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(54) Title: TREATMENT OF CONTACT DERMATITIS

(57) Abstract: The present invention provides a method for treating contact dermatitis by the topical administration of a caspase-1 inhibitor. The present invention further provides a pharmaceutical composition comprising a caspase-1 inhibitor for the treatment of contact dermatitis.



**WO 2015/144183 A1**

## Treatment of contact dermatitis

### Field of invention

- 5       The present invention relates to a method of treating contact dermatitis by topical administration of caspase-1 inhibitors.

### Background of invention

- 10       The skin is one of the first lines of defense against foreign threats like pathogens and chemicals. If the immune system of the skin responds to non-harmful substances like proteins for plants or metals like nickel it is called a hypersensitivity reaction or an allergic reaction. The two major forms of allergic reactions in the skin are type I and type IV reactions. The type 1 reactions includes atopic dermatitis and urticaria and are immunologically driven by T-helper 2-type effector cells producing IL-4, IL-5 and IL-13
- 15       and involves IgE and mast cells and the reaction can be seen within minutes after allergen exposure. The allergens inducing type I allergy are protein allergens like birch pollen allergen betv1 and house dust mite allergen Der p2 and Der f2. In contrast, to type I allergy, type IV allergy is induced by small chemicals (Haptens) that need to modify self-proteins to induce an immune response. Type 4 allergy reactions are
- 20       mediated by T cells and therefore the reactions are normally seen 24-72 hrs after allergen exposure. The classical example of a type IV reaction is allergic contact dermatitis.

- 25       The present invention relates in one embodiment to treatment of contact dermatitis. Contact dermatitis is a localized inflammation of the skin caused by contact with a foreign substance (irritants or allergens). Contact dermatitis can be subdivided into irritant contact dermatitis and allergic contact dermatitis based on the inducing substance. Inflammation of the affected tissue is present in the epidermis (the outermost layer of skin) and the outer dermis (the layer beneath the epidermis). Unlike urticaria, in
- 30       which a rash appears within minutes of exposure and fades away within minutes to hours, allergic contact dermatitis takes days to fade away. Even then, contact dermatitis fades only if the skin no longer comes in contact with the allergen or irritant. Contact dermatitis results in large, burning, and itchy rashes, and these can take anywhere from several days to weeks to heal. Chronic contact dermatitis can develop
- 35       when the removal of the offending agent no longer provides the expected relief.

Allergic contact dermatitis is mediated by CD8+ T-cells and T-helper 1-type effector cells and T-helper 17-type effector cells. IL-1 $\beta$  have been shown to play a central role in the inflammatory response induced by contact allergens. IL-1 $\beta$  is produced by both  
5 keratinocytes and Langerhans cells in the epidermis following skin exposure to allergens. IL-1 $\beta$  leads to activation of the Langerhans cells and induce the migration of this to the draining lymph node. Furthermore, IL-1 $\beta$  is involved in the local inflammation in the skin. The present invention relates to the use of caspase-1 inhibitors for treatment of contact dermatitis. Caspase-1 is required for the cleavage of pro-IL-1 $\beta$  into  
10 the active IL-1 $\beta$ . The present invention relates to the discovery that topically administered caspase-1 inhibitors show a significantly better treatment result for contact dermatitis than the traditional treatment of topically administered steroids.

US2002/0058630 describes compositions comprising caspase inhibitors in general for  
15 treatment of IL-1 mediated disease in general. A long list of diseases to be treated is mentioned in the document; however contact dermatitis is not mentioned. The document does not describe the use of topical administration of a caspase-1 inhibitor in for use in the treatment of contact dermatitis.

20 Li et al. describe the effect of administering caspase-1 inhibitors prior to the subjection to allergen. This is very different from the real life situation where one would not administer caspase-1 inhibitors prophylactically, but would rather wait with the administration of any treatment until symptoms of contact dermatitis have occurred. Li et al. accordingly do not describe that caspase-1 inhibitors have a significantly better  
25 effect than the traditional treatment using topical administration of steroids.

WO 1998/41232 describes a method of modulating responsiveness to corticosteroids in a subject. This method involves in one embodiment that the corticosteroid is combined with an inhibitor of a caspase family protease, preferably an ICE inhibitor  
30 (caspase-1 inhibitor). A preferred composition comprises an ICE inhibitor, a corticosteroid and a pharmaceutically acceptable carrier. The document does not describe topical administration of a caspase-1 inhibitor for treatment of contact dermatitis.

Contact dermatitis is traditionally treated with topical or systemic administered glucocorticosteroids, which until now has been the only effective method of treating contact dermatitis. The use of steroid products is connected with several unwanted side effects and thus a new treatment method is needed.

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### Summary of invention

The present inventors have found that topical administration of a caspase-1 inhibitor for the treatment of contact dermatitis has a surprisingly high efficacy. The topical administration of caspase-1 inhibitors does not have the unwanted side-effects of the current treatments that typically comprise the administration of steroids.

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Thus in one embodiment the present invention relates to a caspase-1 inhibitor for use in the treatment of contact dermatitis by topical administration. Said inhibitor may be selected from the group comprising: Ac-YVAD-cmk, z-WEHD-FMK, YVAD-CHO, VX-765 and Ac-YVAD-FMK. The inhibitor may be used for the treatment of any type of contact dermatitis including allergic contact dermatitis, irritant contact dermatitis and/or photocontact dermatitis.

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In another embodiment the present invention relates to a pharmaceutical composition for topical administration comprising a caspase-1 inhibitor. Said inhibitor may be selected from the group comprising: Ac-YVAD-cmk, z-WEHD-FMK, YVAD-CHO, VX-765 and Ac-YVAD-FMK. The composition may furthermore comprise a second active ingredient, which may be the active ingredient of the treatments of contact dermatitis currently in use and thus be selected from the group comprising steroids, vitamin D and/or tacrolimus. The composition may furthermore comprise a second active ingredient which may be an inhibitor of the inflammasome and thus be one or more compounds selected from the group comprising glyburide, 16673-34-0, Parthenolide, Bay 11-7082, VX-740, VX-765, and/or a 1Cys-LT receptor antagonist (as described in Ema Ozaki et al. 2015)

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The inflammasome is a large molecular platform that triggers the activation of caspases. The inflammasome contains a pattern recognition receptor, which upon recognition of pathogen- or damaged-associated molecular patterns, oligomerizes with the adaptor protein ASC and procaspase-1 leading to activation of caspase-1.

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### Description of Drawings

Figure 1: A diagram showing the ear swelling (%) in a mouse after treatment with Group 1) control; Group 2) DNFB alone; Group 3) DNFB + Hydrocortison 1%; Group 4) DNFB + caspase-1 inhibitor 0.1%.

Figure 1a: A diagram showing the change in ear thickness after treatment with various treatments as in figure 1. Cas (Selleckchem): Caspase-1 inhibitor (Belnacasan (VX-765) (cat no. S2228). Cas (Santa Cruz): Caspase-1 inhibitor (Caspase-1 Inhibitor II (Ac-YVAD-CMK) (cat no. sc-300323). Cas (Sigma): Caspase-1 inhibitor (AC-YVAD-CMK) (cat no. SML0429).

Figure 2: Groups of C57bl/6 mice were treated for three consecutive days and after a minimum of 21 days challenged as indicated. After 24 hours, changes in ear-swelling were measured as a sign of inflammation. Mice were treated with (1) Vaseline (Control), (2) 0.15% DNFB plus vaseline, (3) 0.15% DNFB plus Mildison, (4) 0.15% DNFB plus Locoid, (5) 0.15% DNFB plus 0.1% Caspase-1 inhibitor (Ac-YVAD-cmk (Sigma-Aldrich, Denmark)), and (6) 0.15% DNFB plus 0.5% Caspase-1 inhibitor (Ac-YVAD-cmk (Sigma-Aldrich, Denmark)).

Figure 3: Groups of C57bl/6 mice were treated for three consecutive days and after a minimum of 21 days challenged as indicated. After 24 hours, the local inflammation was evaluated by H&E stainings. Mice were treated with (1) Vaseline (Control), (2) 0.15% DNFB plus vaseline, (3) 0.15% DNFB plus Mildison, (4) 0.15% DNFB plus Locoid, (5) 0.15% DNFB plus 0.1% Caspase-1 inhibitor (Ac-YVAD-cmk (Sigma-Aldrich, Denmark)), and (6) 0.15% DNFB plus 0.5% Caspase-1 inhibitor (Ac-YVAD-cmk (Sigma-Aldrich, Denmark)).

