The endocannabinoid system as a key mediator during liver diseases: new insights and therapeutic openings

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Chronic liver diseases represent a major health problem due to cirrhosis and its complications. During the last decade, endocannabinoids and their receptors have emerged as major regulators of several pathophysiological aspects associated with chronic liver disease progression. Hence, hepatic cannabinoid receptor 2 (CB2) receptors display beneficial effects on alcoholic fatty liver, hepatic inflammation, liver injury, regeneration and fibrosis. Cannabinoid receptor 1 (CB1) receptors have been implicated in the pathogenesis of several lesions such as alcoholic and metabolic steatosis, liver fibrogenesis, or circulatory failure associated with cirrhosis. Although the development of CB1 antagonists has recently been suspended due to the high incidence of central side effects, preliminary preclinical data obtained with peripherally restricted CB1 antagonists give real hopes in the development of active CB1 molecules devoid of central adverse effects. CB2-selective molecules may also offer novel perspectives for the treatment of liver diseases, and their clinical development is clearly awaited. Whether combined treatment with a peripherally restricted CB1 antagonist and a CB2 agonist might result in an increased therapeutic potential will warrant further investigation.

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Abbreviations
CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis

Alcohol abuse, viral hepatitis and non-alcoholic fatty liver disease (NAFLD) represent the major causes of chronic liver injury, resulting in progressive accumulation of fibrosis within the liver parenchyma. Progression to cirrhosis exposes patients to life-threatening complications of portal hypertension liver failure and hepatic encephalopathy, and to a high risk of developing hepatocellular carcinoma. Overall, chronic liver diseases represent a major health problem with an estimated rate of death in the range of 1 400 000 per year worldwide (Lotersztajn et al., 2005). Recent findings have revealed a role of endocannabinoids and their receptors in the pathogenesis of several key steps of acute and chronic liver injury, therefore identifying pharmacological modulation of cannabinoid receptors as an attractive strategy for the...
manipulation of morbidity related to liver injury (Mallat et al., 2007; Mallat and Lotersztajn, 2008a,b).

The endocannabinoid system

Cannabis Sativa has a long-standing history of recreational and therapeutic use, starting over 200 years ago. Understanding of pathways involved in the pharmacological properties of cannabinoids has only emerged with the identification of an endocannabinoid system that comprises at least two specific G-protein coupled receptors [cannabinoid receptor 1 (CB₁) and cannabinoid receptor 2 (CB₂)], their endogenous lipidic ligands (endocannabinoids), and enzymes involved in endocannabinoid synthesis and degradation (for reviews see Pacher et al., 2006; Pertwee et al., 2010).

Cannabinoid receptors

Classical cannabinoid receptors, CB₁ and CB₂ share 44% amino acid sequence homology. CB₁ is the most abundant G-protein coupled receptor in the brain, but is also expressed at lower levels in a large number of peripheral tissues (Mallat and Lotersztajn, 2006; Pacher et al., 2006; Pertwee et al., 2010). Accordingly, CB₁ receptors are exclusively responsible for central psychotrophic and behavioural effects of cannabinoids, and additionally regulate several peripheral processes such as energy homeostasis, cardiovascular function or reproduction (Di Marzo, 2008). In contrast, CB₂ receptors are mainly expressed in the periphery, although they have recently been detected at low levels in the central nervous system (Van Sickle et al., 2005). CB₂ receptors are predominantly expressed by immune cells and play a key role in the modulation of innate immunity in several settings such as inflammatory bowel disease or atherosclerosis (Klein, 2005; Lotersztajn et al., 2008; Patel et al., 2010), they are also involved in the regulation of bone mass and display antitumor properties (Klein, 2005; Lotersztajn et al., 2008; Patel et al., 2010). Endocannabinoids may also bind other receptors, such as the putative non-CB₁ non-CB₂ cannabinoid receptor GRP55, ligand-gated ion channels, transient receptor potential channels or nuclear receptors such as peroxisome proliferator-activated nuclear receptors. In addition, CB₁ receptors heterodimerize with several G-protein coupled receptors, including the angiotensin II type 1 receptor, dopamine, orexin or opioid receptors, although the physiological relevance of these interactions has not been clearly demonstrated. Finally, endocannabinoids promote receptor-independent effects that may be linked to their high lipophilicity (for a detailed review, see Pertwee et al., 2010).

