Review Article

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Role of Cannabinoids in the Development of Fatty Liver (Steatosis)

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Abstract. Emerging evidence suggests that cannabinoids play an important role in the modulation of fatty liver, which appears to be mediated via activation of cannabinoid receptors. Steatogenic agents such as ethanol and high-fat diet can upregulate the activity of cannabinoid 1 (CB1) receptors via increasing synthesis of endocannabinoids, 2-arachidonoylglycerol, and anandamide. CB1 receptors can also be upregulated by obesity. CB1 receptor activation results in upregulation of lipogenic transcription factor, sterol regulatory element-binding protein 1c and its target enzymes, acetyl-CoA carboxylase-1, and fatty acid synthase and concomitantly, downregulation of carnitine palmitoyltransferase-1. This leads to increased *de novo* fatty acid synthesis as well as decreased fatty acid oxidation, culminating into the development of fatty liver. High-fat diet, in addition to CB1 receptor activation, appears to activate CB2 receptors that may also contribute to fatty liver. In non-alcoholic fatty liver disease, CB2 receptor activation is associated with the development of fatty liver. Cannabis smoking can increase the severity of fatty liver in hepatitis C patients although the precise mechanism is unknown. As the mechanisms involved in endocannabinoid receptor signaling are being increasingly well understood and the biosynthetic regulatory elements elucidated, these present good opportunity for the pharmaceutical scientists to design drugs to treat liver diseases, including steatosis, based on the cannabinoids, endocannabinoids, and related templates.

KEY WORDS: cannabinoids; CB1 receptor; CB2 receptor; fatty liver.

INTRODUCTION

Fatty liver (steatosis) is characterized by an excess accumulation of triglycerides within hepatocytes, which are parenchymal cells of the liver. It is an initial stage of many liver diseases including alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), and hepatitis C. Excess hepatic fat accumulation could result from the following mechanisms: (a) increased *de novo* fatty acid synthesis; (b) decreased fatty acid oxidation; (c) increased transport of fatty acids from the peripheral organs to the liver; and (d) blunted transport of fatty acids (triglycerides) from the liver to the general circulation and peripheral organs. Steatosis is reversible, and it has been considered as a benign condition for a long time; however, increasing evidence suggests that it is a potentially pathologic condition. If the causal agent is not eliminated or injury persists, steatosis may progress to inflammation,

fibrosis, and even cirrhosis of the liver, especially in the presence of hepatitis C virus, diabetes, and obesity. Considering potential clinical relevance, researchers were prompted to discern the underlying mechanisms of steatosis. Some of the proposed mechanisms of fatty liver are activation of lipogenic transcription factor sterol regulatory element-binding protein 1c (SREBP1c) (1), impaired function of lipolytic transcription factor peroxisome proliferator-activated receptor- α (2), inhibited AMP-activated protein kinase activity (3), decreased adiponectin levels (4), sirtuin-1 inhibition (5,6), insulin resistance, interleukin-6 deficiency, increased complement C3 levels, and other factors (7,8).

Emerging evidence suggests that cannabinoids play an important role in the modulation of fatty liver. The endocannabinoid system is primarily comprised of three components: endocannabinoids, endocannabinoid receptors, and endocannabinoid-metabolizing enzymes. Endocannabinoids (endogenous cannabinoids) are lipid mediators that interact with cannabinoid receptors to produce effects similar to those of delta 9-tetrahydrocannabinol (THC), which is the main psychoactive component of cannabis. The two main endocannabinoids discovered are arachidonoyl ethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), and two main cannabinoid receptors identified to date are cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). CB1 receptors are expressed at high levels in the brain, but they are also present in peripheral tissues, though at much

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lower concentrations in most of the peripheral tissues (9,10). On the other hand, CB2 receptors are expressed predominantly in immune and hematopoietic cells (9). Cannabinoid receptors have also been identified in the liver of rodents (11–13) and humans (13–15). In addition, liver has been shown to produce endocannabinoids—anandamide (11) and 2-AG (12).

Animal studies have shown a link between cannabinoid system and the development of fatty liver associated with various agents such as ethanol (12), obesity (16), and high-fat diet (11,17). Similarly, recent human studies have also revealed an association between cannabinoids and fatty liver present in patients with hepatitis C (18) and NAFLD (15). This review summarizes the role of the cannabinoid system in the development of fatty liver.