Figure 4: Groups of C57bl/6 mice were treated for three consecutive days and after a minimum of 21 days challenged as indicated. After 24 hours, ears were collected and snap-frozen. Proteins were purified from the skin and the levels of IL-1b were measured by ELISA. Mice were treated with (1) Vaseline (Control), (2) 0.15% DNFB plus vaseline, (3) 0.15% DNFB plus Mildison, (4) 0.15% DNFB plus Locoid, (5) 0.15% DNFB plus 0.1% Caspase-1 inhibitor (Ac-YVAD-cmk (Sigma-Aldrich, Denmark)), and

(6) 0.15% DNFB plus 0.5% Caspase-1 inhibitor (Ac-YVAD-cmk (Sigma-Aldrich, Denmark)).

## 5 Detailed description of the invention

### ***Definitions***

As used herein, the term “patient” and “individual in need” refers to a mammal, such as for example rats, mice, dogs, cats, horses, guinea pigs, and primates such as humans.

10 “Treatment” can be performed in several different ways, including curative and ameliorating. Curative treatment generally aims at curing a clinical condition, such as a disease or an infection, which is already present in the treated individual. Ameliorating treatment generally means treating in order to improve, in an individual, an existing clinical condition.

15 % by weight as used herein is intended to be used in its normal meaning, i.e. grams (g) of caspase-1 inhibitor in gram (g) of the medicament or composition.

20 The term “Locoid” as used herein refers to a Locoid® Lipocream comprising 0.1% w/w Hydrocortisone butyrate from Astellas. Known Locoid side effects are: Thinning of the skin, redness of the skin, bruising and stretch marks. Facial spots, redness of the skin around the mouth. Worsening of the skin condition after treatment has stopped. Discolouration of skin. Suppression of the release of the stress hormones.

25 The term “Mildison” as used herein refers to a Mildison® Lipocream 1% cream comprising 1% w/w Hydrocortisone. Known Mildison side effects are: Redness of the skin, rash/inflammation, bruising, stretch marks and skin thinning. Facial spots, redness of the skin around the mouth. Worsening of the skin condition after treatment has stopped. Discolouration of skin. Skin irritation.

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### ***Caspase-1 and caspase-1 inhibitors***

Caspases or cysteine-aspartic proteases or cysteine-dependent **aspartate**-directed proteases are a family of cysteine proteases that play essential roles in apoptosis (programmed cell death), necrosis, and inflammation.

Caspases are essential in cells for apoptosis, or programmed cell death, during development and most other stages of adult life, and have been termed "executioner" proteins for their roles in the cell. Some caspases are also required in the immune system for the maturation of lymphocytes.

5 Caspase-1 was the first identified caspase, and is also known as interleukin converting enzyme (ICE). Caspase-1 converts precursor interleukin-1beta (IL-1 $\beta$ ) to the pro-inflammatory active form by specific cleavage of pIL-1 $\beta$  between Asp-116 and Ala-117. Caspase-1 therefore plays a key role in the inflammatory response.

10 The utility of caspase inhibitors to treat a variety of mammalian diseases associated with an increase in cellular apoptosis has been demonstrated using caspase inhibitors.

Caspase-1 inhibitors according to the invention have the ability to inhibit the activity of caspase-1. Preferred caspase-1 inhibitors of the invention are highly selective,  
15 competitive, cell-permeable, and irreversible inhibitors of caspase-1.

A caspase-1 inhibitor according to the invention inhibits the activity of caspase-1 and shows a higher preference for caspase-1 over other caspases. The activity of caspase-1 may be measured using a caspase-1 assay kit, an assay that can measure the  
20 specific caspase-1 activity by using YVAD labeled with the chromophore p-nitronilide (p-NA). Upon cleavage of the substrate pNA can be quantified using a spectrophotometer (e.g. cat.no. 39470, abcam). In addition, the activity can be measured indirectly by either measuring the formation of caspase-1 from pro-caspase-1 or IL-1beta from pro-IL-1beta by Western blot or by measuring the level of IL-1beta by ELISA.

25 The caspase-1 inhibitor of the present invention should be able to inhibit caspase-1 activity to such a degree that the activity is significantly lower than the activity observed without added caspase-1 inhibitor.

30 According to the present invention caspase-1 is considered to be inhibited when the activity of caspase-1 as measured in an appropriate assay in the presence of a caspase-1 inhibitor is at least 4 times lower than the activity measured without the presence of the inhibitor, such as at least 3 times lower, such as at least 2 times lower, such as at least 1.5 times lower, such as at least 1 time lower. In one embodiment the  
35 activity of caspase-1 is completely inhibited.

In one embodiment the inhibitor of the present invention is specific for caspase-1. With this it is meant that the inhibitor of the present invention is more specific for caspase-1 than for other caspases. The inhibitor of the present invention should have specificity  
5 for caspase-1 which is at least 2 times higher than for any other caspase, such as at least 3 times higher, such as at least 4 times higher, such as at least 5 times higher, such as at least 10 times higher.

In an embodiment of the present invention the caspase-1 inhibitor is selected from the  
10 group consisting of Ac-YVAD-cmk (Sigma-Aldrich), z-WEHD-FMK (R&D), YVAD-CHO (Santa Cruz), Ac-YVAD-FMK (Santa Cruz), VX-765 (Selleckchem) and Ac-YVAD-AOM, Z-YVAD-FMK, Z-D-CH2-DCB (all from Merck) or a combination hereof. Preferably the caspase-1 inhibitor is Ac-YVAD-cmk (e.g. from (Sigma-Aldrich). In an embodiment the caspase-1 inhibitor is z-WEHD-FMK (e.g. from R&D). In an  
15 embodiment the caspase-1 inhibitor is YVAD-CHO (e.g. from Santa Cruz). In an embodiment the caspase-1 inhibitor is Ac-YVAD-FMK (e.g. from Santa Cruz). In an embodiment the caspase-1 inhibitor is VX-765 (e.g. from Selleckchem). In an embodiment the caspase-1 inhibitor is Ac-YVAD-AOM (e.g. from Merck). In an embodiment the caspase-1 inhibitor is Z-YVAD-FMK (e.g. from Merck). In an  
20 embodiment the caspase-1 inhibitor is Z-D-CH2-DCB (e.g. from Merck).

### ***Contact dermatitis***

Contact dermatitis is a clinical term used to describe an inflammatory reaction in the skin which is characterized by a wide range of symptoms, and patients are typically  
25 described as having an inflammatory reaction (dermatitis) with intensely pruritic erythema, oedema and even vesicles at sites where chemical contact the skin. Substances responsible for contact dermatitis after single or multiple exposures are low-molecular weight compounds, that induce skin inflammation through activation of innate skin immunity (irritant contact dermatitis) or both innate and acquired specific  
30 immunity (allergic contact dermatitis). There are over 2,800 chemicals that have the potential to cause contact dermatitis.

In humans, allergic contact dermatitis usually occurs in response to low-molecular weight compounds (i.e. haptens) after many sub-thresholds exposures that do not  
35 initially induce allergic signs or symptoms. This is termed the "afferent" or "sensitizing"

phase of allergic contact dermatitis. The afferent phase may take weeks and months of repeated exposures to the sensitizing chemical. Gradually the patient becomes allergic to the offending compound, and dermatitis occurs on further exposures to the allergen. This is the "efferent", "elicitation" or "challenge" phase of allergic contact dermatitis.

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Allergic contact dermatitis is a T-cell-mediated skin inflammation caused by repeated skin exposures to contact allergens. Allergic contact dermatitis, also referred to as contact hypersensitivity, is mediated by both CD4+ and CD8+ T cells, which are primed in lymphoid organs during the sensitization phase and are recruited to the skin upon re-exposure to the hapten. In addition, subsets of CD4+ T cells endowed with suppressive activity are responsible for both the down-regulation of eczema in allergic patients and the prevention of priming to haptens in nonallergic individuals.

