic hepatitis C infection, daily cannabis smoking was an independent risk factor for steatosis severity but not for obesity.

Although lipogenesis via hepatic CB₁ receptors may contribute to lipid accumulation, extrahepatic CB₁ must play a major role because liver-specific CB₁ mice are only partially protected from steatosis, whereas CB₁ mice with selective transgenic re-expression of CB₁ in hepatocytes, similar to their global CB₁ knockout littersmates, remain largely resistant to the steatotic effect of a high-fat diet. The source of liver fat may be adipose tissue because the activation of CB₁ receptors in adipocytes promotes lipogenesis, and the released fatty acids may be taken up and converted to triglycerides (TGs) by the liver.

On the other hand, the rapid depletion of excess hepatic TGs after CB₁ blockade may involve hepatic CB₁ receptors, as indicated by the increased rate of secretion of TG-rich very low density lipoprotein (VLDL) from the livers of both DIO and ob/ob mice after treatment with a peripherally restricted CB₁ antagonist (see Fig. 2).

Endocannabinoids are also involved in the diet-induced decrease in fatty acid oxidation. The activity of hepatic carnitine palmitoyltransferase 1 (CPT1), the rate-limiting enzyme in mitochondrial fatty acid β-oxidation, is suppressed by either a high-fat diet or treatment with a CB₁ agonist, and both effects are prevented by rimonabant. Conversely, hepatic CPT1 activity is increased in CB₁ mice and in DIO mice after chronic CB₁ blockade.

Adiponectin is a key stimulator of fatty acid β-oxidation, and CB₁ blockade increases plasma adiponectin. The improved insulin sensitivity following CB₁ blockade has been found to have both adiponectin-dependent and adiponectin-independent components, although the role of adiponectin in the effects of CB₁ blockade on hepatic mitochondrial function and fatty acid oxidation has not been explored. Increased energy expenditure due to increased fat oxidation after CB₁ blockade has been documented with indirect calorimetry in rats and mice. These effects likely contribute to the food intake–independent sustained weight loss as well as the reversal of hepatic steatosis after chronic CB₁ blockade.
The DIO-related hypertriglyceridemia was modestly attenuated, whereas the accompanying increase in plasma LDL cholesterol and decrease in high-density lipoprotein cholesterol were absent in both CB1−/− and LCB1−/− mice on a high-fat diet. This suggests that hepatic CB1 mediates diet-induced changes in hepatic lipoprotein metabolism and/or secretion. In a recent study, the treatment of mice with an inhibitor of monoglyceride lipase resulted in elevated hepatic levels of 2-AG, increased hepatic expression of sterol regulatory element binding protein 1c (SREBP1c) and FAS, hypertriglyceridemia, and an accumulation in plasma of apolipoprotein E (ApoE)–depleted, TG-rich apolipoproteins. These changes were absent in CB1−/− and ApoE−/− mice and could be prevented by CB1 blockade. Furthermore, despite the elevated hepatic lipogenic gene expression, TG secretion rates were unchanged, but TG clearance from plasma was inhibited. In contrast, in DIO mice with long-term exposure of male mice to a low-fat, liquid ethanol diet for 4 weeks increased hepatic CB1 expression and 2-AG levels but not AEA levels. 2-AG was increased in HSCs,23 and CB1/CB2 agonists on glucose homeostasis. The well-documented insulin sensitization by chronic CB1 blockade may be due to a reversal of the action of AEA, which has low CB2 efficacy. This is also consistent with findings that high-fat diet–induced glucose intolerance and insulin resistance are associated with increases in hepatic AEA levels but not 2-AG levels.

In a recent study, CB2 expression was strongly induced by both steatosis and nonalcoholic steatohepatitis, and this suggests CB2 involvement in hepatic fat metabolism. Indeed, a modest increase in CB2 expression was reported in hepatocytes from both ob/ob and DIO mice. On the other hand, CB2−/− mice were resistant to diet-induced steatohepatitis and were less insulin-resistant than wild-type littermates on the same diet. Furthermore, JWH-133 increased the hepatic accumulation of TGs in DIO mice. The CB2−/− mice remained glucose-tolerant and insulin-sensitive,24 as discussed previously.

