

METHODS OF TREATING CHRONIC WOUNDS USING CASPASE INHIBITORS

Technical Field

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The present invention relates generally to wound treatment and more particularly to compositions and methods for treating wounds involving the use of caspase inhibitors.

Background

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The integrity of healthy skin plays a crucial role in maintaining the physiological homeostasis of the human body and protecting against infection. The process of wound healing is a finely tuned sequence of events involving multiple cell types and signalling pathways. On wounding, inflammatory cells are recruited to protect against infection. A fibrin clot is formed to seal the wound until a new skin barrier can be constructed. Fibroblasts migrate to the wound bed and form granulation tissue, while activated keratinocytes at the wound edge migrate across the wound bed until the epithelial tongues meet at the wound centre. From here, keratinocytes differentiate to form a new skin barrier. On epidermal wounding, keratinocytes at the wound edge undergo an epithelial-to-mesenchymal transition (EMT) from a non-motile epithelial state to a mesenchymal-like state where they lose cell-cell contacts and become motile. Migrating cells reorganise their actin cytoskeleton and secrete proteases to remodel the dermal extracellular matrix (ECM) and enable migration across the wound. Directly behind the migrating cells, keratinocytes rapidly proliferate to provide enough cells to cover the wound. These events are coordinated through the transient regulation of multiple signalling pathways. Failure or delay to initiate these events can lead to chronic or non-healing wounds.

Older adults often suffer from underlying medical conditions, such as vascular disease, venous insufficiency and diabetes mellitus, which predispose them to non-healing or slow healing chronic wounds. Chronic wounds like diabetic foot ulcers often lead to lower extremity amputations in the elderly. With an increasingly ageing population and a high incidence of diabetes globally, chronic wounds have become a heavy societal and medical burden. The involvement of multiple signalling and regulatory pathways in wound healing means that current wound therapies, many of which target a single pathway or protein, are

mostly ineffective at treating chronic wounds. There is thus a need for more effective therapies for chronic wounds.

It would be desirable to ameliorate at least one of the above-described problems, or at least
5 to provide a useful alternative.

Summary

Disclosed herein is a method of treating a wound in a subject, the method comprising
10 administering a therapeutically effective amount of a caspase-3 inhibitor to the subject.

Disclosed herein is a caspase-3 inhibitor, for use in treating a wound in a subject, wherein a
therapeutically effective amount of the caspase-3 inhibitor is to be administered to the
subject.

15 Disclosed herein is the use of a caspase-3 inhibitor in the manufacture of a medicament for
treating a wound in a subject, wherein a therapeutically effective amount of the caspase-3
inhibitor is to be administered to the subject.

20 Disclosed herein is a method of promoting re-epithelialisation of a wound in a subject, the
method comprising administering a therapeutically effective amount of a caspase-3 inhibitor
to the subject.

Disclosed herein is a method of detecting a chronic wound in a subject, the method
25 comprising detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample
from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3
activity, relative to a reference, indicates that the subject has a chronic wound.

Disclosed herein is a method of treating a chronic wound in a subject, the method
30 comprising: (a) detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample
from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3
activity, relative to a reference, indicates that the subject has a chronic wound; and (b)
administering a therapeutically effective amount of a caspase-3 inhibitor to the subject with
a chronic wound.

Disclosed herein is a pharmaceutical composition comprising (a) a caspase-3 inhibitor and (b) spermine and/or spermidine.

- 5 Disclosed herein is a pharmaceutical combination comprising (a) a caspase-3 inhibitor and (b) spermine and/or spermidine.

Disclosed herein is a pharmaceutical composition or combination as defined herein, for use as a medicament.

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Disclosed herein is a method of treating a wound in a subject, the method comprising administering a pharmaceutical composition or combination as defined herein to the subject.

- Disclosed herein is a pharmaceutical composition or combination as defined herein, for use
15 in treating a wound in a subject.

Disclosed herein is the use of a pharmaceutical composition or combination as defined herein in the manufacture of a medicament for treating a wound in a subject.