10

Common skin contact allergens include, but are not limited to, metals like nickel, gold and cobalt, fragrance components like cinnamal and eugenol, hair dye chemicals like para-phenylenediamine and para-toluenediamine, the oily coating from plants of the Toxicodendron genus like poison ivy, poison oak, and poison sumac, preservative components like MI and the experimental allergens urushiol, 2,4-Dinitrophenol (2,4-DNP), dinitrofluorobenzene (DFNB), trinitrochlorobenzene (TNCB), fluorescein isothiocyanate (FITC), and dinitrothiocyanobenzene (DNTB).

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There are three types of contact dermatitis: irritant contact dermatitis (ICD), allergic contact dermatitis (ACD), and photocontact dermatitis. Photocontact dermatitis is divided into two categories that is, phototoxic and photoallergic.

Irritant contact dermatitis (ICD) can be divided into forms caused by chemical irritants and those caused by physical irritants. Common chemical irritants implicated include solvents (alcohol, xylene, turpentine, esters, acetone, ketones, and others); metal-working fluids (neat oils, water-based metalworking fluids with surfactants); latex; kerosene; ethylene oxide; surfactants in topical medications and cosmetics (sodium lauryl sulfate); alkalis (drain cleaners, strong soap with lye residues). Physical irritant contact dermatitis may most commonly be caused by low humidity from air conditioning. Also, many plants directly irritate the skin. It is an object of the present invention to treat irritant contact dermatitis.

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Although less common than ICD, allergic contact dermatitis (ACD) is accepted to be the most prevalent form of immunotoxicity found in humans. By its allergic nature, this form of contact dermatitis is a hypersensitive reaction. The mechanisms by which these reactions occur are complex, with many levels of fine control. Their immunology centres on the interaction of immunoregulatory cytokines and discrete subpopulations of T lymphocytes. It is an object of the present invention to treat allergic contact dermatitis.

Photocontact dermatitis (PCD), sometimes termed "photoaggravated" dermatitis, is divided into two categories, phototoxic and photoallergic dermatitis. PCD is the eczematous condition which is triggered by an interaction between an otherwise unharmed or less harmful substance on the skin and ultraviolet light (320-400 nm UVA) (ESCD 2006), therefore manifesting itself only in regions where the sufferer has been exposed to such rays. Without the presence of these rays, the photosensitiser is not harmful. For this reason, this form of contact dermatitis is usually only associated with areas of skin which are left uncovered by clothing, and it can be soundly defeated by avoiding exposure to sunlight. The mechanism of action varies from toxin to toxin, but is usually due to the production of a photoproduct. Toxins which are associated with PCD include the psoralens. Psoralens are in fact used therapeutically for the treatment of psoriasis, eczema and vitiligo. It follows that it is an object of the present invention to treat photocontact dermatitis.

An embodiment of the present invention thus relates to the use of a caspase-1 inhibitor for the treatment of any type of contact dermatitis such as allergic contact dermatitis, irritant contact dermatitis and/or photocontact dermatitis. Another embodiment of the present invention relates to a pharmaceutical composition for topical administration comprising at least one caspase-1 inhibitor, such as at least two caspase-1 inhibitors, such as at least three caspase-1 inhibitors, such as at least four caspase-1 inhibitors, for the treatment of any type of contact dermatitis such as allergic contact dermatitis, irritant contact dermatitis and/or photocontact dermatitis.

The skin can be divided into two broad anatomical units: The epidermis and the dermis. The epidermis is a stratified epithelial layer that is composed primarily of keratinocytes that produce the water-impermeable stratum corneum. The dermis is a cell-poor stromal layer below the epidermis. Several immune cell types reside within the skin.

Langerhans cells are the only dendritic cell (DC) population resident in the epidermis. In the dermis, there are at least two subsets of DC's, which can be distinguished based on the expression of langerin (langerin<sup>+</sup> dermal DC's and langerin<sup>-</sup> dermal DC's).

5 Langerin<sup>+</sup> dermal DC's (also referred to as CD103<sup>+</sup> dermal DC's) are a distinct DC subset and although they represent only a small percentage (app. 3%) of the total DC population in the dermis, langerin<sup>+</sup> dermal DC's are continually replaced by new recruits from the blood. Langerin<sup>-</sup> dermal DC's (also referred to as classic dermal DC's) make up the bulk of dermal DC's (app. 80%) and are heterogeneous. The dermis also contains mast cells and memory T cells.

10 Although the precise sequence of events that occurs shortly after an encounter with an antigen has not been rigorously established, it is clear that most cells in the skin participate in the innate recognition of haptens and other cutaneous antigens. Keratinocytes secrete a large number of factors, including pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumour necrosis factor (TNF). These pro-inflammatory  
15 mediators activate neighbouring cells, thereby amplifying the immune response. DC's also have the intrinsic ability to sense haptens, and they then secrete pro-inflammatory cytokines and probably participate in escalating the inflammatory cascade. A combination of direct sensing of the hapten and the inflammatory milieu causes the activation of Langerhans cells in the epidermis and DC's in the dermis, resulting in their migration to  
20 the skin-draining lymph nodes. In the lymph node, migratory DC's present antigens acquired in the skin to naïve and memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Langerin<sup>+</sup> dermal DC's are the only skin-derived DC subset specialized for the cross-presentation of antigens to CD8<sup>+</sup> T cells. These DC's also present antigens to naïve CD4<sup>+</sup> T cells and promote the differentiation of these cells into T helper 1-type effector cells. Presenta-  
25 tion of antigens by Langerhans cells to naïve CD4<sup>+</sup> T cells promotes the differentiation of these cells into T helper 17-type effector cells.

### ***Treatment of contact dermatitis***

The present invention relates to a pharmaceutical composition comprising at least one  
30 caspase-1 inhibitor, such as at least two caspase-1 inhibitors, such as at least three caspase-1 inhibitors, such as at least four caspase-1 inhibitors, and the use thereof.

IL-1 $\beta$  plays a central role in allergic contact dermatitis, which however has not yet been fully understood. Caspase-1 is required for the formation of IL-1 $\beta$  from pro-IL-1 $\beta$ . By

blocking the IL-1 $\beta$  signalling in the skin it has been shown that a beneficial effect is achieved in relation to allergic contact dermatitis.

5 The present invention thus relates to the surprising finding that caspase-1 inhibitors may be used in the treatment of contact dermatitis by simple topical administration. The effect of topical administration of caspase-1 inhibitors is significantly better than the effect achieved by administration of the traditional treatment with glucocorticosteroids (see Example 1 and Figure 1).

10 Caspase-1 inhibitors according to the invention have the ability to inhibit the activity of caspase-1 as described herein above in the section "caspase-1 and caspase-1 inhibitors".

15 Preferred caspase-1 inhibitors of the invention are highly selective, competitive, cell-permeable, and irreversible inhibitors of caspase-1.

In one embodiment of the present invention the caspase-1 inhibitor is selected from the group consisting of Ac-YVAD-cmk (Sigma-Aldrich), z-WEHD-FMK (R&D), YVAD-CHO (Santa Cruz), and Ac-YVAD-FMK (Santa Cruz), VX-765 (Selleckchem) or a  
20 combination hereof.

According to the present invention the individual in need of treatment is a mammal, preferably a human being, suffering from contact dermatitis as described herein above in the section "contact dermatitis".

25 In one embodiment of the present invention the caspase-1 inhibitor of the invention is administered in connection with the administration of traditional drugs for treatment of contact dermatitis and in another embodiment the caspase-1 inhibitor is administered alone. Traditional drugs for the treatment of contact dermatitis include but are not  
30 limited to steroids such as corticosteroids and synthetic glyco steroids. Non-limiting examples hereof include but are not limited to dexamethasone, prednisone, and hydrocortisone.

By the phrase "administered in connection with" is meant the combined administration  
35 of a caspase-1 inhibitor and a suitable pharmaceutical composition usually used for

treatment of the disease in question (e.g. glucocorticosteroids and oral antihistamines for treatment of contact dermatitis). Administration of these therapeutic agents in combination is typically carried out over a defined time period. The combined administration of the present invention is to be understood as meaning that the individual components can be administered simultaneously or more or less simultaneously or respectively in succession. As an example, in one embodiment one therapeutic agent could be administered in the morning and the other could be administered later in the day. Or in another embodiment one therapeutic agent could be administered once daily and the other twice daily. The skilled person would know which dosing regime would be the most beneficial and will administer the therapeutic agents accordingly. It follows that it is an object of the present invention to administer a caspase-1 inhibitor and a steroid, such as a corticosteroid and/or an antihistamine, for simultaneous, separate or sequential use for the treatment of contact dermatitis.

