

# The Role of Endocannabinoids System in Fatty Liver Disease and Therapeutic Potentials

Khalid A. Alswat

Department of Medicine,  
Liver Disease Research Center,  
College of Medicine, King Saud  
University, Riyadh, Saudi Arabia

**Address for correspondence:**

Dr. Khalid A. Alswat,  
Department of Medicine,  
College of Medicine,  
Liver Disease Research  
Center, King Saud University,  
P.O. Box 7805, Riyadh 11472,  
Saudi Arabia.  
E-mail: kalswat@ksu.edu.sa

## ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver morbidity and mortality with no proven effective therapy as of yet. Its prevalence is increasing globally in parallel with obesity and metabolic syndrome pandemic. The endocannabinoid (EC) system has been implicated in the pathogenesis of several diseases, including fatty liver diseases. This system refers to the cannabinoid receptors type 1 (CB1) and type 2 (CB2), with both their endogenous ligands and machinery dedicated to EC synthesis and degradation. There is accumulating evidence on the role CB1 as a key mediator of insulin resistance and liver lipogenesis in both animals and humans. On the other hand, CB2 receptors have been shown to promote inflammation with anti-fibrogenic properties. The pharmacological modulation of the EC system activity for the treatment of metabolic syndrome and NAFLD are promising yet premature. The initial limited success due to deleterious central nervous system side-effects are likely to be bypassed with the use of peripherally restricted drugs.

**Key Words:** Cannabinoid receptors type 1, cannabinoid receptors type 2, endocannabinoids, endocannabinoids system, fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver morbidity and mortality. Its prevalence varies widely depending on the population studied and the definition used.<sup>[1,2]</sup> The prevalence of histologically-defined NAFLD has been reported to a range from 20% to 51%.<sup>[3,4]</sup> However, the reported prevalence of NAFLD when defined by liver ultrasound ranged from 17% to 46%, again, depending on the population studied.<sup>[2]</sup> In Saudi Arabia, the prevalence of NAFLD is estimated to be around 16.6%.<sup>[5]</sup> NAFLD is as a spectrum of disease ranging from simple steatosis to inflammatory non-alcoholic steatohepatitis (NASH) with varying degrees of fibrosis. The latter stage is associated with an activation of fibrogenic pathways and carries a 10-20% risk of cirrhosis after 10-20 years.<sup>[6]</sup> As shown in different studies, NASH is associated with increased

liver-related mortality due to end-stage liver disease and/or development of hepatocellular carcinoma.<sup>[7]</sup>

Mechanisms of steatosis are related to: (a) increased de novo fatty acid synthesis; (b) increased transport of fatty acids from the peripheral organs to the liver; (c) decreased fatty acid oxidation; and (d) impaired transport of fatty acids (triglycerides (TGs)) from the liver to the circulation and peripheral organs.<sup>[8]</sup> The detailed pathways of liver steatosis pathogenesis are beyond the scope of this review.

Management of NAFLD remains a major challenge as no treatment has been approved yet for this indication. Most of the current therapeutic strategies aim to decrease insulin resistance as well as the processes leading to necroinflammation and hepatic fibrosis. In this context, modulation of cannabinoid receptors is emerging as a potential novel therapeutic approach.<sup>[9]</sup> These receptors are part of a novel signaling pathway, known as the endocannabinoid (EC) system that is increasingly incriminated in a variety of physiological and pathological conditions.

The *in vitro* and *in vivo* studies reported efficacies of cannabis extracts and its individual compounds in a variety of conditions

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such as; analgesic,<sup>[10]</sup> anti-inflammatory,<sup>[11]</sup> anti-emetic,<sup>[12]</sup> anxiolytic,<sup>[13]</sup> anti-psychotic,<sup>[14]</sup> and anti-cancer.<sup>[15]</sup>

Regarding liver disease, accumulating evidence indicates that the cannabinoid system plays a crucial role in the pathophysiology of many liver diseases, both as a key player in hepatic injury and as a mediator of complications of cirrhosis.<sup>[16]</sup> The present review will focus on the role of ECs on fatty liver disease.

