

## Review article

## Recent advances in the understanding of the role of the endocannabinoid system in liver diseases

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## ABSTRACT

Endocannabinoids are ubiquitous signalling molecules that exert their effects through a number of specific cannabinoid receptors. Recent studies have indicated that this endocannabinoid system is involved in the pathophysiological processes associated with both acute and chronic liver diseases as well as in the complications that arise from these diseases such as hepatic encephalopathy and cardiac problems. Targeting this signalling system has been useful in ameliorating some of the symptoms and consequences in experimental models of these liver diseases. This review summarises the recent advances into our knowledge and understanding of endocannabinoids in liver diseases and highlights potential novel therapeutic strategies that may prove useful to treat these diseases.

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## 1. Introduction

Marijuana and its derivatives have been used in medicine for centuries; however, it was not until the isolation of the psychoactive component of *Cannabis sativa* ( $\Delta^9$ -tetrahydrocannabinol; THC) and the subsequent discovery of the endogenous cannabinoid signalling system that research into the therapeutic value of cannabinoids re-emerged. Ongoing research is determining that regulation of the endocannabinoid system may be effective in the treatment of pain [1,2], glaucoma [3], and neurodegenerative disorders such as Parkinson's disease [4] and multiple sclerosis [5]. In addition, cannabinoids might be effective antitumoral agents because of their ability to inhibit the growth of various types of cancer cell lines in culture [6–9] and in laboratory animals [10].

The endogenous cannabinoid system consists of the cannabinoid receptors, their endogenous ligands (endocannabinoids) and the proteins for their synthesis and inactivation [11]. The cannabinoid receptors are seven-transmembrane-domain proteins coupled to  $G_{i/o}$  type G-proteins [11]. To date, there are two definitive cannabinoid receptors, Cb1 and Cb2, as well as a putative involvement of the vanilloid receptor VR1. More recently, the orphan receptor GPR55 was shown to function as a novel cannabi-

noid receptor [12]. Cb1 receptors are found predominantly in the central nervous system, but they can also be found in most peripheral tissues including immune cells, the reproductive system, the gastrointestinal tract and the lungs [13–15]. Cb2 receptors are found predominantly in the immune system; i.e. tonsils, spleen, macrophages and lymphocytes [13–15].

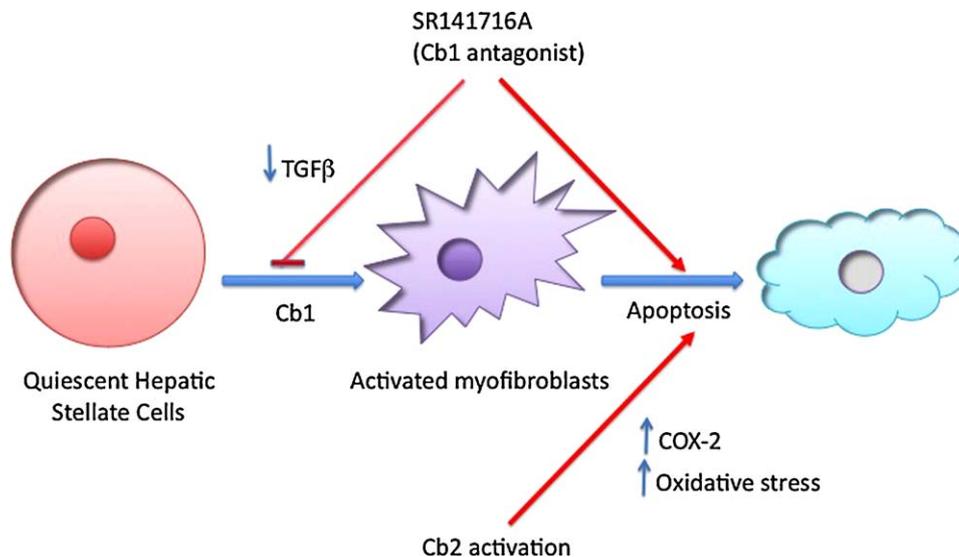
To date, many endocannabinoids, all of which are lipid molecules, have been identified with varying affinities for the receptors. Anandamide (AEA) was the first endogenous ligand to be identified [13], which acts as a partial Cb1 agonist and weak Cb2 agonist. It has also been shown to activate the GPR55 receptor [12]. Whilst the physiological roles of many of the other ligands have not yet been fully clarified, AEA has been implicated in a wide variety of physiological and pathological processes.

