Molecular Immunology 54 (2013) 482–492

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Preventive and therapeutic oral administration of the pentacyclic triterpene α,β-amyrin ameliorates dextran sulfate sodium-induced colitis in mice: The relevance of cannabinoid system☆

Israel Matos[1], Allisson Freire Bento[1], Rodrigo Marcon, Rafaela Franco Claudino, João B. Calixto*

Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, 88049-900, Florianópolis, SC, Brazil

ARTICLE INFO

Article history:
Received 16 October 2012
Received in revised form 28 January 2013
Accepted 30 January 2013
Available online 1 March 2013

Keywords:
α,β-Amyrin
Inflammatory bowel disease
Dextran sodium sulfate
Cannabinoid system
Inflammatory cytokines

ABSTRACT

The pentacyclic triterpene α,β-amyrin has been previously reported as an effective compound in the treatment of several inflammatory conditions. Recent evidence indicates that α,β-amyrin displayed its effects through interaction with the cannabinoid pathway. We assessed the anti-inflammatory effects of the α,β-amyrin in the dextran sulfate sodium (DSS)-induced colitis in mice and investigated whether its effects were associated with the interaction with the cannabinoid system. Our results showed that the oral preventive or therapeutic treatment with α,β-amyrin significantly reduced disease activity, body weight loss, colonic damage, as well as colonic myeloperoxidase and *N*-acetylglucosaminidase activities. Moreover, α,β-amyrin decreases the colonic pro-inflammatory mediators tumor necrosis factor (TNF)-α, interleukin (IL)-1β and keratinocyte-derived chemokine (CXCL1/KC), while up-regulating the IL-4 levels. Additionally, we also observed that the α,β-amyrin caused a significant reduction of the adhesion molecules mRNA expression for intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), platelet cell adhesion molecule 1 (PCAM-1), β_2-integrin and protein expression for proliferation marker Ki67, the macrophage molecule CD68 and for adhesion molecule P-selectin. Interestingly, our results also showed that the cannabinoid receptor 1 (CB_1), but not CB_2, pharmacological blockade significantly reversed the beneficial effects of α,β-amyrin in DSS-induced colitis. Besides, our data demonstrated that mRNA expression for both the endocannabinoid hydrolase monoglyceride lipase 1 (MGL1) and fatty acid amide hydrolase (FAAH) were significantly reduced in the colon of α,β-amyrin-treated mice. Altogether, these results suggest that the α,β-amyrin might possess potential therapeutic interest for the treatment of IBD, and also provide new insights for the underlying mechanisms.

1. Introduction

Inflammatory bowel disease (IBD) is a generic term used to describe the two major clinically defined forms, which are Crohn's disease (CD) and ulcerative colitis (UC). CD is a chronic remittent or progressive inflammatory condition that may affect the entire gastrointestinal tract, whilst UC is a relapsing inflammatory disease that is restricted to the colon (Baumgart and Sandborn, 2007; Kaser et al., 2010). The currently most effective treatment for IBD is anti-TNF antibodies (Baumgart and Sandborn, 2007); however, the mechanistic basis of their effectiveness still remains unclear and their use is related with many side effects (Kaser et al., 2010; Perrier and Rutgeerts, 2011). Thus, finding by a new effective therapy for the treatment of IBD has been the focus of studies through the years, especially with regard to active compounds from plant origins.

Natural products are attractive therapeutic sources for the development of new drugs for the management of several diseases (Calixto et al., 2004). In this context, the pentacyclic triterpenes

Abbreviations: COX-2, cyclooxygenase-2; DAI, disease activity index; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; KC, keratinocyte-derived chemokine; MPO, myeloperoxidase; NAG, *N*-acetylglucosaminidase; PMN, polymorphonuclear; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF, tumor necrosis factor interleukin (IL); IFN, interferon; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; PCAM, platelet cell adhesion molecule; CB, cannabinoid receptor; MGL1, monoglyceride lipase 1; FAAH, fatty acid amide hydrolase; CD, Crohn's disease; UC, ulcerative colitis.