## CANNABINOIDS SYSTEM

The cannabinoid system refers to the cannabinoids, cannabinoid receptors, and machinery dedicated to EC synthesis and degradation.<sup>[17]</sup> Cannabinoids are a class of diverse chemical compounds that activate cannabinoid receptors, including phytocannabinoids (found in cannabis and some other plants), the ECs (produced naturally in the body by humans and animals), and synthetic cannabinoids (produced chemically by humans).<sup>[18]</sup> The famous plant "*Cannabis sativa L*" is a unique source of at least 66 cannabinoids.<sup>[19]</sup> These plant-derived cannabinoids have long been used for recreational and therapeutic purposes. The principal psychoactive constituent (or cannabinoid) of the cannabis plant is tetra-hydro-cannabinol (THC). This compound was first isolated, identified and synthesized in 1964.<sup>[20]</sup> Its discovery subsequently led to the identification of cannabinoid receptors and their endogenous legends' ECs. A number of therapeutic actions of these compounds have been reported and thought to be mediated via EC system. Unfortunately, THC-based drugs produce both therapeutic and undesirable psychotropic actions by activating cannabinoid receptors type 1 (CB1) in the central nervous system (CNS). Interestingly, some other components such as cannabidiol (CBD) are devoid of the typical psychological effects. CBD constitutes up to 40% of cannabis extracts with some pharmacological effects without the undesirable psychoactive side effects.<sup>[21]</sup>

## Endocannabinoids receptors

EC receptors are G-protein-coupled receptors that react to a variety of cannabinoid (exogenous and endogenous) ligands.<sup>[22]</sup> The first cannabinoid receptor (CB1) has been isolated and subsequently cloned almost three decades after the discovery of the active component of the plant *Cannabis sativa* (THC).<sup>[23]</sup> Consequently, cannabinoid receptors type 2 (CB2) receptor was identified and isolated.<sup>[24,25]</sup>

Both CB1 and CB2 receptors share low (44%) sequence homology and a similar ligand binding profile. CB1 receptors are located throughout the body, with the highest density expressed in the CNS "forebrain, thalamus, and basal ganglia." This distribution correlates with known clinical and psychological effects of cannabinoids.<sup>[26]</sup> Peripherally, CB1 receptors are localized in most internal organs and glands.<sup>[26,27]</sup> CB2 receptors on the other hand are primarily expressed in the cells of the immune system in the periphery, although they were recently detected in the brain, especially during inflammatory conditions.<sup>[27,28]</sup> Table 1 showed a comparison between both types of receptors and their role in fatty liver disease.

## Endocannabinoids

The cloning of the CB1 receptor was followed by the discovery that mammalian tissues can synthesize ECs and release them on cannabinoid receptors. ECs are endogenous arachidonic acid-derived mediators synthesized from membrane phospholipids "on demand," and are released from cells immediately after production to activate the cannabinoid receptor to elicit a biological response, after which they are inactivated through reuptake.<sup>[26]</sup> The first of ECs was identified in 1992, and designated as 2-arachidonylethanolamine (Anandamide),<sup>[29]</sup> followed by 2-arachidonyl-glycerol (2-AG). Several other ligands with cannabinoid receptor binding activity were reported since then, e.g., noladine and virodhamine (O-arachidonoyl ethanolamine).<sup>[30]</sup> Among these, the anandamide and 2-AG are best studied. Biological functions however of most of

**Table 1: Comparison between CB1 and CB2 receptors**

Parameters	CB1	CB2
Distribution in the body	Throughout the body, highest density CNS	Cells of the immune system
Expression in normal liver	Faint	Faint/absent
Expression in liver pathology	Hepatocytes, endothelial cells, hepatic myofibroblasts	Kupffer cells, hepatic myofibroblasts
Main roles	Mood, appetite, emesis control, memory, spatial coordination muscle tone and analgesia	Immune-modulatory, anti-inflammatory, pain, bone loss
Role in fatty liver and metabolic syndrome	Liver steatosis, food intake/body weight, insulin resistance, lipogenesis, splanchnic, vasodilation	Inflammation, fibrogenesis (chronic liver diseases, ischaemia/reperfusion induced liver injury) hepatic encephalopathy (acute liver failure)
Role in liver fibrosis	Profibrogenic	Antifibrogenic
Therapeutics potentials	CB1 antagonist (Rimonabant) in obesity, diabetes mellitus, fatty liver, hyperlipidemia (several trials and the meta-analysis)	Pre-clinical in liver fibrosis, ascites

