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Adelmidrol, a palmitoylethanolamide analogue, as a new pharmacological treatment for the management of acute and chronic inflammation

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ABSTRACT

The aim of study was to examine the anti-inflammatory and analgesic effects of adelmidrol, an analogue of palmitoylethanolamide (PEA), in animal models of acute and chronic inflammation [carrageenan-induced paw edema (CAR) and collagen-induced arthritis (CIA)].

In order to elucidate whether the action of adelmidrol is related to activation of peroxisome proliferator-activated receptors (PPAR-α or PPAR-γ), we investigated the effects of PPAR-γ antagonist, GW9662 on adelmidrol mechanism. CAR induced paw edema, hyperalgesia and the activation of pro-inflammatory NF-κB pathway were markedly reduced by treatment with adelmidrol. GW9662, (administered prior to adelmidrol treatment), antagonized the effect of adelmidrol abolishing its positive action. On the contrary, the genetic absence of PPAR-α receptor did not modify the beneficial results of adelmidrol treatment in the acute model of inflammation.

In addition, for the first time, we demonstrated that adelmidrol was able to ameliorate both the clinical signs and the histopathology of the joint and the hind paw during chronic inflammation. In particular, the degree of oxidative damage and proinflammatory cytokines and chemokines production were significantly reduced in adelmidrol-treated mice. Moreover, in CIA model, the effect of GW9662 pre-treatment on adelmidrol mechanism was also confirmed. Thus, in this study, we report that adelmidrol reduces the development of acute and chronic inflammation and could represent a novel therapeutic approach for inflammation and pain.

1. Introduction

Inflammation and pain have been attributed in large part to elevated levels of prostaglandins, TNF-α and interleukins [1] and for this reason most anti-inflammatory agents possess analgesic activity [1]. CAR-induced paw edema is a common experimental model to study the acute phase of inflammation. The acute inflammation is characterized by increased vascular permeability, leukocyte infiltration, neutrophil-derived active oxygen species nitric oxide, cytokines and prostaglandins [2].

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic joint inflammation, chronic pain, destruction of joint cartilage and bone erosion. Type II collagen-induced arthritis (CIA) is a useful animal model of RA [3]. The pathogenesis of CIA is characterized by the host's response to type II collagen and production of auto-antibodies that recognize collagen [3]. Moreover, the activation of neutrophils, macrophages, lymphocytes into joint tissues and the formation of the pannus are important events in the pathogenesis of both CIA and human RA. Several laboratories have reported that mast cells (MCs) also have a role in the pathogenesis of inflammatory conditions and joint disorders [4].

In that regard, palmitoylethanolamide (PEA) and some of its analogues showed great efficacy in the treatment of pain and inflammation. Several of our works demonstrated the beneficial

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effects of PEA alone and in combination in different models of inflammation and pain [5,6] and in a mouse model of CIA [7]. The exact mechanism of action of PEA is not well known although PEA could interact with peroxisome proliferator-activated receptor (PPAR)-α, which are involved in its anti-inflammatory effects and with the orphan G-protein-coupled receptor, GPR55 [8]. Interestingly, a previous study of ours also indicated that PPAR-δ and PPAR-γ can contribute to the anti-inflammatory activity of PEA [9]. The genetic absence of the PPAR-α receptor significantly blocked the effect of PEA treatment in a model of SCI [9]. Pretreatment with GW9662 a potent PPAR-γ antagonist, or GSK0660 a PPAR-δ antagonist, counteracted the actions of PEA [9]. In the same time, mutant PPAR-α mice failed to respond to GW7647 (a synthetic PPAR-α agonist) or to PEA treatments in CAR induced paw inflammation model [10]. Moreover, the anti-inflammatory and analgesic effects of PEA have been reported and may be due to the ability to down-modulate MCs activation and MCs mediators release [8].

Adelmidrol, (International Nonproprietary Name INN) of the diamide derivative of azelaic acid is one of PEA analogues that belongs to ALIAmides family (Autacoid Local Injury Antagonist Amides) and the effects of adelmidrol may depend on the control of mast cell activation [8]. Several studies showed that topical treatment with adelmidrol increased MCs granular density, suggesting a decrease in their degranulation [11,12]. In addition, this compound showed some beneficial effects in a pilot study on mild atopic dermatitis [13].