Exogenous and endogenous ligands

Ligands for CB receptors include phytocannabinoids and fatty acid-derived endocannabinoids with predominantly autocrine/paracrine effects, owing to their lipophilic properties (Pacher et al., 2006; Di Marzo, 2008). ∆₉-tetrahydrocannabinol, the major psychoactive ingredient of Cannabis Sativa, binds CB₁ and CB₂ receptors with similar affinity (Pertwee et al., 2010). Among endocannabinoids, anandamide (arachidonoyl ethanolamide, AEA) and 2-arachidonoyl glycerol (2-AG) are the two best studied. Anandamide is a partial agonist for CB₁ receptors and shows low affinity for CB₂ receptors, whereas 2-AG is a full agonist for both CB₁ and CB₂ receptors. Endocannabinoids are locally synthesized on demand and contribute to endogenous activation of receptors, even though CB₁ and CB₂ receptors also display a high constitutive activity (Pacher et al., 2006; Di Marzo, 2008; Pertwee et al., 2010). Both AEA and 2-AG are synthesized from membrane phospholipid precursors, via parallel pathways involving phospholipase D for AEA and diacylglycerol lipase for 2-AG. Clearance of AEA relies on its cellular uptake by a mechanism that may involve a specific AEA transporter (Moore et al., 2005), or additional pathways (Piomelli et al., 2000; Di Marzo et al., 2004). Catalysis of 2-AG is catalysed by monoacylglycerol lipase and that of AEA by fatty acid amide hydrolase (FAAH), although recent data demonstrate that FAAH may also operate in reverse way, and synthesize AEA via conjugation of arachidonic acid and ethanolamine (Mukhopadhyay et al., 2011). Aside from cannabinoid compounds, cannabinoid receptors may also bind other lipid mediators such as noladin ether, virodhamine or N-arachidonoyl dopamine, but their functions remain poorly characterized.

Pharmacomodulation of cannabinoid receptors

Cannabinoid receptor 1 antagonists have undergone clinical development for the management of obesity, in light of their beneficial effect on food intake and energy expenditure. Rimobabant was initially approved in Europe for the management of overweight and related cardiometabolic disorders. Unfortunately, the drug was withdrawn after 2 years, given an alarming rate of adverse central side effects, therefore interrupting other therapeutic developments that had been expected (Van Gaal et al., 2008). This major drawback may nevertheless soon be overcome with the emerging availability of CB₁ antagonists devoid of brain effects (Kunos et al., 2009) (see below).

Pharmacomodulation of CB₂ receptors remains at a pre-clinical stage at the present time, although meaningful therapeutic applications of CB₂ agonists are anticipated in the management of atherosclerosis (Steffens et al., 2005), osteoporosis (Ofek et al., 2006), chronic liver disease (Lotersztajn et al., 2008) (Mallat and Lotersztajn, 2008a) (see below) or as analgesic or anti-allergic compounds (Patel et al., 2010).

The hepatic cannabinoid system

Basal hepatic expression of cannabinoid receptors is faint, with low levels of CB₁ receptors in Kupffer cells and of CB₂ receptors in endothelial cells and hepatocytes. Nonetheless, anandamide and 2-AG are present at substantial levels, and hepatic expression of FAAH and monoacylglycerol lipase indicates local degradation of endocannabinoids in the liver. As shown in other tissues, liver injury is associated with an increased endocannabinoid tone in several pathological settings (Mallat and Lotersztajn, 2008b). Thus, CB₂ receptors may undergo significant up-regulation in Kupffer cells and hepatic myofibroblasts, whereas CB₁ receptors are induced in hepatocytes, hepatic myofibroblasts and endothelial cells (Batkai et al., 2007b; Mallat et al., 2007; Mallat and Lotersztajn, 2008a). Increases in liver concentrations of endocannab-
in rodents are also frequently observed, with varying patterns depending on the nature of the liver insult (see below). AEA can be produced both by hepatocytes, Kupffer cells and endothelial cells, whereas increases of 2-AG by hepatic stellate cells and hepatocytes has been reported in response to acute or chronic liver injury (Batkai et al., 2007b; Mallat et al., 2007; Jeong et al., 2008; Mallat and Lotersztajn, 2008b).

