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Adelmidrol, a palmitoylethanolamide analogue, reduces chronic inflammation in a carrageenin-granuloma model in rats

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Abstract

Palmitoylethanolamide (PEA) and some of its analogues have shown great efficacy in the treatment of pain and inflammation. Adelmidrol – the International Nonproprietary Name (INN) of the di-amide derivative of azelaic acid – is one of these analogues. The anti-inflammatory and analgesic effects of PEA and adelmidrol are hypothesized to be mediated, at least in part, by mast cell down-modulation. Mast cell mediators released at early stage of the inflammatory process drive the inflammatory reaction to chronicity as it happens in λ-carrageenin-induced granulomatous tissue formation. In the present study, the choice of testing adelmidrol depends upon the physicochemical properties of the compound, *i.e.* the amphipatic feature, that make it more easily soluble than PEA. In this study, we investigated the effect of adelmidrol on granuloma formation induced by λ-carrageenin-soaked sponge implant in rats. Our results show that the local administration of the compound under study significantly decreases weight and neo-angiogenesis in granulomatous tissue. The anti-inflammatory effect was due to the modulation of mast cells degranulation, as shown by histological analysis and by the inhibition of the release of several pro-inflammatory and pro-angiogenic enzymes (*e.g.* iNOS, chymase and metalloproteinase MMP-9), and mediators (*e.g.* nitric oxide and TNF-α). The results indicate that adelmidrol, given locally, may represent a potential therapeutic tool in controlling chronic inflammation.

Keywords: inflammation • mast cells • palmitoylethanolamide • adelmidrol

Introduction

Adelmidrol is the International Nonproprietary Name (INN) of a synthetic derivate of azelaic acid, a naturally occurring saturated dicarboxylic acid, that is found in some whole grains and in trace amounts in the human body [1], its plasma levels normally ranging from 20 to 80 ng/ml. Chemically, ademidrol is the N,N′-bis (2-hydroxyethyl) non anediamide and it is an amphiphilic or amphipathic compound, possessing both hydrophilic and hydrophobic properties, that favour its solubility both in aqueous and organic media. The physicochemical properties of the compound make it particularly suitable for topical application and an adelmidrol (2%) emulsion has recently shown some benefit in a pilot study on mild atopic dermatitis [2]. The effect of adelmidrol has been shown to depend, at least in part, on the control of mast cell activation. Densitometric and morphometric analyses of skin biopsies from experimental skin wounds showed that treatment with adelmidrol led to an increase in the mast cell granular density, thereby suggesting a decrease in their degranulation [3, 4]. According to chemical structure and cellular mechanism of action, adelmidrol hence belongs to the family of ALIAmides (Autacoid Local Injury Antagonist Amides), *i.e.* fatty acid amides, whose purported mechanism of action is the down-modulation of mast cell degranulation [5–7]. Palmitoylethanolamide (PEA) is considered to be the parent molecule of ALIAmides. PEA is naturally present both in animal and vegetable tissues [8, 9] and is able to enhance both the cannabinoid and vanilloid signalling systems [10], down-regulating the degradation pathways of endocannabinoid and endovanilloid compounds [11, 12]. The exact

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mechanism of action of PEA is not yet well known although PEA may interact with peroxisome proliferator-activated receptor-α, which seems to be involved in some of its anti-inflammatory effects [13] and with the orphan G-protein-coupled receptor, GPR55 [14]. The anti-inflammatory and analgesic effects of PEA have been repeatedly reported [15–19] and are thought to be due, at least in part, to its ability to down-modulate mast cell activation and mast cell mediator release both *in vitro* [5, 20, 21] and *in vivo* [22, 23]. Mast cells are highly specialized immune effector cells that, according with their granule content, may be divided into two subpopulations: mucosal mast cells mainly present in the mucosa of respiratory and intestinal tracts, and connective mast cells placed preferentially in skin [24]. Following either classical immunological IgE-dependent activation or in response to a variety of stimuli [25], mast cells release their stored and newly synthesized mediators, including cytokines (TNF-α), histamine and pro-inflammatory and pro-angiogenic mediators such as chymase, collagenase, nitric oxide, IL-1 and IL-6 (for a review see [26]). Mast cells are now considered a master player in the promotion and perpetuation of chronic skin inflammation [27] and have recently been defined as a 'central cellular switchboard of pruritogenic skin inflammation' [28]. We have shown that mast cell mediators, released at early stage of the inflammatory process, play a pivotal role in a classical model of chronic inflammation, *i.e.* the λ-carrageenin-induced granuloma formation [29]. In fact, during granuloma, mast cell-derived vasoactive mediators trigger the angiogenic process essential for the maintenance of tissue perfusion and for sustaining cellular traffic [30]. Both these events are strictly required for the development of the cutaneous granulomatous tissue [31]. On all these assumptions, the aim of the present study is to evaluate the effect of adelmidrol, which for its chemical structure is prearranged to control dermatological pathologies, in a model of chronic cutaneous inflammation sustained by mast cells, *i.e.* granuloma induced by λ-carrageenin-soaked sponge implantats.