☆ This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Apoio à Pesquisa Científica Tecnológica do Estado de Santa Catarina (FAPESC) (Brazil). AFB and RFC are Postdoctoral students, RM is a PhD student and IM is an undergraduate student; all received grants from CNPq.

* Corresponding author at: Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina (UFSC), Campus Universitário, Trindade, 88049-900, Florianópolis, SC, Brazil. Tel.: +55 48-33313414.
E-mail addresses: calixto@farmaco.ufsc.br, calixto2@terra.com.br (J.B. Calixto).
[1] These authors contributed equally to this study.

0161-5890/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.molimm.2013.01.018

are good candidates due their wide range of pharmacological activities, such as anti-inflammatory, anti-carcinogenic, antiviral, antibacterial, anti-nociceptive, gastroprotective, hepatoprotective, cytotoxic, and cardioprotective properties (Baltina et al., 2003; Katerere et al., 2003; Oliveira et al., 2004a, 2004b, 2004c; Ukiya et al., 2002). The pentacyclic triterpene α,β-amyrin is a mixture (1:1) of two isomers, which has been associated with orally anti-nociceptive and anti-inflammatory effects, mainly through the down-regulation of pro-inflammatory mediators, the reduction of mast cell degranulation and inflammatory cell transmigration (Medeiros et al., 2007; Oliveira et al., 2004a, 2005). Recent studies have demonstrated that anti-inflammatory and anti-nociceptive properties of α,β-amyrin are owing to its ability to interact with the cannabinoid system (Chicca et al., 2012; da Silva et al., 2011).

Cannabinoids have been used for thousands of years for their psychoactive properties (Tanasescu and Constantinescu, 2010); however, in the past 20 years the identification of the two cannabinoid receptors, type 1 (CB_1) and type 2 (CB_2), which are G protein-coupled receptors, has greatly contributed to the elucidation of important biological functions of the cannabinoid system (Croxford and Yamamura, 2005). Although the functions of cannabinoids are not yet completely understood, it is now well recognized that the CB_1 receptor is abundant in the central nervous system and its activation has been associated with the control of neurological disorders, most notably pain control (Croxford, 2003; Paszcuk et al., 2011). On the other hand, CB_2 receptor were found most commonly in the periphery, especially in immune cells, and they seem to play an important role in the immune system in both the peripheral and central nervous system (Croxford, 2003; Croxford and Yamamura, 2005).

Accumulated evidence has demonstrated that the activation of either CB_1 or CB_2 receptors by selective cannabinoid agonists is able to protect against experimental intestinal inflammation in mice (Kimball et al., 2006). Furthermore, a study by our group showed that the sesquiterpene β-caryophyllene, a full CB_2 agonist, given orally greatly improves dextran sodium sulfate (DSS)- and oxazolone-induced colitis (Bento et al., 2011). In the present study, we investigated further the possible anti-inflammatory effects of oral treatment with α,β-amyrin in DSS-induced colitis in mice, and determined whether this effect was associated with its ability to interact with cannabinoid pathway. The findings presented here indicate that the α,β-amyrin given orally to mice greatly improve intestinal inflammation caused by DSS treatment, with its effect associated with the CB_1-mediated mechanism and also via the inhibition of endocannabinoid hydrolases.

2. Material and methods

2.1. Animals

Male CD1 mice (8 weeks old, 25–30 g) were bred and obtained in the Laboratory of Experimental Pharmacology at the Federal University of Santa Catarina (Laboratório de Farmacologia Experimental, Universidade Federal de Santa Catarina) and housed collectively (5 animals per box) at a controlled temperature ($22 \pm 1\,^{\circ}C$), humidity (50–80%), under a 12-h light/dark cycle (lights on at 07:00 h). Access to laboratory chow and tap water was ad libitum. Animals were acclimatized to the laboratory settings for at least 1 week before the start of the experimental protocols. The number of mice used in this work was the minimum possible to determine consistent effects of the drug treatments. All protocols were submitted and approved by the local Ethics Committee in accordance with the Brazilian regulations on animal welfare.

