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Abstract
SUMMARY

Prevalence of non alcoholic steatohepatitis (NASH) rises steadily in Western countries with the obesity epidemics. NASH is associated with activation of liver fibrogenesis and exposes to cirrhosis and associated morbi-mortality. The cannabinoid system is increasingly emerging as a crucial mediator of acute and chronic liver injury. Recent experimental and clinical data indicate that peripheral activation of cannabinoid CB1 receptors promotes insulin resistance and liver steatogenesis, two key steps in the pathogenesis of non alcoholic fatty liver disease. Moreover, CB1 receptors also enhance progression of liver fibrogenesis. These findings provide a strong rationale for the use of CB1 antagonists in the management of NASH.

MESH Keywords Arachidonic Acids ; therapeutic use ; Cannabinoids ; therapeutic use ; Fatty Liver ; drug therapy ; physiopathology ; prevention & control ; Humans ; Insulin Resistance ; physiology ; Polyunsaturated Alkamides ; therapeutic use ; Receptor, Cannabinoid, CB1 ; drug effects ; physiology ; Receptor, Cannabinoid, CB2 ; drug effects ; physiology ; Receptors, Cannabinoid ; drug effects

Author Keywords fatty liver, non alcoholic steatohepatitis, endocannabinoids, cannabinoid receptors, liver fibrosis

Preparations of the hemp plant Cannabis Sativa have been used for medicinal purposes over centuries. THC was identified in 1964 as the predominant cannabinoid compound responsible for psychoactive effects of marijuana. Thereafter, cloning of cannabinoid receptors CB1 and CB2 in the early nineties constituted a determinant milestone in the characterization of a novel biological system with a wide array of biological functions. Moreover, improvement in the understanding of the signaling mechanism responsible for cannabinoid actions has fostered research efforts in the development of therapeutic applications. Consequently, capsules of THC and its synthetic analog nabilone are approved in several countries for the management of chemotherapy-induced nausea and vomiting (1) and rimonabant, a selective CB1 receptor antagonist has been available since 2 years in Europe as an adjunctive treatment of obesity or overweight with associated type 2 diabetes or dyslipidemia (2 –4).

In this context, accumulating experimental and clinical data have stressed the crucial role of the cannabinoid system in the pathogenesis of non alcoholic fatty liver disease (NAFLD). NAFLD is closely linked to the metabolic syndrome and the obesity epidemic (5) and currently accounts for a rising cause of liver injury, with a 20–30% prevalence in Western countries. The spectrum of the disease ranges from simple steatosis, a condition generally associated to a benign liver outcome, to steatohepatitis, an entity that associates steatosis, liver inflammation, hepatocellular injury. The latter stage is associated with an activation of fibrogenic pathways and carries a 10 –20 % risk of cirrhosis after 10 to 20 years. As shown in several recent studies, NASH is entailed with increased liver-related mortality, due to end-stage liver disease or development of hepatocellular carcinoma (6). The present review will depict evidences suggesting that cannabinoid receptor antagonism may offer novel therapeutic approaches for the management of NAFLD.

THE ENDOCANNABINOID SYSTEM

The endocannabinoid system comprises endogenous lipidic ligands, specific G-protein coupled receptors (CB1 and CB2) and proteins that are responsible for their biosynthesis, cellular uptake and degradation (7 –9).

CB1 receptor was originally cloned from a rat brain library owing its high level of expression in the central nervous system (10), and subsequent studies have shown its presence at lower levels in many peripheral tissues. Expression of CB2 receptors predominates in the immune system, and although more restricted, is increasingly demonstrated in several cells (8 , 11 , 12). Recent reports also suggest the existence of additional cannabinoid receptors.