Thus in an embodiment of the present invention the caspase-1 inhibitor is administered simultaneously, separately or sequentially with a second active ingredient. The second active ingredient may be but is not limited to any medicament typically administered to treat contact dermatitis. Examples of such medicaments may be but are not limited to: steroids such as corticosteroids and synthetic glycosteroids, dexamethasone, prednisone, hydrocortisone, vitamin D and/or tacrolimus and/or antihistamines.

The a second active ingredient may be an inhibitor of the inflammasome and thus be one or more compounds selected from the group comprising glyburide, 16673-34-0, Parthenolide, Bay 11-7082, VX-740, VX-765, and/or a 1Cys-LT receptor antagonist.

The inflammasome is a large molecular platform that triggers the activation of caspases. The inflammasome contains a pattern recognition receptor, which upon recognition of pathogen- or damaged-associated molecular patterns, oligomerizes with the adaptor protein ASC and procaspase-1 leading to activation of caspase-1.

The one or more caspase-1 inhibitor of the present invention is to be administered after contact dermatitis has been determined or diagnosed. The diagnosis may be pronounced by a medical doctor. That is, the pharmaceutical composition is for use after the allergic reaction has occurred in the skin and contact dermatitis has been diagnosed as described in the section "contact dermatitis". In another embodiment the

caspase-1 inhibitor is administered as a precautionary treatment before working with products and/or materials which may cause contact dermatitis. Thus it is an object of the present invention to provide a topical treatment for contact dermatitis to an individual in need thereof. The need is either the presence of contact dermatitis of the prophylactic treatment hereof.

In one embodiment of the present invention the caspase-1 inhibitor is administered at least once daily, such as at least twice daily, such as at least three times daily, such as at least four times daily, such as at least five time daily, such as at least six times daily, such as at least seven times daily.

The person skilled in the art will know when to administer the composition of the invention and would also know when to end the treatment. It should be understood that a specific dosage and treatment regime for any particular patient will depend upon a variety of factors, including the activity of the specific compound used, the age of the patient, the body weight of the patient, general health of the patient, sex of the patient, diet, time of administration, rate of excretion, drug combination and the evaluation of the treating physician and the severity of the particular contact dermatitis case being treated.

Thus the present invention relates to a method of treating contact dermatitis by topical administration of a caspase-1 inhibitor. It follow that it is an object of the present invention covers the use of a caspase-1 inhibitor for the manufacture of a medicament for the treatment of contact dermatitis.

#### ***Pharmaceutical composition***

In a preferred embodiment of the present invention the pharmaceutical composition is administered topically. Topical administration may be used when the target of treatment includes areas or organs readily accessible by topical administration, including diseases of the eye, the skin, the ear, mucous membranes, the mouth, the vagina and/or the lower intestinal tract. The skilled person will know how to prepare topical formulations for each of these areas or organs.

Topical application for the lower intestinal tract may be effected in a rectal suppository formulation or in a suitable enema formulation. Topical application for the vagina may be effected in a vaginal tablet or capsule or in a vaginal gel or suspension.

- 5 Thus an embodiment of the present invention is at least one caspase-1 inhibitor, such as at least two caspase-1 inhibitors, such as at least three caspase-1 inhibitors, such as at least four caspase-1 inhibitors, for use in the treatment of contact dermatitis, wherein the inhibitor is applied topically to the skin, the ear, the eye, and/or mucous membranes such as but not limited to the mouth, the vagina and/or the lower intestinal  
10 tract. The at least one inhibitor may be administered in a pharmaceutical composition / medicament formulated according with the surface to which it is to be applied.

- For topical application, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or  
15 more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax, petroleum jelly, natural plant oil and water. Alternatively, the pharmaceutical compositions may be formulated in a suitable lotion or cream containing the active component suspended or  
20 dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, petroleum jelly and water. Natural plant oils include, but are not limited to, jojoba oil, argan oil, lavender oil, sweet almond oil, apricot kernel oil, coconut oil, avocado oil, babussa oil, borage oil,  
25 canola oil, carrot root oil, castor bean oil, evening primrose oil, grapeseed oil, hemp seed oil, olive oil, palm oil, sesame seed oil, and sunflower oil. In one embodiment the carrier used for topical administration is petroleum jelly. The composition may comprise a surfactant, such as a wetting agent. In a preferred embodiment the pharmaceutical composition is formulated with ingredients that are safe, i.e. that are not known to  
30 cause contact dermatitis.

The pharmaceutical composition may furthermore comprise a penetration enhancer, i.e. a substance that alters the skin structure thereby allowing other chemical substances to penetrate deeper into the skin or other tissue in question. The penetration

enhancer may be selected from the group consisting of alcohol, propylene glycol, panthenol, behenyl alcohol, hyaluronic acid and mixtures thereof.

5 The amount of compound present in the abovementioned compositions should be sufficient to cause a detectable decrease in the severity of the contact dermatitis which is treated as measured by techniques well known in the art. For example the effect may be evaluated as described in Example 1 and example 5.

10 The amount of active ingredients will depend on the particular compounds and possibly other therapeutic agents in the composition.

The pharmaceutical composition of the present invention is for topical administration and in one embodiment may include a pharmaceutically acceptable carrier adapted for topical administration. Thus, the composition may take the form of a suspension,  
15 solution, ointment, lotion, sexual lubricant, cream, foam, aerosol, spray, suppository, implant, inhalant, tablet, capsule, dry powder, syrup, balm, lozenge, pad, sponge, powder, flakes, paste and/or a medical adhesive patch (i.e. patch) for example. In another embodiment the caspase-1 inhibitor and/or the composition comprising said inhibitor is comprised in a transdermal patch. Preferably, the pharmaceutical  
20 composition is in the form of a cream, a gel, a lotion, a suspension, a spray, an ointment or foam.

Preferably, the formulation will comprise about 0.05% to 95% by weight of the active ingredient(s) (i.e. at least one caspase-1 inhibitor) with the remainder consisting of  
25 suitable pharmaceutical excipients and/or carriers as described herein.

In one embodiment the formulation will comprise about 0.05% to 95% by weight of the active ingredient(s) (i.e. at least one caspase-1 inhibitor). In another embodiment the formulation will comprise about 0.05% to 85% by weight of the active ingredient(s),  
30 such as 0.05% to 75% by weight of the active ingredient(s), such as 0.05% to 65% by weight of the active ingredient(s), such as 0.05% to 55% by weight of the active ingredient(s), such as 0.05% to 45% by weight of the active ingredient(s), such as 0.05% to 35% by weight of the active ingredient(s), such as 0.05% to 25% by weight of the active ingredient(s), such as 0.05% to 20% by weight of the active ingredient(s),

such as 0.05% to 10% by weight of the active ingredient(s), such as 0.05% to 5% by weight of the active ingredients.

5 In a preferred embodiment the formulation comprises 0.05% to 20% by weight of the active ingredient(s).

Pharmaceutically acceptable salts of the caspase-1 inhibitor described in the present invention, where they can be prepared, are also intended to be covered by this invention. These salts will be ones which are acceptable in their application to a  
10 pharmaceutical use. By that it is meant that the salt will retain the biological activity of the parent compound (the caspase-1 inhibitor) and the salt will not have untoward or deleterious effects in its application and use in treating diseases.

Pharmaceutically acceptable salts are prepared in a standard manner. If the parent  
15 compound is a base it is treated with an excess of an organic or inorganic acid in a suitable solvent. If the parent compound is an acid, it is treated with an inorganic or organic base in a suitable solvent.

Thus an embodiment of the present invention is a medicament for use in the treatment  
20 of contact dermatitis comprising at least one caspase-1 inhibitor, such as at least two caspase-1 inhibitors, such as at least three caspase-1 inhibitors, such as at least four caspase-1 inhibitors. Correspondingly the present invention relates to the use of at least one caspase-1 inhibitor, such as at least two caspase-1 inhibitors, such as at least three caspase-1 inhibitors, such as at least four caspase-1 inhibitors for the  
25 manufacture of a medicament for the treatment of contact dermatitis.