Cannabinoid receptors and NAFLD

Non-alcoholic fatty liver disease, the hepatic hallmark of the metabolic syndrome, is an increasingly common finding in clinical practice with a 20–30% prevalence in Western countries (Cortez-Pinto et al., 2006; Parekh and Anania, 2007). The spectrum of the disease ranges from simple steatosis (triglyceride accumulation into hepatocytes) to steatohepatitis, a condition that associates steatosis, liver inflammation, hepatic cellular injury and activation of fibrogenic pathways with a 10–20% risk of cirrhosis after 10–20 years (Ong and Younossi, 2007).

Steatosis is tightly associated with insulin resistance and results from several alterations of lipid metabolism including: (i) increased lipolysis of peripheral fat stored in adipose tissue that flow to the liver as non-esterified fatty acids; (ii) increased lipogenesis and impaired fatty acid oxidation in hepatocytes; and (iii) reduced fat export from the liver in the form of very low density lipoprotein (Postic and Girard, 2008). The transition from steatosis to non-alcoholic steatohepatitis (NASH) is poorly understood and appears multifactorial. Recent studies have revealed a role for lipotoxic fatty acid metabolites originating from the adipose tissue or from de novo lipogenesis in the development of hepaticcellular injury. Moreover, enhanced cytokine production by infiltrating macrophages in adipose tissue and the liver is also implicated in the progression of injury (Tilg and Moschen, 2010).

CB1 receptors promote metabolic steatosis and insulin resistance

A large body of evidence has demonstrated that administration of CB1 antagonists to obese animals reduces food intake and increases energy expenditure, thereby inducing weight loss (Mallat and Lotersztajn, 2010). Not surprisingly, these effects are associated with improvement of other features of the metabolic syndrome. Thus, CB1-deficient mice exposed to a high fat diet show neither insulin resistance nor fatty liver in contrast to wild-type counterparts (Osei-Hyiaman et al., 2005). Along the same lines, genetically obese fa/fa rats treated with the CB1 receptor antagonist rimonabant show reversal of hepatic steatosis and improved insulin sensitivity (Gary-Bobo et al., 2007). Interestingly, obese mice fed a high fat diet display marked induction of CB1 receptors in hepatocytes and enhanced hepatic production of anandamide, suggesting that beneficial effects of CB1 receptor antagonism also result from interaction with peripheral receptors (Osei-Hyiaman et al., 2005). This hypothesis has recently been confirmed owing to the use of mice bearing an hepatocyte-specific deletion of CB1 receptors. Upon high fat feeding, these animals do become obese but are protected from steatosis, dyslipidaemia and insulin resistance (Osei-Hyiaman et al., 2008). Further studies in hepatocyte culture have shown that activation of CB1 receptors increases lipid accumulation by several mechanisms, including enhanced hepatocyte lipogenesis, reduced fatty acid oxidation and blockade of the hepatic production and release of triglyceride-rich very low density lipoprotein (Osei-Hyiaman et al., 2005; 2008; Tam et al., 2010). Finally, data from mice subjected to a high fat/high sucrose diet indicate that adipose tissue CB1 receptors contribute to steatosis by enhancing the influx of free fatty acids to the liver (Jourdan et al., 2010). Collectively, these data show that peripheral overactivation of CB1 receptors promotes obesity-associated fatty liver and insulin resistance, suggesting that selective targeting of peripheral CB1, with peripherally restricted molecules may be an efficient therapeutic strategy for the management of NAFLD. The feasibility of this approach has recently been validated with the development of AM6545, an orally bioavailable CB1 antagonist with limited brain penetration (Cluny et al., 2010; Tam et al., 2010). The therapeutic impact of AM6545 was investigated in two models of obesity-associated NAFLD. Mice exposed to AM6545 displayed reversal of steatosis, as well as improved dyslipidaemia and glycaemic control. As anticipated from previous studies, in vivo and in vitro studies demonstrated that AM6545 reduced the impairment in liver and adipose tissue metabolism (Tam et al., 2010).

