US 20170209395A1

(19) **United States**

(12) **Patent Application Publication**

Della Valle et al.

(10) **Pub. No.: US 2017/0209395 A1**

(43) **Pub. Date: Jul. 27, 2017**

(54) **USE OF ADELMIDROL IN THE TREATMENT OF EPITHELIAL DYSFUNCTIONS**

(71) Applicant: **Epitech Group S.r.l.**, Milano (IT)

(72) Inventors: **Maria Federica Della Valle**, Milano (IT); **Francesco Della Valle**, Milano (IT); **Vincenzo Di Marzo**, Milano (IT); **Stefania Petrosino**, Milano (IT); **Barbara Costa**, Milano (IT); **Gabriele Marcolongo**, Milano (IT); **Daniele Grassi**, Milano (IT)

(73) Assignee: **Epitech Group S.r.l.**, Milano (IT)

(21) Appl. No.: **15/482,129**

(22) Filed: **Apr. 7, 2017**

Related U.S. Application Data

(63) Continuation of application No. 14/793,165, filed on Jul. 7, 2015.

(30) **Foreign Application Priority Data**

Jul. 8, 2014 (IT) MI2014A001245

Publication Classification

(51) **Int. Cl.**

A61K 31/16	(2006.01)
A61K 31/201	(2006.01)
A61K 31/728	(2006.01)
A61K 45/06	(2006.01)
A61K 31/421	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/16*** (2013.01); ***A61K 45/06*** (2013.01); ***A61K 31/421*** (2013.01); ***A61K 31/728*** (2013.01); ***A61K 31/201*** (2013.01)

(57) **ABSTRACT**

Described herein is the use of Adelmidrol in the treatment of epithelial dysfunctions. In particular, described herein is Adelmidrol for use in the treatment of epithelial tissue dysfunctions in a human being or animal, wherein said Adelmidrol causes an increase of the endogenous levels of Palmitoylethanolamide without inhibiting the activity of the Palmitoylethanolamide-degrading FAAH and NAAA enzymes.