20 **Brief description of the drawings**

Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

- 25 **Fig 1.** is a schematic of a proposed model of caspase-3 mediated control of wound healing and the potential effect of caspase-3 inhibitors on chronic wounds. (A) Caspase-3 is transiently inhibited in acute wound healing while is persistently activated in chronic wounds. (B) In acute wounds, transiently inhibited caspase-3 promotes the expression of pro-healing mRNAs, resulting in increased cell migration and efficient healing. (C) In
30 chronic wounds, persistently activated caspase-3 prevents expression of pro-healing mRNAs resulting in deficient healing.

Fig. 2 shows caspase-3 activity in human *ex vivo* wounded skin samples. (A) Representative human *ex vivo* wounds (scale bar, 5 mm). (B) Caspase-3 activity in non-wounded and

wounded human *ex vivo* skin samples. Error bar, mean $\pm$ SD of $n = 3$ experiments. P values are from one-way ANOVA. *, $p < 0.05$, **, $p < 0.01$. Bottom * indicates p values from statistical analysis between wound and non-wound at corresponding time point. Top * indicates p values from statistical analysis between different time points and 0 hour after wounding.

Fig. 3 shows caspase-3 activity in monolayer keratinocyte scratch model. Error bar, mean $\pm$ SD of $n = 3$ experiments. P values are from one-way ANOVA. *, $p < 0.05$, **, $p < 0.01$.

Fig. 4 shows caspase-3 activity in acute and perturbed rat wound models. (A) Representative acute and perturbed rat wounds (scale bar, 10 mm). (B) Caspase-3 activity in acute and perturbed rat wound models. Error bar, mean $\pm$ SD of $n = 3$ experiments. P values are from two-way ANOVA. *, $p < 0.05$, **, $p < 0.01$.

Fig. 5 shows topical addition of the caspase-3 inhibitor Z-DEVD-FMK and spermine into perturbed rat wound models. (A) Representative perturbed rat wounds under treatment (scale bar, 10 mm). (B) Relative rates of wound closure of perturbed rat wounds under different treatments. (C) H&E staining of perturbed rat wounds with and without Z-DEVD-FMK treatment. Arrow shows re-epithelialisation of rat wounds. (D) Caspase-3 activity in perturbed rat wounds at Day 3 with and without Z-DEVD-FMK treatment. (E) Expression of key factors of wound healing in perturbed rat wounds with and without Z-DEVD-FMK ($n = 6$ animals, per each treatment). Data analysis by Mann-Whitney U Test. *, $p < 0.05$; **, $p < 0.01$.

Detailed description

Caspases are an evolutionarily conserved family of cysteine-dependent proteases which are essential for apoptosis. The enzymes are produced as inactive proenzymes which undergo proteolytic processing at conserved aspartic acid residues to produce two subunits. The two subunits dimerise, and two dimers further assemble to form the functional heterotetrameric enzyme. Caspase-3, which is known as an executioner caspase of apoptosis, has been found to harbour various non-apoptotic functions in development, cell proliferation and migration,

cell fate decision, cytoskeletal reorganisation and signalling transduction by cleaving a variety of substrate factors.

The inventors have discovered that caspase-3 functions as a master regulator of wound healing by inhibiting the expression of pro-healing mRNAs. Caspase-3 activity undergoes a transient decrease in the early stages of wound healing, which promotes the expression of multiple key factors required for cell migration and wound closure. Higher levels of caspase-3 activity in chronic wounds can prevent upregulation of these factors and lead to delayed healing. Conversely, inhibition of caspase-3 can improve wound healing, even for non-healing or slow healing wounds. Unlike conventional wound healing therapies which target a single pathway or protein, targeting a master regulator of wound healing such as caspase-3 is advantageous as it can trigger multiple pathways and processes to accelerate wound healing. The inventors have also found that the native polyamines spermine and spermidine can promote cell migration and improve wound healing. Thus, the use of caspase-3 inhibitors in combination with spermine and/or spermidine can further promote the healing of chronic wounds.

Accordingly, provided in this disclosure are methods of treating wounds in a subject, including chronic wounds, using caspase-3 inhibitors. The caspase-3 inhibitor may be used alone or in combination with spermine and/or spermidine. Also provided are methods of detecting wounds based on the caspase-3 levels in a sample from a subject.

Disclosed herein is a method of treating a wound in a subject, the method comprising administering a therapeutically effective amount of a caspase-3 inhibitor to the subject.