Endocannabinoids are hydrophobic fatty-acid derived compounds with predominantly autocrine/paracrine effects, among which anandamide (arachidonoyl ethanolamide) and 2-arachidonoyl glycerol (2-AG) are best known. Both compounds are synthetized on demand and are rapidly degraded by fatty acid amide hydrolase (FAAH) or monoacyglycerol lipase, following ligand binding and cellular reuptake (8 , 9 , 11 , 12). Anandamide shows a higher affinity for CB1 than CB2 receptors and is therefore considered a major endogenous CB1 ligand, whereas 2-arachidonoyl glycerol binds both receptors similar affinity (13). In addition, both compounds also induce CB1 and

CB2-independent effects. Lipid mediators other than anandamide and 2-AG have been reported to bind CB receptors, but their biological significance remains undetermined.

MODULATORS OF CANNABINOID RECEPTORS AS THERAPEUTIC AGENTS

Rimonabant has been the first CB1 antagonist to reach the market in Europe (2 –4). The drug was initially developed for the treatment of obesity, in light of the positive impact of phyto-and endocannabinoids on central appetite regulating pathways. It soon became clear that CB1 anatagonism produces metabolic effects beyond those expected from weight loss alone, including improvement in dyslipidemia, insulin resistance or diabetes (14). In keeping with clinical data, experimental studies have established that multiple peripheral mechanisms contribute to the beneficial effects of CB1 antagonism by enhancing energy expenditure, peripheral lipolysis and insulin sensitivity among others (15 , 16). Accordingly, trials are under way to further define the impact of CB1 antagonism on dyslipidemia, type 2 diabetes and cardiovascular morbidity. Other therapeutic applications under evaluation also include management of alcohol and nicotine-dependence or neurodegenerative disorders (9). The safety of CB1 antagonists in obesity has been questioned given the occurrence of modest rates of anxiety and depression in susceptible individuals (14). As a result, the FDA denied approval of rimonabant pending additional data, whereas Merck recently suspended the development of taranabant in obesity, due to safety concerns. In this context, the development of peripherally restricted CB1 antagonists could prove of interest, by avoiding central adverse effects.

Although selective agonists and antagonists of CB2 receptors have not reached a clinical stage yet, preclinical studies nevertheless suggest meaningful therapeutic applications as anti-inflammatory, analgesic or anti-allergic compounds (9 , 17). Of interest, such compounds should be devoid of central adverse effects.

Identification of cannabinoid receptors as potential therapeutic targets for the management of liver diseases (7) has emerged recently with the demonstration that CB1 receptors contribute to the pathogenesis of cirrhotic portal hypertension (18 , 19). Soon after, additional studies uncovered a key role of cannabinoids in metabolic and ethanol-induced fatty liver, ischemia reperfusion, or the scarring process associated with chronic liver disease (20 –25).

PATHOGENESIS OF NAFLD

It is now admitted that metabolic steatosis and promoted by insulin resistance are in tight relationship (26). Thus, rodent models have shown that resistance to insulin promotes lipolysis in the adipose tissue, thereby increasing delivery of free fatty acids to the liver (26). Moreover, in the liver, hyperinsulinemia triggers de novo fatty acid and impairs β-oxidation and lipid disposal. Conversely however, steatosis may also contribute to hepatic insulin resistance (26). The transition from steatosis to NASH is poorly understood and appears multifactorial. Excessive accumulation of free fatty acids leads to increased oxidative stress and lipid peroxidation, thereby resulting in cellular injury. Moreover, enhanced cytokine production by infiltrating macrophages in adipose tissue and the liver are also incriminated in the progression of injury (5).

CANNABINOID RECEPTOR ANTAGONISM REDUCES DEVELOPMENT OF NAFLD

CB1 receptors promote metabolic steatosis and insulin resistance

Recent findings have shown that the hepatic cannabinoid system is activated in NAFLD. Thus, in the experimental model of diet-induced obesity, hepatic anandamide levels are increased following inhibition of its degradation by FAAH and CB1 receptor expression is strongly induced in hepatocytes (23).