Materials and methods

Sponge implantation

Male Wistar rats (Harlan, Italy), weighing 200–220 g, were used in all experiments. Animals were provided with food and water *ad libitum*. The light cycle was automatically controlled (on 07 hrs 00 min.; off 19 hrs 00 min.) and the room temperature thermostatically regulated to 22 ± 1°C with 60 ± 5% humidity. Prior to the experiments, animals were housed in these conditions for 3–4 days to become acclimatized. Sponges were implanted as previously described by De Filippis *et al.* [31]. Briefly, two polyether sponges (0.5 × 1.5 × 2.0 cm) weighing 0.035 ± 0.002 g were implanted subcutaneously on the back of rats (n = 12–18 for each group) under general anaesthesia with pentobarbital (60 mg/kg). Sponges and surgery tools were sterilized by autoclaving for 20 min. at 120°C. λ-carrageenin (1% w/v) (Sigma) was dissolved in pyrogen-free saline and injected into each sponge in presence or absence of 100 μl of different adelmidrol solution (15, 30, 70 mg/ml) in final volume of 0.5 ml/sponge; saline (0.5 ml/sponge) was used as control. In some experiments GW6471 (5 mg/ml), a well-known antagonist of PPAR-alpha receptor was added in the presence of the highest concentration of Adelmidrol. Ninety-six hours after sponge implant, rats were killed in atmosphere of CO_2. The granulomatous tissue around the sponge was dissected by using a surgical blade, weighted, quickly frozen in liquid nitrogen, and stored at −80°C. Animal care as well as all experiments were in accordance with European Community Council Directive 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animals used.

Evaluation of angiogenesis

Angiogenesis was evaluated by both haemoglobin content measurement and histological investigations.

Haemoglobin content measurement

The granulomatous tissue, *i.e.* the new formation tissue encapsulating the sponge, was collected and measured with a balance weighting min 0.02 to max 300 g (KERN EG300-EM) always by the same person who was blinded for the treatments. In some experiments, the granulomatous tissue was homogenized on ice with the Polytron PT300 tissue homogenizer in 1 × PBS (4 ml each g of wet weight). Briefly, after centrifugation at 2500 × *g* for 20 min. at 4°C, the supernatants were further centrifuged at 5000 × *g* for 30 min. and haemoglobin concentration in the supernatant was determined spectrophotometrically at 450 nm performed with the haemoglobin assay kit (Sigma Diagnostic). The haemoglobin content was expressed as mg haemoglobin/g of wet weight.

Histological investigation

After 96 hrs from sponge implants, the granulomatous tissue around the sponge was removed and fixed in 10% formalin. Paraffin-wax sections were cut at 4–6 μm and stained with haematoxylin and eosin for the evaluation of blood vessels.

Mast cell counting

Thin (0.5 μm) paraffined section were prepared and stained with toluidine blue according to Iuvone *et al.* [25] and then processed for light microscopy examination. MC counting was performed on five randomly selected sections using a x100 objective lens.

MPO activity

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear (PMN) accumulation, was determined. Granulomatous tissues were homogenized in a solution containing 0.5% (w/v) hexadecyl-trimethyl-ammonium bromide dissolved in 10-mm potassium phosphate buffer (pH 7) and centrifuged for 30 min. at 20,000 *g* at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mm) and 0.1 mm H_2O_2. The rate of change in absorbance was measured

spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 mmol of hydrogen peroxide/min at 37°C and was expressed in units per gram weight of wet tissue.

Nitrite assay

Nitrite production, as the stable metabolites of nitric oxide, was measured in 24-hrs-cultured granulomatous tissues supernatants. Briefly, the granulomatous tissues were weighted and plated in a 6 multi-wells plate according to Iuvone *et al.* [32]. After 24 hrs, the medium of cultured granulomatous tissue (0.1 ml) was added to an equal amount of Griess reagent (1% sulphanilamide, 0.1% naphtylendiammine, 2.5% H_3PO_4) and allowed at room temperature for 10 min. The absorbance of constituted chromophore was determined using a UV/visible spectrophotometer at 550 nm. Nitrite levels were determined using a sodium nitrite standard curve and expressed as μmol/l.