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Also disclosed herein is a method of promoting re-epithelialisation of a wound in a subject, the method comprising administering a therapeutically effective amount of a caspase-3 inhibitor to the subject.

30 Disclosed herein is a caspase-3 inhibitor, for use in treating a wound in a subject and/or promoting re-epithelialisation of a wound in a subject, wherein a therapeutically effective amount of the caspase-3 inhibitor is to be administered to the subject.

Disclosed herein is the use of a caspase-3 inhibitor in the manufacture of a medicament for

treating a wound in a subject and/or for promoting re-epithelialisation of a wound in a subject, wherein a therapeutically effective amount of the caspase-3 inhibitor is to be administered to the subject.

5 As used herein, the term “wound” refers to an injury to a tissue. Wounds include both open wounds (in which the underlying tissue is exposed to the outside environment, such as, for example, a laceration, puncture, burn or surgical incision) and closed wounds (in which the underlying tissue is not exposed to the outside environment, such as, for example, pressure sores, wounds induced by blunt trauma, and wounds caused by surgical implants). The
10 wound may be an acute or a chronic wound. Wounds can display a spectrum of healing rates, whereby acute and non-healing wounds lie at opposite ends of the spectrum. The skilled person will be able to determine expected timeframes for wound healing based on, for example, the severity of the wound, the site of the wound, the type of wound (e.g., open or closed), and the age and health condition of the wounded subject.

15 An “acute wound” herein is an injury which occurs rapidly (such as a cut, laceration, contusion, burn, etc.) which typically heals quickly and is expected to move through the normal stages of the healing process at the expected rate, ultimately resulting in complete closure of the wound. Acute wounds may be expected to heal within three months. Acute
20 wounds can happen anywhere on the body and range from superficial scratches to deep injuries that damage the blood vessels, nerves, and muscle tissue.

A “chronic wound” herein is a non-healing or slow-healing wound that fails to progress through the usual phases of healing in an orderly way or at the expected rate. Wounds that
25 do not heal within three months, for example, may be considered chronic. A chronic wound may be characterised at least in part by one or more of (1) a prolonged self-perpetuating state of wound inflammation; (2) a deficient, defective and/or slow-forming wound extracellular matrix; (3) poorly responding (senescent) cells at the wound site, especially fibroblasts, limiting extracellular matrix production; and (4) failure or a decreased rate of re-
30 epithelialisation or wound closure. Chronic wounds may be slow-healing wounds that heal at a slower rate than expected but still demonstrate some healing over time. Chronic wounds may also be non-healing wounds which do not show improvement or closure despite appropriate care.

As used herein, the terms “treatment”, “treating” and the like, refer to obtaining a desired pharmacological and/or physiological effect. The effect may be therapeutic in terms of a partial or complete cure for a disease or condition and/or adverse effect attributable to the disease or condition. These terms also cover any treatment of a condition or disease in a subject and include: (a) inhibiting the disease or condition, i.e., arresting its development; or (b) relieving the disease or condition, i.e., causing regression of the disease or condition.

By “therapeutically effective amount” or “effective amount”, in the context of treating a disease or condition, is meant the administration of an amount of active agent to a subject, either in a single dose or as part of a series or slow release system, which is effective for the treatment of that disease or condition. The effective amount will vary depending upon the health and physical condition of the subject and the taxonomic group of subject to be treated, the severity of the disease or condition, the formulation of the active agent or pharmaceutical composition, the assessment of the medical situation, and other relevant factors. The skilled person would be able to determine an effective amount of an active agent with consideration of, for example, a subject’s age, weight, and clinical condition.

A “subject” herein refers to an organism that is to be treated using the methods of this disclosure. In some embodiments the subject is a mammal, including both human and non-human mammals such as a dogs, cats, pigs, bovids, equines, rodents or primates. In one embodiment, the subject is a human. The subject can be a patient.