Currently, there are two biosynthesis pathways for AEA. The first involving the remodelling of an existing membrane phosphoglyceride. This happens through the calcium-dependent *N*-transacylation of phosphatidylethanolamine with arachidonic acid to form *N*-arachidonyl-phosphatidyl-ethanolamine, which is then hydrolysed to AEA [11,16]. The enzyme responsible for the catalysis of this pathway is phospholipase D [11]. The second pathway is via the *de novo* synthesis of AEA from arachidonic acid and ethanolamine by the enzyme anandamide amidohydrolase catalysing the reverse reaction from high levels of ethanolamine [16]. After synthesis, AEA is rapidly inactivated via a tightly controlled series of events involving sequestration by cells and enzymatic hydrolysis. The mechanism of AEA uptake is largely unknown, with some data suggesting that it is via passive diffusion and other data indicating that it is through the presence of an active transporter [17]. Regardless of the mechanism, this uptake is a rapid

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**Fig. 2.** Schematic diagram depicting the effects of cannabinoids and cannabinoid receptor activation of hepatic stellate cell activation and the resulting liver fibrosis; Cb, cannabinoid receptor; COX-2, cyclooxygenase 2; TGFb, transforming growth factor b.

Conversely, Cb2 receptor activation appears to exert antifibrogenic effects [24]. Using Cb2 genetic knockout mice, Julien et al. [24] demonstrated an augmented fibrogenic response to carbon tetrachloride-induced liver injury [24]. Activation of Cb2 receptors on activated hepatic stellate cells inhibited growth via a COX-2-dependent pathway and increased apoptosis via a mechanism involving increased oxidative stress [24]. Our knowledge of the effects of cannabinoids on liver fibrosis is summarised in Fig. 2.

#### 4. Endocannabinoids mediate hepatic/ischemia reperfusion injury

Ischemia reperfusion injury occurs during myocardial infarction, stroke and organ transplant. The mechanism of injury involves the acute generation of reactive oxygen and nitrogen species that follows the reoxygenation of the tissue, which results in direct tissue injury, cell death and ultimately organ failure [26]. In the liver, ischemia reperfusion injury occurs during liver transplant or during surgery to treat extensive hepatic trauma or resect large intra-hepatic lesions [27]. Upon restoring the blood supply, the liver is subjected to further insult, aggravating the injury already caused by the ischemia [27]. Activation of Cb2 by a synthetic agonist in a mouse model of hepatic ischemia reperfusion injury significantly attenuated the extent of liver damage (assessed by serum levels of liver enzymes) [28]. Associated with this Cb2-mediated protection was a decrease in tumour necrosis factor- $\alpha$  in tissue and serum, tissue lipid peroxidation, hepatocyte apoptosis and inflammatory cell infiltration [28].

#### 5. Endocannabinoids attenuate cholangiocyte proliferation during cholestatic liver diseases

During the course of chronic cholestatic liver diseases such as primary sclerosing cholangitis, primary biliary cirrhosis, liver allograft rejection and graft-versus-host disease, cholangiocytes exhibit marked proliferative capacity followed by cholangiocyte loss [29]. As mentioned above, the expression of FAAH is decreased in cholestatic liver disease, indicating that AEA is probably increased during the course of these diseases. The role of endocannabinoid signalling in the progression of this process is largely unknown. Using a mouse model of cholestatic liver disease, we have previously shown that chronic AEA treatment *in*