CNS: Central nervous system, CB1: Cannabinoid receptors type 1, CB2: Cannabinoid receptors type 2

the other compounds remain largely unknown. Anandamide shows higher affinity for CB1 over CB2 and also binds the vanilloid VR1 receptors, whereas 2-AG binds both CB1 and CB2 receptors with similar affinity. Both anandamide and 2-AG are generated on demand via phospholipid-dependent distinct pathways in response to a rise in intracellular calcium or metabotropic receptor activation.<sup>[18]</sup> Once released, they remain largely membrane-associated because of their hydrophobic nature. Clearance of ECs relies on cellular uptake and enzymatic degradation (for anandamide through membrane-associated fatty acid amide hydrolase (FAAH)<sup>[31]</sup> while 2-AG by monoacylglycerol lipase).<sup>[32]</sup>

### Hepatic endocannabinoids system

In the normal liver, the expression of CB1 and CB2 receptors is modest, which probably explains why the focus of research on the role of ECs in the liver pathophysiology has come only recently. Indeed, early studies of brain CB1 receptors used the liver as a negative control.<sup>[33]</sup> However, during liver pathology, endocannabinoid system is activated, and CB1 and CB2 receptors undergo marked up-regulation in the cirrhotic liver, most particularly in stellate cells, and hepatic endothelial cells<sup>[34,35]</sup> as well in monocytes (R-33).<sup>[36,37]</sup>

## THE ROLE OF ENDOCANNABINOIDS IN FATTY LIVER DISEASE

### Endocannabinoids role in maintaining body weight and energy homeostasis

Maintenance of body weight and energy homeostasis involves the coordinated regulation of appetitive behavior and peripheral energy metabolism.<sup>[38]</sup> Historically, the well-known craving side-effect of smoking marijuana particularly for sweets and palatable foods, are well- documented in the literature. This has led to the investigation of the possible role of ECs in the control of food intake and body weight.

The orexigenic effect of ECs is regulated through complex central and peripheral mechanisms. Centrally, multiple hypothalamic circuits are connected to the different areas of the brain such as the brainstem (which detects and responds to hunger and satiety signals through sensory and vagal fibers) and the brain reward system, mainly the mesolimbic pathway (which modulates the motivation to obtain food).<sup>[39]</sup> This effect is regulated through the activation of CB1 receptors in these areas, in addition to the interaction with other hormones, such as leptin, insulin, and ghrelin.<sup>[40]</sup> Experiments in laboratory animals revealed that THC, as well as the ECs anandamide and 2-AG increased food intake when administered orally, subcutaneously, or centrally and antagonized by CB1 blockade.<sup>[41,42]</sup>

### Central and peripheral effects of endocannabinoids in obesity and liver steatosis

Several observations suggested that reduction of food

intake alone cannot fully explain anti-obesity effects of CB1 antagonists. Investigators showed that CB1 knockout mice (CB1<sup>-/-</sup>) are lean with resistance to diet-induced obesity (DIO) even though their total caloric intake is similar to that of wild-type littermates, which became obese on the same diet.<sup>[43]</sup> This finding has been supported by other experiments, where treatment of DIO mice with Rimonabant, a CB1 antagonist induced a transient reduction of food intake on week 1 and a marked, but sustained reduction of body weight and adiposity of these animals.<sup>[43]</sup> Furthermore, CB1<sup>-/-</sup> mice display only a temporary hypophagia in the first few weeks of life but maintained a lean phenotype throughout adulthood.<sup>[44]</sup> These observations concluded that EC system affects energy balance, weight changes, and steatosis via central orexigenic drive and peripheral metabolic effects.

