Expert Opinion

CB_1 cannabinoid receptor antagonism for treating inflammation and arthritis

Pfizer: WO2007039797

Didier M Lambert
Université catholique de Louvain, Groupe Cannabinoïdes & Endocannabinoïdes, Unité de Chimie pharmaceutique et de Radiopharmacie, Ecole de Pharmacie, Faculté de Médecine, Avenue E. Mounier 73, UCL-CMFA 73.40, B-1200 Bruxelles, Belgique

The application evaluated here describes the treatment of inflammation and arthritis as two new potential indications for CB_1 cannabinoid receptor antagonists. While these derivatives are known for their actions on food intake, energy metabolism and addictions, little is known on their anti-inflammatory properties. Here, the effects of three representative CB_1 antagonists, SR141716A, AM251 and a pyrazolo[1,5-a][1,3,5]triazine, are described in rodent *in vivo* models of inflammation. CB_1 receptor blockade decreased the lipopolysaccharide-induced cytokines production, the carrageenan-induced paw inflammation and hyperalgesia, and the signs of arthritis following complete Freund's adjuvant injection. However, no CB_1 knockout mice were used to confirm the involvement of the CB_1 receptors in the described effects. Thus, additional research is needed to confirm the usefulness of CB_1 antagonist in the claimed indications.

Keywords: arthritis, carrageenan, CB_1 cannabinoid receptor, cytokines, inflammation, TNF-α.

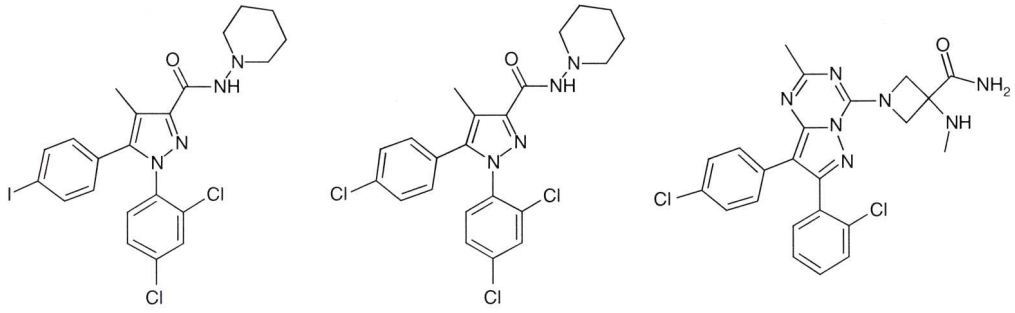
Expert Opin. Ther. Patents (2007) 17(8):1027-1031

1. Introduction

Cannabinoids, either plant-derived or of synthetic origin, mainly exert their effects by binding to two G-protein-coupled receptors (GPCRs), the CB_1 and the CB_2 cannabinoid receptors [1,2]. Over the last 15 years, a considerable drug development effort was made on the CB_1 cannabinoid receptor. This is well illustrated by the blooming of patents and papers disclosing CB_1 cannabinoid receptor antagonists/inverse agonists [3-5].

The CB_1 cannabinoid receptor is a 472-amino acid GPCR widely distributed throughout the body. It is highly expressed in the brain, where it is thought to be the most highly expressed GPCR [6]. The highest density of the receptor is found in the cerebellum, the basal ganglia, the substantia nigra pars compacta and in some regions of the globus pallidus. It is also present in peripheral organs, such as the adrenal glands, liver, gastrointestinal tract, adipose tissue, bone marrow, lungs, testis and uterus. Agonist stimulation of this GPCR activates several signal transduction pathways, mainly through a $G_{i/o}$ type G protein. The signal transduction includes adenylyl cyclase inhibition, mitogen-activated protein kinase (MAPK) and phospholipase C (PLC) activation, as well as N- and P/Q-type calcium channel inhibition and K_{ir} channel activation [7,8]. Evidence indicates that activation of a presynaptic CB_1 cannabinoid receptor can result in inhibition of neurotransmitter (e.g., acetylcholine, noradrenaline, GABA, glutamate) release. In this endocannabinoid retrograde signalling system, endocannabinoids are synthesised in a postsynaptic cell, crossed by an unknown mechanism the synaptic cleft and activate the cannabinoid receptors at the presynaptic cell.

informa
healthcare



1 AM251 **2** SR141716A **3**

Figure 1. Structures of the CB_1 cannabinoid receptor antagonists used in the patent. Compound **3** has been described in a patent from Pfizer [103].