2.2. Induction and assessment of DSS-induced colitis

The protocol was conducted as previously described (Ghia et al., 2008). In short, the experimental colitis was induced in male CD1 mice (5–7 per group) offering a 3% (w/v) DSS (36–50 kDa) solution in the drinking water for 5 days, which was replaced every other day. At the end of day 5, animals received only normal drinking water instead, until day 7 when they were euthanized and their colons were collected and analyzed. Control mice received only drinking water from day 0 to day 7. Animals were examined once a day when each mouse received a score for stool consistency, the presence of fecal blood and weight loss. These features were used together to determine the Disease Activity Index (DAI) as previously described (Bento et al., 2011; Ghia et al., 2008). The scores were defined as follows: weight loss was graded 0 if body weight increased or remained within 1% of the baseline, 1 for a 1–5% loss, 2 for a 5–10% loss, 3 for a 10–15% loss, or 4 for weight loss >15%. The stool consistency was graded 0 for no diarrhea, 2 for loose stool that did not stick to the anus, and 4 for liquid stool that did stick to the anus. The presence of fecal blood received a value of 0 when assigned for none, 2 for moderate, and 4 for gross bleeding. At the end of day 7, the colons were analyzed considering the consistency of the stool found within, the thickness of the colon and its length (from 1 cm from the anus to the top of the cecum). These three aspects were used to measure the macroscopic damage of each animal. In addition, the distal portion of each colon was excised and immediately fixed in 4% formaldehyde solution, embedded in paraffin wax and then sectioned at 5 μm thickness before being deparaffinized. Slices were stained using H&E standard techniques and the microscopic score was determined as described previously (Kimball et al., 2004).

2.3. Pharmacological treatments

The following series of treatment were designed to investigate the anti-inflammatory properties of the mixture of α,β-amyrin in the DSS-induced colitis in mice. The 1:1 mixture of α,β-amyrin was diluted in 5% Tween 80 plus 5% ethanol in phosphate-buffered saline (PBS) (Vitor et al., 2009). Mice were orally treated by gavage with 1, 3 or 10 mg/kg from day 0 to day 7 (preventive treatment) or with 10 mg/kg from day 3 to day 7 (therapeutic treatment). To further explore a possible link between α,β-amyrin and the cannabinoid system, another set of experiments was performed in which the mice received the selective CB_1 antagonist AM251 (10 mg/kg, i.p.) or the selective CB_2 antagonist AM630 (10 mg/kg, i.p.) once a day, given 30 min before the first treatment with α,β-amyrin (10 mg/kg) from day 0 to day 7. The doses of α,β-amyrin and for AM251 and AM630 were selected based on previous studies (Bento et al., 2011; Storr et al., 2009). Control groups received only vehicle solution.

2.4. MPO and NAG assays

Neutrophil and macrophage infiltration in the colon was assessed indirectly by the measurement of myeloperoxidase (MPO) and *N*-acetylglucosaminidase (NAG) activities, respectively. Briefly, animals were sacrificed on day 7 and colon tissue segments were homogenized in 5% EDTA/NaCl buffer (pH 4.7) and centrifuged at $10{,}000 \times g$ for 15 min at $4\,^{\circ}C$. The pellet was re-suspended in 0.5% hexadecyltrimethyl ammonium bromide buffer (pH 5.4), and the samples were frozen in liquid nitrogen and thawed three times. Upon thawing, the samples were re-centrifuged ($10{,}000 \times g$, 15 min, $4\,^{\circ}C$), and 25 μL of the supernatant was used for the MPO. On final thawing, the samples were similarly centrifuged, and 25 μL of the supernatant was used for MPO and NAG assays. The enzymatic reaction was assessed by the addition of 1.6 mM