The mechanism underlying these effects was demonstrated in animal experiments which showed that hepatocytes express CB1, stimulation of which induces the expression of the lipogenic transcription factor sterol regulatory element-binding protein (SREBP)-1c and its target enzymes: Acetyl coenzyme-A carboxylase-1 and fatty acid synthase (FAS) as well as increased de novo fatty acid synthesis.<sup>[45]</sup> The same molecular target for CB1 has been demonstrated in the hypothalamus, where inhibitors of FAS have been reported to cause anorexia.<sup>[46,47]</sup> Thus, the fatty acid biosynthetic pathway likely represents a common pathway for the central and peripheral effects of ECs.

## ROLE OF CB1 RECEPTORS IN FATTY LIVER DISEASE: (EXPERIMENTAL EVIDENCE)

### Role of CB1 receptors in high fat diet-induced fatty liver

Animal studies showed that high-fat diet increases hepatic levels of anandamide, CB1 density, and basal rates of fatty acid synthesis, and the latter is reduced by CB1 blockade.<sup>[48]</sup> In DIO mice model, treatment with rimonabant reduced fat mass, insulin levels, and liver TGs. The expression of CB1, which was strongly increased in the liver and adipose tissue of high-sucrose high-fat mice, was totally normalized by the treatment.<sup>[49]</sup> Furthermore, treatment with a CB1 agonist also increases de novo fatty acid synthesis in the liver or in isolated hepatocytes, which express CB1.<sup>[45]</sup> These studies support clearly the role of CB1 receptors in fatty acid synthesis and DIO and fatty liver. In order to determine the exact target tissue for these effects, Ossei *et al.*, examined the effect of high fat diet on three groups of mice: Wild type, global CB1 receptor deficient mice CB<sub>1</sub> (CB1<sup>-/-</sup>) and liver-specific CB<sub>1</sub> knockout (LCB1<sup>-/-</sup>) mice. They observed that LCB1<sup>-/-</sup> mice are susceptible to DIO similar to wild type, but unlike (CB1<sup>-/-</sup>); however, they are resistant to diet-induced steatosis, changes in plasma lipid, leptin and insulin levels. Furthermore, they found CB1 receptor agonist (HU-210) increased hepatic de novo lipogenesis in

wild-type chow-fed mice, but not in (CB1<sup>-/-</sup>) or LCBI<sup>-/-</sup>.<sup>[50]</sup> This experiment supported the contention that high-fat diet induces fatty liver primarily via activation of hepatic CB1 receptors, and these receptors are required for the development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance. Overall, these findings strongly support role for hepatic CB1 receptors in diet-induced liver steatosis, and associated hormonal and metabolic changes.

### Role of CB1 receptors in obesity-associated fatty liver

Gary-Bobo *et al.*, investigated the role of CB1 receptors in the development of fatty liver in Zucker rats (genetically obese with defective leptin receptors and have severe hepatic steatosis), and showed that rimonabant reduces obesity-associated hepatic steatosis, features of metabolic syndrome including reduction of elevated liver enzymes, tumor necrosis factor- $\alpha$  and increased the anti-inflammatory hormone adiponectin.<sup>[51]</sup> Furthermore, 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 pair-fed rats, which consumed the same amount of food as that of rimonabant-treated rats, steatosis, and hepatomegaly were not significantly reduced. This suggests that Rimonabant, and not reduced food intake, was responsible for reducing steatosis.<sup>[51]</sup> Taken together, these results indicate that in the obese rats, development of fatty liver is mediated via activation of CB1 receptors.