Activation of the cannabinoid receptors results in a decreased release of neurotransmitters, probably through inhibition of calcium influx [9,10].

Blocking the CB_1 cannabinoid receptor presents several therapeutic outcomes. The most reported remains the management of obesity and associated metabolic syndrome (recent extensive reviews have been published [11-14]), together with the treatment of addictions (smoking, alcohol and drug of abuse) [15]. Rimonabant (SR141716A) from sanofi (now sanofi-aventis) was the first CB_1 receptor antagonist to enter clinical trials and has been approved in 42 countries and marketed in 20 to treat obesity and overweight patients with associated cardiovascular risk factors [16,17].

Recently, new indications have been identified for this class of compounds. For example, INSERM and sanofi-aventis described the use of rimonabant in the treatment of hepatic diseases such as liver fibrosis [101,18]. sanofi-aventis claimed the use of pyrazole derivatives (i.e., rimonabant derivatives) in the prevention and treatment of chronic bronchitis and chronic obstructive bronchopneumopathy [102]. The patent application evaluated here claims the usefulness of CB_1 cannabinoid receptor antagonism in treating inflammation and arthritis. The data are shortly summarized and discussed in the Expert opinion section.

2. Chemistry

No original structure has been described in the patent. The three CB_1 cannabinoid receptor antagonists used by the applicant to exemplify the invention are the reference compounds: AM251 or *N*-(1-piperidyl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl])-4-methyl-1*H*-pyrazole-3-carboxamide (**1**), rimonabant or *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (**2**, SR141716A) and 1-[7-2-chlorophenyl)-8-(4-chlorophenyl)-2-methyl-pyrazolo[1,5-*a*][1,3,5]triazin-4-yl]-3-methylamino-azetidine-3-carboxylic amide (**3**) [103]. The structures are illustrated in the Figure 1.

3. Biology

The *in vivo* potency of the three CB_1 cannabinoid receptor antagonists was assessed in two different models of inflammation using two murine species and in a rheumatoid arthritis model in mice.

First, an acute inflammation model consisting of lipopolysaccharide-induced TNF-α production in BALB/c mice was used. One hour after an intravenous injection, the three CB_1 cannabinoid receptor antagonists (dose not given) decreased the plasma levels of the three cytokines monitored: TNF-α, macrophage inflammatory protein-1α (MIP-1α, also named chemokine ligand 3) and IL-10. A similar injection of the CB_2 cannabinoid receptor antagonist AM630 did not elicit changes in the three cytokine levels. Compound **3** was then tested at 3 and 5 mg/kg and was found to decrease prostaglandin E_2 (PGE_2), TNF-α and swelling. Taking these results together, the patent's authors claim that CB_1 cannabinoid receptor antagonists might be beneficial in inflammatory diseases such as arthritis, inflammatory bowel disease and congestive obstructive pulmonary disorders.

In a second *in vivo* model, the carrageenan-induced paw inflammation and hyperalgelsia in rats, following intraperitoneal administration (3 and 5 mg/kg) compound **3** reduced both the thermal and mechanical hyperalgesia.

For the arthritis model, DBA/1 mice were immunised with type II collagen in complete Freund's adjuvant, which resulted in the development of an arthritis after 22 days. Following administration of compound **3** (30 mg/kg), a daily follow-up for 10 days indicated that the compound reduces the signs and symptoms as well as the progression of the arthritis. All the results have been obtained in wild type animals and were only compared with the administration of a CB_2 selective antagonist AM630.

