CB₁ cannabinoid receptor antagonism for treating inflammation and arthritis

Pfizer: WO2007039797

Didier M Lambert
Université catholique de Louvain, Groupe Cannabinoides & Endocannabinoides,
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 CB₁ 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 CB₁ antagonists, SR141716A, AM251 and a pyrazol[1,5-a][1,3,5]triazine, are described in rodent in vivo models of inflammation. CB₁ 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 CB₁ knockout mice were used to confirm the involvement of the CB₁ receptors in the described effects. Thus, additional research is needed to confirm the usefulness of CB₁ antagonist in the claimed indications.

Keywords: arthritis, carrageenan, CB₁ 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 CB₁ and the CB₂ cannabinoid receptors [1,2]. Over the last 15 years, a considerable drug development effort was made on the CB₁ cannabinoid receptor. This is well illustrated by the blooming of patents and papers disclosing CB₁ cannabinoid receptor antagonists/inverse agonists [3-5].

The CB₁ 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⁺ channel activation [7,8]. Evidence indicates that activation of a presynaptic CB₁ 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.
Activation of the cannabinoid receptors results in a decreased release of neurotransmitters, probably through inhibition of calcium influx [9,10].

Blocking the CB₁ cannabinoid receptor presents several therapeutic outcomes. The most reported remains the management of obesity and associated metabolic syndrome (recent extensive reviews have been published [11-13], together with the treatment of addictions (smoking, alcohol and drug of abuse) [14]. Rimonabant (SR141716A) from sanofi (now sanofi-aventis) was the first CB₁ 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 [16,18]; 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 CB₁ 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 CB₁ 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-1H-pyrazole-3-carboxamide (1), rimonabant or N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-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 CB₁ 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 CB₁ 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 CB₂ 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 E₂ (PGE₂), TNF-α and swelling. Taking these results together, the patent’s authors claim that CB₁ 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 hyperalgesia 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 CB₂ selective antagonist AM630.

4. Expert opinion

The Pfizer application presents new potential indications for CB₁ 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 CB₁ and CB₂ cannabinoid receptors subtypes are present in immune cells (for a review [20]), with a predominance of the CB₂ over the CB₁ 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 CB₁ and CB₂ receptors have been observed. Similarly, depending of the cannabinoid (the partial agonist Δ⁹-tetrahydrocannabinol, the non-psychotropic cannabinoid) used and the type of cells studied (macrophages, monocytes, T cells, PMBC, 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 difficult to figure out how these molecules really work: are they antagonising the endogenous endocannabinoids acting at the CB₁ cannabinoid receptor? In contrast, do they promote an endocannabinoid signalling at CB₂ 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 CB₁ cannabinoid receptor mediated effect. Nevertheless, the definitive proof-of-concept would require the use of CB₁⁻⁻⁻ CB₂⁻⁻⁻ knockout and double knockout mice. On the other hand, the absence of effect of a selective CB₂ cannabinoid receptor antagonist as well as the predominant involvement of the CB₂ cannabinoid receptors in inflammatory pain [21-25] are in favour of a CB₂ endocannabinoid signalling hypothesis. Recently, GlaxoSmithKline reported the discovery of 2-[(2,4-dichlorophenyl)amino]-N-[(tetrahydro-2H-pyran-4-yl)methyl]-4-[(trifluoromethyl)-5-pyrimidin-carboxamide, a selective CB₂ receptor agonist for the treatment of inflammatory pain [26]. To confirm the CB₂ involvement, the use of double knockout mice or of a putative non-selective CB₁/CB₂ antagonist would have been helpful to delineate the precise mechanism of action of the CB₁ antagonists used here. Conversely, the use of a CB₁ selective agonist or at least the combination of a non-selective agonist and a CB₂ antagonist on the cytokine plasmatic levels could provide some clues to precise the mode of action.

However, the use of a CB₁ selective antagonist instead of a CB₂ 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 CB₁ 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 antinoceptive effects by reducing thermal hyperalgesia and mechanical allodynies. 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 CB₂ cannabinoid receptors, a desensitisation of the transient receptor potential vanilloid type 1 (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 CB₁ 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.
CB₂, cannabinoid receptor antagonism for treating inflammation and arthritis

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


   ** An excellent review on the medicinal chemistry strategies applied to the CB₁ cannabinoid receptor antagonists/inverse agonists.


   - An in-depth review on the CB₁ and CB₂ receptors antagonists/inverse agonists.


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


   ** Describes the positive effects of rimonabant in liver fibrosis models suggesting that blocking the CB₁ receptor might be beneficial in this pathology.


   • The paper describes the local actions of cannabinoids, through distinct CB₁ and CB₂ mechanisms, to suppress mechanical hypersensitivity present in chronic inflammation states.


27. CROCI T, ZARINI E: Effect of the cannabinoid CB₁ receptor antagonist rimonabant on nociceptive responses and adjuvant-induced arthritis in obese and
150:559-566.

**This paper describes the effects of
rimonabant (SR14716A) 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
150:535-537.

29. PETROSINO S, PALAZZO E, DE N V
et al.: Changes in spinal and supraspinal
endocannabinoid levels in neuropathic rats.

**Patents**

101. INST. NAT. SANTE RECH. MED.: 

102. SANOFI-AVENTIS; SEBILLE M: 

103. PFIZER PROD., INC.; GRIFFITH DA: 

**Affiliation**

Didier M Lambert PhD
Université catholique de Louvain, 
Groupe Cannabinoides & Endocannabinoides, 
Unité de Chimie pharmaceutique et de 
Radiopharacie, 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