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REVIEW

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

A Mallat[1,2,3], F Teixeira-Clerc[1,2], V Deveaux[1,2], S Manin[1,2] and S Lotersztajn[1,2,3]

[1]*INSERM, U955, Créteil, France,* [2]*Université Paris-Est, Faculté de Médecine, UMR-S955, Créteil, France, and* [3]*AP-HP, Groupe Henri Mondor-Albert Chenevier, Dept d'Hépatology et de Gastroenterology, Créteil, France*

Correspondence
Dr Sophie Lotersztajn, Inserm U955, Institut Mondor de Recherche Biomédicale, Hôpital Henri Mondor, 94010 Créteil, France. E-mail: sophie.lotersztajn@inserm.fr

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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 (CB_2) receptors display beneficial effects on alcoholic fatty liver, hepatic inflammation, liver injury, regeneration and fibrosis. Cannabinoid receptor 1 (CB_1) 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 CB_1 antagonists has recently been suspended due to the high incidence of central side effects, preliminary preclinical data obtained with peripherally restricted CB_1 antagonists give real hopes in the development of active CB_1 molecules devoid of central adverse effects. CB_2-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 CB_1 antagonist and a CB_2 agonist might result in an increased therapeutic potential will warrant further investigation.

LINKED ARTICLES

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Abbreviations

CB_1, cannabinoid receptor 1; CB_2, 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

management 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 (CB_1) and cannabinoid receptor 2 (CB_2)], 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, CB_1 and CB_2, share 44% amino acid sequence homology. CB_1 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, CB_1 receptors are exclusively responsible for central psychotropic and behavioural effects of cannabinoids, and additionally regulate several peripheral processes such as energy homeostasis, cardiovascular function or reproduction (Di Marzo, 2008). In contrast, CB_2 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). CB_2 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-CB_1 non-CB_2 cannabinoid receptor GRP55, ligand-gated ion channels, transient receptor potential channels or nuclear receptors such as peroxisome proliferator-activated nuclear receptors. In addition, CB_1 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). Δ^9-tetrahydrocannabinol, the major psychoactive ingredient of *Cannabis Sativa,* binds CB_1 and CB_2 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 CB_1 receptors and shows low affinity for CB_2 receptors, whereas 2-AG is a full agonist for both CB_1 and CB_2 receptors. Endocannabinoids are locally synthesized on demand and contribute to endogenous activation of receptors, even though CB_1 and CB_2 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). Catabolism 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. Rimonabant 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 CB_1 antagonists devoid of brain effects (Kunos *et al.*, 2009) (see below).

Pharmacomodulation of CB_2 receptors remains at a preclinical stage at the present time, although meaningful therapeutic applications of CB_2 agonists are anticipated in the management of atherosclerosis (Steffens *et al.*, 2005), osteoperosis (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 CB_2 receptors in Kupffer cells and of CB_1 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, CB_2 receptors may undergo significant up-regulation in Kupffer cells and hepatic myofibroblasts, whereas CB_1 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-

inoids 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, hepatocellular 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 hepatocellular 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).

CB_1 receptors promote metabolic steatosis and insulin resistance

A large body of evidence has demonstrated that administration of CB_1 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, CB_1-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 CB_1 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 CB_1 receptors in hepatocytes and enhanced hepatic production of anandamide, suggesting that beneficial effects of CB_1 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 CB_1 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 CB_1 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 CB_1 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 CB_1 receptors promotes obesity-associated fatty liver and insulin resistance, suggesting that selective targeting of peripheral CB_1 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 CB_1 antagonist with limited brain penetrance (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 CB_1 receptors may also participate to the inflammatory response and liver injury associated with NASH (Tilg and Moschen, 2010). Thus, CB_1-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 CB_2 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 CB_2 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, CB_2 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 CB_2-selective agonist JWH-133 enhanced insulin resistance and steatosis in these animals. In contrast, CB_2-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 CB_2 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 CB_1 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 CB_1 antagonists has been suspended due to central side effects, indirect evidences from randomized phase III clinical trials of rimonabant suggest blockade of CB_1 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 CB_1 receptors in patients with NAFLD.

Alcoholic liver disease

Chronic alcohol abuse, a leading cause of liver-related morbimortality 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 liposaccharide (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).

CB_1 antagonism prevents the development of alcohol-induced fatty liver

Jeong *et al.* recently demonstrated that an increased CB_1-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 CB_1 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 CB_1 receptors were resistant to alcohol-induced fatty liver (Jeong *et al.*, 2008). The mechanism underlying CB_1-mediated steatogenesis was ascribed to enhanced production of 2-AG by hepatic stellate cells, resulting in paracrine activation of CB_1 receptors in neighbouring hepatocytes with subsequent activation of lipogenesis and inhibition of fatty acid oxidation (Jeong *et al.*, 2008). Altogether, these results suggest that CB_1 antagonism may reduce the development of alcohol-induced fatty liver. Whether CB_1 inactivation might also interfere with the deleterious inflammatory reaction elicited by alcohol abuse remains to be investigated.