4. Expert opinion

The Pfizer application presents new potential indications for CB_1 cannabinoid antagonists in inflammation, including

inflammatory pain and arthritis, probably by reducing the levels of some cytokines. The modulation of immunity observed in chronic cannabis smokers has been known since the 1970s [19]. It constituted the first clue that the cannabinoid receptors might be involved in the immune system. Nowadays, it is well established that both CB_1 and CB_2 cannabinoid receptors subtypes are present in immune cells (for a review [20]), with a predominance of the CB_2 over the CB_1 cannabinoid receptors. The level of cannabinoid receptor expression has been shown to be dependent upon the activation state of the cell and the activating stimuli or the cell at the origin of the activating stimuli. Also, upregulation and downregulation of cannabinoid CB_1 and CB_2 receptors have been observed. Similarly, depending of the cannabinoid (the partial agonist Δ^9-tetrahydrocannabinol, the non-pschytropic cannabidiol) used and the type of cells studied (macrophages, monocytes, T cells, PBMC, splenocytes, NK cells), the reported effects of cannabinoids on cytokine production seem to be controversial as the increase of IL-1, IL-4 and IL-6 levels, decrease of IFN-γ, IL-2 and IL-10 levels and both increase and decrease of TNF-α and IL-12 levels have been described.

All together, the results described by Pfizer in the application and the analysis of the state-of-the-art about cannabinoids and cytokines modulation raise the question of the exact mechanism of action. With the results given and the methods used, as well as the choice of wild type rodents, it is quite difficult to figure out how these molecules really work: are they antagonising the endogenous endocannabinoids acting at the CB_1 cannabinoid receptor? In contrast, do they promote an endocannabinoid signalling at CB_2 cannabinoid receptors? Are these effects independent of cannabinoid receptors interaction? To rule out this latter hypothesis, the fact that compound **3** is quite structurally different from the diarylpyrazoles **1** and **2** is a first point in favour of a CB_1 cannabinoid receptor mediated effect. Nevertheless, the definitive proof-of-concept would require the use of $CB_1^{-/-}$ $CB_2^{-/-}$ knockout and double knockout mice. On the other hand, the absence of effect of a selective CB_2 cannabinoid receptor antagonist as well as the predominant involvement of the CB_2 cannabinoid receptors in inflammatory pain [21-25] are in favour of a CB_2 endocannabinoid signalling hypothesis. Recently, GlaxoSmithKline reported the discovery of 2-[(2,4-dichlorophenyl)amino]-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]-4-(trifluoromethyl)-5-pyrimidinecarboxamide, a selective CB_2 receptor agonist for the treatment of inflammatory pain [26]. To confirm the CB_2 involvement, the use of double knockout mice or of a putative non-selective CB_1/CB_2 antagonist would have been helpful to delineate the precise mechanism of action of the CB_1 antagonists used here. Conversely, the use of a CB_1 selective agonist or at least the combination of a non-selective agonist and a CB_2 antagonist on the cytokine plasmatic levels could provide some clues to precise the mode of action.

However, the use of a CB_1 selective antagonist instead of a CB_2 selective agonist presents a great therapeutic advantage by avoiding the possibility (e.g., in the case of overdosage of a cannabinoid agonist) to activate the central CB_1 cannabinoid receptors responsible for the psychotropic and mood disturbances side effects.

Recently, Croci and Zarini [27] from sanofi-aventis evidenced the anti-inflammatory and antiallodynic activities of rimonabant in lean and diet-induced obese female rats with complete Freund's adjuvant-induced arthritis. Obesity favours the severity of the induced arthritis, with observed increased leptin and TNF-α levels and decreased adiponectin levels. Rimonabant (**2**), given orally, was able to reduce the inflammation associated with the obesity. As described here in the patent application, chronic use of rimonabant exhibits antinociceptive effects by reducing thermal hyperalgesia and mechanical allodynia. Once again, the effects were markedly enhanced in obese rats. In her commentary paper B. Costa [28] raised the possibility that, in addition of an activation of CB_2 cannabinoid receptors, a desensitisation of the transient receptor potential vanilloid type I (TRPV1) occurs, both hypotheses supported by the increased levels of anandamide and 2-arachidonoylglycerol, the major endocannabinoids, in spinal and supra spinal areas of neuropathic rats (rats with chronic constriction injury of the sciatic nerve) [29].