Nitrite and nitrate assay

Nitrite and nitrate production were evaluated in granulomatous tissues. Briefly, tissues were cooled in ice-cold distilled saline and homogenized. The crude homogenate was centrifuged at 21.000 *g* for 20 min. at 4°C, and aliquots of the supernatant were used to calculate NOx levels. Nitrite present in the samples was determined by reducing nitrate enzymatically performed with the enzyme nitrate reductase (Sigma-Aldrich, Dorset, UK) and Nicotinamide-Adenine Dinucleotide Phosphate (NADPH) at room temperature (RT) for 3 hrs. The amount of NOx was measured following the Griess reaction Iuvone *et al.* [25].

Western blot analysis

Granulomatous tissues were weighted and rapidly homogenized in 60 μl of ice-cold hypotonic lysis buffer (10 mm HEPES, 1.5 mm $MgCl_2$, 10 mm KCl, 0.5 mm phenylmethylsulfonyl fluoride, 1.5 μg/ml soybean trypsin inhibitor, pepstatin A 7 μg/ml, leupeptin 5 μg/ml, 0.1 mm benzamidine, 0.5 mm dithiothreitol [DTT]) and incubated in ice for 45 min. After this time, the cytoplasmic fractions were then obtained by centrifugation at 13,000 *g* for 1 min. and protein concentration in the samples was determined with Bio-Rad assay kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Immunoblotting analysis of inducible nitric oxide synthase (iNOS), tumour necrosis factor-α (TNF-α) Chymase and MMP-9, CD-31, Tubulin were performed on a cytosolic fraction of cultured specimens. Cytosolic fraction proteins were mixed with gel loading buffer (50 mm Tris, 10% SDS, 10% glycerol 2-mercaptoethanol, 2 mg bromophenol/ml) in a ratio of 1:1 v/v, boiled for 5 min. and centrifuged at 10,000 *g* for 10 min. Protein concentration was determined and equivalent amounts (50 μg) of each sample were separated under reducing conditions in 12% SDS-polyacrylamide minigel. The proteins were transferred onto nitrocellulose membrane according to the manufacturer's instructions (Bio-Rad Laboratories). Depending upon the experiments, the membranes were blocked by incubation at 4°C overnight in high salt buffer (50 mm Trizma base, 500 mm NaCl, 0.05% Tween-20) containing 5% bovine serum albumin; they were then incubated for 1 hr with anti-iNOS (1:2000 v/v) (BD Biosciences, Pharmingen, Italy), anti-TNF-α (1:250 v/v) (Sigma Aldrich), anti-Chymase (1:500 v/v) (NeoMarkers, Fremont, CA, USA), anti-MMP-9 (1:100 v/v) (NeoMarkers), anti-CD-31 (1:1000v/v) (Diaclone, France) and anti-beta Tubulin (1:1000) (Sigma Aldrich) for 2 hrs at room temperature, followed by incubation with specific horseradish peroxidase (HRP)-conjugate secondary antibody (Dako, Glostrup, Denmark). The immune complexes were developed performed with enhanced chemiluminescence detection reagents (Amersham, Italy), according to the manufacturer's instructions and exposed to Kodak X-Omat film. The protein bands of proteins on X-ray film were scanned and densitometrically analysed with a GS-700 imaging densitometer (Bio-Rad Laboratories).

Statistics

Results were expressed as the mean ± S.E.M. of *n* animals where each value is the average of responses in duplicate sites. Statistical comparisons were made by one-way ANOVA followed by Bonferroni's test for multiple comparisons. $P < 0.05$ was considered to be significant.

In all set of experiments, analysis of linear regression was performed to evaluate a concentration-dependent relationship.

Results

Effect of adelmidrol on granuloma formation

The implant of λ-carrageenin-soaked sponges on the back of rats caused a significant increase of granulomatous tissue formation around the sponge at 96 hrs, evaluated as the wet weight of tissue around the sponge (1.67 ± 0.079 g *versus* saline 0.61 ± 0.042 g; $P < 0.001$). Single administration, at time 0 (*i.e.* the implantation time), of adelmidrol (15, 30, 70 mg/ml) resulted in a significant and concentration-dependent decrease in granuloma formation (by 15, 28, 44% respectively; $P < 0.001$). The ademidrol effect, on granulomatous tissue formation, was not reversed by the usage of GW6471, a potent and selective antagonist of PPAR-alpha receptor (Fig. 1A).

Effect of adelmidrol on leucocyte infiltration

Adelmidrol (15, 30, 70 mg/ml) decreased in a significant and concentration-dependent way the leucocyte infiltration in the granulomatous tissue, at 96 hrs, evaluated as myeloperoxidase activity (21, 44, 72% inhibition, respectively; $P < 0.001$) as compared to λ-carrageenin alone (1.8 ± 0.54 mU/100 mg wet tissue; $P < 0.001$ *versus* saline) (Fig. 1B).