Caspase-3 inhibitors

As used herein, the term “caspase-3 inhibitor” refers to any molecule which partially, substantially or completely prevents or blocks caspase-3 production and/or activity. The term “caspase-3 inhibitor” encompasses inhibitors of caspase-3 production as well as of inhibitors of caspase-3 activity. Caspase-3 inhibitors include small molecules, peptides (e.g., allosteric antagonists or substrate mimetics), proteins (e.g., antibodies), nucleic acids (e.g., aptamers, antisense oligonucleotides and small interfering RNA), glycans and conjugates or combinations of two or more of such. The inhibitor may be a naturally isolated molecule or a synthetic molecule (for example, a chemically or biologically synthesized molecule). The inhibitor may be a nucleic acid encoding an inhibitory peptide or polypeptide. The inhibitor may inhibit caspase activity directly (e.g., by preventing interaction with a substrate or by

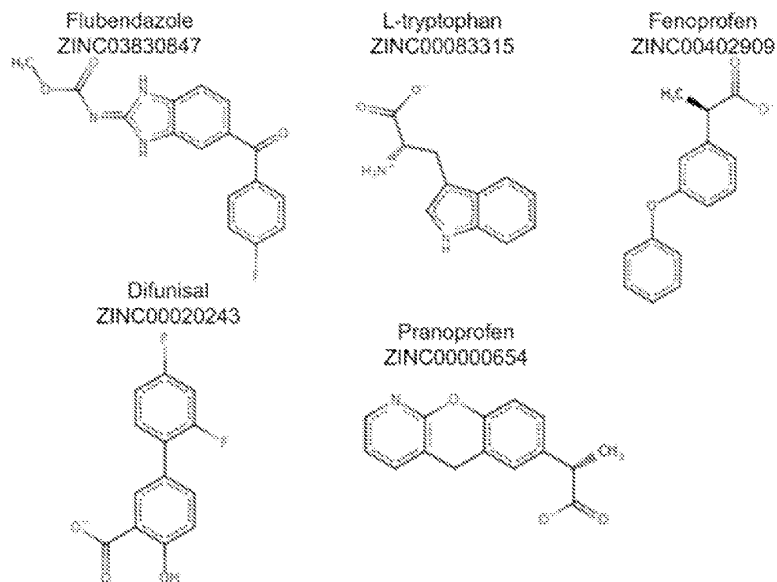
altering the structure of the enzyme) or indirectly (e.g., by affecting the subcellular localisation of the enzyme). The caspase-3 inhibitor can be targeted, for example, to epithelial cells or keratinocytes. One or more inhibitors may be used for the methods herein.

- 5 An inhibitor of production can be any molecule negatively affecting the synthesis, processing or maturation of caspase-3. The inhibitor can be, for example, a suppressor of gene expression of caspase-3; an antisense oligonucleotide or double-stranded RNA like small interfering RNA or microRNA which reduces or prevents the transcription of caspase-3 mRNA or degrades caspase-3 mRNA; a protein impairing correct folding of caspase-3; a
10 protease which degrades caspase-3, once it has been synthesized; or a compound that inhibits cleavage of procaspase-3 and thus prevents generation active caspase-3 (cleaved caspase-3).

- An inhibitor of caspase-3 activity can be an antagonist of caspase-3. Antagonists can bind to or sequester the caspase-3 molecule itself with sufficient affinity and specificity to
15 partially or substantially neutralise caspase-3 activity. Inhibitors of caspase-3 activity can also be caspase-3-specific antibodies, such as polyclonal or monoclonal antibodies, or any other molecule which prevents binding of caspase-3 to its targets, thus diminishing or preventing triggering of the reactions mediated by caspase-3.

- 20 In some embodiments, the caspase-3 inhibitor targets both caspase-3 and caspase-7, which are structurally similar and have structurally similar allosteric sites. In other embodiments, the caspase-3 inhibitor is a selective caspase-3 inhibitor, i.e., it preferentially targets or binds to caspase-3 over other caspases.

- 25 In some embodiments, the caspase-3 inhibitor is a small molecule inhibitor. The small molecule inhibitor may have a molecular weight of about 1000 Da or less. Small molecule inhibitors include but are not limited to metabolites, metabolic analogues, amino acids, amino acid analogues, nucleotides, nucleotide analogues, hetero-organic and organometallic compounds. Non-limiting examples of small molecule caspase-3 inhibitors include
30 flubendazole, L-tryptophan, fenoprofen, diflunisal, pranoprofen, emricasan, VX-166, M826, M867, isatin-based sulphonamides, 5-fluoro-1H-indole-2-carboxylic acid (2-mercaptoethyl) amide) (FICA), 2-(2,4-dichlorophenoxy-N-(2-mercapto-ethyl)-acetamide) (DICA), and pharmaceutically acceptable salts and solvates thereof. The structures of some of these compounds are provided below.