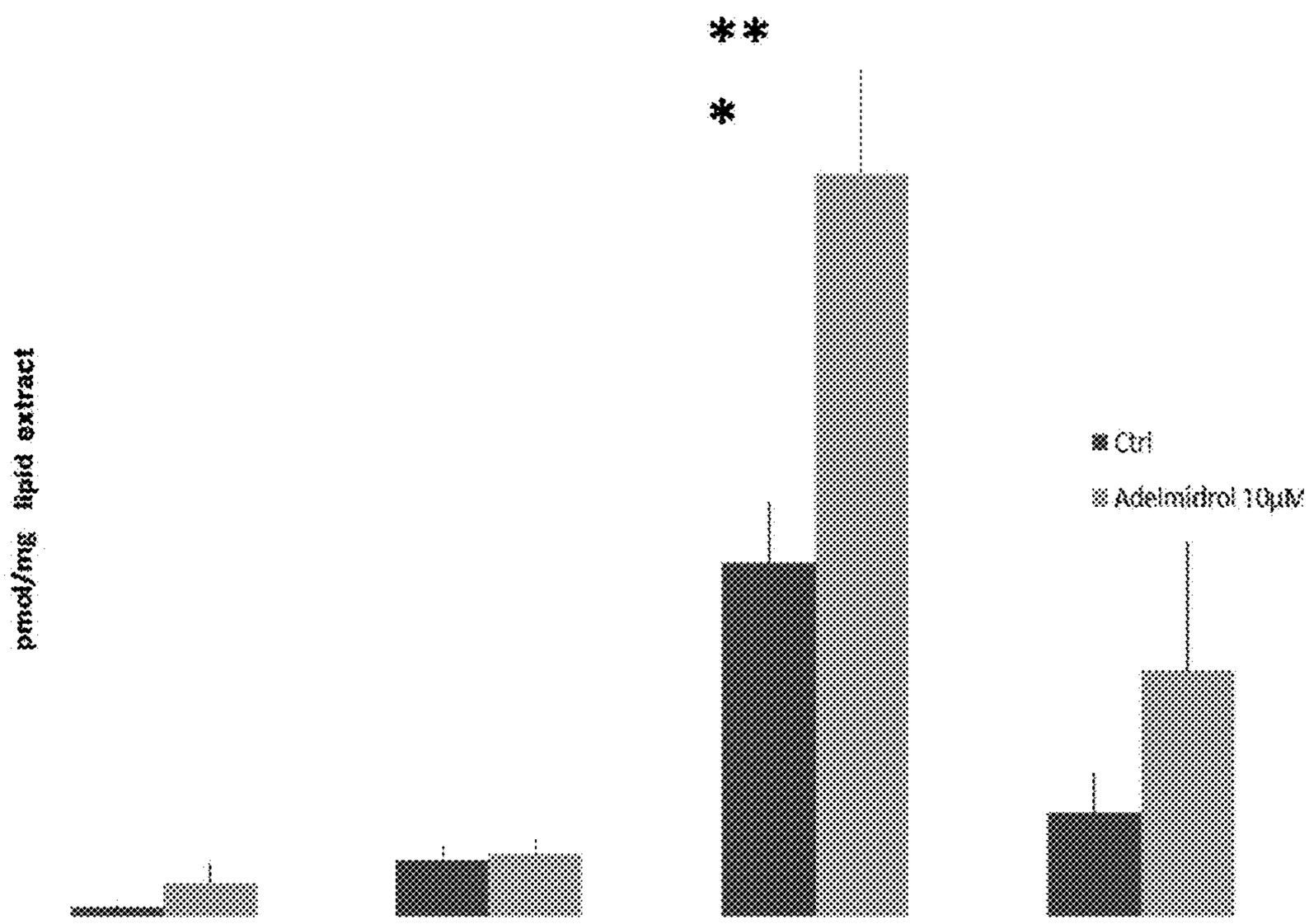

**
*
pmol/mg lipid extract
Ctrl
Adelmidrol 10µM

Fig. 1

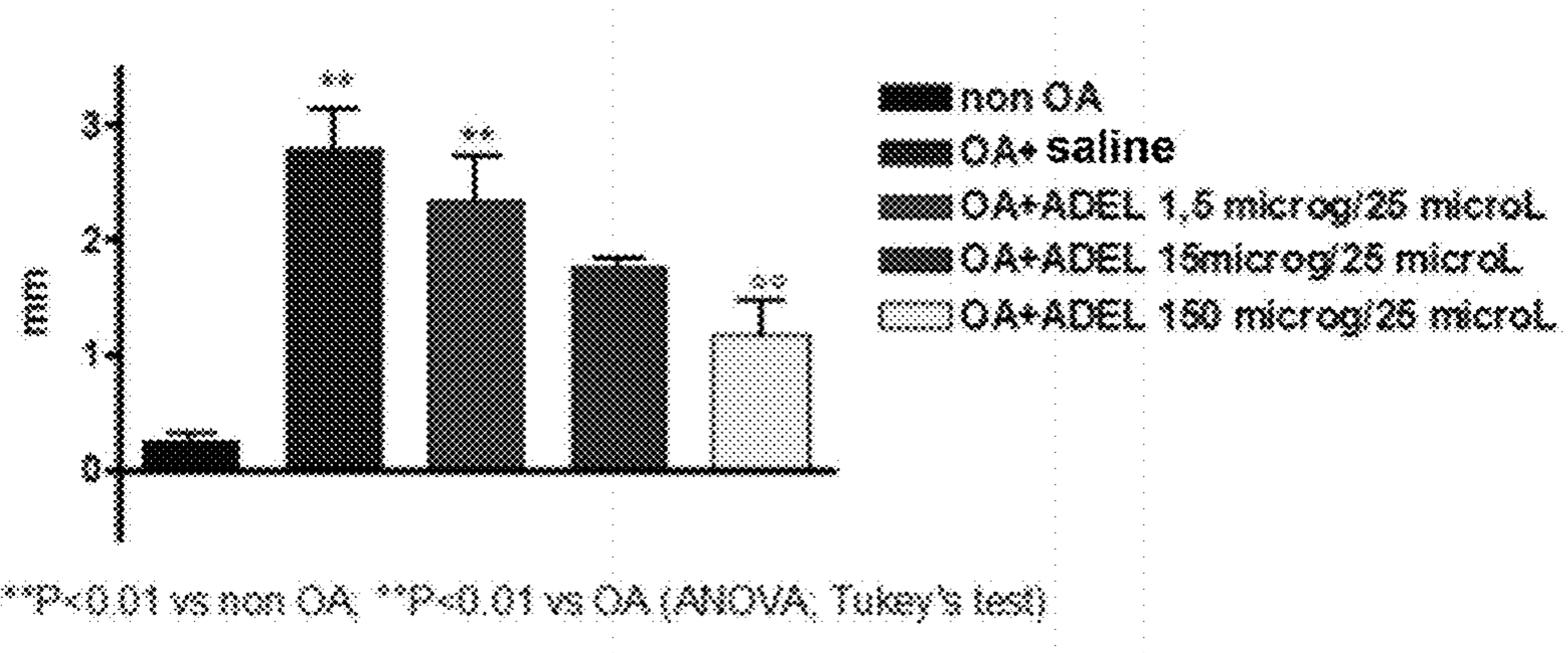
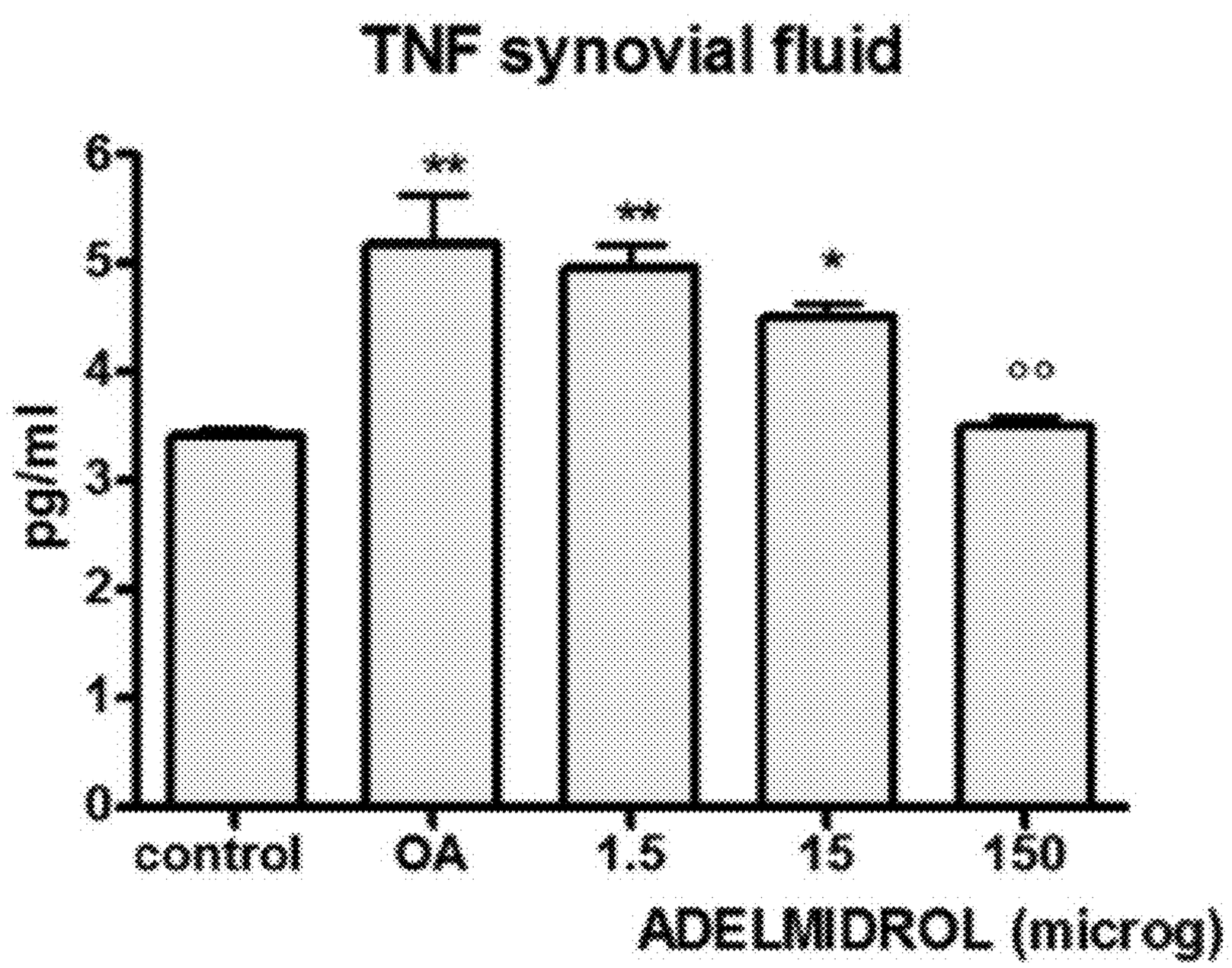