Effect of adelmidrol on pro-inflammatory markers

The effect of adelmidrol on two of the most studied pro-inflammatory markers was evaluated. Adelmidrol (15, 30, 70 mg/ml) showed a significant and concentration-dependent inhibition

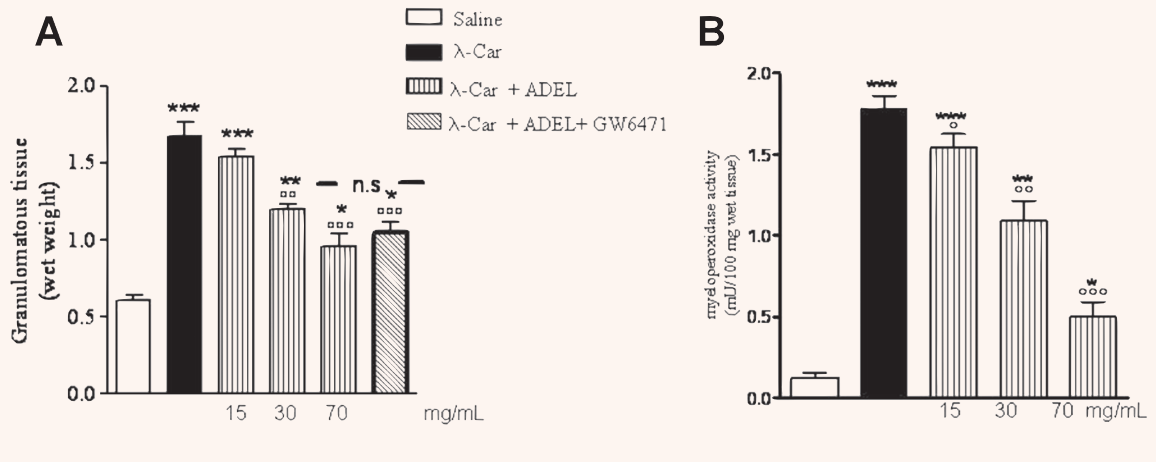

Fig. 1 (**A**) Effect of adelmidrol on λ-carrageenin-induced granulomatous tissue formation. Adelmidrol was administrated at the time of implantation (t 0). The administration of GW6471, a PPAR-alpha antagonist, did not reverse the effect of adelmidrol. Granulomatous tissue formation was evaluated 96 hrs after implantation as wet weight of tissue around the sponge. (**B**) Effect of adelmidrol on λ-carrageenin-induced leucocytes infiltration evaluated as myeloperoxidase activity. Data are expressed as mean ± S.E.M. of $n = 3$ separate experiments; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* saline; $^{\circ\circ}P < 0.01$; $^{\circ\circ\circ}P < 0.001$ *versus* λ-carrageenin alone.

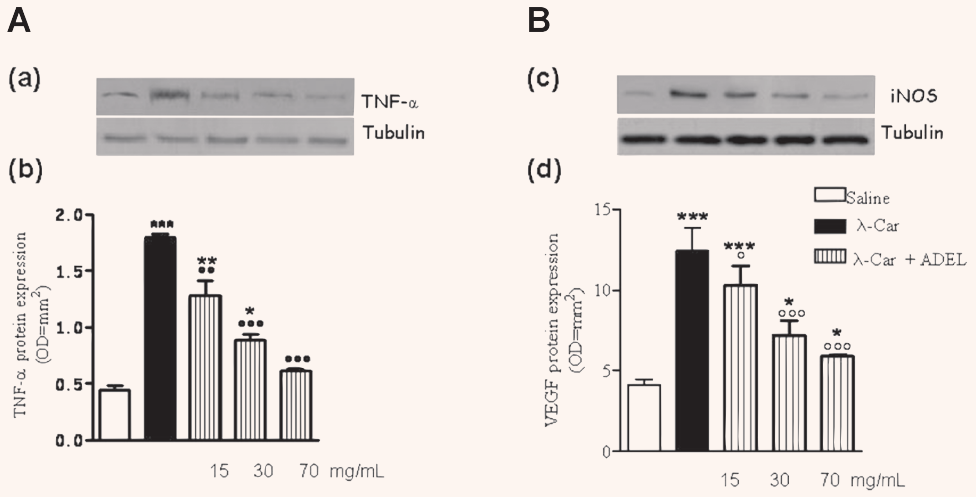

Fig. 2 Effect of adelmidrol on λ-carrageenin-induced TNF-α, iNOS expression in granulomatous tissue at 96 hrs. Representative Western blot analysis and relative densitometric analysis of (**A**) TNF-α and (**B**) iNOS. Tubulin expression is shown as control. Data are representative of 3 separate experiments. Results are expressed as mean ± S.E.M. of 3 experiments; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* saline; $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$ *versus* λ-carrageenin alone.