High-fat diets also result in elevated plasma insulin and leptin levels accompanied by hyperglycemia, which indicates insulin resistance. Interestingly, both CB1−/− and LCB1−/− mice remained glucose-tolerant and insulin-sensitive and did not display the hyperleptinemia associated with high-fat diets. Moreover, the insulin and leptin resistance of DIO mice was normalized by the peripheral CB1 antagonist AM6545. There is also evidence that THC induces glucose intolerance in humans and rodents via activation of CB1 receptors. Thus, endocannabinoids and hepatic CB1 play an important role in diet-induced insulin and leptin resistance.

Diet-induced insulin resistance involves adipose tissue, skeletal muscle, and the liver as well as interactions between the three tissues through neurogenic and/or humoral factors. In mice, a high-fat diet induces CB1 expression in skeletal muscle, and CB1 blockade increases insulin-induced glucose uptake and phosphorylation in the skeletal muscle of genetically obese mice. The possibility that the activation of hepatic CB1 may influence the insulin sensitivity of extrahepatic tissues via the release of soluble mediators remains to be explored.

CB2 receptors may also be involved in diet-induced hormonal and metabolic changes. In rats, the selective CB2 agonist JWH-133 improved glucose tolerance, whereas the CB2 antagonist AM630 had the opposite effect and also prevented the effect of JWH-133. These effects are the opposite of the glucose intolerance induced by CB1 receptor activation (discussed previously) and could minimize the effects of mixed CB1/CB2 agonists on glucose homeostasis. The well-documented insulin sensitization by chronic CB1 blockade may be due to a reversal of the action of AEA, which has low CB2 efficacy. This is also consistent with findings that high-fat diet–induced glucose intolerance and insulin resistance are associated with increases in hepatic AEA levels but not 2-AG levels.

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### Alcoholic Fatty Liver

Chronic alcoholism may lead to steatosis that can further progress to steatohepatitis, liver cirrhosis, and HCC. Ethanol enhances hepatic lipogenesis and decreases fatty acid oxidation. The similar mechanisms of diet-induced and alcohol-induced steatosis, together with ethanol’s ability to increase endocannabinoid levels, at least in the brain, suggest ECS involvement in the alcoholic fatty liver. Indeed, the exposure of male mice to a low-fat, liquid ethanol diet for 4 weeks increased hepatic CB1 expression and 2-AG levels but not AEA levels. 2-AG was increased in HSCs but not in hepatocytes. The expression of diacylglycerol lipase β was also increased in HSCs, and this suggests increased biosynthesis of 2-AG. Rimonabant treatment attenuated ethanol-induced steatosis without affecting alcohol intake and blood ethanol levels, and this suggests CB1 involvement. This was further supported by the resistance of both CB1−/− and LCB1−/− mice to ethanol-induced steatosis.

The hepatic nuclear expression of SREBP1c and its target FAS was increased, whereas CPT1 expression and activity decreased in ethanol-fed mice, in agreement with earlier findings. In both CB1−/− and LCB1−/− mice, the effects of ethanol on SREBP1c, FAS, and CPT1 were blunted or absent. Furthermore, CPT1 activity was increased and resistant to suppression by ethanol in both CB1 knockout strains. This supports the notion that in alcoholic fatty liver disease...
CB1 receptors. This paracrine interaction, together with ethanol increases 2-AG exclusively in HSCs. This suggests a paracrine mechanism by which HSC-derived 2-AG activates CB1 receptors on adjacent HSCs. This suggests a paracrine mechanism by which steatosis, whereas ethanol increases 2-AG exclusively in the treatment of both AFLD and NAFLD.

Endocannabinoids and I/R Injury

I/R injury may develop in conditions in which the blood and oxygen supply to a tissue is transiently disrupted and then restored. Hepatic I/R injury is a potentially fatal complication of liver surgery (including liver transplantation). Substances that improve hypoxia tolerance may also protect against I/R injury. CBs induce hypomotility and hypothermia, both of which result in reduced oxygen demand. The metabolic effects of CBs that promote energy storage and reduce energy expenditure (discussed previously) may also reduce oxygen demand. There is evidence that endocannabinoids acting via CB1 protect against hepatic I/R injury.