A preferred embodiment of the present invention is a medicament for use in the treatment of contact dermatitis comprising Ac-YVAD-cmk (Sigma-Aldrich Denmark). Another embodiment of the present invention is a medicament for use in the treatment  
30 of contact dermatitis comprising Ac-YVAD-cmk (Sigma-Aldrich Denmark) and z-WEHD-FMK (R&D). Another embodiment of the present invention is a medicament for use in the treatment of contact dermatitis comprising Ac-YVAD-cmk (Sigma-Aldrich Denmark) and YVAD-CHO (Santa Cruz). Yet an embodiment of the present invention is a medicament for use in the treatment of contact dermatitis comprising Ac-YVAD-  
35 cmk (Sigma-Aldrich Denmark) and Ac-YVAD-FMK (Santa Cruz). Another embodiment

of the present invention is a medicament for use in the treatment of contact dermatitis comprising Ac-YVAD-cmk (Sigma-Aldrich Denmark) and VX-765 (Selleckchem).

Another embodiment of the present invention is a medicament for use in the treatment of contact dermatitis comprising Ac-YVAD-cmk (Sigma-Aldrich Denmark) and Ac-

5 YVAD-AOM (Merck). Another embodiment of the present invention is a medicament for use in the treatment of contact dermatitis comprising Ac-YVAD-cmk (Sigma-Aldrich Denmark) and Z-YVAD-FMK (Merck). Another embodiment of the present invention is a medicament for use in the treatment of contact dermatitis comprising Ac-YVAD-cmk (Sigma-Aldrich Denmark) and Z-D-CH<sub>2</sub>-DCB (Merck).

10

Correspondingly, the present invention relates to the use of at least one caspase-1 inhibitor, such as at least two caspase-1 inhibitors, such as at least three caspase-1 inhibitors, such as at least four caspase-1 inhibitors for the manufacture of a medicament for the treatment of contact dermatitis.

15

In a preferred embodiment the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) for the manufacture of a medicament for the treatment of contact dermatitis. Another embodiment of the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) and z-WEHD-FMK (R&D) for the manufacture

20

of a medicament for the treatment of contact dermatitis. Another embodiment of the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) and YVAD-CHO (Santa Cruz) for the manufacture of a medicament for the treatment of contact dermatitis. Another embodiment of the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) and Ac-YVAD-FMK (Santa Cruz) for the

25

manufacture of a medicament for the treatment of contact dermatitis. Another embodiment of the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) and VX-765 (Selleckchem) for the manufacture of a medicament for the treatment of contact dermatitis. Another embodiment of the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) and Ac-YVAD-AOM (Merck) for the manufacture of a medicament for the treatment of contact dermatitis.

30

Another embodiment of the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) and Z-YVAD-FMK (Merck) for the manufacture of a medicament for the treatment of contact dermatitis. Another embodiment of the present invention relates to the use of Ac-YVAD-cmk (Sigma-Aldrich Denmark) and Z-D-CH<sub>2</sub>-

DCB (Merck) for the manufacture of a medicament for the treatment of contact dermatitis.

## 5 Examples

### Example 1

Mice were divided in four groups with four mice in each group and were treated as outlined in Table 1. Control mice (group 1) were exposed to 0.1 g petroleum jelly (Apotekets Vaseline, Apotekernes A.m.b.a) on the dorsal side of both ears for three consecutive days (day 0-2). On day 23, the same treatment was repeated once.

To induce contact dermatitis (group 2), mice were painted with 25 µl 0.15% DNFB in olive oil: acetone (OOA) followed by 0.1 g petroleum jelly on the dorsal side of both ears for three consecutive days (day 0-2). On day 23, the same treatment was repeated once.

To study the effect of hydrocortisone on contact dermatitis (group 3), mice were painted with 25 µl 0.15% DNFB in OOA and within 30 min upon this mice were treated with 0.1 g 1% Mildison Lipid crème from Astellas on the dorsal side of both ears for three consecutive days (day 0-2). On day 23, the same treatment was repeated once.

To study the effect of the caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark) on contact dermatitis (group 4), mice were painted with 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.1% Ac-YVAD-cmk in petroleum jelly on the dorsal side of both ears for three consecutive days (day 0-2). On day 23, the same treatment was repeated once.

All mice were euthanized 24 hours after the challenge and ear thickness was measured on both ears using an engineer's micrometer (Mitutoyo, Tokyo, Japan).

**Table 1:**

|                                    | Day 0-2                                                   | Day 23                                                    | Day 24                |
|------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|-----------------------|
| <b>Group 1</b> (control)           | 0.1 g petroleum jelly                                     | 0.1 g petroleum jelly                                     | Measure ear thickness |
| <b>Group 2</b> (without treatment) | 25 µl 0.15% DNFB in OOA followed by 0.1 g petroleum jelly | 25 µl 0.15% DNFB in OOA followed by 0.1 g petroleum jelly | Measure ear thickness |

|                                                        |                                                                                       |                                                                                       |                       |
|--------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|-----------------------|
|                                                        |                                                                                       |                                                                                       |                       |
| <b>Group 3</b> (treated with 1% hydrocortisone)        | 25 µl 0.15% DNFB in OOA followed by 0.1 g 1% hydrocortisone (Mildison)                | 25 µl 0.15% DNFB in OOA followed by 0.1 g 1% hydrocortisone (Mildison)                | Measure ear thickness |
| <b>Group 4</b> (treated with 0.1% caspase-1 inhibitor) | 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.1% caspase-1 inhibitor in petroleum jelly | 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.1% caspase-1 inhibitor in petroleum jelly | Measure ear thickness |

The results can be seen in figure 1. In figure 1 the ear swelling observed in group 2 mice (no treatment) is set to 100% and the reduction in ear swelling seen in group 3 (hydrocortisone) and group 4 (caspase 1 inhibitor) mice demonstrates the efficacy of the administered treatment.

## Example 2

Using the same experimental setup as described herein above in Example 1 the following additional experiments were performed.

In order to investigate the role of the concentration of the caspase-1 inhibitor different amounts of caspase-1 inhibitor was added to petroleum jelly. The following concentrations of caspase-1 inhibitor were tested: 0.1%, 0.2%, 0.5% and 1 %.

The effect was evaluated as described herein above in example 1.

All mice were euthanized 24 hours after the challenge and ear thickness was measured on both ears using an engineer's micrometer (Mitutoyo, Tokyo, Japan).

## Example 3

In order to investigate the role of the vehicle different types of vehicles were evaluated in order to examine whether the vehicle used has an effect on the efficacy of the caspase-1 inhibitor.

The effect was evaluated as described herein above in example 1.

All mice were euthanized 24 hours after the challenge and ear thickness was measured on both ears using an engineer's micrometer (Mitutoyo, Tokyo, Japan).

#### 5      **Example 4**

In order to investigate the potential of different caspase-1 inhibitors the following caspase-1 inhibitors were investigated for their effect:

- Ac-YVAD-cmk from Sigma-Aldrich, irreversible, cell permeable
- z-WEHD-FMK from R&D, irreversible, cell permeable
- 10      - YVAD-CHO from Santa Cruz, reversible, cell permeable
- Ac-YVAD-FMK from Santa Cruz, irreversible, cell permeable
- VX-765 from Selleckchem, cell permeable

15      The effect was evaluated as described herein above in example 1.

All mice were euthanized 24 hours after the challenge and ear thickness was measured on both ears using an engineer's micrometer (Mitutoyo, Tokyo, Japan).

#### **Example 5**

20      A patient suffering from contact dermatitis is treated with the pharmaceutical composition of the invention. The pharmaceutical composition comprises a casapse-1 inhibitor in an efficient amount suitable for the patient to be treated and the severity of the contact dermatitis.

25      The pharmaceutical composition is in the form of a cream or ointment and is topically applied to the areas of the skin showing signs of inflammation/contact dermatitis. The administration is repeated if necessary.

30      The treatment is considered successful when the signs of contact dermatitis has diminished to such a degree that the skilled person would evaluate the patient as being healthy.

#### **Example 6**

35      Mice were divided in six groups with four mice in each group and were treated as outlined in Table 2. Control mice (group 1) were exposed to 0.1 g petroleum jelly (Apotekets Vaseline, Apotekernes A.m.b.a) on the dorsal side of both ears for three

consecutive days (day 0-2). On day 23, the same treatment was repeated once.