of TNF-α protein levels (29, 61, 76% inhibition, respectively; $P < 0.001$) as compared to carrageenin alone (1.8 ± 0.30 OD = mm^2; $P < 0.001$ *versus* saline) and iNOS (21, 43, 63% inhibition respectively $P < 0.001$) as compared to carrageenin alone (12.45 ± 1.41 OD = mm^2; $P < 0.001$ *versus* saline) into the granulomatous tissue at 96 hrs, evaluated by Western blot and densitometric analysis (OD = mm^2) of relative bands (Fig. 2A and B).

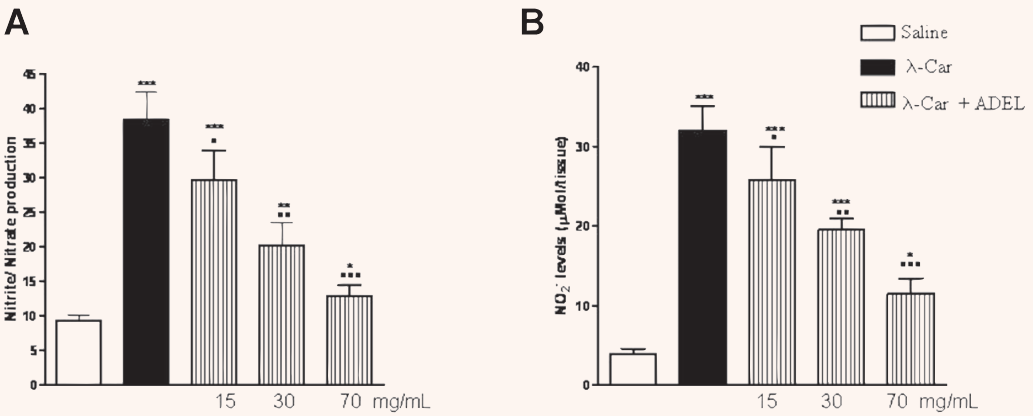

Fig. 3 Effect of adelmidrol on λ-carrageenin-induced nitric oxide production. Nitric oxide production was determined by measuring the accumulation of (**A**) nitrite and nitrate in granulomatous tissues and (**B**) in supernatant of 24-hrs-cultured granulomatous tissues. Data are expressed as mean ± S.E.M. of 3 experiments; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* saline; $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$ *versus* λ-carrageenin alone.

Effect of adelmidrol on nitrite production

Adelmidrol strongly reduced nitrite and nitrate, stable metabolites of nitric oxide, evaluated both in homogenates from granulomatous tissue at the explant time (26, 40, 73% inhibition *versus* carrageenin alone 37 ± 0.600 μmoli/tissue; $P < 0.001$) and released from tissue cultured in plates for further 24 hrs (23, 46, 69% inhibition *versus* carrageenin alone 33 ± 0.4 μmol/tissue; $P < 0.001$) (Fig. 3A and B).

Effect of adelmidrol on mast cells

Fig. 4A shows the toluidine blue stained granulomatous tissue sections. Treatment with λ-carrageenin (b) induced a significant mast cell degranulation (light blue stained cells, *i.e.* degranulated cells, in comparison to the deep blue stained cells, *i.e.* non-degranulated mast cells); moreover, histological analyses showed that mast cells are predominantly located in close proximity to blood vessels. Adelmidrol (c) was able to reduce mast cell degranulation. Moreover, histological analysis of granulomatous tissue showed that adelmidrol (70 mg/ml), locally administered at time 0, reduced the number of mast cells by 52% ($P < 0.001$) in comparison to carrageenin alone (104.66 ± 1.67 number of mast cells $P < 0.001$ *versus* saline) (Fig. 4B).

The results are paralleled to the Western blot analysis for both chymase (Fig. 4C) and MMP-9 (Fig. 4D) protein expression that were found to be significantly and concentration-dependently reduced by adelmidrol (15, 30, 70 mg/ml), respectively by 24, 39, 57% ($P < 0.001$ in comparison to carrageenin alone 9.6 ± 0.85 OD = mm^2, $P < 0.001$ *versus* saline) and by 21, 39, 60% ($P < 0.001$ in comparison to carrageenin alone 5.33 ± 0.19 OD = mm^2, $P < 0.001$ *versus* saline), respectively.