In one embodiment, the caspase-3 inhibitor is flubendazole, L-tryptophan, fenoprofen,
 5 diflunisal, pranoprofen, or a pharmaceutically acceptable salt or solvate thereof.

In some embodiments, the caspase-3 inhibitor is a peptide or a peptidomimetic. Peptide and
 peptidomimetic inhibitors can compete with caspase-3 substrates for enzyme binding.
 Caspase-3 recognises the tetrapeptide motif Asp-X-X-Asp, wherein X may be any amino
 10 acid. The C-terminal Asp is absolutely required while variations at other three positions can
 be tolerated. This substrate specificity may be used to design caspase-3 inhibitors. For
 example, caspase-3 is known to cleave the peptide sequence DEVDG (Asp-Glu-Val-Asp-
 Gly) between D and G *in vitro*. Peptides WEHD, VDVAD and DEVD are other examples
 of peptides that bind caspase-3. It is possible to generate reversible or irreversible inhibitors
 15 of caspase activation by coupling caspase-specific peptides to certain aldehyde, nitrite or
 ketone compounds. Fluoromethyl ketone (FMK)- or chloromethyl ketone (CMK)-
 derivatised peptides, such as Z-IETD-FMK, act as effective irreversible inhibitors. Inhibitors
 synthesized with a benzyloxycarbonyl group (also known as BOC or Z) at the N-terminus
 and O-methyl side chains exhibit enhanced cellular permeability thus facilitating their use

in vivo. Non-limiting examples of peptide inhibitors of caspase-3 include Z-VAD-FMK, Z-DEVD-FMK and Ac-DEVD-CMK.

In some embodiments, the caspase-3 inhibitor is a nucleic acid inhibitor. Non-limiting
5 examples of nucleic acid inhibitors include antisense compounds (e.g., antisense oligonucleotides (ASOs), gapmers and the like); nucleic acid molecules mediating RNA interference, including but not limited to short hairpin RNA (shRNA), small interfering RNA (siRNA) and variants and precursors of such (e.g., small segmented siRNA, small interfering ribonucleic neutrals, Dicer substrate siRNA, etc.), and microRNA (miRNA) and precursors;
10 and guide RNAs (gRNAs) mediating sequence-specific base editing, gene or RNA editing, or DNA or RNA cleavage in association with a Cas protein. The inhibitor may be a vector (e.g., plasmid vectors, viral vectors, etc.) encoding a nucleic acid inhibitor.

In some embodiments, the caspase-3 inhibitor reduces caspase-3 production or activity by
15 at least about 10%. For example, the caspase-3 inhibitor may reduce caspase-3 production or activity by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 100%.

It will be appreciated that additional bioactive agents may be screened for caspase-3
20 inhibitory activity. The term “candidate agent” or “candidate inhibitor” as used herein describes any molecule, e.g., small molecule, peptide, polypeptide, glycan, carbohydrate (including polysaccharides), polynucleotide, lipid, etc. Generally, a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a
25 negative control, i.e., at zero concentration or below the level of detection. In addition, positive controls, i.e. the use of agents known to inhibit caspase-3, may be used.

Candidate agents can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and
30 directed synthesis of a wide variety of organic compounds and biomolecules, including peptides, polypeptides and oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Candidate agents may undergo *in silico* screening (such as by computational docking of the agent to a target molecule or target molecular site) in addition to screening using bioactivity

assays. The ZINC database provides a collection of commercially available chemical compounds for *in silico* screening.

Combination therapy

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This disclosure also provides combination therapies for treating wounds, comprising the administration of (a) a caspase 3 inhibitor, and (b) one or more polyamines such as spermine or spermidine. The inventors have found that intracellular levels of spermine and spermidine are upregulated at wound sites and these polyamines promote cell behavioural changes that enable wound re-epithelialisation. The use of spermine and/or spermidine in combination with a caspase-3 inhibitor for wound treatment thus has the advantage of further accelerating wound closure, especially of chronic wounds.