Accumulating experimental evidences indicate that CB1 receptors contribute to metabolic steatosis and the related insulin resistance (23 , 24 , 27). CB1 receptor knockout mice are resistant to high fat-diet (HFD) induced obesity and steatosis and to the associated increase in hepatic lipogenesis; moreover, HFD-fed CB1 ablated mice display reduced insulin resistance (23 , 28). In keeping, genetically-obese fa/fa rats treated with rimonabant show reversal of hepatic steatosis and improved insulin sensitivity (27). Interestingly, mice bearing a selective deletion of CB1 receptors in hepatocytes become obese under a high-fat diet but are protected from hepatic steatosis and impaired glucose tolerance (24). Finally, characterisation of functions of upregulated hepatic CB1 receptors during steatogenesis suggests combined enhancement of lipogenesis and inhibition of fatty acid β-oxydation (23 , 24). Collectively, these data indicate that peripheral overactivation of the cannabinoid system promotes obesity-associated fatty liver and insulin resistance. Beyond its contribution to steatogenesis, CB1-dependent endogenous cannabinoid tone may also favour the inflammatory response associated to NASH. Thus, it has been shown that endogenous CB1 activation reduces secretion of adiponectin (29), an adipocytokine with potent anti-inflammatory effects in the liver (30). In keeping with these observations, administration of rimonabant to genetically obese rats induces a significant improvement in the hepatic inflammatory response (27). Clinical studies also indirectly support the potential role of the endocannabinoids and their receptors in the pathogenesis NAFLD. Analysis of pooled 1-year data from four pivotal trials in overweight patients indicates that rimonabant reduces alanine aminotransferase levels, a marker of NAFLD (14). In addition, we recently investigated the impact of cannabis use on steatosis grade in 307 patients with chronic hepatitis C and found that daily cannabis consumption is an independent predictor of severe steatosis (31). Overall, these results provide a strong evidence for a steatogenic role of cannabinoids in humans.

CB receptors regulate liver fibrogenesis

As stated previously, transition from steatosis to NASH is associated with activation of fibrogenic pathways and exposes to the development of liver fibrosis (32). We recently found that expression of CB1 and CB2 receptors is markedly upregulated in cirrhotic liver samples, predominantly in liver fibrogenic cells and demonstrated that CB1 and CB2 receptors display potent pro and anti-fibrogenic properties, respectively (22 , 25). Antifibrogenic properties of CB2 receptors were established in CB2 knockout mice repeatedly exposed to carbon tetrachloride, based on the findings that these mice show enhanced liver fibrosis and increased accumulation of liver fibrogenic cell compared to wild type animals (22). The function of CB1 receptors in liver fibrogenesis was assessed in three different experimental models (chronic carbon tetrachloride or thiacetamide administration and bile duct ligation). Administration of rimonabant or genetic inactivation of CB1 receptors significantly reduced progression of fibrosis (25). Profibrogenic properties of CB1 receptors were ascribed to the overactivation of CB1 receptors expressed by liver fibrogenic cells, leading to a combined enhancement of cell proliferation and decrease in apoptosis rate.

The clinical relevance of these experimental findings was confirmed in an epidemiological study of the impact of cannabis use on fibrosis severity in HCV-infected individuals. Daily cannabis use was documented as an independent predictor of fibrosis severity, suggesting that CB1 signaling dominates over CB2 during chronic hepatitis C (33). A subsequent independent study in a canadian cohort reported similar findings (34).

Emerging role of CB2 receptors in the pathogenesis of NAFLD

Several studies have shown that obesity generates a low grade inflammatory state that contributes to the development of insulin resistance and NAFLD (35 –37). CB2 receptors are potent regulators of innate immunity (38) and we recently investigated their potential role in the pathogenesis of NAFLD. Compared to wild type counterparts, mice invalidated for CB2 receptors are less prone to high fat diet –induced obesity (39). Moreover, CB2 knock out mice are resistant to steatosis and display improved glucose tolerance. The mechanism underlying steatogenic effects of CB2 receptors appears to involve proinflammatory effects of upregulated CB2 receptors in adipose tissue.