To induce contact dermatitis (group 2), mice were painted with 25 µl 0.15% DNFB in olive oil: acetone (OOA) followed by 0.1 g petroleum jelly on the dorsal side of both ears for three consecutive days (day 0-2). On day 23, the same treatment was repeated once.

To study the effect of hydrocortisone (group 3) and hydrocortisone butyrate (group 4) on contact dermatitis, mice were painted with 25 µl 0.15% DNFB in OOA followed by 0.1 g 1% Mildison Lipid crème from Astellas (group 3) or by 0.1 g 0.1% Locoid crème from Astellas (group 4) on the dorsal side of both ears for three consecutive days (day 0-2). On day 23, the same treatment was repeated once.

To study the effect of the caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark) on contact dermatitis (group 5 and 6), mice were painted with 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.1% (group 5) or 0.5% (group 6) Ac-YVAD-cmk in petroleum jelly on the dorsal side of both ears for three consecutive days (day 0-2). On day 23, the same treatment was repeated once.

All mice were euthanized 24 hours after the challenge and ear thickness was measured on both ears using an engineer's micrometer (Mitutoyo, Tokyo, Japan) (Figure 2). Furthermore, one ear from each mouse was analyzed histologically (figure 3) and protein was purified from the other ear to analyze IL-1β levels by ELISA (figure 4).

**Table 2:**

|                                                       | Day 0-2                                                   | Day 23                                                      | Day 24                                               |
|-------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------------|
| <b>Group 1</b> (control)                              | 0.1 g petroleum jelly                                     | 0.1 g petroleum jelly                                       | Measure ear thickness<br>H&E staining<br>IL-1β level |
| <b>Group 2</b> (without treatment)                    | 25 µl 0.15% DNFB in OOA followed by 0.1 g petroleum jelly | 25 µl 0.15% DNFB in OOA followed by 0.1 g petroleum jelly   | Measure ear thickness<br>H&E staining<br>IL-1β level |
| <b>Group 3</b> (treated with 1% Mildison lipid creme) | 25 µl 0.15% DNFB in OOA followed by 0.1 g 1%              | 25 µl 0.15% DNFB in OOA followed by 0.1 g 1% hydrocortisone | Measure ear thickness<br>H&E staining<br>IL-1β level |

|                                                                                             |                                                                                                                            |                                                                                                                            |                                                             |
|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
|                                                                                             | hydrocortisone<br>(Mildison)                                                                                               | (Mildison)                                                                                                                 |                                                             |
| <b>Group 4</b> (treated with 0.1% Locoid creme)                                             | 25 µl 0.15% DNFB in OOA followed by 0.1 g 1% hydrocortisone butyrate(Locoid)                                               | 25 µl 0.15% DNFB in OOA followed by 0.1 g 1% hydrocortisone butyrate(Locoid)                                               | Measure ear thickness<br>H&E staining<br>IL-1 $\beta$ level |
| <b>Group 5</b> (treated with 0.1% caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark)) | 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.1% caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark) in petroleum jelly | 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.1% caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark) in petroleum jelly | Measure ear thickness<br>H&E staining<br>IL-1 $\beta$ level |
| <b>Group 6</b> (treated with 0.5% caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark)) | 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.5% caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark) in petroleum jelly | 25 µl 0.15% DNFB in OOA followed by 0.1 g 0.5% caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark) in petroleum jelly | Measure ear thickness<br>H&E staining<br>IL-1 $\beta$ level |

The results can be seen in figure 2, 3 and 4. In figure 2 the ear swelling observed in group 2 mice (no treatment) is set to 100% and the reduction in ear swelling seen in group 3 and 4 (hydrocortisone/hydrocortisone butyrate) and group 5 and 6 (caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, Denmark)) mice demonstrates the efficacy of the administered treatment. Treatment with 0.5% caspase-1 inhibitor (group 6) was at least as or even more effective than hydrocortisone butyrate treatment (Locoid, group 4) in reducing the inflammatory response as measured by ear-swelling. In figure 3 the histological changes are shown in H&E stained sections. These stainings confirm that treatment with 0.5% Caspase-1 inhibitor is as effective in reducing inflammation as hydrocortisone/hydrocortisone butyrate treatments. Figure 4 shows the level of IL-1 $\beta$  in the ear skin measured by ELISA. A reduced level of IL-1 $\beta$  was found both following hydrocortisone/hydrocortisone butyrate treatment and caspase-1 inhibitor treatment.

**References:**

Ozaki E et al. 2015. J Inflamm Res. Jan 16;8:15-27. doi: 10.2147/JIR.S51250.  
eCollection 2015. Targeting the NLRP3 inflammasome in chronic inflammatory diseases: current perspectives.

## Claims

1. At least one caspase-1 inhibitor for use in the treatment of contact dermatitis by topical administration.
- 5 2. The at least one caspase-1 inhibitor for use according to claim 1, wherein the inhibitor is selected from the group consisting of: Ac-YVAD-cmk, z-WEHD-FMK, YVAD-CHO, VX-765, Ac-YVAD-FMK, Ac-YVAD-AOM, Z-YVAD-FMK, and Z-D-CH2-DCB.
- 10 3. The at least one caspase-1 inhibitor for use according to any one of the preceding claims, wherein the contact dermatitis to be treated is allergic contact dermatitis, irritant contact dermatitis and/or photocontact dermatitis.
- 15 4. The at least one caspase-1 inhibitor for use according to any one of the preceding claims, wherein the inhibitor is applied topically to the skin, the ear, the eye, and/or mucous membranes such as but not limited to the mouth, the vagina and/or the lower intestinal tract.
- 20 5. The at least one caspase-1 inhibitor for use according to any one of the preceding claims, wherein the inhibitor is comprised in a pharmaceutical composition.
- 25 6. The at least one caspase-1 inhibitor for use according to claim 5, wherein the pharmaceutical composition comprises 0.05% to 20% by weight of the caspase-1 inhibitor, such as 0.05% to 10% by weight, such as 0.05% to 0.5% by weight.
- 30 7. The at least one caspase-1 inhibitor for use according to any one of claims 5-6, wherein the composition is in the form of a cream, gel, lotion, suspension, spray, ointment, foam, pad, sponge, powder, flakes, paste and/or a patch.
8. The at least one caspase-1 inhibitor for use according to any one of claims 5-7, wherein the composition further comprises a wetting agent.
- 35 9. The at least one caspase-1 inhibitor for use according to any one of the claims 5-8, wherein the composition comprises a component from the group consisting

of: petroleum jelly, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax, natural plant oil and water.

- 5           10. The at least one caspase-1 inhibitor for use according to any one of the claims 5-9, wherein the composition comprises 0.1% w/w Ac-YVAD-cmk and petroleum jelly as pharmaceutically acceptable carrier.
- 10           11. The at least one caspase-1 inhibitor for use according to any one of the preceding claims, wherein the inhibitor is administered simultaneously, separately or sequentially with a second active ingredient.
- 15           12. The at least one caspase-1 inhibitor for use according to claim 11, wherein the second active ingredient is selected from the group of steroids, vitamin D and tacrolimus.
13. A pharmaceutical composition for topical administration comprising at least one caspase-1 inhibitor.
- 20           14. The composition according to claim 13, wherein the at least one caspase-1 inhibitor is selected from the group consisting of: Ac-YVAD-cmk, z-WEHD-FMK, YVAD-CHO, VX-765, Ac-YVAD-FMK, Ac-YVAD-AOM, Z-YVAD-FMK, and Z-D-CH<sub>2</sub>-DCB.
- 25           15. The composition according to any one of claims 13-14, wherein the composition comprises 0.05% to 20% by weight of the at least one caspase-1 inhibitor, such as 0.05% to 10% by weight, such as 0.05% to 0.5% by weight.
- 30           16. The composition according to any one of claims 13-15, wherein the composition is in the form of a cream, gel, lotion, suspension, spray, ointment, foam, pad, sponge, powder, flakes, paste and/or a patch.
17. The composition according to any one of the claims 13-16, wherein the composition further comprises a wetting agent.