mm
0
1
2
3
**
**
**
non OA
OA+ saline
OA+ADEL 1,5 microg/25 microL
OA+ADEL 15microg/25 microL
OA+ADEL 150 microg/25 microL
*P<0.01 vs non OA; °P<0.01 vs OA (ANOVA, Tukey's test)

Fig. 2



*** P<0.001, ** P<0.01, * P<0.05 vs ctrl+saline
°° P<0.01, ° P<0.05 vs OA+saline
(one-way ANOVA Tukeys' test)

Fig. 3

USE OF ADELMIDROL IN THE TREATMENT OF EPITHELIAL DYSFUNCTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a continuation application of U.S. application Ser. No. 14/793,165, filed on Jul. 7, 2015, published; which claims priority to Italian Application Number MI2014A001245, filed on Jul. 8, 2014. The entire disclosures of the aforementioned applications are expressly incorporated herein by reference for all purposes.

DESCRIPTION

[0002] Technical Field of Invention

[0003] The object of the present invention is Adelmidrol in the treatment of epithelial dysfunctions.

[0004] Background Art

[0005] Palmitoylethanolamide (PEA) is an endogenous lipid N-acylamidic substance of which an effect on neuroinflammation and pain has been widely demonstrated [Calignano A. et al *Europ. J. Pharmacol.* 2001; 419:191-198; Skaper S. D. et al *Mol Neurobiol.* 2013; 48:340-352; Skaper S. D. et al. *Inflammopharmacology.* 2014; 22:79-94]. On the pharmacological level, the increase in the endogenous levels of PEA is currently considered important to determine the control of the neuroinflammation and pain mechanism due to different etiopathogenetic causes and associated with many diseases both of human beings and animals [Petrosino S. et al *WSAVA/FECAVA World Small Animal Congress* 2008; Richardson D. et al. *Arthritis Research & Therapy* 2008; 10 (2); R43; Ghafouri N. et al *PLoS ONE* 2011; 6(11); Naccarato M. et al *Lipids in Health and Disease* 2010; 9:47]. Two different pharmacological methods have currently been suggested to obtain this increase.

[0006] The first method is based on the systemic oral or sublingual administration of PEA: in this case, PEA must be administered in micronized (particle size in the range between 2 and 10 microns) or ultra-micronized (particle size in the range between 0.8 and 6 microns) form due to the high insolubility in water of the lipid molecule [EP 1207870 B1; WO 2011/027373 A1].

[0007] On the other hand, the second method is based on the inhibition of the activity of the specific PEA-degrading enzyme activity, i.e. FAAH (Fatty Acid Amide Hydrolase) and NAAA (N-Acylethanolamine Acid Amidase); this inhibition was obtained with the systemic administration of synthetic inhibitors of said hydrolases [Piomelli D. et al *CNS Drug Reviews* 2006; 12:21-38; Fiasella A. *ChemMedChem* 2014 Epub of Print] able to block the degradation of PEA. However, this method implies a serious problem since blocking the degradation of PEA through the block of specific degradation enzymes corresponds to preventing reuse, which is essential, of the PEA components, ethanolamine and palmitic acid, needed to return the phospholipid from which PEA is biologically synthesized "on demand" to the cell, through the phospholipid synthesis. There is also the problem that, while the systemic oral administration of PEA in micronized or ultramicronized form has been found to be effective and safe [Skaper S. D. et al *Inflammopharmacol.* 2014; 22:79-94; Esposito E. et al., *Mini Rev Med Chem.* 2013; 13:237-55], the topical use, in the form of a cream or solution, is very difficult and poorly effective because of the difficulty of producing, in this manner, pharmacologically effective amounts of PEA as a result of the high hydrophobicity of this important lipid molecule.