ROLE OF CB1 RECEPTORS IN THE DEVELOPMENT OF FATTY LIVER

The role of CB1 receptors has been investigated in the development of fatty liver induced by ethanol, high-fat diet, and obesity.

a. Role of CB1 receptors in the development of alcoholic fatty liver:

Chronic ethanol consumption is known to induce fatty liver, which is an initial stage of alcoholic liver disease characterized by fatty liver, steatohepatitis, fibrosis, and cirrhosis. Jeong *et al.* (12) investigated the role of CB1 receptors in the development of ethanol-induced fatty liver using a mouse model of alcoholic liver injury. Ethanol exposure of male mice in a low-fat diet induced fatty liver as assessed by histological evaluation and hepatic triglyceride levels. In this study, ethanol was administered in Lieber–DeCarli low-fat liquid diet comprised of 27% ethanol calories and 12% fat calories. In contrast to ethanol-exposed mice, pair-fed control mice did not develop fatty liver. Ethanol-induced fatty liver was associated with increased hepatic expression of the gene encoding for CB1 receptors. In these mice, ethanol increased hepatic levels of 2-AG (but not anandamide), which was selectively produced by hepatic stellate cells. Treatment of mice with rimonabant (SR141716), a CB1 receptor antagonist, prevented the development of fatty liver by ethanol. Consistent with rimonabant findings, mice with global or hepatocyte-specific CB1 receptor knockout were resistant to ethanol-induced steatosis. These findings suggest that ethanol induces fatty liver via hepatocyte CB1 receptor activation.

In order to understand the underlying mechanisms of CB1 receptor-mediated induction of fatty liver, these researchers measured the expression/level of a lipogenic transcription factor—SREBP-1c, fatty acid synthase (FAS), and carnitine palmitoyltransferase (CPT-1), which is the rate-limiting enzyme for fatty acid oxidation. Ethanol exposure increased the nuclear level of SREBP-1c in the liver of wild-type mice but not in the liver of global or hepatocyte-specific CB1 receptor knockout mice. Consistent with this, ethanol increased FAS protein levels in the liver of wild-type mice, but this effect was absent or blunted in both global and hepatocyte-specific CB1 receptor knockout mice. On the other hand, ethanol exposure decreased both hepatic CPT-1 protein level and CPT-1 activity in wild-type mice, but these

decreases were not seen in hepatocyte-specific CB1 receptor knockout mice. In global CB1 receptor knockout mice, although ethanol did not decrease CPT-1 activity, it did decrease CPT-1 protein levels compared to pair-fed control. Coculture of hepatic stellate cells from ethanol-fed mice with hepatocytes from pair-fed mice upregulated gene expression of CB1 receptor, SREBP-1c, and FAS in hepatocytes. In contrast, in hepatocytes from hepatocyte-specific CB1 receptor knockout mice cocultured with hepatic stellate cells from ethanol-fed mice, CB1 mRNA was absent, and the induction of SREBP-1c and FAS expression was blunted. Taken together, these results suggest that ethanol-induced increase in 2-AG from stellate cells activates hepatocyte CB1 receptors in a paracrine manner, which leads to steatosis via increasing fatty acid synthesis and decreasing fatty acid oxidation.

b. Role of CB1 receptors in the development of high-fat diet-induced fatty liver:

Consumption of a high-fat diet is associated with obesity and development of hepatic steatosis. Osei-Hyiaman *et al.* (11) used this approach to investigate the role of CB1 receptors in the development of fatty liver. Feeding of high-fat diet (60% of total calories) to wild-type mice for 14 weeks made these animals obese, and they developed fatty liver as diagnosed by histological examination and triglyceride accumulation. In contrast, global CB1 receptor knockout mice on high-fat diet remained lean, and they did not develop fatty liver despite consuming caloric intake similar to that of wild-type mice on the high-fat diet. In addition, high-fat diet upregulated hepatic expression of CB1 receptors in wild-type mice and increased hepatic levels of anandamide, but not 2-AG, in both wild-type and CB1 receptor knockout mice. Furthermore, in wild-type mice, high-fat diet increased basal rates of fatty acid synthesis, which was blocked by the CB1 antagonist rimonabant. Taken together, these results suggest that high-fat diet-induced steatosis resulting from increased fatty acid synthesis is mediated via anandamide-induced CB1 receptor activation. Mechanistic studies by these investigators further revealed that activation of CB1 receptors by CB1 agonist (HU-210) in wild-type mice, maintained on regular chow diet, increased hepatic gene expression of the lipogenic transcription factor SREBP-1c and its target enzymes, acetyl-CoA carboxylase-1 (ACC-1) and FAS. These effects were blocked or prevented by CB1 antagonist. Consistently, treatment with CB1 agonist also increased *de novo* fatty acid synthesis in the liver or in isolated CB1 receptor-expressing hepatocytes, and this effect was also blocked or prevented by CB1 antagonist, further corroborating the role of CB1 receptor activation in fatty acid synthesis.