- 5 18. The composition according to any one of the claims 13-17, wherein the pharmaceutically acceptable carrier is selected from the group consisting of: petroleum jelly, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax, natural plant oil and water.
- 10 19. The composition according to any one of the claims 13-18, wherein the composition comprises 0.1% w/w Ac-YVAD-cmk and petroleum jelly as pharmaceutically acceptable carrier.
- 20 20. The composition according to any one of the claims 13-19, wherein the composition further comprises a second active ingredient.
- 15 21. The composition according to any one of the claims claim 13-20, wherein the second active ingredient is selected from the group consisting of: steroids, vitamin D and tacrolimus.
- 20 22. The composition according to any one of claims 13-21 for use according to any one of claims 1-12.
23. A method of treating contact dermatitis by topical administration of at least one caspase-1 inhibitor.
- 25 24. Use of at least one caspase-1 inhibitor for the manufacture of a medicament for the treatment of contact dermatitis.

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Figure 1

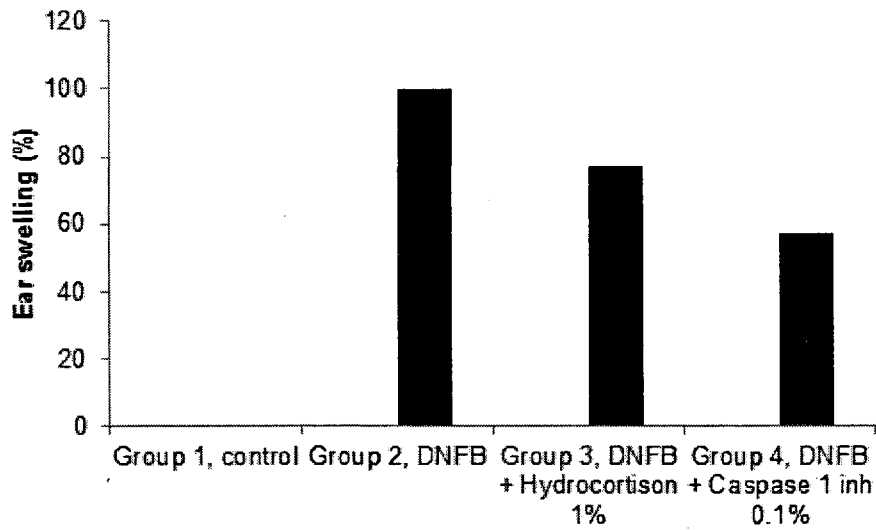
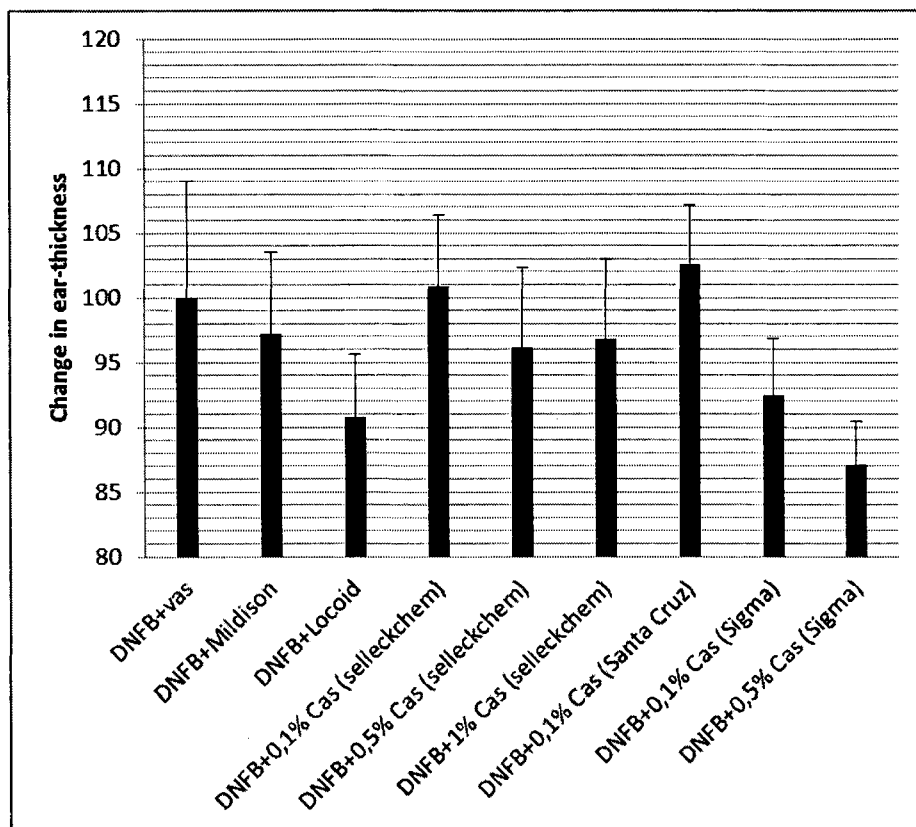
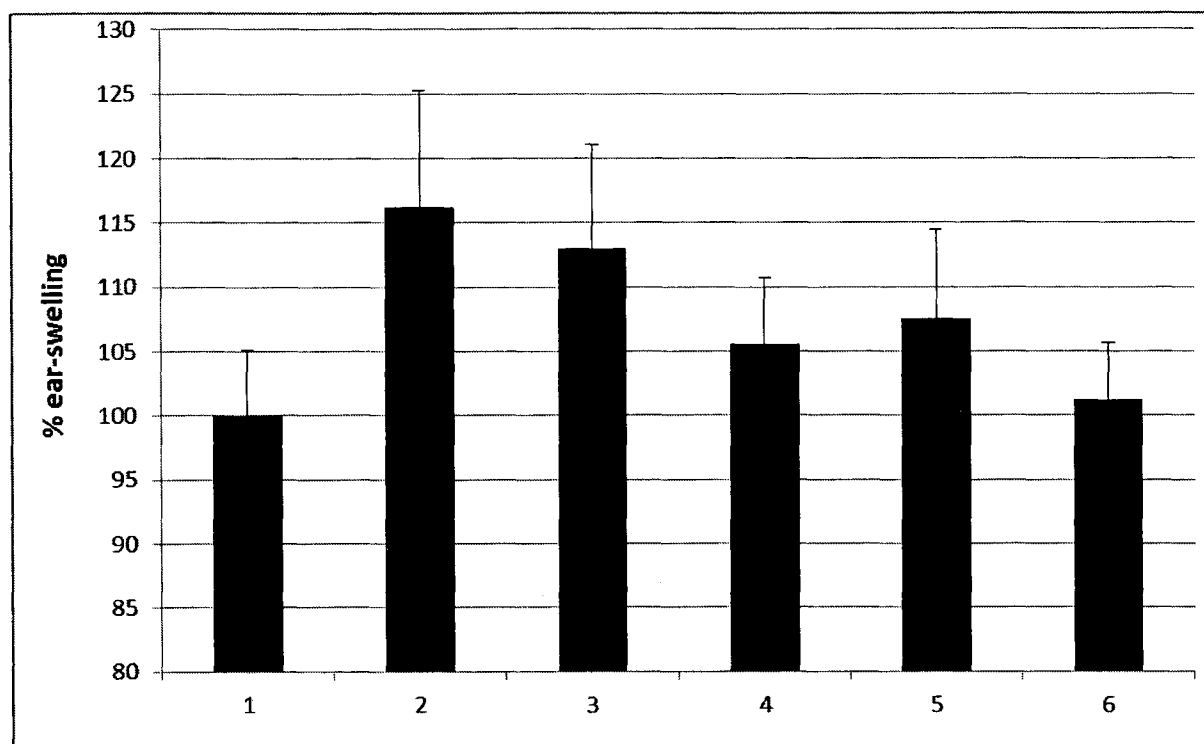