Effect of adelmidrol on angiogenesis

We studied the effect of adelmidrol on vessel formation, evaluated both as haemoglobin (Hb) content and expression of CD31 protein, a marker of endothelial cells. As shown in Fig. 5A, adelmidrol (15, 30, 70 mg/ml) significantly and concentration-dependently reduced Hb content in granulomatous tissue by 23, 42, 60% ($P < 0.001$) in comparison to carrageenin alone (53.4 ± 1.72 mg Hb/g tissue; $P < 0.001$ *versus* saline). Immunoblotting analysis of CD31 is illustrated in Fig. 5B and shows that adelmidrol significantly and concentration-dependently inhibited λ-carrageenin-induced CD31 protein expression in granulomatous tissue by 24, 43, 60% in comparison to carrageenin alone (8.45 ± 0.78 OD = mm^2). The results were confirmed by histological analysis of granulomatous tissue stained with haematoxylin/eosin showing a deeply decrease of vessel number after adelmidrol treatment (Fig. 5C).

Discussion

In the present study, we investigated the potential protective effect of adelmidrol, an analogue of PEA, in a model of chronic inflammation, *i.e.* the λ-carrageenin-induced granuloma in rats. Although PEA is the most thoroughly researched ALIAmide, several previous studies have pointed to the use of PEA analogues and homologues, trying to exploit their different physical and chemical properties and the relative effects [33–35]. In line with this idea, in the present study we have chosen to evaluate the effect of the PEA analogue adelmidrol, whose physicochemical properties favour its use in skin disorders. In fact, adelmidrol has good solubility both in aqueous and organic media, which provides good transepidermal absorption.

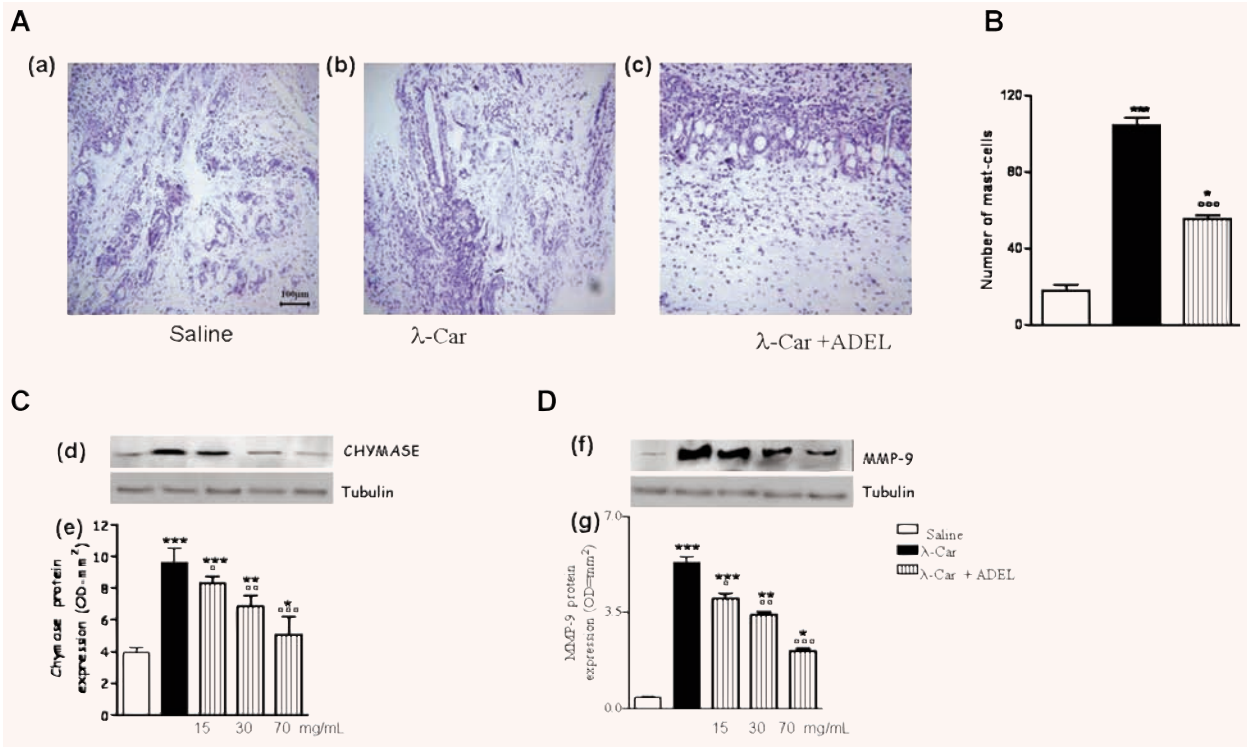

Fig. 4 Effect of adelmidrol on λ-carrageenin-induced mast cell activation. (**A**) Mast cell degranulation was evaluated on microscopically visible connective mast cells stained with 0.05% (w/v) toluidine blue and counterstained with 0.1% (w/v) nuclear fast red (magnification 100x). A differentiation between not degranulated (deep blue) and degranulated (light blue) mast cell was valuable. In (**B**) is shown the counting of mast cell number. Representative Western blot analysis and relative densitometric analysis of (**C**) Chymase and (**D**) MMP-9. Tubulin expression is shown as control. Data are representative of 3 separate experiments. Results are expressed as mean ± S.E.M. of 3 experiments; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* saline; $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$ *versus* λ-carrageenin alone.