[0008] Adelmidrol is a synthetic N-acylamidic molecule as well (N,N'-Bis(2-hydroxyethyl)nonandiamide) with a high solubility in water as well as a good solubility in lipids.

SUMMARY OF INVENTION

[0009] The inventors of the present patent have surprisingly found that Adelmidrol, when brought in contact with epithelial cells such as keratinocytes, is able to cause an important increase of the endogenous levels of PEA while not interfering with the activity of the PEA-degrading enzymes (FAAH and/or NAAA). This discovery allows an effective pharmacological action on external (skin, mucocutaneous genital tissues, oral mucosa) and/or internal epithelia (bladder urothelium, ureter mucosa, mucous membranes of the seminal vesicles, mucous membranes of the digestive system, endothelial layer of synovial membranes, mucous membranes of the respiratory tract), with suitable pharmaceutical forms for the various uses, on diseases of the epithelia of different organs, both in human beings and in animals.

[0010] It may also be useful to enhance the activity of Adelmidrol on increasing the local levels of Palmitoylethanolamide by associating the same Adelmidrol with substances capable of modulating (but not blocking) the activity of PEA-degrading enzymes. In particular, PEA Oxazoline, described in the international application published with number WO 2013/121449 A1, may be used.

[0011] Therefore, an object of the present invention therefore is Adelmidrol for use in the treatment of epithelial tissue dysfunctions in a human being or animal.

[0012] A further object of the invention is a pharmaceutical formulation for use in the treatment of epithelial tissue dysfunctions in a human being or animal, the formulation containing Adelmidrol, optionally in association with an active ingredient selected from the group consisting of an oxazoline derivative of Palmitoylethanolamide, an antimicrobial agent, trans-traumatic acid, and hyaluronic acid or derivatives thereof.

[0013] The invention is defined by the appended claims.

[0014] Further features and advantages of the invention will become apparent from the following description of preferred embodiments thereof, provided by way of non limiting example.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. **1** shows the endogenous levels of AEA, 2-AG, PEA and OEA in HaCaT cells stimulated for 24 hours with 10 μM Adelmidrol. Ctrl (n=6) vs Adelmidrol 10 μM (n=6). $P<0.0001$ (***);

[0016] FIG. **2** shows the evaluation of knee edema using a digital caliper—From left to right: non OA: OA+ saline; OA+Adelmidrol 1.5 microgr/25 microL; OA+Adelmidrol 15 microgr/25 microL; OA+Adelmidrol 150 microgr/25 microL;

[0017] FIG. **3** shows the evaluation of the biochemical marker TNF-α in the synovial fluid.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The invention relates to Adelmidrol for use in the treatment of epithelial tissue dysfunctions in a human being or animal.

[0019] Adelmidrol is the International Non-proprietary Name (INN) of a synthetic derivative of azelaic acid, a naturally-occurring saturated dicarboxylic acid. In fact, azelaic acid has been found in the human body and its plasma levels are in the range between 20-80 ng/ml.

[0020] Chemically, Adelmidrol is N,N'-bis(2-hydroxyethyl)nonandiamide; the molecule has an amphiphile behavior as it has both hydrophilic and lipophilic properties able to promote solubility both in water and in organic solvents. These features, combined with the possibility of hot-sterilizing solutions containing Adelmidrol, make the molecule highly suitable for topical application on external surfaces and internal epithelia.

[0021] Epithelial dysfunctions treated with Adelmidrol according to the present invention are preferably selected from the group consisting of: oropharyngeal and esophageal Dysphagia with different etiology; gastroesophageal reflux; Cricopharyngeal Achalasia; esophageal Achalasia; stomatites with different etiology; Presbiphagia in the elderly; feline gingivostomatitis; periodontal disease also related to endodontics/orthodontics and dental implantology interventions; Burning Mouth Syndrome (BMS); Eyelid Edema; Blepharitis, Blepharoconjunctivitis; inward turning (entropion) and eversion (ectropion) of the eyelid; keratitis and keratitoconjunctivitis with different etiology (e.g., superficial punctate keratitoconjunctivitis); corneal lesions with different etiology; quali-quantitative alterations of the tear film; Dacryocystitis; Uveitis; Glaucoma; diseases of the ceruminous glands of the ear; ear hematomas in dog and cat; fly strike irritations in dog and cat; Pododermatites; Rhinites, Rhinotracheites, acute and chronic rhinopharyngites; acute and chronic Pharyngites; acute and chronic bronchites; Sinusites, Rinosinusites; bronchial asthma; Alopecia; nasal dermatoses in dog and cat; Acute and chronic sialadenites; neuropathic itch; bladder pain syndrome with different etiology attributable to alterations of the urothelium and in particular interstitial cystitis, cystitis due to systemic chemotherapeutics, cystitis due to bladder instillation of local chemotherapeutics, such as Epirubicin or Mitomycin, cystitis due to pelvic radiotherapy; chronic and/or recurrent Cystites; Gastrointestinal disorders attributable to alterations of the epithelium; Diseases of the ano-rectal segment, particularly posterior rectocele, proctites, muco-rectal prolapse, hemorrhoids, anal rhagades, perianal itching, diseases of the anal sacs in dog, and other perineal dysfunctions; small and medium vessel Vasculites and particularly granulomatous Vasculites, Vasculites due to immunocomplexes; Inflammations of the secondary sexual glands and particularly of the seminal vesicles and the seminal ducts; orofacial pain syndromes in the human and veterinary field; synovites associated with rheumatoid arthritis and osteoarthrosis.