Activation of CB1 receptors may also participate to the inflammatory response and liver injury associated with NASH (Tilg and Moschen, 2010). Thus, CB1-dependent increase in adipose tissue TNF-α expression is associated with reduced secretion of adiponectin (Bensaid et al., 2003), an adipokine with potent anti-inflammatory effects in the liver (Xu et al., 2003). In keeping with these observations, administration of rimonabant to genetically obese rats reduces liver inflammation (Bensaid et al., 2003).

Pro-inflammatory effects of CB2 receptors in fat tissue participate to the pathogenesis of NAFLD

It is well established that low grade adipose tissue inflammation associated with obesity is a key determinant in the pathogenesis of insulin resistance and NAFLD (Tilg and Moschen, 2010). As CB2 receptors are potent regulators of innate immunity, we recently evaluated their impact on adipose tissue inflammation and the related hallmarks of the metabolic syndrome (Deveaux et al., 2009). The study was conducted in mice fed a high fat diet and in leptin-deficient ob/ob mice, two well-characterized models of insulin resistance and metabolic fatty liver. In obese mice, CB2 receptors were markedly up-regulated in the stromal vascular fraction of epididymal adipose tissue, and their activation by JWH-133 promoted fat inflammation (Deveaux et al., 2009; Agudo et al., 2010). Moreover, treatment of obese mice with the CB2-selective agonist JWH-133 enhanced insulin resistance and steatosis in these animals. In contrast, CB2-deficient animals showed improved insulin sensitivity and resistance to fatty liver. These results have been recently confirmed in aged mice (Agudo et al., 2010). Collectively, these data indicate that adipose tissue CB2 receptors contribute to fat inflammatory response thereby enhancing insulin resistance and liver steatogenesis associated with obesity.
Cannabinoid receptors and NAFLD: clinical evidence

In keeping with preclinical data, enhanced endocannabinoid tone has been reported in obese patients prone to the development of the metabolic syndrome. Thus, in three studies, obese individuals displayed elevated serum levels of 2-AG, compared with lean individuals (Engeli et al., 2005; Bluher et al., 2006; Cote et al., 2007). A recent study also analysed the relationship between splanchnic endocannabinoids levels and steatosis severity in nine overweight women with NAFLD (Westerbacka et al., 2010). There was a positive correlation between fasting arterial and hepatic venous concentrations of 2-AG and liver fat content (Westerbacka et al., 2010).

The impact of the cannabinoid system on liver steatosis has also been investigated in patients with chronic hepatitis C. In this setting, prevalence of steatosis ranges between 30% and 70%, as a result of associated metabolic disturbances and/or direct steatogenic effect of genotype 3 hepatitis C virus. We performed a prospective study in 315 untreated patients with chronic hepatitis C, in order to evaluate whether stimulation of cannabinoid receptors by exogenous phytocannabinoids might influence the severity of steatosis in liver biopsies (Hezode et al., 2008). Daily cannabis use over the 6 month period preceding biopsy was identified as an independent predictor of severe steatosis (Hezode et al., 2008). Moreover, in 88 patients with chronic hepatitis C, hepatic CB1 expression correlated with the extent of steatosis and was significantly up-regulated in those with increased steatosis grade (van der Poorten et al., 2010).

Although clinical development of CB1 antagonists has been suspended due to central side effects, indirect evidences from randomized phase III clinical trials ofrimonabant suggest blockade of CB1 receptor may prove useful in the management of NAFLD. Analysis of pooled 1 year data from four pivotal trials in overweight patients showed a significant decrease in serum alanine aminotransferase in patients under rimonabant, compared with placebo-treated individuals, suggesting a beneficial impact on fatty liver (Van Gaal et al., 2008). The ADAGIO-Lipids trial in 800 patients with abdominal obesity and dyslipidaemia also reported similar findings (Despres et al., 2005). Moreover, a computed tomography sub-study evaluated the distribution of fat depots and showed a reduction of liver steatosis in treated patients versus controls (Despres et al., 2005). Overall, clinical data provide consistent evidences for a steatogenic role of endocannabinoids and CB1 receptors in patients with NAFLD.

