(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2001 (15.02.2001)

PCT

(10) International Publication Number
WO 01/10434 A1

(51) International Patent Classification[7]: A61K 31/16, A61P 37/00

(21) International Application Number: PCT/IT99/00259

(22) International Filing Date: 6 August 1999 (06.08.1999)

(25) Filing Language: Italian

(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **INNOVET ITALIA S.R.L.** [IT/IT]; Via Egadi, 7, I-20144 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **COMELLI, Cristina** [IT/IT]; Via dei Mille, 24, I-35141 Padova (IT). **DELLA VALLE, Maria, Federica** [IT/IT]; Via Lepanto, 9, I-35141 Padova (IT). **DELLA VALLE, Francesco** [IT/IT]; Via Cerato, 14, I-35122 Padova (IT). **MARCOLONGO, Gabriele** [IT/IT]; Via Strade Interne, 3, I-35020 Due Carrare (IT).

(74) Agents: **LONG, Giorgio** et al.; Jacobacci & Perani S.p.A., Via Senato, 8, I-20121 Milano (IT).

(81) Designated States *(national)*: JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/10434 A1

(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING N-PALMITOYLETHANOLAMIDE AND USE THEREOF IN THE VETERINARY FIELD

(57) Abstract: The present invention relates to pharmaceutical compositions containing N-palmitoylethanolamide (palmidrol) for use in the veterinary field, particularly for the treatment of the eosinophilic skin condition in felines which is normally known as the Eosinophilic Granuloma Complex, and of tendonous keloids in horses. Pharmaceutical compositions containing from 20 mg to 4 g of N-palmitoylethanolamide per 100 g of composition for use in the cat and from 15 g to 40 g of N-palmitoylethanolamide per 100 g of composition for use in the horse are described.

PHARMACEUTICAL COMPOSITIONS CONTAINING N-PALMITOYLETHANOLAMIDE AND USE THEREOF IN THE VETERINARY FIELD

The present invention relates to pharmaceutical compositions containing N-palmitoylethanolamide (palmidrol) for use in the veterinary field, particularly for the treatment of the eosinophilic skin condition in felines which is normally known as Eosinophilic Granuloma Complex, and of tendonous keloids in horses.

The eosinophilic condition in felines (Moriello K et al, 1997, Handbook of Small Animal Dermatology, pp. 205-208, Pergamon Press) has clinical signs such as erythema, pruritis and alopecia, and skin symptoms which are recognizable in the form of eosinophilic plaque (EP), eosinophilic granuloma (EG), and miliary or papulo-scabby dermatitis, which can appear in the animal individually or simultaneously or at different times.

EP is a circumscribed area of erosion and exudation associated with clinical signs such as erythema, pruritis and auto-induced alopecia. Although they may appear anywhere on the skin surface, the lesions are located preferentially in the inguinal or perianal regions or in the medial region of the upper rear leg. The

characteristic hystopathology of EP shows considerable cell infiltration in the perivascular spaces, associated with epidermal hyperplasia, spongiosis and ulceration.

EG appears as an erythematous, alopecic and raised area generally located on the caudal face of the rear paws on the extremities (the claw bed and the pads), in the oral cavity, or on the chin. The characteristic hystopathology of EG appears as a diffuse granulomatous dermatitis associated with areas of collagenolysis.

Miliary dermatitis is similar to EP but less extensive and with the formation of scabs.

Regardless of whether it is in the form of EP or EG, eosinophilic granuloma with lesions, is a highly recurrent condition. For this reason, animals suffering from eosinophilic granuloma, in the form either of EP or of EG, are subject throughout their lives, to intermittent or continuous treatments with antihistamines and corticosteroids the side effects of which, particularly in treatment of long duration, are known and documented.

The identification of active ingredients which can alleviate or resolve the inflammatory picture, at the same time ensuring maximum tolerability and an absence of adverse reactions, is an objective of considerable

interest in veterinary treatment.

Tendonous keloids represent one of the most common chronic manifestations of tendinitis which arise in horses - and in particular in competition horses - as a result of acute inflammations of the tendons, as well as partial or complete traumatic lesions and also lesions associated with haemorrhage and oedema.

Tendonous keloids appear as soft, easily palpable masses of variable sizes and are constituted by fibrous tissue with thickenings and adhesions in the peritendonous area. Horses with chronic tendon symptoms of this type notice pain with clear lameness which greatly comprise their competitive performance.

The treatment of these symptoms, when they are chronic, is particularly complex since the anti-inflammatory drugs normally used (FANS and corticosteroids) are more effective in the acute phases than in the consequent chronic manifestations.