Accordingly, in one embodiment of the disclosed methods, the caspase-3 inhibitor is administered with a therapeutically effective amount of spermine and/or spermidine to the subject to treat the wound in the subject.

Disclosed herein is a pharmaceutical combination comprising (a) a caspase-3 inhibitor and (b) spermine and/or spermidine. The caspase-3 inhibitor and spermine and/or spermidine may be comprised in a single or in separate compositions. Additionally, the caspase-3 inhibitor and spermine and/or spermidine may be formulated for administration via the same route or via different routes. In one embodiment, the caspase-3 inhibitor and spermine and/or spermidine are comprised in a single composition. In one embodiment, the caspase-3 inhibitor and spermine and/or spermidine are comprised in separate compositions.

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Disclosed herein is a pharmaceutical composition comprising (a) a caspase-3 inhibitor and (b) spermine and/or spermidine.

Disclosed herein is a pharmaceutical composition or combination as defined herein, for use as a medicament.

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Disclosed herein is a method of treating a wound in a subject, the method comprising administering a pharmaceutical composition or combination as defined herein to the subject.

Disclosed herein is a pharmaceutical composition or combination as defined herein, for use in treating a wound or promoting re-epithelialisation of a wound in a subject.

Disclosed is the use of a pharmaceutical composition or combination as defined herein in the manufacture of a medicament for treating a wound or for promoting re-epithelialisation of a wound in a subject.

Disclosed herein is a method of treating a wound or promoting re-epithelialisation of a wound in a subject, the method comprising administering a therapeutically effective amount of a caspase-3 inhibitor in combination with a therapeutically effective amount of spermine and/or spermidine to the subject.

The terms “a combination” and “in combination with” are not intended to imply that the therapy or the therapeutic agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope described herein. The therapeutic agents in the combination can be administered concurrently with, prior to, or subsequent to, one or more other additional therapies or therapeutic agents. The therapeutic agents or therapeutic protocol can be administered in any order. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. It will further be appreciated that the additional therapeutic agent utilised in the combination may be administered together or separately in different compositions. In general, it is expected that therapeutic agents utilised in combination be utilised at levels that do not exceed the levels at which they are utilised individually. In some embodiments, the levels utilised in combination are lower than those utilised individually.

The therapeutic agents may be administered simultaneously or sequentially depending on, for example, the nature of the wound to be treated, the composition of the agents or the desired administration route. As used herein, “simultaneously” is used to mean that two or more therapeutic agents are administered concurrently or in a substantially concurrent manner (such as immediately after each other). “Sequentially” refers to the administration of two or more therapeutic agents at different times. In sequential administration, the caspase-3 inhibitor may be administered before or after the spermine and/or spermidine. A time delay may exist between sequential administration of the therapeutic agents. The time interval may be any pre-determined time interval, but is preferably one that provides for a

cooperative effect of the caspase-3 inhibitor and the polyamine. The therapeutic agents may be administered simultaneously or sequentially via the same route or via different routes.

In one embodiment, the caspase-3 inhibitor and spermine and/or spermidine are administered simultaneously. In an alternative embodiment, the caspase-3 inhibitor and spermine and/or spermidine are administered sequentially.

Combination therapies herein may also include other therapeutic agents, for example, anti-microbial agents (e.g., antiseptics, antibiotics or antifungals), analgesics (both narcotic and non-narcotic), anti-inflammatory agents (e.g., steroids), growth factors (e.g., PDGF), anti-histamines, and/or vitamins (e.g., vitamin B, C or E). These agents may be administered together with (e.g., at the same time, or in the same composition) or sequentially to the caspase-3 inhibitor and/or polyamine.

15 Pharmaceutical compositions

The caspase-3 inhibitor and spermine or spermidine may be comprised in a suitable pharmaceutical composition for administration.