Alcoholic liver disease

Chronic alcohol abuse, a leading cause of liver-related morbidity in Western countries, is associated with several patterns of liver injury presenting certain similarities to metabolic liver disease, ranging from isolated fatty liver to alcoholic hepatitis, cirrhosis and hepatocellular carcinoma (Mandayam et al., 2004; Lucey et al., 2009). Compelling evidences indicate that resident hepatic macrophages (Kupffer cells) play a key role in the initiation of alcoholic liver disease (Mandrekar and Szabo, 2009). The currently accepted model stipulates that alcohol-induced enhancement of gut permeability increases translocation of bacterial lipopolysaccharide (LPS)-endotoxin to the liver. Alcohol also sensitizes Kupffer cells to LPS by increasing oxidative stress, and primed Kupffer cells respond to LPS by polarization towards a pro-inflammatory M1 phenotype, characterized by up-regulation of a number of pro-inflammatory mediators, including cytokines and chemokines, as well as their cognate receptors (Mandrekar and Szabo, 2009).

CB1 antagonism prevents the development of alcohol-induced fatty liver

Jeong et al. recently demonstrated that an increased CB1-dependent tone is involved in the pathogenesis of alcohol-induced fatty liver (Jeong et al., 2008). Mice fed an ethanol diet were found to display a marked induction of hepatocyte CB1 receptors and increased levels of 2-AG. The authors also showed that administration of rimonabant prevents the development of fatty liver elicited by chronic alcohol feeding. In addition, mice bearing an hepatocyte-specific deletion of CB1 receptors were resistant to alcohol-induced fatty liver (Jeong et al., 2008). The mechanism underlying CB1-mediated steatogenesis was ascribed to enhanced production of 2-AG by hepatic stellate cells, resulting in paracrine activation of CB1 receptors in neighbouring hepatocytes with subsequent activation of lipogenesis and inhibition of fatty acid oxidation (Jeong et al., 2008). Altogether, these results suggest that CB1 antagonism may reduce the development of alcohol-induced fatty liver. Whether CB1 inactivation might also interfere with the deleterious inflammatory reaction elicited by alcohol abuse remains to be investigated.

CB2 receptor activation reduces alcohol-induced liver inflammation and fatty liver

Treatment of alcohol-induced liver disease should ideally reduce oxidative stress, macrophage activation and steatogenesis. In this respect, CB2 receptors appear as attractive targets, given their well-demonstrated role in the control of innate immunity. We therefore investigated their impact in mice exposed to chronic alcohol feeding. We found that in alcohol-exposed mice, endogenous or exogenous activation of CB2 receptors prevents the switch of Kupffer cells to a pro-inflammatory M1 phenotype and the accumulation of triglycerides in hepatocytes (Louv et al., 2010). In vitro experiments demonstrated that CB2 receptor activation regulates macrophage polarization, by preventing the pro-inflammatory M1 response and inducing polarization towards an anti-inflammatory M2 phenotype (Louv et al., 2010). Exposure of Kupffer cells to the CB2-selective agonist JWH-133 prevented the pro-inflammatory response to LPS, following inhibition of NF-kB. Moreover, there was a causal relationship between activation of macrophage CB2 receptors and inhibition of hepatocyte steatogenesis. Indeed, mice lacking CB2 receptors exhibited exacerbated steatosis, while animals treated with the CB2-selective agonist JWH-133 showed no steatosis upon alcohol feeding (Louv et al., 2010). Because CB2 receptors are not expressed in hepatocytes (Deveaux et al., 2009; Teixeira-Clerc et al., 2010), these data suggested that the anti-steatogenic signal may originate from Kupffer cells. In vitro experiments demonstrated that preventing M1 polarization in CB2-stimulated macrophages reduces
Opposite effects of CB₁ and CB₂ receptors on liver fibrogenesis

Chronic liver diseases are characterized by prolonged liver injury resulting in the chronic activation of an altered wound-healing with progressive accumulation of fibrosis in the liver parenchyma, eventually leading to liver cirrhosis, portal hypertension and liver failure. Progression of fibrosis combines enhanced production of extracellular matrix by hepatic myofibroblasts and impaired matrix turnover (Lotersztajn et al., 2005). Effective antifibrotic treatments are not available in humans as yet, and numerous efforts are directed at the development of liver-specific antifibrotic therapies. Studies from our lab have revealed the major impact of the endocannabinoid system in the regulation of liver fibrogenesis. Indeed, we found that CB₁ and CB₂ receptors are markedly up-regulated in cirrhotic liver samples, primarily in hepatic myofibroblasts, and demonstrated that endogenous activation of CB₁ receptors enhances fibrogenesis, whereas, conversely, stimulation of CB₂ receptors counteracts progression of fibrosis (Julien et al., 2005; Teixeira-Clerc et al., 2006).