We demonstrated, for the first time, that adelmidrol exerts an anti-inflammatory effect in the studied model of chronic inflammation and that this effect may be due to the modulation of mast cell activation and mediator release. We showed that local administration of adelmidrol into the sponge at time 0, *i.e.* at implantation time, significantly and concentration-dependently reduced granulomatous tissue formation and leucocyte infiltration (evaluated as MPO content), both of which have been shown to significantly increase after carrageenin-soaked sponge implant [36, 37]. Furthermore, adelmidrol significantly reduced levels of pro-inflammatory markers (*i.e.* iNOS, TNF-α and nitric oxide) in a concentration-dependent manner. Interestingly, we have previously shown, although in different inflammatory models both *in vitro* and *in vivo*, that endogenous and synthetic cannabinoids are able to inhibit several inflammatory markers, such as iNOS protein expression and nitric oxide production, as well as TNF-α, COX-2, IL-1beta, VEGF [31, 32, 38–42]. The involvement of TNF-α and nitric oxide in inflammation is not limited to certain aspects but encompasses the overall inflammatory process, including angiogenesis [43, 44], which is required to allow cellular traffic and tissue perfusion and to sustain chronicity [30]. In our study, adelmidrol showed also an antiangiogenic effect as it was evidenced by histological analysis of blood vessels, Western blot analyses of an endothelial cell marker (*i.e.* CD31) and haemoglobin content into the granulomatous tissue. Finally, histological analysis of toluidine blue stained granulomatous tissue sections, evidenced that local administration of adelmidrol down-modulates mast cells degranulation, in agreement with previous studies [3, 4], and also prevents the λ-carrageenin-induced increase of mast cell number. It is well recognized the relevant role played by mast cells in orchestrating inflammation [27, 45, 46] since, by the release of their granule content along the whole process these cells contribute to trigger the inflammation and, afterwards, bring it into the chronicity. We administered, therefore, adelmidrol at the starting of the inflammatory process concomitantly to carrageenin insult more than when the inflammatory process was already started. The down-modulation of mast cell