Up to now, various treatments have been tried with little success; in particular, it has been noted that the intratendonous administration of corticosteroids is contraindicated. Up to now, use has been made of very questionable operations such as superficial burning, percutaneous splitting of the tendons, or implantation of

carbon fibres.

Basically, up to the present time, there has been no pharmacological treatment which has solved the chronic symptomatic manifestations of tendinitis in horses.

It has now surprisingly been found that N-palmitoylethanolamide (common international name : palmidrol) is effective in the treatment of eosinophilic granuloma in cats, for both EP and EG lesions, and of tendonous keloids in horses. In the latter case in particular, a complete recovery of the animal with the possibility of a return to the competition circuit has been confirmed.

The subject of the present invention is therefore the use of n-palmitoylethanolamide, preferably in micronized and/or co-micronized form, for the preparation of pharmaceutical compositions for veterinary use, particularly for the treatment of eosinophilic granuloma in cats, for both EP and EG lesions, and of tendonous keloids in horses.

A further subject of the present invention is pharmaceutical compositions containing N-palmitoylethanolamide in micronized and/or co-micronized form.

The treatment of cats with N-palmitoylethanolamide

provides for the administration of the drug in quantities of from 1 to 50 mg/kg/die for a period of between 15 and 60 days. A preferred treatment scheme provides for a daily administration of 10 mg/kg of body weight for 30 consecutive days.

The treatment of horses with N-palmitoylethanolamide provides for the administration of the drug in quantities of from 0.5 to 5 g/die, preferably 2 g/die for a period of between 20 and 150 consecutive days, preferably between 30 and 120 days.

The following examples explain the invention and the preferred method of implementing it without, however, being limiting thereof.

BIOLOGICAL EXAMPLES

Example A - Effect of oral treatment with N-palmitoylethanolamide in eosinophilic skin condition in cats

Method

Included in the investigation were 15 cats of European race with short hair, of which 9 were female and 6 were male, with ages of between 7 and 123 months. All of the animals had symptoms of the eosinophilic condition, such as pruritis, alopecia and erythema, and the skin manifestations associated therewith and, more

precisely, 6 subjects had EP, 5 had EG and 4 had milary dermatitis (scabs). A numerical evaluation relating to the intensity and location of the signs and symptoms was assigned to each individual animal in accordance with the P.A.S.I. (psoriasis area severity index) "score" (Marks R. et al., 1989, Arch. Dermatol., 125: 235-240). The improvements in the clinical signs and in the associated lesions were evaluated on the 15th and 30th days of treatment. The treatment consisted of a preparation in accordance with Example 3 of the pharmaceutical preparations given below, containing 120 mg of micronized N-palmitoylethanolamide. The active ingredient was administered in a proportion of 10 mg/kg/die for 30 days.

Table 1 below summarizes the results of the test (PEA = N-palmitoylethanolamide):

	SYMPTOMS pruritis-erythema-alopecia %			SIGNS plaque-granuloma-miliary scabs %		
	improved	unchanged	worsened	improved	unchanged	worsened
not treated						
T15	0	80	20	0	85	15
T30	0	70	30	0	80	20
treated with composition of Example 3						
T15	14,3	85,7	0	30	70	0
T30	67	33	0	66,7	33,3	0
treated with non-micronized PEA (10 mg/kg/die)						
T15	8,2	91,8	0	14,6	85,4	0
T30	52	48	0	51,4	48,6	0
treated with cortisones						
T15	28	72	0	40	60	0
T30	65	35	0	64,8	35,2	0

Example B - Effect of oral treatment with N-palmitoylethanolamide in the treatment of tendonous keloids in competition horses

CASE 1

On initial examination, an 8 year-old thoroughbred male chestnut racehorse had a large keloid, soft to the touch, in the palmar region of the metacarpal of the lower left-hand limb in a proximal position on the profound flexor tendon. The horse had undergone an operation on the above-mentioned tendon 6 months previously for serious traumatic lesion of the sheath.

The animal had a pronounced limp and was practically immobilized.

Treatment for 30 days with the preparation of Example 1 of the pharmaceutical compositions given below, involving administration of 2 g/die of micronized N-palmitoylethanolamide, brought about a regression of the keloid by between 90 and 95%.

The animal was able to recommence competitive activity.

CASE 2

Upon examination, a 3-year-old half-breed female bay racehorse had a spheroidal formation the size of a lemon, of soft connective tissue in the latero-palmar region of the metacarpal of the left-hand front limb. The keloidal formation was connected to the profound flexor tendon. The tendon had been burnt about four months previously. After treatment for 30 days with the preparation of Example 1 of the pharmaceutical preparations given below, involving the administration of 2 g/die of micronized N-palmitoylethanolamide, the formation regressed completely and the functionality of the tendon was completely restored.