In order to discern the specific role of liver CB1 receptors in the development of steatosis, researchers have used liver-specific CB1 knockout mice along with global knockout mice (17). In this study, global CB1 receptor knockout mice were totally resistant to high-fat-induced fatty liver and obesity. In liver-specific CB1 knockout mice, high-fat diet induced obesity and increased hepatic triglyceride levels to some extent, but this increase was significantly less (about 75% over pair-fed control) than that observed in wild-type mice (about 200% over pair-fed control) maintained on high-fat diet. In this study, high-fat diet was also shown to

increase CB1 expression in hepatocytes in wild-type mice (17). Furthermore, CB1 receptor agonist (HU-210) increased hepatic *de novo* lipogenesis in wild-type chow-fed mice, but not in global CB1 knockout or liver-specific CB1 knockout mice. These findings support the contention that high-fat diet induces fatty liver primarily via activation of hepatic CB1 receptors.

In addition to hepatic lipogenesis, CB1 receptor activation also appears to regulate hepatic fatty acid oxidation by modulating the activity of hepatic CPT-1, the rate-limiting enzyme in fatty acid β -oxidation. High-fat diet, compared to chow-fed control, significantly reduced the activity of hepatic CPT-1 in wild-type mice, but not in global or liver-specific CB1 receptor knockout mice. In addition, rimonabant treatment of high-fat diet-fed mice increased protein levels of CPT1A, the hepatic isoform of CPT1. Furthermore, in wild-type chow-fed mice, CB1 receptor agonist (HU210) significantly decreased hepatic CPT-1 activity, whereas rimonabant significantly increased CPT-1 activity and prevented the inhibitory effect of subsequently administered HU-210. Taken together, these results suggest that CB1 activation decreases fatty acid oxidation via decreasing CPT-1 activity, which may contribute, in part, to high-fat diet-induced steatosis.

c. Role of CB1 receptors in obesity-associated fatty liver:

Obesity is a chronic metabolic disorder characterized by increased body weight and development of adipose tissue with excessive fat storage, and it is invariably associated with fatty liver. The Zucker fatty rats (fa/fa) are genetically obese with defective leptin receptors. These rats have elevated plasma leptin levels and are resistant to exogenous leptin administration. In addition, they have severe hepatic steatosis characterized by accumulation of fat within hepatocytes. Gary-Bobo *et al.* (16) investigated the role of CB1 receptors in the development of fatty liver in Zucker rats by administering rimonabant (SR141716), a CB1 receptor antagonist. In this study, oral treatment of obese (fa/fa) rats with rimonabant (30 mg/kg) daily for 8 weeks abolished hepatic steatosis and attenuated hepatomegaly. Liver slices from the obese (fa/fa) rats treated with rimonabant were found to be histologically comparable to those from lean rats. In contrast, in paired rats, which consumed the same amount of food as that consumed by rimonabant-treated rats, steatosis and hepatomegaly were not significantly reduced. This suggests that rimonabant, and not reduced food intake, was responsible for reducing steatosis. Taken together, these results indicate that in the obese rats, development of fatty liver is mediated via activation of CB1 receptors.

ROLE OF CB2 RECEPTORS IN THE DEVELOPMENT OF FATTY LIVER

Compared with CB1 receptors, the role of CB2 receptors in the development of fatty liver is underinvestigated. CB2 receptors are predominantly expressed in immune cells and hematopoietic cells (9), and their role in the development of fatty liver is not clear. The expression of CB2 receptors has been investigated in the liver of patients with NAFLD, which is characterized by fatty liver and steatohepatitis (15). In this study, CB2 receptors were upregulated in all of the liver samples from patients with steatosis and steatohepatitis.

Specifically, receptors were localized in hepatocytes, cholangiocytes, and hepatic stellate cells. In contrast, liver samples obtained from normal subjects did not express CB2 receptors. Expression of CB2 receptors in fatty liver, but not in normal liver, is an indication of CB2 activation in NAFLD, which is associated with obesity, insulin resistance, type 2 diabetes, and hypertriglyceridemia. In another CB2 receptor study, the role of CB2 receptors was investigated in the development of high-fat diet-induced fatty liver in mice (19). In this study, high-fat diet feeding for 15 weeks induced severe fatty liver in wild-type mice as shown by histological changes and triglyceride levels. In contrast, hepatic CB2 knockout mice exhibited minimal hepatic steatosis. Consistent with these results, administration of CB2 agonist (JWH-133) enhanced liver triglyceride accumulation in wild-type mice fed with a high-fat diet for 6 weeks. Taken together, these findings indicate a mediating role for CB2 receptor activation in high-fat diet-induced fatty liver.