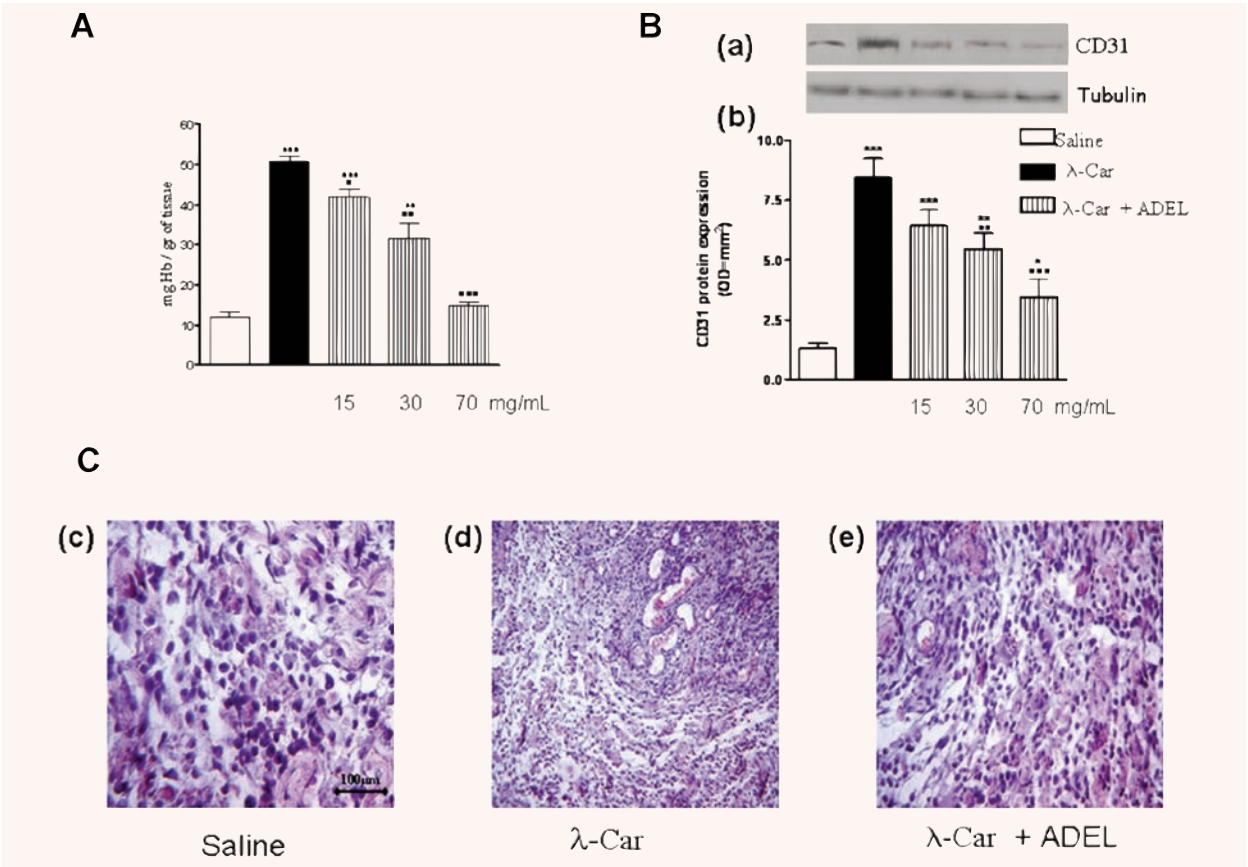

Fig. 5 Effect of adelmidrol on λ-carrageenin-induced angiogenesis. New vessel formation was evaluated as (**A**) haemoglobin content (Hb); (**B**) CD31 protein expression, a marker of endothelial cells. Results are expressed as mean ± S.E.M. of 3 experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* saline; $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$ *versus* λ-carrageenin alone. (**C**) Representative histological analysis of granulomatous tissue stained with haematoxylin and eosin. Fields are representative of 3 separate experiments. Original magnification, 100x.

activation justified also the anti-angiogenic effect of adelmidrol, since mast cell density within skin tissues correlates with the density of blood vessels [47] and activation of mast cells can be associated with the degradation of connective tissue, therefore suggesting the active participation of these cells in angiogenesis-dependent diseases occurring in the skin. The importance of mast cells is confirmed here, in agreement with the literature, by the observation that these cells are present in granulomatous tissue in close proximity to blood vessels, thus suggesting their role in directing the new vessel sprouting by the release of selected mediators.

In the present study, the adelmidrol-mediated modulation of mast cells was further established by the reduction in the λ-carrageenin-induced chymase expression. Chymases are peptidases with chymotrypsin-like activity, fairly selectively expressed by mast cells [48]. Not only chymases contribute directly to matrix destruction by cleaving proteins (*e.g.* fibronectin and collagens), but they also cooperate indirectly at activating matrix metalloproteases (MMPs) [49]. MMPs are a family of enzymes involved in matrix degradation [50] that are now considered important mediators in the advancement of the vessels into the tissue [51], as it happens in several inflammatory diseases, such as granuloma and other dermatological conditions including atopic dermatitis [52]. In our study, adelmidrol was shown to specifically reduce MMP-9, a member of the MMP family, which is suggested to play a crucial role in skin inflammation [53] and to be produced by mast cells under inflammatory conditions contributing to local tissue damage [54]. Moreover, MMP-9 not only participates in the degradation of elastic tissue but also is associated with neoangiogenesis [55]. Depending on their chymase content, mast cells, unlike other MMP-9-secreting cells, provide a complete tissue injuring package: they secrete, activate and disinhibit MMP-9 and can thus immediately start degrading matrix, without the help from other cellular sources reviewed in

[49]. Although the present study corroborates previous evidences showing that mast cell is a cellular target of adelmidrol [3, 4], the exact mechanism of action of the compound is not yet fully elucidated. However, we have ruled out a PPAR-α receptor involvement in the anti-inflammatory and antiangiogenetic effects of adelmidrol in our model. In conclusion, although further studies are needed to address the molecular mechanisms and pathways underlying the effects of adelmidrol, we demonstrated that local application of the compound reduces λ-carrageenin-induced chronic inflammation, likely through down-modulating the number and the extent of degranulation of mast cells. These findings shed new light on the understanding of the favourable outcomes of previous human and veterinary trials evaluating adelmidrol in patients with skin inflammation [2, 56], which are known to be strictly dependent upon mast cells, such as wound healing [57, 58] and atopic dermatitis [59]. By providing new data on the mechanisms underlying the anti-inflammatory effect of adelmidrol, our results may pave the way for the development of a new disease-oriented approach to a variety of chronic inflammatory diseases.

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