CB_2 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, CB_2 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 CB_2 receptors prevents the switch of Kupffer cells to a pro-inflammatory M1 phenotype and the accumulation of triglycerides in hepatocytes (Louvet *et al.*, 2010). *In vitro* experiments demonstrated that CB_2 receptor activation regulates macrophage polarization, by preventing the pro-inflammatory M1 response and inducing polarization towards an anti-inflammatory M2 phenotype (Louvet *et al.*, 2010). Exposure of Kupffer cells to the CB_2-selective agonist JWH-133 prevented the pro-inflammatory response to LPS, following inhibition of NF-κB. Moreover, there was a causal relationship between activation of macrophage CB_2 receptors and inhibition of hepatocyte steatogenesis. Indeed, mice lacking CB_2 receptors exhibited exacerbated steatosis, while animals treated with the CB_2-selective agonist JWH-133 showed no steatosis upon alcohol feeding (Louvet *et al.*, 2010). Because CB_2 receptors are not expressed in hepatocytes (Deveaux *et al.*, 2009; Teixeira-Clerc *et al.*, 2010), these data suggested that the antisteatogenic signal may originate from Kupffer cells. *In vitro* experiments demonstrated that preventing M1 polarization in CB_2-stimulated macrophages reduces

fat accumulation in hepatocytes (Louvet *et al.*, 2010). Altogether, these data demonstrate that CB_2 receptors display beneficial effects on alcohol liver disease by limiting hepatic inflammation and steatosis via autocrine and paracrine effects. This study identifies CB_2 receptor agonism as a potential promising approach in the management of alcohol-induced liver injury.

Opposite effects of CB_1 and CB_2 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 CB_1 and CB_2 receptors are markedly up-regulated in cirrhotic liver samples, primarily in hepatic myofibroblasts, and demonstrated that endogenous activation of CB_1 receptors enhances fibrogenesis, whereas, conversely, stimulation of CB_2 receptors counteracts progression of fibrosis (Julien *et al.*, 2005; Teixeira-Clerc *et al.*, 2006).

Antifibrogenic properties of CB_2 receptors

Antifibrogenic properties of CB_2 receptors were established using the carbon tetrachloride model, based on the findings that CB_2-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 CB_2-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 CB_2 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 CB_1 receptors

The role of CB_1 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 CB_1 receptors were both associated with a significant reduction in fibrosis progression (Teixeira-Clerc *et al.*, 2006). Rimonabant-treated or CB_1 knock-out mice also displayed reduced hepatic expression of the profibrogenic cytokine TGF-β1, and a decrease in the number of fibrogenic cells. Antifibrogenic properties of the CB_1-selective antagonist were ascribed to antiproliferative and apoptotic properties of the compound in hepatic myofibroblasts. The antifibrogenic potential of CB_1 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 CB_1-selective antagonist, AM251 (Yang *et al.*, 2007).

Overall, these data strongly suggest that selective CB_2 agonists and peripherally restricted CB_1 antagonists may prove useful for the management of hepatic fibrosis.

Receptor-independent effects of endocannabinoids on liver fibrogenesis

Aside from CB_1- and CB_2-mediated effects on liver fibrogenesis, endocannabinoids may also modulate the fibrogenic process by CB_1- and CB_2-independent pathways, although the latter are less fully characterized. In cultured fibrogenic cells, apoptotic effects of AEA and 2–AG are not blocked by CB_1 or CB_2 antagonists (Julien *et al.*, 2005; Siegmund *et al.*, 2005).

Clinical data

The opposite effects of CB_1 and CB_2 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 CB_1 signalling dominates over CB_2 for exogenous cannabinoid ligands during chronic hepatitis C (Hezode *et al.*, 2005). In keeping, it has recently been shown that CB_1 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 CB_1 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 CB_1-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 CB_1-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 CB_2 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 CB_2 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 CB_2-deficient mice exposed to carbon tetrachloride, compared with wild-type littermates. In contrast, treatment of wild-type animals with the CB_2-selective agonist JWH-133 protected from the apoptotic effects of carbon tetrachloride (Teixeira-Clerc *et al.*, 2010). Results from CB_2-deficient mice and mice treated with JWH-133 also demonstrated that CB_2 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 CB_2 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 CB_2 receptors on hepatocyte survival and regeneration following an acute insult.