In mice, segmental ischemia followed by reperfusion (but not ischemia alone) markedly increased the hepatic levels of AEA and 2-AG, which correlated with the severity of tissue damage. I/R-induced tissue damage, including neutrophil infiltration and lipid peroxidation, was attenuated by pretreatment with JWH-133 in wild-type mice but not in CB2−/− mice, in which the damage was more severe than that in wild-type littersmates. Another potent and selective CB2 agonist, HU-308, caused similar effects and also attenuated I/R-induced hepatocyte apoptosis and mitigated the tumor necrosis factor-α-induced expression of cell adhesion molecules (intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1) in hepatic sinusoidal endothelial cells. Thus, CB2 agonists may afford protection at multiple levels against I/R injury; this highlights their therapeutic potential.

CB1 receptor blockade also protects the liver from I/R injury and superimposed endotoxaemia. In one study, rats subjected to lipopolysaccharide plus I/R had an immediate increase in CB1 expression in perisinusoidal hepatocytes. Rimonabant treatment reduced both tissue necrosis and serum alanine aminotransferase levels in the late phase of reperfusion and attenuated the oxidative injury. Further studies with peripherally restricted CB1 receptor antagonists could reinforce the therapeutic potential of this approach.

Hepatic Encephalopathy and Autoimmune Hepatitis

Hepatic encephalopathy is a neuropsychiatric syndrome that may accompany acute liver failure. The underlying mechanisms are not completely understood, although there is evidence for the pathogenic role of ammonia, alterations in various central neurotransmitter systems, and altered cerebrovascular function. Mice with thioacetamide-induced fulminant liver failure have elevated brain 2-AG levels. The treatment of such mice with 2-AG or the CB2 agonist HU-308 improved the neurological score and cognitive function, and these effects were blocked by a CB2 antagonist. The beneficial effects of CB2 agonists could be mimicked by treatment with the CB1 antagonist rimonabant. In another study by the same group, thioacetamide treatment or bile duct ligation induced CB2 expression in the brain and also resulted in the activation of adenosine monophosphate-activated protein kinase. The absence of both effects in CB2−/− mice indicated the role of CB2 receptors, although there is also evidence for the additional involvement of transient receptor potential cation channel V1 receptors.

Cannabidiol (CBD) is a nonpsychoactive constituent of marijuana with no significant CB1 or CB2.
activity. CBD was found to improve cognitive and motor function as well as the neuroinflammation found in hepatic encephalopathy. The cerebral inflammatory response of mice to bile duct ligation was reduced by CBD treatment, and the effect was attributed to indirect activation of hippocampal A2A adenosine receptors. It is possible that a combined treatment with a CB2 agonist and CBD would offer additive therapeutic benefits to patients with hepatic encephalopathy.

In a murine model of concanavalin A–induced autoimmune hepatitis, THC was found to attenuate the hepatitis on the basis of decreased plasma levels of liver enzymes and inflammatory cytokines and reduced tissue injury. Interestingly, FAAH−/− mice responded with reduced hepatic damage to concanavalin A treatment, and this suggests hepatoprotection by endogenous AEA. In contrast, the results of another study suggest that hepatoprotection may be achieved by blocking CB1 receptors.

Concluding Remarks

The ECS is present in the liver and is involved in the control of various hepatic functions with important therapeutic implications. Increased CB1 activity contributes to the hemodynamic abnormalities and promotes fibrosis in liver cirrhosis, whereas CB1 blockade attenuates and delays these changes. Endocannabinoids acting via hepatic CB1 receptors have emerged as mediators of both diet-induced fatty liver and alcoholic fatty liver, which together account for the majority of cirrhosis cases in Western societies. Additionally, hepatic CB1 activation contributes to obesity-related insulin and leptin resistance and dyslipidemias. This provides a strong rationale for the therapeutic use of CB1 antagonists in patients with these conditions. Although neuropsychiatric side effects limit the therapeutic potential of brain-penetrating CB1 antagonists, the recent emergence of second-generation, peripherally restricted CB1 antagonists may mitigate this problem. Additionally, nonpsychostimulatory CB2 agonists may offer therapeutic benefits by attenuating liver injury and promoting tissue repair in the fibrotic liver.

References

33. Teixeira-Clerc F, Belot MP, Manin S, Deveaux V, Cadoudal T, Cho...
32. Buckley NE, Hansson S, Harta G, Mezey E. Expression of the CB1...
31. Mendez-Sanchez N, Zamora-Valdes D, Pichardo-Bahena R, Barredo-Prieto...
30. Moezi L, Gaskari SA, Liu H, Baik SK, Dehpour AR, Lee SS. Ananda-
28. Biecker E, Sagesser H, Reichen J. Vasodilator mRNA levels are...