[0022] The concentration of Adelmidrol in pharmaceutical forms for topical application (creams, gels, patches) intended for use in a human being and in an animal is in the range between 0.2% and 7.0%.

[0023] In solutions for application on internal epithelia (endovesical instillations, infusions in seminal vesicles, introduction in joint cavity, nebulizer solutions), Adelmidrol should be used in a concentration in the range between 0.3% and 5.0%.

[0024] The amounts of pharmaceutical forms for topical application (creams, gels, patches) for use both in a human being and in an animal are in the range between 0.01 and 0.5 ml per cm^2 of epithelium (e.g. skin, mucous membranes); thereby, the administered dose of Adelmidrol does not exceed the LD_{50} of the molecule by more than 10%, which is calculated in experimental animals and by oral administration, in 2-3 g/kg body weight.

[0025] The amount of Adelmidrol to be administered in the form of solutions intended for internal epithelia (endovesical instillations, infusions in seminal vesicles, introduction in joint cavity, nebulizer solutions) is in the range between 0.5 and 20 mg/Kg body weight.

[0026] It should be considered that it may be necessary to make continual changes in the dosage depending on the patient's age and weight and on the clinical severity of the condition being treated. Finally, the exact dose and route of administration will be at the discretion of the treating physician or veterinarian.

[0027] Adelmidrol may be administered in combination with an active ingredient selected from the group consisting of an oxazoline derivative of palmitoylethanolamide, an anti-microbial agent, trans-traumatic acid and hyaluronic acid or derivatives thereof.

[0028] The anti-microbial agent is preferably selected from *Echinacea purpurea* extract, *Usnea barbata* extract, usnic acid, phytosphingosine, bronopol and mixtures thereof.

[0029] In case of combination of Adelmidrol with hyaluronic acid and trans-traumatic acid, preferably the latter two active ingredients will be present in the sodium hyaluronate trans-traumatate double salt form.

[0030] When Adelmidrol is administered in combination with an active substance as defined above, a joint (i.e. in the same pharmaceutical formulation), separate or sequential administration may be provided.

[0031] A pharmaceutical formulation according to the invention may have the following composition by weight, the balance being related only to the active ingredients (thus, excluding carriers and excipients):

Adelmidrol	50-100%
Oxazoline derivative of PEA	0-5%
Hyaluronic acid or derivative thereof	0-5%
Trans-traumatic acid	0-5%
Anti-microbial agent	0-1%

[0032] The treatment with Adelmidrol is topical (on external epithelia or internal epithelia).

[0033] The inventive formulation can thus contain pharmaceutically acceptable additives and excipients, selected according to the selected pharmaceutical form, such as solvents, viscous carriers, tackifying agents (acrylic polymers), buffering agents, preservatives, antioxidants, gelling agents, thickeners and so on.

[0034] Pharmaceutical formulations suitable for both human and veterinarian use can be preferably selected from: instillation solutions, solutions for inclusion in the joint capsule, gels for internal or external use, spray solutions, eye drops, creams, salves, patches and ointments.

[0035] According to the present invention, the compounds can also be formulated as rectal formulations such as suppositories, retention enemas or micro-enemas, for example containing the basic components of common suppositories such as cocoa butter or other glycerides.

[0036] In addition to the formulations described above, the compounds may also be formulated as deposition preparations. Such long-acting formulations may be administered by implantation (e.g. subcutaneously or by transdermal or intramuscular route). Therefore, for example, the compounds according to the present invention may be formulated with appropriate hydrophobic or polymeric materials (e.g. in the form of an emulsion in a suitable oil) or ion exchange resins or as minimally soluble derivatives, for example as minimally soluble salt.

[0037] The formulations described above may be prepared according to conventional methods, such as those described in Remington's Pharmaceutical Sciences Handbook, Mack Pub. Co., N.Y., USA.

EXPERIMENTAL PART

[0038] In Vitro Test on Isolated Cells

[0039] Methods

[0040] Cultured human keratinocytes, HaCaT, were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with glutamine (2 mM), penicillin (400 U/ml), streptomycin (50 mg/ml), 10% FBS (Fetal Bovine Serum), in the presence of 5% CO 2 and at 37° C. Thereafter, cells plated in 6-well multi-wells (9×10^5/well), once 70% confluence had been reached, were stimulated with Adelmidrol, 10 μM, or carrier (Ctrl, 0.05% methanol) for 24 hours in the presence of 5% CO_2 at 37° C. After 24 hours, the cells and the surnatants were homogenized in 5 vol. of a chloroform/methanol TRIS-HCl 50 mM pH 7.4 (2:1:1) solution containing 10 pmol of $[^2H]_8$anandamide (AEA) and 50 pmol of $[^2H]_5$2-arachidonylglycerol (2-AG), $[^2H]_4$palmitoylethanolamide (PEA) and $[^2H]_2$oleoylethanolamide (OEA) (Bisogno et al., 1997). The organic-lipidic phase obtained from the four extractions with chloroform was subjected to purification by silica column chromatography, eluted by increasing concentrations of methanol in chloroform. The chloroform/methanol 90:10 fraction containing AEA, 2-AG, PEA and OEA was analyzed by liquid chromatography coupled to mass spectrometry using chemical ionization at atmospheric pressure (LC-APCI-MS) (Marsicano et al., 2002). The equipment used includes a Shimadzu HPLC (LC-10ADVP) coupled with a Shimadzu spectrometer (LCMS-2010) via a Shimadzu APCI interface. The ionization source temperature is 400° C. and a Phenomenex reverse phase HPLC column (C-18, 5 microns, 150×4.6 mm) is used. The mobile phase, consisting of a mixture of methanol/water/acetic acid (85/15/0.1%), passes through the column at a rate of 1 ml/min. The mass spectrum determination is carried out according to the selected ion monitoring (SIM) [Di Marzo et al. *Nature* 2001; 410:822-825]. Selected ions correspond to mass/charge values (m/z) of 356 and 348 (molecular ions of deuterated and non-deuterated AEA), 384.35 and 379.35 (molecular ions of deuterated and non-deuterated 2-AG), 304 and 300 (molecular ions of deuterated and non-deuterated PEA), 328 and 326 (molecular ions of deuterated and non-deuterated OEA). The quantity expressed as pmol/mg of lipid extract were compared using ANOVA followed by the Student-Newman-Keuls test.