### Role of CB1 receptors in alcoholic fatty liver

Chronic alcohol use is a major cause of liver steatosis. Similar to diet induced liver steatosis, alcohol induced steatosis is associated with enhanced hepatic lipogenesis and decreased fatty acid oxidation.<sup>[52]</sup>

Jeong *et al.*, has shown that mice fed with ethanol developed marked hepatic expression of CB1 receptors and high levels of 2-AG and its biosynthetic enzymes selectively in hepatic stellate cells. Concurrent administration of CB1 receptor blocker Rimonabant resulted in attenuation of steatosis in mice chronically fed with ethanol diet. Consistent with Rimonabant effect, 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. The mechanism underlying this CB1 mediated steatogenesis is likely related to enhanced paracrine effect of 2-AG produced by hepatic stellate cells. This is supported by the finding that in controls co-culture with stellate cells from ethanol-fed mice resulted in up-regulation of CB1 receptors and lipogenic gene expression.<sup>[34]</sup> From these experiments, paracrine activation of hepatic CB1 receptors by stellate cell-derived ECs 2-AG mediates alcoholic fatty liver. Collectively, these data support the concept

that activation of CB1 receptors promotes liver steatosis associated with high fat diet, obesity and alcohol in addition to other metabolic effects such as insulin resistance and that targeting of CB1 may be an efficient therapeutic strategy for the management of NAFLD.

## ROLE OF CB1 RECEPTORS IN FATTY LIVER DISEASE: (CLINICAL EVIDENCE)

### Dysregulated endocannabinoids system in subjects with obesity

Enhanced EC tone has been reported in obese patients prone to develop fatty liver disease and metabolic syndrome. In several studies, obese individuals displayed higher serum levels of ECs than lean individuals. There was a strong association between high plasma EC levels and visceral obesity, high TGs, low high-density lipoprotein (HDL) cholesterol, and insulin resistance in obese as well as type 2 diabetes mellitus patients.<sup>[53-56]</sup> In a study of 60 non-diabetic Caucasian patients who underwent open abdominal surgery, circulating 2-AG, and not anandamide, was significantly correlated with body fat ( $r = 0.45$ ,  $P = 0.03$ ), visceral fat mass ( $r = 0.44$ ,  $P = 0.003$ ), and fasting plasma insulin concentrations ( $r = 0.41$ ,  $P = 0.001$ ) in obese subjects compared with lean subjects.<sup>[53]</sup>

In another study involving 62 untreated asymptomatic men with varying body mass index (BMI), plasma concentrations of 2-AG levels correlated positively with BMI, waist girth, intra-abdominal adiposity (IAA), fasting plasma TG and insulin levels, and negatively with HDL cholesterol and adiponectin levels. Furthermore, obese men with similar BMI values and who markedly differed in their amount of IAA exhibited higher 2-AG levels in the presence of high IAA. However, no difference in 2-AG concentrations was observed between obese men with low levels of IAA versus non-obese controls.<sup>[54]</sup>

Effects of exercise on ECs level were assessed in 49 viscerally obese men (average age 49 years, BMI 30.9 kg/m<sup>2</sup>, waist 107.3 cm) who underwent a 1 year lifestyle modification program. Plasma levels of 2-AG and anandamide were measured in addition to the anthropometric and metabolic risk factors. Most risk factors were improved by the intervention, which led to a significant decrease in body weight ( $-6.4$  kg,  $P < 0.0001$ ), waist circumference ( $-8.0$  cm,  $P < 0.0001$ ) Visceral adipose tissue (VAT) ( $-30\%$ ,  $P < 0.0001$ ), plasma 2-AG ( $-62.3\%$ ,  $P < 0.0001$ ) and anandamide ( $-7.1\%$ ,  $P = 0.005$ ) levels. In this study, the decreased levels of 2-AG correlated with decreases in VAT and TG levels, and with the increase in HDL-cholesterol levels. Multivariate analysis suggested that decreases in 2-AG and VAT were both independently associated with decreases in TGs.<sup>[55]</sup>



### Dysregulated endocannabinoids system in subjects with liver steatosis

The relationship between splanchnic EC levels and liver steatosis has been analyzed in a recent study. A total of 9 subjects with various degrees of hepatic steatosis underwent hepatic venous catheterization in combination with infusion of ( $^2\text{H}_2$ ) palmitate in the fasting state and during a low-dose insulin infusion. There was a positive correlation between liver fat content and splanchnic free fatty acids (FFA) extraction during hyperinsulinemia, with a concomitant increase in the arterial and hepatic venous concentrations of 2-AG.<sup>[57]</sup> This indicates that the human fatty liver takes up 2-AG and overproduces triacylglycerols containing saturated fatty acids, which might reflect increased *de novo* lipogenesis.