These two independent observations made by sanofi-aventis and Pfizer open new perspectives for the use of CB_1 cannabinoid receptors antagonists in inflammatory states. Inflammatory pain, inflammation accompanying obesity and arthritis may all benefit from these new therapeutics, although more fundamental research is highly needed to understand the molecular events responsible of these effects.

Bibliography

Papers of special notes have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. HOWLETT AC, BREIVOGEL CS, CHILDERS SR, DEADWYLER SA, HAMPSON RE, PORRINO LJ: Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* (2004) **47**(Suppl. 1):345-358.
2. LAMBERT DM, FOWLER CJ: The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. *J. Med. Chem.* (2005) **48**:5059-5087.
3. LANGE JHM, KRUSE CG: Medicinal chemistry strategies to CB_1 cannabinoid receptor antagonists. *Drug Discov. Today* (2005) **10**:693-702.

•• **An excellent review on the medicinal chemistry strategies applied to the CB_1 cannabinoid receptor antagonists/inverse agonists.**

4. MUCCIOLI GG, LAMBERT DM: Current knowledge on the antagonists and inverse agonists of cannabinoid receptors. *Curr. Med. Chem.* (2005) **12**:1361-1394.

• **An in-depth review on the CB_1 and CB_2 receptors antagonists/inverse agonists.**

5. MUCCIOLI GG, LAMBERT DM: Latest advances in cannabinoid receptor antagonists and inverse agonists. *Expert Opin. Ther. Patents* (2006) **16**:1405-1423.
6. MACKIE K: Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb. Exp. Pharmacol.* (2005) 299-325.
7. MACKIE K: Cannabinoid receptors as therapeutic targets. *Ann. Rev. Pharmacol. Toxicol.* (2006) **46**:101-122.

•• **Recent review on the therapeutic potential of modulating the endocannabinoid system. Both agonists and antagonists strategies are reviewed.**

8. HOWLETT AC, BARTH F, BONNER TI *et al.*: International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacologic. Rev.* (2002) **54**:161-202.
9. WILSON RI, NICOLL RA: Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* (2001) **410**:588-592.
10. WILSON RI, NICOLL RA: Endocannabinoid signaling in the brain. *Science* (2002) **296**:678-682.
11. HENNESS S, ROBINSON DM, LYSENG-WILLIAMSON KA: Rimonabant. *Drugs* (2006) **66**:2109-2119.
12. BIFULCO M, GRIMALDI C, GAZZERRO P, PISANTI S, SANTORO A: Rimonabant: just an antiobesity drug? Current evidence on its pleiotropic effects. *Mol. Pharmacol.* (2007) **71**:1445-1456.
13. PATEL PN, PATHAK R: Rimonabant: a novel selective cannabinoid-1 receptor antagonist for treatment of obesity. *Am. J. Health Syst. Pharm.* (2007) **64**:481-489.
14. LAFONTAN M, PIAZZA PV, GIRARD J: Effects of CB_1 antagonist on the control of metabolic functions in obese Type 2 diabetic patients. *Diabetes Metab.* (2007) **33**:85-95.
15. LE FOLL B, GOLDBERG SR: Cannabinoid CB_1 receptor antagonists as promising new medications for drug dependence. *J. Pharmacol. Exp. Ther.* (2005) **312**:875-883.
16. VAN GAAL LF, RISSANEN AM, SCHEEN AJ, ZIEGLER O, ROSSNER S: 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**:1389-1397.
17. SCHEEN AJ, FINER N, HOLLANDER P, JENSEN MD, VAN GAAL LF: Efficacy and tolerability of rimonabant in overweight or obese patients with Type 2 diabetes: a randomised controlled study. *Lancet* (2006) **368**:1660-1672.
18. TEIXEIRA-CLERC F, JULIEN B, GRENARD P *et al.*: CB_1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nat. Med.* (2006) **12**:671-676.