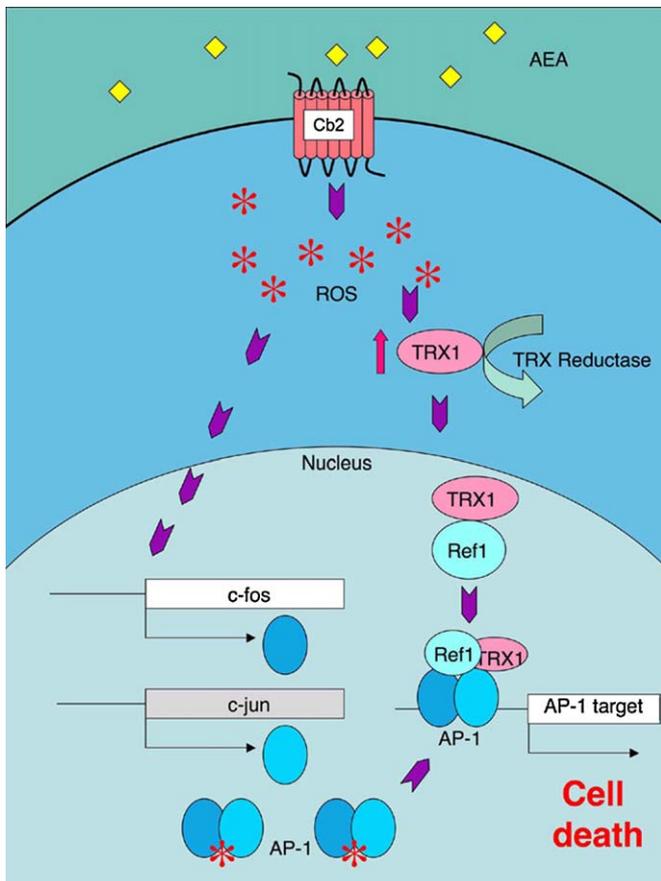
*in vivo* inhibited biliary growth after bile duct ligation, which could be inhibited by specific Cb2 antagonists [30]. Coupled to the effects of AEA on cholangiocyte proliferation was an increased accumulation of reactive oxygen species [30]. This, in turn, results in an increase in expression and nuclear translocation of Thioredoxin 1 where it interacts with Ref1 [30]. In addition, increased reactive oxygen species result in an upregulation of c-Fos and c-Jun expression, which together constitute the AP-1 DNA-binding activity. The AEA-induced AP-1 transcriptional activity is inhibited in the absence of the TRX1/Ref1 complex, thus suggesting post-translational control of Thioredoxin 1/Ref1 in AP-1 transcriptional activity. A schematic diagram summarizing these data can be found in Fig. 3. These data suggest that therapies designed to modulate the endocannabinoid system may prove beneficial in regulating the cholangiocyte proliferation resulting from biliary obstruction, which can be an early event in cholestatic liver diseases.

#### 6. The effects of cannabinoids on complications of end-stage liver disease

##### 6.1. Hepatic encephalopathy

Hepatic encephalopathy is a complication of both acute and chronic liver failure characterised by neurological symptoms ranging from shortened attention span to stupor and coma [31]. Traditionally, hepatic encephalopathy was thought to involve ammonia neurotoxicity due to its inefficient removal by the damaged liver [32]. However, more recently it has been acknowledged that hepatic encephalopathy involves the dysregulation of many neurotransmitter systems, including the monoaminergic [33–35], opioidergic [36] and GABA-ergic [37,38] systems.

The endocannabinoid 2-AG has been found to be elevated in the brains of mice 3 days after the injection of the hepatotoxin thioacetamide [39]. In addition, treatment with 2-AG ameliorated the neurological symptoms of hepatic encephalopathy in these mice [39], which was more pronounced with co-administration with the Cb1 antagonist SR141716A. This suggests that concerted activation of Cb2 and inhibition of Cb1 receptor function may be an effective treatment for hepatic encephalopathy [39]. In another study, researchers showed an increase in both Cb1 and Cb2 expression in the hippocampus using the same mouse model of hepatic encephalopathy as above [40]. Associated with this increase was



**Fig. 3.** Schematic diagram of the potential cell signalling mechanisms responsible for the AEA-induced cell death of proliferating cholangiocytes after BDL. Activation of Cb2 by AEA results in increased intracellular ROS accumulation (red asterisks). This, in turn, results in an increased expression and nuclear translocation of TRX1 (but not TRX2) where it interacts with Ref1. In addition, increased ROS results in an upregulation of c-Fos and c-Jun expression, which together constitute the AP-1 DNA-binding activity. However, this transcription factor, under oxidised conditions such as that seen here (i.e., in the presence of increased ROS) fails to retain AP-1 transcriptional activity. This apparent dichotomy is resolved by the reducing properties of the TRX1/Ref1 complex, which restores the AP-1 complex to its reduced form thereby allowing DNA-binding activity and the subsequent transcription of AP-1 target genes that are responsible for the AEA-induced cell death.

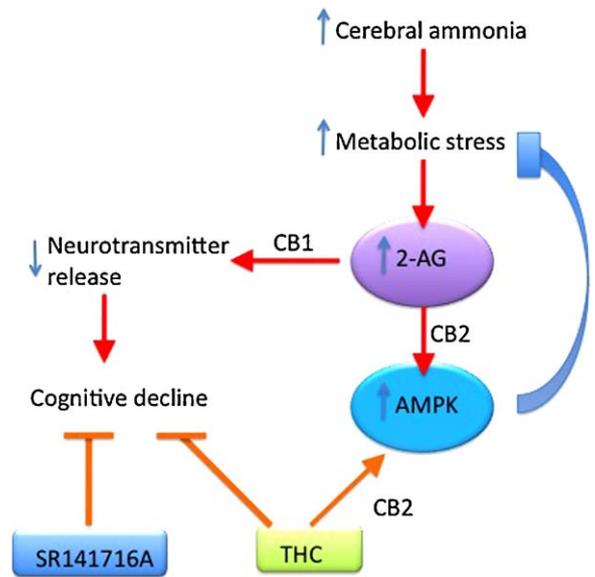
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an activation of AMP-activated protein kinase [40]. Treatment of these animals with the psychoactive component of marijuana, THC, ameliorated the cognitive and motor function decline seen after thioacetamide treatment [40]. The protective effects of THC were abolished in Cb2-deficient mice [40]. A summary of these findings can be found in Fig. 4.