Another support of the steatogenic role of ECs in humans is that exogenous phytocannabinoids affects the severity of steatosis. In a prospective study of 315 untreated patients with chronic hepatitis C, daily cannabis smoking over the 6 month period preceding biopsy was identified as an independent predictor of severe steatosis (odds ratio [OR], 2.1; 95% confidence interval [CI], 1.01-4.5).<sup>[58]</sup> Interestingly, in another cohort of 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, suggesting CB1 receptor activation and signaling. This association was highly significant for genotype 3, but not 1. Moreover, in genotype 3 patients, CB1 expression correlated strongly with the lipogenic transcription factor SREBP-1c and its downstream target FAS, a finding not observed in genotype one patients.<sup>[59]</sup>

### Clinical trials on endocannabinoids and fatty liver

Interventions through modulation of EC pathways have been conducted, the most famous drug is Rimonabant (Acomplia-Sanofi-Aventis), which was the first selective CB1 antagonist introduced into clinical practice. The efficacy and safety of Rimonabant in weight reduction and improving metabolic syndrome parameters in different populations have been assessed in several trials. The four large 'Rimonabant in obesity' (RIO) studies involved over 6600 participants, composed of four separate trials: RIO North America,<sup>[60]</sup> RIO Europe,<sup>[61]</sup> RIO Diabetes,<sup>[62]</sup> and RIO Lipids,<sup>[63]</sup> in addition to other randomized double-blind Rimonabant-placebo controlled trials, namely the Serenade,<sup>[64]</sup> Stradivarius,<sup>[65]</sup> Arpeggio<sup>[66]</sup> and Adagio-lipids studies.<sup>[67]</sup>

In all these trials, there was a clear benefit of Rimonabant on weight reduction, abdominal obesity, liver steatosis and improvement of other cardiometabolic syndrome parameters, including improved insulin sensitivity, elevated plasma adiponectin and HDL cholesterol, and reduced plasma TG and low-density lipoprotein cholesterol levels.

Interestingly, the most worrying adverse event of this drug was the increased incidence of psychiatric disorders: depression, anxiety, irritability, and aggression.<sup>[68]</sup> According to a meta-analysis of randomized trials, Rimonabant caused significantly more adverse events than did placebo (OR = 1.4;  $P = 0.0007$ ; number needed to harm = 25 individuals [95% CI 17-58]), and 1.4 times more serious adverse events (OR = 1.4;  $P = 0.03$ ; number needed to harm = 59 [27-830]).<sup>[69]</sup> Furthermore, two suicide deaths were reported in patients taking this drug. In the US Food and Drug Administration analysis of the four major trials as well as unpublished trials, psychiatric adverse events were found to be more common with Rimonabant (20 mg/day) than placebo Rimonabant. The drug was never approved in the United States for the treatment of obesity. Consequently, the marketing approval for Rimonabant has since been removed by the European Regulatory Authorities.<sup>[70]</sup>

### ROLE OF CB2 RECEPTORS IN FATTY LIVER DISEASE

As mentioned earlier, the role of CB2 receptors has been recognized in the last few years as a critical modulator of inflammation, pain, bone loss and in liver pathophysiology, especially liver inflammation and fibrogenesis associated with chronic liver diseases, ischaemia/reperfusion (I/R)-induced liver injury, and hepatic encephalopathy-associated with acute liver failure.<sup>[71-73]</sup>

### Role of CB2 receptors in the development of fatty liver

In contrast to CB1 receptors, the role of CB2 receptors in the development of fatty liver is under-investigated. Normal adult liver shows weak expression of CB2 receptors, which is up-regulated in pathological conditions.