Antifibrogenic properties of CB₂ receptors

Antifibrogenic properties of CB₂ receptors were established using the carbon tetrachloride model, based on the findings that CB₂-deficient mice show enhanced survival of liver fibrogenic cells resulting in increased fibrosis (Julien et al., 2005). In line with our results, a subsequent study in rats with established cirrhosis showed that administration of the CB₂-selective agonist JWH-133 improves liver fibrosis, decreases the inflammatory infiltrate and reduces the density of hepatic myofibroblasts following increased apoptosis (Munoz-Luque et al., 2008). Interestingly, antifibrogenic properties of CB₂ receptors have also been recently demonstrated in other organs, as shown in models of cardiac fibrosis (Defer et al., 2009) and systemic sclerosis (Servettaz et al., 2010).

Profibrogenic effects of CB₁ receptors

The role of CB₁ receptors in liver fibrosis was examined in models of carbon tetrachloride or thioacetamide intoxication and in bile duct ligated animals. Administration of rimonabant to wild-type mice or genetic inactivation of CB₁ receptors were both associated with a significant reduction in fibrosis progression (Teixeira-Clerc et al., 2006). Rimonabant-treated or CB₁ knock-out mice also displayed reduced hepatic expression of the profibrogenic cytokine TGF-ß1, and a decrease in the number of fibrogenic cells. Antifibrogenic properties of the CB₁-selective antagonist were ascribed to antiproliferative and apoptotic properties of the compound in hepatic myofibroblasts. The antifibrogenic potential of CB₁ antagonism was also confirmed in a murine model of prolonged high fat feeding characterized by histological features of NASH including significant fibrosis (DeLeve et al., 2008), in rats with established cirrhosis (Domenicali et al., 2009), and in rats submitted to bile duct ligation and treated with another CB₁-selective antagonist, AM251 (Yang et al., 2007).

Overall, these data strongly suggest that selective CB₁ antagonists and peripherally restricted CB₁ antagonists may prove useful for the management of hepatic fibrosis.

Receptor-independent effects of endocannabinoids on liver fibrogenesis

Aside from CB₁- and CB₂-mediated effects on liver fibrogenesis, endocannabinoids may also modulate the fibrogenic process by CB₁- and CB₂-independent pathways, although the latter are less fully characterized. In cultured fibrogenic cells, apoptotic effects of AEA and 2-AG are not blocked by CB₁ or CB₂ antagonists (Julien et al., 2005; Siegmund et al., 2005).

Clinical data

The opposite effects of CB₁ and CB₂ receptors on experimental liver fibrosis raised the question as to the resulting effect in a clinical setting. We investigated this issue in a cohort of patients with chronic hepatitis C, by evaluating the impact of cannabis use on fibrosis progression. We identified daily cannabis smoking over the course of the disease as a strong independent predictor of fibrosis severity. Similar findings were reported in another cohort of patients (Ishida et al., 2008). These data therefore suggest that CB₁ signalling dominates over CB₂ for exogenous cannabinoid ligands during chronic hepatitis C (Hezode et al., 2005). In keeping, it has recently been shown that CB₂ expression is enhanced in the liver of patients with chronic hepatitis C, as compared with patients with chronic hepatitis B, a finding that was ascribed to induction of CB₂ expression by hepatitis C virus in experiments performed in an hepatocyte cell line infected with an hepatitis C virus subreplicon (van der Poorten et al., 2010).