[0041] The maximum reduction of the activity of the palmitoylethanolamide-degrading enzymes (NAAA and FAAH) was also measured using homogenates rat brain membranes [Ueda N et al *Chem Phys Lipids*. 2000 November; 108(1-2):107-21; Tsuboi K. Et al *J Biol Chem*. 2005 Mar. 25; 280(12):11082-92].

[0042] Results

[0043] The results obtained show that the levels of the cannabinomimetic PEA are significantly higher in HaCaT cells stimulated with Adelmidrol 10 μM (66.4±9.6) compared to basal values (31.6±5.6) (FIG. **1**).

[0044] No significant variation is observed in the levels of endocannabinoids (AEA, 2-AG) and other cannabinomimetics (OEA) (FIG. **1**).

[0045] Adelmidrol does not inhibit the activity of catabolic enzymes for PEA (FAAH and NAAA).

Maximum inhibition of the enzymatic activity (expressed as IC_{50})	Control	Treatment with Adelmidrol 50 μM
Enzyme FAAH	21.22 ± 3.18%	20.15 ± 4.12%
Enzyme NAAA	11.43 ± 1.72%	11.35 ± 2.26%

[0046] In Vivo Tests in Animals

[0047] Osteoarthritis Induced by Sodium Monoiodoacetate

[0048] Method

[0049] The experiments were conducted using adult male rats of the Wistar strain (weight 200-250 grams) supplied by Harlan Italy, put in enclosures for a week in standard dietary and environmental conditions (temperature 21±1° C., humidity 60±10%, light 12 hours a day and water and food ad libitum) before being used in the experiments. Knee osteoarthritis was induced in rats by a single intra-articular injection of monosodium iodoacetate (MIA) at a dose of 2 mg/254, in the infrapatellar area of the right knee, based on the method suggested by Kolbhen but revisited. MIA acts locally by inhibiting glycolysis, destroying the metabolism of chondrocytes and producing cartilage degeneration. At the time of the induction of osteoarthritis, MIA was dissolved in sterile saline. Before carrying out the intra-articular administration, the rats were anesthetized with pentobarbital sodium dissolved in saline at the dose of 60 mg/kg, in an administration volume of 0.2 ml/hg intraperitoneally (i.p.). The animals that received the MIA injection in their right knee represent the osteoarthritic (OA) group. A second group of rats subjected to an intra-articular injection of solvent only in the same right knee is instead the control group.

[0050] Behavioral Evaluation of Pain

[0051] Evaluation of Mechanical Allodynia

[0052] For the measurements of mechanical allodynia (painful response to normally painless stimuli), the Von Frey test (Ugo Basile, Varese, Italy) was used, an instrument consisting of a tactile stimulator that can be moved on a base Perspex platform, a metal grid supported by four columns placed at the corners of the base platform, two compartments further subdivided into which the animals are placed at least 15-30 minutes before the measurement, and an electronic microprocessor. The tactile stimulator is positioned inside an aluminum cylinder provided with a handle that allows the operator to move it on the platform. Inside the cylinder there is an electronic trigger that causes the lifting of a 0.5 mm diameter steel filament, positioned above the cylinder; the activation button of this mechanism is located on both sides of the handle. Thanks to a mirror positioned above the cylinder, alongside the filament, the stimulation can be applied in the correct point of the plantar surface and the movements of the foot can be monitored. The microproces-

sor is provided with an LCD display that shows the latency time (in seconds) up to the removal of the paw from the mechanical stimulation and the force applied by the filament on the paw (in grams). For measurements in mice, a maximum latency time of 20 seconds and a maximum force corresponding to 5 grams were set.

[0053] Evaluation of Knee Edema Through Digital Caliper

[0054] In order to evaluate the formation of the edema in the right knee of the animals after MIA injection, a digital manual slide caliper (measuring capacity 0-150 mm; resolution: 0.01 mm; ROHS Compliant Electronic Digital Caliper—2 Biological Instruments SNC, Italy) was used. The measurement was done manually by evaluating the diameter (expressed in mm) of the left and right knees of each animal at the kneecap. The edema was calculated as the difference between the volume of the right knee and left knee. The data are shown in FIG. **2**.