Animal studies have shown that CB2 receptor expression undergoes a strong induction in adipose tissue that correlated with increased fat inflammation, and a moderate induction in the liver of ob/ob mice (genetically leptin-deficient mice) and mice fed a high fat diet. This expression arises mainly from the macrophage-enriched stromal vascular fraction of the adipose tissue and the non-parenchymal liver cells. The potential role of CB2 in the pathogenesis of fatty liver has been supported by the finding that the administration of JWH-133 (CB2 agonist) enhanced liver TG accumulation, insulin resistance and potentiated fat inflammation in wild-type mice fed with a high-fat diet for 6 weeks.<sup>[74]</sup> In contrast, genetic or pharmacological inactivation of CB2 receptors decreased adipose tissue macrophage infiltration, protected mice from both age-related and diet-induced insulin resistance.<sup>[75]</sup> In human studies, CB2 receptors are expressed in all liver samples from patients with steatosis and steatohepatitis. Specifically, receptors were localized

in hepatic stellate cells, hepatocytes and cholangiocytes. In contrast, biopsies from normal liver showed neither parenchymal nor non-parenchymal cells CB2 expression.<sup>[76]</sup> Taken together, it is likely that CB2 receptors have a potential role on liver steatogenesis and fat inflammatory response associated with insulin resistance.

### Role of CB2 receptors in fibrogenesis

The role of CB2 receptors in fibrogenesis has not been well-characterized. However, there is some evidence of a potential anti-fibrogenic role of CB2 activation. Interestingly, CB2 has been proposed to have anti-fibrogenic properties while CB1 has been proposed as a pro-fibrogenic activator.<sup>[71,77]</sup> Selective activation of hepatic CB2 receptors significantly reduced hepatic collagen content in rats with pre-existing cirrhosis and enhanced regenerative response to acute liver injury.<sup>[78]</sup> In line with this observation, treatment with CB2 agonist, JWH-133, reduced the injury and accelerated liver regeneration.<sup>[79]</sup> In contrast, CB2<sup>-/-</sup> mice had enhanced response to fibrogenic stimuli and delayed liver regeneration in response to carbon tetrachloride 14CCl4-induced injury.<sup>[78]</sup> In liver biopsy specimens from patients with active cirrhosis of various etiologies, CB2 receptors are highly up-regulated in the cirrhotic liver, predominantly in hepatic fibrogenic cells. In contrast, CB2 receptors were not detected in normal human liver. Furthermore, they were also detected in cultured hepatic myofibroblasts and in activated hepatic stellate cells.<sup>[71]</sup>

These data support the potential anti-fibrotic role of CB2 receptors and signal the therapeutic potential of non-psychoactive CB2 agonists in the treatment of liver fibrosis.

### CONCLUSIONS – FUTURE PERSPECTIVES

There are overwhelming experimental and clinical data supporting a major role of ECs in the pathogenesis of liver steatosis and other features of chronic liver disease, with potential therapeutic interventions. Tempering these promises, concern for psychiatric safety of EC pathway interventions has unfortunately put an end to the clinical development of some antagonists/inverse agonists that enter the brain. Nevertheless, considering the meaningful clinical benefits expected from therapeutic developments in this line in liver diseases and other fields, research efforts are ongoing in several diseases with significant success.

The use of peripherally restricted compounds with CB1 antagonist properties and limited brain penetration, such as CBD is promising.

A phase-1 pharmacokinetic study of potent and selective CB1 antagonist (cp-945598) in patients with NASH is ongoing.<sup>[80]</sup> Another phase-2 study to assess the effect of

CBD on liver fat levels in subjects with fatty liver disease has been completed and waiting the results.<sup>[81]</sup> Another phase-2 study to assess two cannabinoids GW 42004 and GW42003 alone, or in combination in patients with type 2 diabetes is completed, reports due soon.<sup>[82]</sup> These hopes are in line with previously reported significant results in the treatment of painful diabetic neuropathy and spasticity of multiple sclerosis with the use of nabiximols (Sativex) with a principal active cannabinoid components (THC and CBD). Sativex is the first cannabis-based medicine to be licensed in the UK for multiple sclerosis and it has approval in many European countries, as well as in Canada.<sup>[83,84]</sup>

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