CASE 3

A 5-year-old male bay racehorse, had signs of

recurrent, chronic tendon inflammation on the right-hand front profound flexor. 2 years previously, the subject had undergone a tendonectomy for lesion to the sheath, from which it had never fully recovered. After treatment for 30 days with the preparation of Example 1 of the pharmaceutical preparations given below, involving the administration of 2 g/die of micronized N-palmitoylethanolamide, the tendon was clearly reduced. After a further 30 days with the same treatment regime, the subject was completely cured with considerable benefit to its competitive performance.

CASE 4

A 2 year-old thoroughbred male bay racehorse had acute tendinitis of the right-hand front profound flexor in the full inflammatory phase. As a result of an operation performed for the reduction of tendonous oedema, the animal developed a considerable fibrotic reaction with the production of adhesions. 5 months after the operation, treatment was started with the preparation of Example 1 of the pharmaceutical preparations given below, involving the administration of 2 g/die of micronized N-palmitoylethanolamide. After 30 days a considerable improvement was noted and the treatment was continued for a further 60 days.

After treatment for 90 days, the tendon was perfectly shaped and the adhesions previously found had completely regressed. The animal returned to competitive activity.

It is clear from the results given above that N-palmitoylethanolamide can advantageously be used in the treatment of eosinophilic granuloma in cats, for both EP and EG lesions, and of tendonous keloids in horses, both when these conditions are acute and when they are chronic.

In the latter animal in particular, the treatment with N-palmitoylethanolamide seems to be the only effective cure for tendonous keloids which enables the horse to return to competitive activity.

The treatment of the cat with N-palmitoylethanolamide, on the other hand, gave results comparable to treatment with cortisones, with the substantial advantage that it does not have the serious side effects typical of these drugs.

The use of N-palmitoylethanolamide in micronized and/or co-micronized form (for example, with lactose) is particularly advantageous in bringing about the positive outcome of the treatment.

Clearly, the use of N-palmitoylethanolamide,

preferably in micronized and/or co-micronized form, for the treatment of eosinophilic granuloma in cats, for lesions in both EP and EG forms, and of tendonous keloids in horses, may be extended to all animals, particularly felines and equines, which have conditions of the same type.

PREPARATION OF N-PALMITOYLETHANOLAMIDE (PEA)

PEA is a known compound and can be prepared in accordance with the synthesis method described in EP 0 550 008 which is incorporated herein by reference.

The micronization of PEA and its co-micronization with excipients were performed with compressed-air turbine micronizing apparatus. This apparatus is known and is not therefore described in greater detail.

The product obtained was subjected to analysis of the particles.with Mastersizer, μ version apparatus from Malvern Instruments Co. UK. The final fineness of the PEA particles produced can be summarized as follows:

particle size	quantity %
> 14 μ	traces
< 10 μ	96% approx.
< 6 μ	80%

It should be noted that this result of the micronization method obtained with PEA is surprising since it is

unusual for a molecule of a lipid nature to produce particles with a mean fineness much less than 10 μ. The extreme fineness of the particles can be translated into improved absorption of the drug.

EXAMPLES OF PHARMACEUTICAL PREPARATIONS

Example 1 - oral powder for horses

100 g contained:

micronized N-palmitoylethanolamide	22.22 g
maize starch	77.78 g

Example 2 - oral granules for horses

100 g contained:

N-palmitoylethanolamide co-micronized with lactose	35.0 g
lactose co-micronized with N-palmitoyl-ethanolamide	28.0 g
maize starch	27.0 g
carboxymethyl cellulose	10.0 g

Example 3 - tablets for cats

Each tablet, divisible from 350 g, contained:

micronized N-palmitoylethanolamide	120 mg

maize starch	30 mg
lactose	115 mg
carboxymethyl cellulose	15 mg
microcrystalline cellulose	60 mg
magnesium stearate	10 mg

Example 4 - oily gel for cats

100 g contained:

N-palmitoylethanolamide co-micronized with lactose	2.5 g
lactose co-micronized with N-palmitoylethanolamide	1.5 g
soya lecithin	82.5 g
geleol	12.0 g
Vitamin E acetate	0.5 g

Example 5 - Gel for use on oral mucosae in cats

100 g contained:

micronized N-palmitoylethanolamide	300 mg
hyaluronic acid, sodium salt (titrated in bio-binding epitope)	200 mg
carbomer	280 mg
methyl paraoxybenzoate	200 mg
ethyl paraoxybenzoate	50 mg

fish flavouring	800 mg
sorbitol	20 g
demineralized water to make up to	100 g

In general, a composition for administration to cats contains from 20 mg to 4 g of PEA per 100 g of composition. A composition for administration to horses contains from 15 g to 40 g of PEA per 100 g of composition.