HEPATITIS C, CANNABINOIDS, AND FATTY LIVER

Steatosis is a common histologic finding in patients with chronic hepatitis C (20,21). Hezode *et al.* (18) investigated the impact of cannabis smoking on the severity of steatosis in chronic hepatitis C patients. Steatosis was defined according to the percentage of hepatocytes containing fat vacuoles as absent (<5%), mild (5–10%), moderate (11–29%), and marked (equal to or >30%). In this study, marked steatosis was significantly more frequent in daily cannabis smokers compared with occasional users and nonusers. Using multivariate logistic regression analysis, it was shown that daily cannabis smoking is an independent predictor of steatosis severity, suggesting a steatogenic role of cannabinoid system.

DISCUSSION

Steatogenic agents such as ethanol and high-fat diet appear to be capable of inducing endocannabinoid production in the liver. For example, ethanol exposure *in vivo* increased production of 2-AG by cultured mouse HSC (12), whereas high-fat diet increased anandamide production in the mouse liver (11). Such elevated endocannabinoid levels have been reported in other organs as well. Ethanol has been shown to increase the levels of 2-AG in rat cerebellar granule neurons (22) and in rat nucleus accumbens (23). Similarly, high-fat diet has been shown to increase the levels of either anandamide, or 2-AG, or both in the skeletal muscle, heart, and kidney of mice (24). Increased levels of hepatic endocannabinoids by ethanol or high-fat diet are likely to activate cannabinoid receptors which may lead to the development of fatty liver. In fact, increased expression of CB1 receptors was observed in the liver of high-fat diet (11) or ethanol-treated (12) mice. CB1 receptor activation appears to play an important role in the development of fatty liver, and this is based on the following information:

1. CB1 receptor knockout mice were resistant to ethanol (12) or high-fat diet-induced (11,17) fatty liver.
2. Rimonabant (CB1 receptor antagonist) prevents the development of ethanol-induced fatty liver in mice (12); blocks hepatic fatty acid synthesis induced by

high-fat diet in mice (11); blocks or prevents high-fat diet-induced increases in hepatic expression of SREBP1c, ACC, and FAS in mice (17); and abolishes fatty liver in Zucker fatty rats (fa/fa) (16). Furthermore, rimonabant significantly increased hepatic CPT-1 activity in wild-type chow-fed mice, and it prevented the inhibitory effect of CB1 receptor agonist (HU-210) on CPT-1 activity (17).

In the study by Osei-Hyiaman *et al.* (17), while global CB1 knockout mice were totally resistant to obesity and fatty liver in response to high-fat diet exposure, liver-specific CB1 knockout mice became obese and accumulated some triglycerides in the liver, though much less than that observed in wild-type high-fat diet-exposed mice. Triglycerides accumulated in the liver of liver-specific CB1 knockout mice could be due to import of fat from peripheral adipose tissues and other organs. Alternatively, some triglycerides may have accumulated in the liver of CB1 knockout mice due to activation of hepatic CB2 receptors by high-fat diet as reflected in a study by Deveaux *et al.* (19). In their study, high-fat diet-induced fatty liver was significantly less (in comparison to wild-type mice) in hepatic CB2 receptor knockout mice, suggesting that CB2 receptor activation may also contribute to steatosis. Since in this study (19) hepatic fat was present (though minimal) in the CB2 knockout mice, it is speculated that this minimal fat could have accumulated due to high-fat diet-induced activation of hepatic CB1 receptors or import of fat from the peripheral adipose tissue. Taken together the studies on CB1 and CB2 receptor knockout mice, it appears that both receptors may be contributing to the development of fatty liver, although evidence for the role of CB2 receptors is minimal.

Upon activation of CB1 receptors, steatosis appears to result from increased *de novo* synthesis of fatty acids as well as decreased fatty acid oxidation. This is substantiated by the fact that increased activation or expression of CB1 was associated with increased nuclear level or gene expression of SREBP1c and increased gene expression or protein levels of ACC and FAS (11,12). On the other hand, CB1 activation was associated with decreased hepatic protein levels and activity of CPT-1 (12,17).