•• **Describes the positive effects of rimonabant in liver fibrosis models suggesting that blocking the CB_1 receptor might be beneficial in this pathology.**

19. RACHELEFSKY GS, OPELZ G, MICKEY MR *et al.*: Intact humoral and cell-mediated immunity in chronic marijuana smoking. *J. Allergy Clin. Immunol.* (1976) **58**:483-490.
20. CROXFORD JL, YAMAMURA T: Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? *J. Neuroimmunol.* (2005) **166**:3-18.
21. VALENZANO KJ, TAFESSE L, LEE G *et al.*: Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. *Neuropharmacology* (2005) **48**:658-672.
22. GUTIERREZ T, FARTHING JN, ZVONOK AM, MAKRIYANNIS A, HOHMANN AG: Activation of peripheral cannabinoid CB_1 and CB_2 receptors suppresses the maintenance of inflammatory nociception: a comparative analysis. *Br. J. Pharmacol.* (2007) **150**:153-163.

• **The paper describes the local actions of cannabinoids, through distinct CB_1 and CB_2 mechanisms, to suppress mechanical hypersensitivity present in chronic inflammation states.**

23. GUINDON J, DESROCHES J, BEAULIEU P: The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB_2 receptors. *Br. J. Pharmacol.* (2007) **150**:693-701.
24. ROMERO-SANDOVAL A, EISENACH JC: Spinal cannabinoid receptor type 2 activation reduces hypersensitivity and spinal cord glial activation after paw incision. *Anesthesiology* (2007) **106**:787-794.
25. WHITESIDE GT, LEE GP, VALENZANO KJ: The role of the cannabinoid CB_2 receptor in pain transmission and therapeutic potential of small molecule CB_2 receptor agonists. *Curr. Med. Chem.* (2007) **14**:917-936.
26. GIBLIN GM, O'SHAUGHNESSY CT, NAYLOR A *et al.*: Discovery of 2-[(2,4-dichlorophenyl)amino]-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]-4-(trifluoromethyl)-5-pyrimidinecarboxamide, a selective CB_2 receptor agonist for the ttreatment of inflammatory pain. *J. Med. Chem.* (2007) **50**:2597-2600.
27. CROCI T, ZARINI E: Effect of the cannabinoid CB_1 receptor antagonist rimonabant on nociceptive responses and adjuvant-induced arthritis in obese and

lean rats. *Br. J. Pharmacol.* (2007) **150**:559-566.

•• **This paper describes the effects of rimonabant (SR141716A) on inflammation in obese and lean rats. Rimonabant shows higher efficacy in the diet-induced obese rats compared to lean rats.**

28. COSTA B: Rimonabant: more than an anti-obesity drug? *Br. J. Pharmacol.* (2007) **150**:535-537.

29. PETROSINO S, PALAZZO E, DE N V *et al.*: Changes in spinal and supraspinal endocannabinoid levels in neuropathic rats. *Neuropharmacology* (2007) **52**:415-422.

Patents

101. INST. NAT. SANTE RECH. MED.: WO2005084652 (2005).

102. SANOFI-AVENTIS; SEBILLE M: WO2005099690 (2005).

103. PFIZER PROD., INC.; GRIFFITH DA: WO2004069837 (2004).

Affiliation

Didier M Lambert PhD
Université catholique de Louvain,
Groupe Cannabinoïdes & Endocannabinoïdes,
Unité de Chimie pharmaceutique et de
Radiopharmacie, Ecole de Pharmacie,
Faculté de Médecine, Avenue E. Mounier 73,
UCL-CMFA 73.40, B-1200, Bruxelles, Belgique
Tel: +32 2 764 73 47; Fax: +32 2 764 73 63;
E-mail: didier.lambert@uclouvain.be