## 6.2. Vascular and cardiac abnormalities in cirrhosis

Cirrhosis and portal hypertension are associated with cardiovascular abnormalities [41]. The pathogenesis of cirrhotic cardiomyopathy is unclear. However, several factors including central neural dysregulation [42] and humoral factors such as nitric oxide likely play a pathogenic role. Recent evidence suggests that cannabinoids may also play a role in the pathogenesis of cardiomyopathy [43].

In bile duct-ligated cirrhotic rats, cardiac responsiveness to b-adrenergic stimulation is blunted [44]. This effect can be reversed by the *in vitro* and *in vivo* administration of a Cb1 antagonist [44,45] and was suggested to be a result of the direct effect on cardiac muscle, rather than an indirect effect of Cb1 inhibition on peripheral



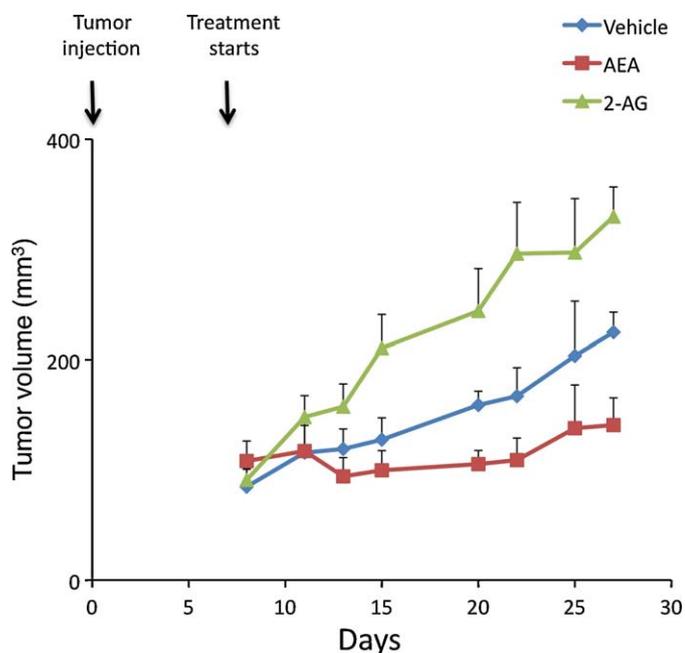
**Fig. 4.** A schematic representation of current knowledge of the effects of endocannabinoids in hepatic encephalopathy. 2-AG, 2-arachidonyl glycerol; AMPK, AMP activated protein kinase; Cb, cannabinoid receptor; THC, tetrahydrocannabinol.

vasculature [45]. It is suggested that in the cirrhotic heart, the local overproduction of anandamide exerts a negative inotropic effect via Cb1 receptors [43]. However, the exact location of the anandamide production in this model is unknown.

## 7. Effects of cannabinoids in liver cancer

### 7.1. Hepatocellular carcinoma

Hepatocellular carcinoma is the most predominant liver cancer: it is the fifth most common malignancy in men and the eighth in women worldwide [46]. Despite the treatment options currently available, the prognosis of hepatocellular carcinoma remains poor [47]. The role of cannabinoids in hepatocarcinogenesis is unclear, although Xu et al. [48] have demonstrated that the expression of cannabinoid receptors Cb1 and Cb2 are increased in cancerous tissue compared to non-malignant liver tissue [48]. The expression of both Cb1 and Cb2 were closely correlated to histopathological grade, with well-differentiated hepatocellular tumours exhibiting a higher expression and poorly differentiated tumours showing low Cb1 immunoreactivity [48]. Furthermore, there was no correlation between cannabinoid receptor expression and survival rate. However, high Cb1 and Cb2 expression correlated with disease-free survival [48]. Modulation of both cannabinoid receptors by the synthetic cannabinoid WIN 55,212-2 (WIN) induces apoptosis in a hepatocellular carcinoma cell line [49]. Associated with the inhibitory effects of WIN was an up-regulation of death-signalling factors Bax and Bcl-Xs and down regulation of the survival factors Survivin, Hsp72 and Bcl-2 [49]. These effects were via the upregulation of PPAR $\gamma$ -mediated transcriptional activity [49]. In parallel, WIN has been shown to sensitise HepG2 cells to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis [50] via the upregulation of the TRAIL receptor (DR5) [50]. Taken together, these data suggest that cannabinoid receptor expression may be useful as prognostic markers for hepatocellular carcinoma, and that the modulation of cannabinoid receptor activity may be an important therapeutic target for the treatment of hepatocellular carcinoma.