Strinkingly, beneficial effects of CB_1 receptors on liver regeneration have also been recently reported (Mukhopadhyay *et al.*, 2011). Indeed, mice undergoing partial hepatectomy showed a marked induction of CB_1 receptors and increased hepatic production of AEA, via FAAH operating a reverse way. Mice administered rimonabant or selectively lacking CB_1 receptors in hepatocytes displayed reduced liver regeneration, as reflected by a weak mitogenesis of hepato-

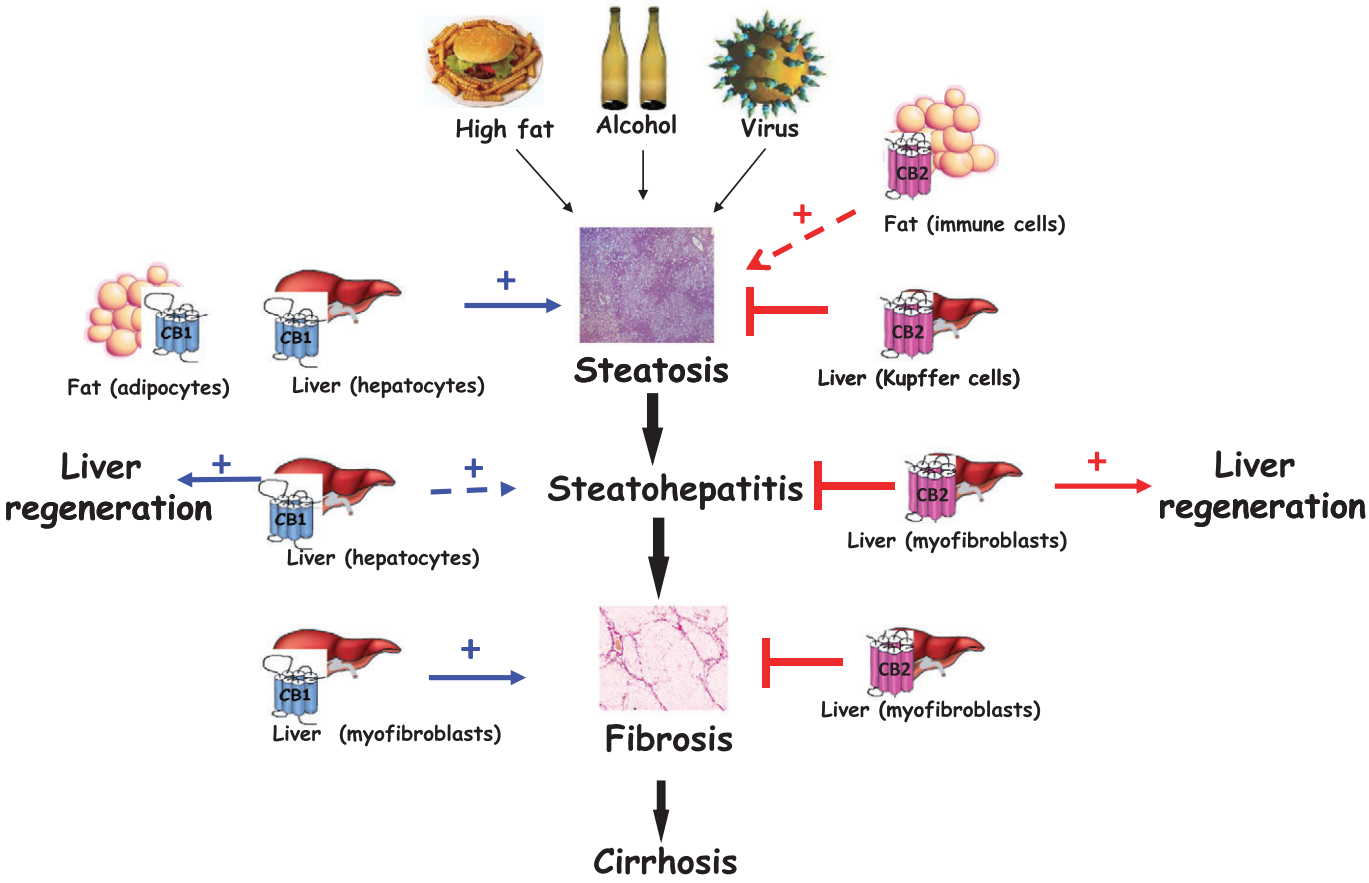

Figure 1

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 show common sequence of events whatever the origin. Studies over the last few years have shown that cannabinoid receptors [cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2)] 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 CB_1 receptors promotes liver regeneration; whether CB_1 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). CB_1 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 CB_2 receptors is emerging as an overall protective pathway with antifibrogenic properties and beneficial effects on liver inflammation, alcoholic fatty liver and hepatocyte survival and regeneration. Exciting therapeutic developments expected with the availability of CB_1 receptor antagonists have been put to a hold, due to the high incidence of central side effects of first generation compounds. Fortunately, CB_1 antagonists devoid of brain penetrance are increasingly being synthetized and initial results suggest that they exhibit beneficial effects expected from previous studies. The clinical development of CB_2-selective agonists is also eagerly awaited.

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Conflicts of interest

The authors have no conflict of interest.

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