Clearly, other pharmaceutical compositions containing a pharmacologically effective dose of N-palmitoylethanolamide together with pharmacologically acceptable excipients may be provided. These compositions may be in the form of capsules, tablets, powders and pellets, and also in gastroresistant formulations for oral administration and may also be produced with the use of preliminary microencapsulation, liposomization or micellization techniques. For topical routes, including the transdermal route, formulations in suppositories, micro-enemas, creams, ointments, sprays, gels, foams, dressings of various thicknesses and patches may be used. All possible pharmaceutical forms indicated for the various administration routes may also be formulated with excipients or by technological processes

suitable for producing fast-release or slow-release medicaments.

CLAIMS

1. Use of N-palmitoylethanolamide for the preparation of a pharmaceutical composition for the treatment of eosinophilic granuloma in Felines, for lesions in both EP and EG forms, and of tendonous keloids in Equines, these conditions being manifested in both acute and chronic form.

2. Use according to Claim 1, in which the Feline is the cat and the Equine is the horse.

3. Use according to Claim 1 or Claim 2, in which the N-palmitoylethanolamide is present in micronized form or is co-micronized with an excipient.

4. A pharmaceutical composition containing N-palmitoylethanolamide in micronized form or co-micronized with an excipient, together with pharmaceutically acceptable excipients.

5. A pharmaceutical composition according to Claim 4, containing from 20 mg to 4 g of N-palmitoylethanolamide per 100 g of composition, for use in the cat.

6. A pharmaceutical composition according to Claim 4, containing from 15g to 40g of N-palmitoylethanolamide per 100 g of composition, for use in the horse.

7. A pharmaceutical composition according to any one of Claims 4, 5 and 6, in the form of an oral powder, oral granules, tablets or gel.

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/IT 99/00259

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/16 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	EP 0 550 008 A (LIFEGROUP SPA) 7 July 1993 (1993-07-07) page 2, column 56 -page 3, column 51 page 6, line 46 -page 7, line 1; claims ---	1-7
X,Y	EP 0 550 006 A (LIFEGROUP SPA) 7 July 1993 (1993-07-07) page 2, line 20-25 page 5, line 8-46 page 15-17 ---	1-7
X,Y	WO 96 18391 A (LIFEGROUP SPA ;DELLA VALLE FRANCESCO (IT); LEON ALBERTA (IT); MARC) 20 June 1996 (1996-06-20) page 6, line 8 -page 8, line 12 page 74-78; claims --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2000	30/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Orviz Diaz, P

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/IT 99/00259

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X,Y	MAZZARI S ET AL: "N-(2-HYDROXYETHYL)HEXADECANAMIDE IS ORALLY ACTIVE IN REDUCING EDEMA FORMATION AND INFLAMMATORY HYPERALGESIA BY DOWN-MODULATING MAST CELL ACTIVATION" EUROPEAN JOURNAL OF PHARMACOLOGY,NL,AMSTERDAM, vol. 300, no. 3, 1 January 1996 (1996-01-01), pages 227-236, XP000573451 ISSN: 0014-2999 the whole document ---	1-7
Y	WO 97 10712 A (MARGOLIN SOLOMON B) 27 March 1997 (1997-03-27) page 3, line 33,34 page 7, line 14-19 page 8, line 19 -----	1-7

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

..formation on patent family members

Inter ..al Application No
PCT/IT 99/00259

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0550008 A	07-07-1993	IT 1252866 B	28-06-1995
		CA 2086441 A	01-07-1993
		JP 5339153 A	21-12-1993
		US 5618842 A	08-04-1997
EP 0550006 A	07-07-1993	IT 1252865 B	28-06-1995
		AT 184193 T	15-09-1999
		CA 2086442 A	01-07-1993
		DE 69229944 D	14-10-1999
		DE 69229944 T	13-01-2000
		JP 5345722 A	27-12-1993
		US 5506224 A	09-04-1996
WO 9618391 A	20-06-1996	IT MI942512 A	14-06-1996
		AU 4344296 A	03-07-1996
		CA 2207850 A	20-06-1996
		EP 0799033 A	08-10-1997
		JP 11500411 T	12-01-1999
		US 5990170 A	23-11-1999
WO 9710712 A	27-03-1997	AU 709178 B	26-08-1999
		AU 7239996 A	09-04-1997
		BR 9610480 A	16-03-1999
		CA 2232191 A	27-03-1997
		EP 0866656 A	30-09-1998
		JP 11512699 T	02-11-1999
		US 5962478 A	05-10-1999