The endocannabinoid system as a mediator of extrahepatic complications of cirrhosis

Vasoregulatory effects of endocannabinoids have been extensively characterized. Several studies indicate that an enhanced peripheral CB₁-dependent cannabinoid tone contributes to the pathogenesis of portal hypertension, via enhanced mesenteric vasodilation (Batkai et al., 2001; Ros et al., 2002). In support of these data, administration of rimonabant to rats with established cirrhosis improves peripheral vascular resistance and blood pressure, thereby preventing the development of ascites (Domenicali et al., 2009). Recent data also suggest that CB₁-mediated stimulation of cardiomyocytes contribute to the cirrhotic cardiomyopathy associated with end-stage cirrhosis (Gaskari et al., 2005; Batkai et al., 2007a; Avraham et al., 2008), and to hepatic encephalopathy, a major complication of acute and chronic liver failure (Avraham et al., 2006; Dagon et al., 2007).
Protective effects of cannabinoid receptors on liver injury and regeneration

Recent data have shown that CB2 receptors decrease the extent of liver injury in models of acute liver insult, as induced by ischaemia-reperfusion, thioacetamide or concanavalin-A (Batkai et al., 2007b; Avraham et al., 2008; Hegde et al., 2008). However, the mechanisms underlying these hepatoprotective effects remained unclear. We therefore investigated the impact of CB2 receptor modulation on hepatocyte survival and liver regeneration in a model of acute hepatitis elicited by a single dose of carbon tetrachloride (Teixeira-Clerc et al., 2010). We found that defective induction of inducible nitric oxide synthase in hepatocytes is responsible for enhanced apoptosis of these cells in CB2-deficient mice exposed to carbon tetrachloride, compared with wild-type littermates. In contrast, treatment of wild-type animals with the CB2-selective agonist JWH-133 protected from the apoptotic effects of carbon tetrachloride (Teixeira-Clerc et al., 2010). Results from CB2-deficient mice and mice treated with JWH-133 also demonstrated that CB2 receptors accelerate the regenerative response that follows acute liver injury in this model. Similar effects were also obtained in another model of liver regeneration, as induced by partial hepatectomy. The beneficial effects of CB2 receptors on liver regeneration were related to an increased production of interleukin-6 from hepatic myofibroblasts, resulting in paracrine mitogenic effects on hepatocytes (Teixeira-Clerc et al., 2010). This study therefore indicates that paracrine mechanisms originating from hepatic myofibroblasts account for beneficial effects of CB2 receptors on hepatocyte survival and regeneration following an acute insult.

Strikingly, beneficial effects of CB1 receptors on liver regeneration have also been recently reported (Mukhopadhyay et al., 2011). Indeed, mice undergoing partial hepatectomy showed a marked induction of CB1 receptors and increased hepatic production of AEA, via FAAH operating a reverse way. Mice administered rimonabant or selectively lacking CB1 receptors in hepatocytes displayed reduced liver regeneration, as reflected by a weak mitogenesis of hepato-

![Diagram]

**Figure 1**

Role of the endocannabinoid system in the progression of chronic liver diseases: In western countries, prevailing causes of cirrhosis include chronic alcohol consumption, hepatitis C virus and obesity. Liver disease progression shows common sequence of events whatever the origin. Studies over the last few years have shown that cannabinoid receptors [cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2)] and their endogenous ligands are highly up-regulated during chronic liver disease and affect multiple common steps, including steatosis, hepatocyte injury and inflammation (steatohepatitis), fibrosis and liver regeneration.
cytes (Mukhopadhyay et al., 2011). Reduced proliferative activity of hepatocytes was the result of the inhibition of cell cycle proteins involved in mitotic progression, including forkhead-box M1 (FoxM1), a transcription factor that is also essential in the development of hepatocellular carcinoma. These data demonstrate that AEA acting on CB1 receptors promotes liver regeneration; whether CB1 receptors may also promote the development of hepatocellular carcinoma warrants further investigation.

Conclusion

Over the past 10 years, the endocannabinoid system has emerged as a major player in the pathogenesis of liver diseases (Figure 1). CB1 receptors have been implicated in the pathogenesis of several lesions such as liver fibrogenesis, alcoholic and metabolic steatosis, or circulatory failure associated with cirrhosis. In contrast, stimulation of hepatic CB2 receptors has been put to a hold, due to the high incidence of central side effects of first generation compounds. Fortunately, CB1 antagonists devoid of brain penetrance are increasingly being expected with the availability of CB1 receptor antagonists expected following thioacetamide-induced acute injury in mice. Am J Physiol Heart Circ Physiol 293: H2210–H2218.


References