Thus, the purpose of our study was to examine the anti-inflammatory effects of adelmidrol treatment during acute and chronic inflammation such as in a classical model of inflammation and hyperalgesia carrageenan-induced paw edema (CAR) [6] and in an animal model of autoimmune disease CIA. Thus, to better understand whether the mechanism of action of adelmidrol treatment could be related to PPAR-α/γ pathways as PEA analogue, we investigated the effect of PPAR-γ antagonist, GW9662 on the protective effects of adelmidrol and whether the genetic absence of PPAR-α receptor could modify its action during acute inflammation.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats (200–230 g, Envigo, Italy) and male mice (6–7 weeks old, 20–27 g) with a targeted disruption of the PPAR-α gene (PPAR-α KO) and littermate wildtype controls (PPAR-α WT) purchased from Jackson Laboratories (Envigo, Italy) and DBA/1 J male mice (9 weeks; Envigo, Italy), were used for studies.

Mice homozygous for the Pparat^{nij}Gonz targeted mutation mice are viable, fertile and appear normal in appearance and behavior. Exon eight, encoding the ligand-binding domain, was disrupted by the insertion of a 1.14 kb neomycin resistance gene in the opposite transcriptional direction. After electroporation of the targeting construct into J1 ES cells, the ES cells were injected into C57BL/6 N blastocysts. This stain was created on B6, 129S4 background and is maintained as a homozygote on a 129S4/SvJae background by brother sister mating.

Water and food were available *ad libitum*. This study was authorized by the University of Messina Review Board for the care of animals. Animal care was in conformity with the new legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU).

2.2. Experimental groups

The study was divided into three steps. First, we studied the analgesic and anti-inflammatory effects of adelmidrol in a model of acute inflammation such as CAR induced paw edema in rats.

Rats were randomly distributed to the following groups:

CAR+saline group*:* rats were subjected to carrageenan-induced paw edema ($n = 20$);
CAR+GW9662 (1 mg/kg)+adelmidrol (10 mg/kg) dissolved in saline: same as the CAR+saline group but GW9662 (1 mg/kg), a potent antagonist of PPAR-γ receptor, was administered intraperitoneally 30 min before CAR injection and adelmidrol was administered intraperitoneally (10 mg/kg) at the same time of CAR injection ($n = 20$);
CAR+adelmidrol (10 mg/kg) dissolved in saline: same as the CAR+saline group but adelmidrol was administered intraperitoneally (10 mg/kg i.p) at the same time of CAR injection ($n = 20$);

Then, to confirm whether the mechanism of action of adelmidrol is related to activation of PPAR-α receptors, we performed new experiments specifically in PPAR-α WT and KO mice.

PPAR-α WT and KO mice were randomly allocated to the following groups:

CAR+saline group: PPAR-α WT and KO mice were subjected to CAR induced paw edema ($n = 20$);
CAR+adelmidrol (10 mg/kg) dissolved in saline: PPAR-α WT and KO mice were subjected to CAR-induced paw edema and adelmidrol was administered intraperitoneally (10 mg/kg i.p) at the same time of CAR injection ($n = 20$);

The doses of adelmidrol were chosen based on a dose–response study carried out in our lab. The sham-operated group was subjected to the same surgical procedures as the CAR group, except that saline or drugs were administered instead of CAR ($n = 20$).

Finally, we tested the analgesic and anti-inflammatory effects of adelmidrol and the relation to PPAR-α and PPAR-γ pathways during chronic inflammation in a mouse model of autoimmune disease (CIA).

Mice were divided into five experimental groups:

CIA-control: mice were subjected to collagen-induced arthritis and administered intraperitoneally with vehicle (saline) every 24 h, starting from day 25 to day 35 ($n = 20$).
CIA+adelmidrol: mice subjected to CIA were administered intraperitoneally with adelmidrol 10 mg/kg dissolved in saline every 24 h, starting from day 25 to day 35 ($n = 20$).
CIA+GW9662+adelmidrol: mice subjected to CIA were administered with the PPAR-γ receptor antagonist GW9662 (1 mg/kg i.p.) 10 min before adelmidrol administration 10 mg/kg dissolved in saline every 24 h, starting from day 25 to day 35 ($n = 20$).
CIA+GW6471+adelmidrol: mice subjected to CIA were administered with the PPAR-α receptor antagonist GW6471 (1 mg/kg i.p.) 10 min before adelmidrol administration 10 mg/kg dissolved in saline every 24 h, starting from day 25 to day 35 ($n = 20$).
CFA-control: mice were injected at the base of the tail with 100 μl of CFA instead of the emulsion containing 100 μg of CII and were treated with saline, every 24 h from day 25 to day 35 ($n = 20$).
Sham-control: mice were injected at the base of the tail with 100 μl of 0.01 M acetic acid instead of the emulsion containing