**Fig. 5.** Opposing effects of the endocannabinoids AEA and 2-AG on cholangiocarcinoma growth occurs in an *in vivo* xenograft model of cholangiocarcinoma. Mz-ChA-1 cells were injected into the flank of athymic mice. After tumours were established, mice were treated with 10 mg/kg/day (ip) AEA, 2-AG or vehicle, 3 days per week for 28 days and tumour volume assessed.

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## 7.2. Cholangiocarcinoma

Cholangiocarcinoma arises from the neoplastic transformation of cholangiocytes and can present as intrahepatic, perihilar or distal extrahepatic tumours [51]. Typically, cholangiocarcinomas are adenocarcinomas and have a poor prognosis and limited treatment options. This is due at least in part, to the late presentation of symptoms and the relative resistance to current treatment options [52]. The incidence of both intra- and extra-hepatic cholangiocarcinoma is typically higher in Asian countries [53]. The mortality rates for intrahepatic cholangiocarcinoma have increased since the 1970s, whereas deaths from extrahepatic cholangiocarcinoma have declined in most countries [53]. There is a slight preponderance for cholangiocarcinoma in males [54] and the incidence in both sexes increases with age [53].

We have previously shown the differential effects of AEA and 2-AG on cholangiocarcinoma growth *in vitro* [55] using a number of cholangiocarcinoma cell lines and in a xenograft model of cholangiocarcinoma (Fig. 5) [55,56]. The growth-promoting effects of 2-AG were found to be via a cannabinoid receptor-independent mechanism involving the disruption of lipid raft structures in the cell membrane [55]. Conversely, the antiproliferative actions of AEA were via a mechanism involving the stabilisation of lipid rafts in the plasma membrane and the recruitment of death receptor complexes into these membrane microdomains [55]. Furthermore, we have shown that AEA suppresses tumour growth *in vivo* using a xenograft model of cholangiocarcinoma [56] and that there was a concomitant activation of the non-canonical Wnt pathway via upregulation of Wnt 5a [56]. More recently, we have demonstrated that the antiproliferative actions of AEA are also associated with an increase in Notch 1 expression and activation, whereas the growth-promoting effects of 2-AG can be associated with an increase in Notch 2 expression and activation [57]. The Notch signal transduction pathways require proteolytic processing of the Notch proteins by a membrane-bound  $\gamma$ -secretase complex

[58–61]. The dependence and recruitment of the  $\gamma$ -secretase complex to lipid raft structures has previously been shown to modulate  $\gamma$ -secretase activity [62,63]. Therefore it is conceivable that agents that stabilise or disrupt lipid raft structures such as cannabinoids [55] may indeed also regulate the Notch signalling pathway. The involvement of lipid rafts in the differential activation of the Notch signalling pathways by endocannabinoids is a topic of ongoing research in our laboratory. Furthermore, activation of the Wnt signalling pathway has been shown to overlap and crosstalk with the Notch signalling pathway [64–66]. Indeed, activation of Notch 1 has been shown to upregulate the expression of Wnt5a in a number of cell models [67].

## 8. Conclusions

From the work described above it is obvious that there remain large gaps in our knowledge concerning the role of cannabinoids in the pathological processes associated with acute and chronic liver diseases. Modulation of endocannabinoid signalling seems to be a promising target for the treatment of not only the type of liver disease in question (e.g. cholestatic liver diseases) but may also alleviate the symptoms arising from the complications of acute and chronic liver diseases (e.g. hepatic encephalopathy). Increased efforts in dissecting the molecular mechanisms by which cannabinoids regulate the pathophysiology of these diseases is necessary to design multifaceted approaches to target key symptoms and consequences of these diseases. In addition, the discovery of the novel endocannabinoid receptor GPR55 brings new and exciting opportunities to target and manipulate the activity of this receptor for the treatment of liver diseases. Research into the role of GPR55 in various liver diseases including liver cancer, is currently underway.

## Conflict of interest statement

None declared.

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