[0055] Evaluation of Biochemical Markers

[0056] Determination of the Tumor Necrosis Factor (TNF-α) Levels in the Spinal Cord

[0057] The determination of TNF-α levels in the synovial fluid was made using an ELISA (Enzyme Linked Immuno-Sorbent-Assay) immunoenzymatic assay using a commercial kit from Biosource International Inc. The method uses a procedure in which the antigen is trapped between two layers of antibodies and for this reason it is called an ELISA sandwich. The sample and the biotinylated antibody are added to the wells of a microtitration plate coated with specific antibodies to TNF-α and the first incubation is carried out, during which the specific cytokine in the sample interacts with both the antigen binding site exposed by the immobilized antibodies on the plate, and with the site of the biotinylated antibody present in solution. After removing the unbound material with a series of washes, the enzyme streptavidin peroxidase is added, which binds to the biotinylated antibody. After a second incubation and subsequent washing to remove the unbound enzyme, a solution containing the substrate (Stabilized Chromogen) is added. Following the enzymatic reaction, a product is generated whose staining intensity is measured spectrophotometrically and is directly proportional to the concentration of TNF-α in the samples.

[0058] Results

[0059] Treatment

[0060] Intra-articular administration of MIA day 0 (T0).

[0061] Intra-articular administration of Adelmidrol days 1, 8, 15. Three doses of Adelmidrol were adopted: 1.5; 15 and 150 μg/injection.

[0062] Evaluation of Inflammatory and Pain Parameters

[0063] The various parameters are measured at different times in relation to the parameter itself (e.g. inflammatory parameters are evaluated only in the first week since the MIA, pain is always evaluated before and 60 min. after the administration of Adelmidrol).

[0064] The results obtained show that Adelmidrol has a strong anti-inflammatory effect, the effect is frankly dose-dependent and evident at all the analyzed times. The regression analysis for the evaluation of the dose response was performed using the area under the curve for each dose used.

[0065] Evaluation of the Biochemical Marker TNF-α in the Synovial Fluid

[0066] The effect on edema is accompanied by a reduction in the levels of TNF-α in the synovial fluid (FIG. **3**).

[0067] In Vivo Tests in Human Beings

[0068] Intravesical instillation in patients with painful bladder syndrome (BPS)

[0069] Method

[0070] A sterile solution containing 2% Adelmidrol and 0.1% hyaluronic acid sodium salt was instilled into the bladder of no. 7 female patients through catheter after the complete emptying of the bladder itself. All patients had a confirmed diagnosis of BPS (Bladder Pain Syndrome). Most of the times, the patients had comorbidity with other pelvic diseases (vulvar vestibulitis 4/7; IBS (irritable bowel syndrome) 2/7; Fibromyalgia Syndrome 1/7, Recurrent Urinary Tract Infections (RUTI) 3/7).

[0071] Treatments were carried out as attack therapy (one intravesical instillation per week for 8 weeks), followed by maintenance therapy (one instillation per month for 6 months).

[0072] Using a dedicated diary, the frequency of urination (measured as the number of urinations in 12 hours), both during the day and at night, and the discomfort with bladder full were controlled, analyzing the pain, the feeling of weight and the burning in the pelvic area (all three parameters were measured by means of numerical scale VAS, before treatment, at the end of the attack therapy and at the end of the maintenance therapy).

[0073] Results

[0074] The results are shown in the following table.

[0075] All the parameters evaluated showed a marked improvement after treatment with Adelmidrol.

			Pat 01	Pat 02	Pat 03	Pat 04	Pat 05	Pat 06	Pat 07
Patient's age			35 years old	27 years old	28 years old	45 years old	72 years old	39 years old	46 years old
Treatment	Attack phase		1 instill/ week × 8 weeks	1 instill/ week × 8 weeks	1 instill/ week × 8 weeks	1 instill/ week × 8 weeks	1 instill/ week × 8 weeks	1 instill/ week × 8 weeks	1 instill/ week × 8 weeks
	Maintenance phase		1 instill/ month × 6 months	1 instill/ month × 6 months	1 instill/ month × 6 months	1 instill/ month × 6 months	1 instill/ month × 6 months	1 instill/ month × 6 months	1 instill/ month × 6 months
Urination frequency	Before treatment	Day	8x	7x	7x	8x	10x	18x	12x
		Night	2x	0x	1x	0x	1x	6x	2x
	At the end of attack phase	Day	6x	6x	6x	7x	7x	7x	7x
		Night	0x	0x	0x	0x	0x	1x	1x
	At the end of maintm. phase	Day	4x	5x	4x	5x	5x	5x	5x
		Night	0x	0x	0x	0x	0x	0x	0x

-continued

			Pat 01	Pat 02	Pat 03	Pat 04	Pat 05	Pat 06	Pat 07
Discomfort with bladder full	Pain in the pelvic area	Before treatm.	9	10	9	7.5	9	9	8.5
		At the end of attack phase	4	2	5	2.5	3	3	2
		At the end of maintm. phase	3	1	4	2	2	2	1
	Feeling of weight in the pelvic area	Before treatm.	6	8	7	7	6	9	9
		At the end of attack phase	3	1	4	3	4	4	5
		At the end of maintm. phase	1	1	2	1	3	2	3
	Burning in the pelvic area	Before treatm.	6	7	6	6	7	9	9
		At the end of attack phase	3	0	3	3	4	5	5
		At the end of maintm. phase	2	0	1	0	2	1	1

[0076] Examples of Formulation

Example 1—Sterile Solution for Intravesical Instillation

[0077] Each 50 ml vial contains:

Adelmidrol	1000 mg
Palmitoylethanolamide oxazoline	500 mg
Hyaluronic acid sodium salt	50 mg
Trans-traumatic acid	50 mg
Distilled water	as needed to 50 ml

Example 2—Sterile Anti-Adhesion Gel for Internal Use

[0078] A 500 ml tube contains:

Adelmidrol	15,000 mg
Hyaluronic acid sodium salt	2,500 mg
Apyrogenic distilled water	as needed to 500 ml