CONCLUSION

Accumulating data indicate that the endocannabinoid system is upregulated in NAFLD and plays an important role in the pathogenesis of steatosis and insulin resistance via peripheral pathways. CB1 antagonism has proven efficient in the control of experimental NAFLD and liver fibrogenesis. Recent clinical trials have also established that inactivation of CB1 receptors not only reduces overweight but also improves several parameters of the metabolic syndrome, including insulin resistance and dyslipidemia (14). These observations undoubtedly provide a strong rationale for the evaluation of CB1 antagonists in the management of NASH, as currently underway in phase III clinical trials. Concern with potential adverse central effects of CB1 antagonists should stimulate ungoing efforts to develop peripherally restricted molecules.

References:

- 1 . Hall W , Christie M , Currow D . Cannabinoids and cancer: causation, remediation, and palliation . Lancet Oncol . 2005 ; 6 : 35 - 42
- 2 . Despres JP , Golay A , Sjostrom L . Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia . N Engl J Med . 2005 ; 353 : 2121 - 34
- 3 . Pi-Sunyer FX , Aronne LJ , Heshmati HM . Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial . Jama . 2006 ; 295 : 761 - 75
- 4 . Van Gaal LF , Rissanen AM , Scheen AJ . Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study . Lancet . 2005 ; 365 : 1389 - 97
- 5 . Cortez-Pinto H , de Moura MC , Day CP . Non-alcoholic steatohepatitis: from cell biology to clinical practice . J Hepatol . 2006 ; 44 : 197 - 208
- 6 . Ratziu V , Poynard T . Assessing the outcome of nonalcoholic steatohepatitis? It's time to get serious . Hepatology . 2006 ; 44 : 802 - 5
- 7 . Mallat A , Lotersztajn S . Endocannabinoids and liver disease. I. Endocannabinoids and their receptors in the liver . Am J Physiol Gastrointest Liver Physiol . 2008 ; 294 : G9 - G12
- 8 . Pacher P , Batkai S , Kunos G . The endocannabinoid system as an emerging target of pharmacotherapy . Pharmacol Rev . 2006 ; 58 : 389 - 462
- 9 . Di Marzo V . Targeting the endocannabinoid system: to enhance or reduce? . Nat Rev Drug Discov . 2008 ; 7 : 438 - 55
- 10 . Matsuda LA , Lolait SJ , Brownstein MJ . Structure of a cannabinoid receptor and functional expression of the cloned cDNA . Nature . 1990 ; 346 : 561 - 4
- 11 . Mallat A , Lotersztajn S . Endocannabinoids as novel mediators of liver diseases . J Endocrinol Invest . 2006 ; 29 : 58 - 65
- 12 . Mallat A , Teixeira-Clerc F , Deveaux V . Cannabinoid receptors as new targets of antifibrosing strategies during chronic liver diseases . Expert Opin Ther Targets . 2007 ; 11 : 403 - 9
- 13 . Lotersztajn S , Teixeira-Clerc F , Julien B . CB2 receptors as new therapeutic targets for liver diseases . Br J Pharmacol . 2008 ; 153 : 286 - 9
- 14 . Van Gaal L , Pi-Sunyer X , Despres JP . Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the Rimonabant in Obesity (RIO) program . Diabetes Care . 2008 ; 31 : (Suppl 2) S229 - 40
- 15 . Di Marzo V , Szallasi A . Rimonabant in rats with a metabolic syndrome: good news after the depression . Br J Pharmacol . 2008 ; 154 : 915 - 7
- 16 . Kunos G , Osei-Hyiaman D , Liu J . Endocannabinoids and the control of energy homeostasis . J Biol Chem . 2008 ;
- 17 . Lunn CA , Reich EP , Fine JS . Biology and therapeutic potential of cannabinoid CB2 receptor inverse agonists . Br J Pharmacol . 2008 ; 153 : 226 - 39
- 18 . Batkai S , Jarai Z , Wagner JA . Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis . Nat Med . 2001 ; 7 : 827 - 32
- 19 . Ros J , Claria J , To-Figueras J . Endogenous cannabinoids: a new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat . Gastroenterology . 2002 ; 122 : 85 - 93
- 20 . Batkai S , Osei-Hyiaman D , Pan H . Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury . Faseb J . 2007 ; 21 : 1788 - 800