Figure 1a



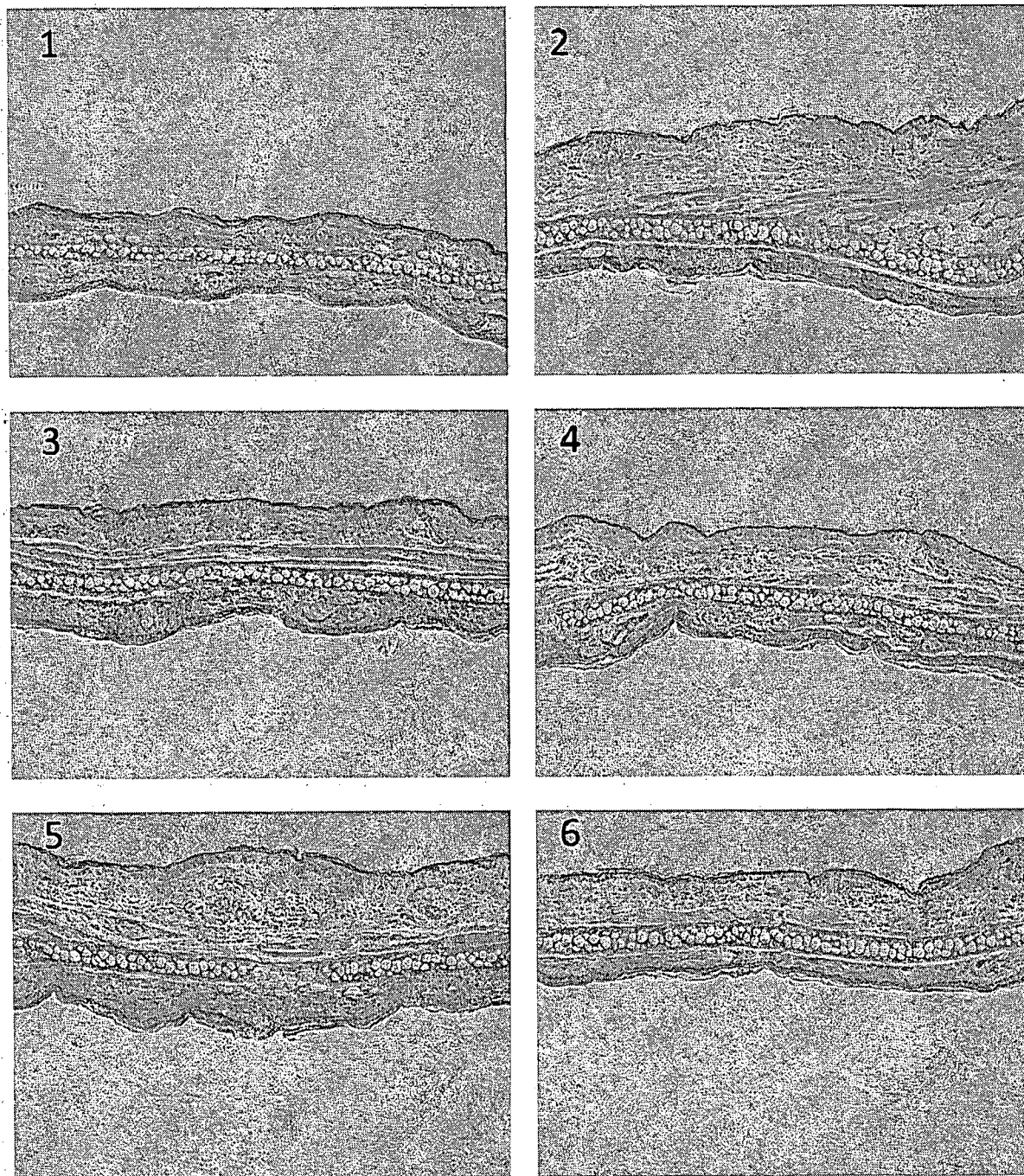
**2/4**

Figure 2



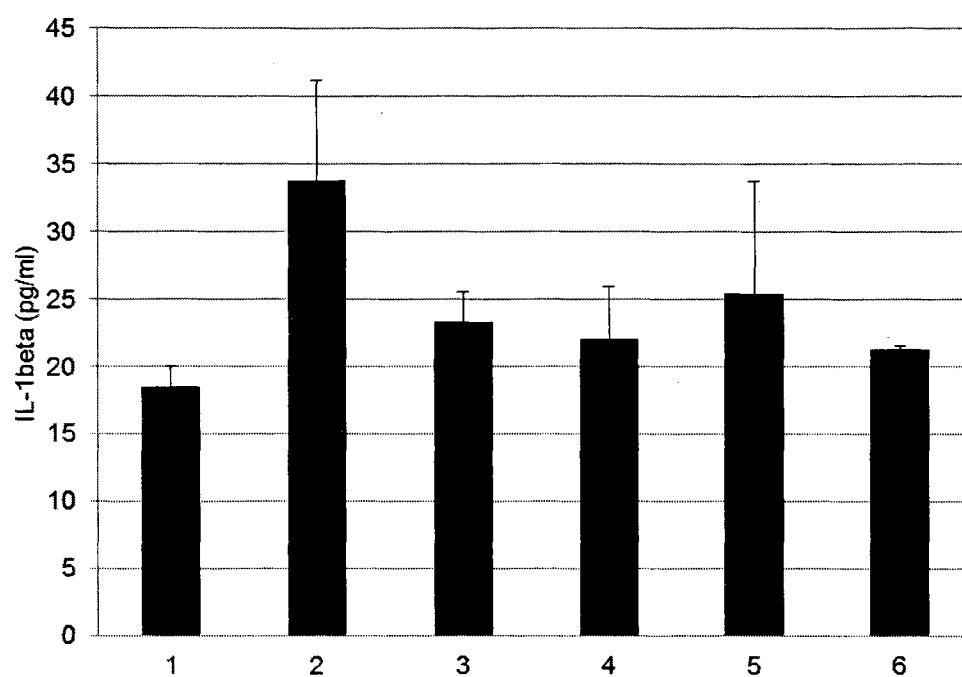
**3/4**

Figure 3



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Figure 4



## INTERNATIONAL SEARCH REPORT

International application No  
PCT/DK2015/050068

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K9/00  
ADD.

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)  
A61K

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 practicable, search terms used)

EPO-Internal, WPI Data, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                      | Relevant to claim No. |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | WO 03/047629 A2 (MOLECULAR SKINCARE LTD [GB]; TAZI-AHNINI RACHID [GB]; WARD SIMON [GB];) 12 June 2003 (2003-06-12)<br>page 4, lines 3-10<br>page 23, line 15 - page 24, line 23<br>page 43, line 10 - line 20<br>page 34 - page 35                                                                                      | 1-24                  |
| A         | -----<br>HOMEY ET AL: "Cytokines and chemokines orchestrate atopic skin inflammation", JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 118, no. 1, 1 July 2006 (2006-07-01), pages 178-189, XP005610118, ISSN: 0091-6749, DOI: 10.1016/J.JACI.2006.03.047 the whole document<br>-----<br>-/-- | 1-24                  |



Further documents are listed in the continuation of Box C.



See patent family annex.

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

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"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

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"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

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Date of the actual completion of the international search

26 June 2015

Date of mailing of the international search report

02/07/2015

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Authorized officer

Ceyte, Mathilde

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/DK2015/050068

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                         |                       |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                      | Relevant to claim No. |
| A                                                    | <p>HAN NA-RA ET AL: "Tryptanthrin ameliorates atopic dermatitis through down-regulation of TSLP", ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, ACADEMIC PRESS, US, vol. 542, 1 December 2013 (2013-12-01), pages 14-20, XP028809876, ISSN: 0003-9861, DOI: 10.1016/J.ABB.2013.11.010<br/>the whole document</p> <p>-----</p>                                                                | 1-24                  |
| A                                                    | <p>ZEPTER K ET AL: "Induction of biologically active IL-1 beta-converting enzyme and mature IL-1 beta in human keratinocytes by inflammatory and immunologic stimuli", THE JOURNAL OF IMMUNOLOGY, THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 159, no. 12, 15 December 1997 (1997-12-15), pages 6203-6208, XP002241669, ISSN: 0022-1767<br/>the whole document</p> <p>-----</p> | 1-24                  |

## INTERNATIONAL SEARCH REPORT

### Information on patent family members

International application No

PCT/DK2015/050068

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)         | Publication<br>date      |
|-------------------------------------------|---------------------|------------------------------------|--------------------------|
| WO 03047629 A2                            | 12-06-2003          | AU 2002365654 A1<br>WO 03047629 A2 | 17-06-2003<br>12-06-2003 |
| -----                                     |                     |                                    |                          |