Regarding CB2 receptors, further studies are required to ascertain their role in the genesis of fatty liver. The limited information, available so far, suggests that these receptors are upregulated in the liver of patients affected with NAFLD (15), and that they appear to contribute to the development of high-fat diet-induced fatty liver (19). How CB2 receptor activation leads to or promotes steatosis needs further investigation. In the study reported by Deveaux *et al.* (19), in addition to enhancing liver triglyceride accumulation, treatment with CB2 agonist JWH-133 also potentiated adipose tissue inflammation in high-fat diet-fed wild-type mice, which prompted the authors to speculate that CB2 receptors may enhance fatty liver at least partly by increasing adipose tissue inflammation; however, this hypothesis needs to be verified. Whether lipogenic transcription factors or enzymes play any role in mediating steatogenic effect of CB2 receptors remains to be investigated.

How does cannabis smoking promote steatosis in hepatitis C patients is also a subject of investigation. Delta 9-

THC, a major active component of marijuana smoke, is known to bind and subsequently activate both CB1 and CB2 receptors. On the basis of information discussed in this report, CB1 receptors activation may be playing a significant role in the genesis of hepatitis C-associated fatty liver, although role of CB2 receptors and receptor-independent effect of cannabinoids cannot be ruled out.

SUMMARY

A summary of the role of cannabinoids in the development of fatty liver is presented in Fig. 1. Steatogenic agents such as ethanol and high-fat diet can upregulate the activity of CB1 receptors via increasing synthesis of endocannabinoids, 2-AG, and anandamide. CB1 receptors can also be upregulated by obesity. CB1 receptor activation results in upregulation of lipogenic transcription factor SREBP1c and its target enzymes ACC and FAS and concomitantly downregulation of CPT-1. This leads to increased *de novo* fatty acid synthesis as well as decreased fatty acid oxidation, culminating into the development of fatty liver. High-fat diet, in addition to CB1 receptor activation, appears to activate CB2 receptors, which may contribute to fatty liver. In NAFLD, CB2 receptor activation is associated with the development of fatty liver. Cannabis smoking can increase the severity of fatty liver in hepatitis C patients.

As the mechanisms involved in endocannabinoid receptor signaling are being increasingly well understood and the biosynthetic regulatory elements elucidated, these present

Fig. 1. Steatogenic agents such as ethanol and high-fat diet can upregulate the activity of cannabinoid 1 (CB1) receptors via increasing synthesis of endocannabinoids, 2-arachidonoylglycerol (2-AG), and anandamide. CB1 receptors can also be upregulated by obesity. CB1 receptor activation results in upregulation of lipogenic transcription factor sterol regulatory element-binding protein 1c (SREBP1c) and its target enzymes acetyl-CoA carboxylase-1 (ACC) and fatty acid synthase (FAS) and concomitantly downregulation of carnitine palmitoyltransferase-1 (CPT-1). This leads to increased *de novo* fatty acid synthesis as well as decreased fatty acid oxidation, culminating into the development of fatty liver. High-fat diet, in addition to CB1 receptor activation, appears to activate CB2 receptors, which may contribute to fatty liver. In non-alcoholic fatty liver disease (NAFLD), CB2 receptor activation is associated with the development of fatty liver. Cannabis smoking can increase the severity of fatty liver in hepatitis C (HepC) patients

good opportunity for the pharmaceutical scientists to design drugs to treat liver diseases, including steatosis, based on the cannabinoids, endocannabinoids, and related templates.

CONCLUSIONS

Upregulation of CB1 receptors plays an important role in the development of fatty liver associated with ethanol intake, high-fat diet, and obesity. CB2 receptor upregulation also appears to contribute to fatty liver; however, this inference needs further confirmation.

FUTURE DIRECTIONS

- Investigate mechanisms by which steatogenic agents such as ethanol and high-fat diet stimulate endocannabinoid production in the liver. In addition, identify the cellular source of endocannabinoids in the liver
- Confirm the role of CB2 receptors and investigate the role of orphan receptors (e.g., GPR 55) in the development of fatty liver.
- Study nonreceptor-mediated effects of cannabinoids on the development of fatty liver.
- Discern the role of cannabinoid system in mediating the development of fatty liver in hepatitis C, NAFLD, and obesity.
- Understand the underlying common molecular mechanisms whereby activation (upregulation) of cannabinoid system mediates the development of fatty liver induced by ethanol intake, high-fat diet, or obesity. This may help identify targets of intervention for the treatment/prevention of fatty liver, which in turn may prevent the development of or progression to severe liver conditions such as hepatitis, fibrosis, and cirrhosis.

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