Example 3—Dense Solution for Rectal Use

[0079] A 10 ml micro-enema contains:

Adelmidrol	200 mg
Tocopherol acetate	5,000 mg
Transcutol	5,000 mg

Example 4—Viscous Endo-Urethral Solution

[0080] A 10 ml single dose squeezable container contains:

Adelmidrol	200 mg
Hyaluronic acid sodium salt	10 mg
Trans-traumatic acid	10 mg
Phytosphingosine	10 mg
Usnic acid	10 mg
Polyvinyl alcohol	20 mg
Noveon AA1	15 mg
Biotin	1 mg

Example 5—Sterile Solution for Intra-Articular Infiltration

[0081] A 2 ml vial contains:

Adelmidrol	30 mg
Palmitoylethanolamide oxazoline	15 mg
Hyaluronic acid sodium salt	10 mg
Phosphate buffer pH 7.0	as needed to 2 ml

Example 6—Neuropathic Anti-Itch Spray Solution for Veterinary Use

[0082] A 100 ml spray bottle contains:

Adelmidrol	2000 mg
Phytosphingosine	20 mg
Trans-traumatic acid	15 mg
Transcutol	as needed to 100 ml

Example 7—Eye Drops for Application on Corneal Abrasions

[0083] Each 1 ml single dose squeezable container contains:

Adelmidrol	25 mg
Hyaluronic acid sodium salt	2.0 mg
Trans-traumatic acid	2.5 mg
Sodium chloride	3.5 mg
Monobasic potassium phosphate	0.5 mg
Water	as needed to 1 ml

Example 8—Gel for Application on Oropharynx Mucosa

[0084] Each 250 ml container contains:

Adelmidrol	6,000 mg
Palmitoylethanolamide oxazoline	2,500 mg
Sodium carboxymethylcellulose	5,000 mg
Noveon AA1	500 mg

[0085] Water with the addition of preservatives as needed to 250 ml.

Example 9—Gel for Corneal Application

[0086] Each 10 ml tube contains:

Adelmidrol	100 mg
Hyaluronic acid sodium salt	100 mg
Trans-traumatic acid	10 mg
Noveon AA1	100 mg
Carbomer Ultrez 10 NF	20 mg
Monobasic potassium phosphate	5 mg
Thimerosal	1 mg
Distilled water	as needed to 10 ml

Example 10—Solution for Spraying with Aerosol

[0087] Each 5 ml sterile vial contains:

Adelmidrol	150 mg
Distilled water	as needed to 5 ml

What is claimed is:

1. A method of increasing the endogenous levels of palmitoylethanolamide without inhibiting the activity of the palmitoylethanolamide-degrading FAAH and NAAA enzymes in a human being or animal affected by an epithelial tissue dysfunction comprising bladder inflammation, the method comprising

administering Adelmidrol, wherein Adelmidrol causes the palmitoylethanolamide endogenous levels to increase.

2. The method according to claim **1**, wherein said Adelmidrol is administered in a combined, sequential, or separate manner in combination with at least one active ingredient selected from the group consisting of 2-pentadecyl-2-oxazoline of palmitoylethanolamide, an anti-microbial agent, trans-traumatic acid and hyaluronic acid or derivatives thereof.

3. The method according to claim **2**, wherein said anti-microbial agent is selected from *Echinacea purpurea* extract, *Usnea barbata* extract, usnic acid, phytosphingosine, bronopol and mixtures thereof.

4. The method according to claim **2**, wherein hyaluronic acid and trans-traumatic acid are present in the sodium hyaluronate trans-traumatate double salt form.

5. The method according to claim **1**, wherein the amount of Adelmidrol to be administered in the form of solutions intended for the epithelia ranges between 0.5 and 20 mg/Kg body weight.

6. A method of increasing the endogenous levels of palmitoylethanolamide without inhibiting the activity of the palmitoylethanolamide-degrading FAAH and NAAA enzymes in a human being or animal affected by an epithelial tissue dysfunction comprising bladder inflammation, the method comprising administering a pharmaceutical formulation containing Adelmidrol and one or more active ingredients selected from the group consisting of 2-pentadecyl-2-oxazoline of palmitoylethanolamide, an anti-microbial agent, trans-traumatic acid, and hyaluronic acid.

7. The method according to claim **6**, wherein said formulation has the following weight composition, the balance being relative only to the active ingredients:

Adelmidrol	50-100%
2-pentadecyl-2-Oxazoline of PEA	0-5%
Hyaluronic acid	0-5%
Trans-traumatic acid	0-5%
Anti-microbial agent	0-1%.

8. The method according to claim **6**, wherein said anti-microbial agent is selected from *Echinacea purpurea* extract, *Usnea barbata* extract, usnic acid, phytosphingosine, bronopol and mixtures thereof.

9. The method according to claim **6**, wherein hyaluronic acid and trans-traumatic acid are present the sodium hyaluronate trans-traumatate double salt form.

10. The method according to claim **6**, wherein the weight concentration of Adelmidrol in the pharmaceutical forms for topic application for use in both human beings and animals ranges between 0.2% and 7.0%, and the weight concentration of Adelmidrol in the solutions for application on internal epithelia ranges between 0.3% and 5.0%.

11. A method of treating bladder inflammation, the method comprising administering to a subject affected by bladder inflammation an effective amount of Adelmidrol so as to increase endogenous levels of palmitoylethanolamide without inhibiting the activity of palmitoylethanolamide-degrading FAAH and NAAA enzymes in the subject, and thereby treating bladder inflammation in the subject, wherein the subject is a human being or animal.

* * * * *