- 21 . Jeong WI , Osei-Hyiaman D , Park O . Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver . Cell Metab . 2008 ; 7 : 227 - 35
- 22 . Julien B , Grenard P , Teixeira-Clerc F . Antifibrogenic role of the cannabinoid receptor CB2 in the liver . Gastroenterology . 2005 ; 128 : 742 - 55
- 23 . Osei-Hyiaman D , Depetrillo M , Pacher P . Endocannabinoid activation at hepatic CB(1) receptors stimulates fatty acid synthesis and contributes to diet-induced obesity . J Clin Invest . 2005 ; 115 : 1298 - 305
- 24 . Osei-Hyiaman D , Liu J , Zhou L . Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice . J Clin Invest . 2008 ; 118 : 3160 - 9
- 25 . Teixeira-Clerc F , Julien B , Grenard P . CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis . Nat Med . 2006 ; 12 : 671 - 6
- 26 . Postic C , Girard J . Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice . J Clin Invest . 2008 ; 118 : 829 - 38
- 27 . Gary-Bobo M , Elachouri G , Gallas JF . Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker fa/fa rats . Hepatology . 2007 ; 46 : 122 - 9
- 28 . Ravinet Trillou C , Arnone M , Delgorge C . Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice . Am J Physiol Regul Integr Comp Physiol . 2003 ; 284 : R345 - 53
- 29 . Bensaid M , Gary-Bobo M , Esclangon A . The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells . Mol Pharmacol . 2003 ; 63 : 908 - 14
- 30 . Xu A , Wang Y , Keshaw H . The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice . J Clin Invest . 2003 ; 112 : 91 - 100
- 31 . Hezode C , Zafrani ES , Roudot-Thoraval F . Daily cannabis use: a novel risk factor of steatosis severity in patients with chronic hepatitis C . Gastroenterology . 2008 ; 134 : 432 - 9
- 32 . Lotersztajn S , Julien B , Teixeira-Clerc F . Hepatic fibrosis: molecular mechanisms and drug targets . Annu Rev Pharmacol Toxicol . 2005 ; 45 : 605 - 28
- 33 . Hezode C , Roudot-Thoraval F , Nguyen S . Daily cannabis smoking as a risk factor for progression of fibrosis in chronic hepatitis C . Hepatology . 2005 ; 42 : 63 - 71
- 34 . Ishida JH , Peters MG , Jin C . Influence of cannabis use on severity of hepatitis C disease . Clin Gastroenterol Hepatol . 2008 ; 6 : 69 - 75
- 35 . Shoelson SE , Herrero L , Naaz A . Obesity, inflammation, and insulin resistance . Gastroenterology . 2007 ; 132 : 2169 - 80
- 36 . Hotamisligil GS . Inflammation and metabolic disorders . Nature . 2006 ; 444 : 860 - 7
- 37 . Kanda H , Tateya S , Tamori Y . MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity . J Clin Invest . 2006 ; 116 : 1494 - 505
- 38 . Klein TW . Cannabinoid-based drugs as anti-inflammatory therapeutics . Nat Rev Immunol . 2005 ; 5 : 400 - 11
- 39 . Deveaux V , Ichitogani Y , Teixeira-Clerc F . CB2 receptor antagonism reduces diet-induced obesity, insulin resistance and hepatic steatosis . Hepatology . 2007 ; 46 : 308A -