Depending on the location of the wound to be treated, the pharmaceutical composition may be administered locally or systemically, such as via an oral, intra-adiposal, intra-arterial, intra-articular, intracranial, intradermal, intra-lesional, intramuscular, intranasal, intraocular, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrarectal, intrathecal, intratracheal, intra-tumoral, intra-umbilical, intravaginal, intravenous, intravesicular, intravitreal, liposomal, local, mucosal, parenteral, subconjunctival, cutaneous, subcutaneous, sublingual, topical, transbuccal or transdermal route, or combinations thereof. The pharmaceutical composition may be administered via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via an implant, via a dressing, or via any combination thereof.

Depending on the intended route of administration, the therapeutic agents, pharmaceutical compositions, combined preparations and medicaments of the invention may, for example, take the form of tablets, caplets, capsules, hard capsules, soft capsules, gelatin capsules, cachets, troches, lozenges, dispersions, suppositories, ointments, creams, gels, hydrogels,

foams, poultices, pastes, powders, dressings, plasters, solutions, patches, aerosols, nasal sprays, inhalers, salves, suspensions, aqueous liquid suspensions, non-aqueous liquid suspensions, oil-in-water emulsions, water-in-oil emulsions, solutions, sterile solids, crystalline solids, amorphous solids, solids for reconstitution, delayed release formulations, sustained release formulations, or combinations thereof. Other suitable formulations include liposomal formulations, nanoparticle formulations, pluronic gel-based formulations, carboxymethylcellulose (CMC)-based formulations, and hydroxypropylmethylcellulose (HPMC)-based formulations. A skilled person may select an appropriate formulation and dosage depending on, for example, the location of the wound, the type and severity of the wound, and the desired route of administration.

Pharmaceutical compositions herein can further comprise a pharmaceutically acceptable carrier and/or a pharmaceutically acceptable salt. Suitable carriers include isotonic saline solutions, for example phosphate-buffered saline. Suitable diluents and excipients also include, for example, water, saline, dextrose, glycerol, and the like, and combinations thereof. In addition, if desired, substances such as wetting, solubilising or emulsifying agents, stabilising or pH buffering agents, viscosity controlling agents, preservatives, antioxidants, emollients, odour controlling or fragrance compounds may also be present.

The term “pharmaceutically acceptable carrier” refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, and amino acid copolymers.

Pharmaceutically acceptable salts can also be present, e.g., mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like.

Topical compositions

In one embodiment, the pharmaceutical composition is a topical composition. The topical composition may be in the form of, for example, a liquid, cream, ointment, gel, emulsion, lotion, paste, jelly, hydrogel, soap, spray, foam, powder, paint, patch or an impregnated

dressing.

- Suitable carrier materials for topical administration include any carrier or vehicle commonly used as a base for creams, ointments, gels, emulsions, lotions, pastes, jellies, sprays, foams, powders, or paints for topical administration, including but not limited to emulsifying agents, inert carriers including hydrocarbon bases, emulsifying bases, water-soluble bases, or combinations thereof. Suitable solvents, emollients and emulsifiers for hydrophobic topical formulations include lanolin, paraffin, beeswax, emulsifying waxes, dimethicones, mineral oils, silicone oils, vegetable oils, fatty acids and alkyl esters of fatty acids or dicarboxylic acids, triglyceride esters, fatty alcohols and fatty alcohol ethers and sterols. Other suitable solvents, emollients and emulsifiers include polyhydric alcohols and polyether derivatives such as glycerol, sorbitol, polyethylene glycols, polypropylene glycols, ionic and zwitterionic surfactants, amphoteric surfactants and non-ionic surfactants.
- Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, or thickening agents. Liquid sprays may be delivered from pressurised packs, for example, via a specially shaped closure.
- In some embodiments, the therapeutic agents herein are formulated with oleaginous bases or ointments to form a semi-solid composition with a desired shape. The composition may be shaped for easy application to, or insertion into, a wound, ulcer, or surgical site. In addition to the active ingredients, these semi-solid compositions may contain dissolved and/or suspended bactericidal agents, preservatives and/or a buffer system. The petrolatum component in these bases can be any paraffin ranging in viscosity from mineral oil employing incorporated isobutylene, colloidal silica, or stearate salts to paraffin waxes. Bases of this class can be made by incorporating high-melting waxes into a fluid mineral oil via fusion or by incorporation of polyethylene into mineral oil at elevated temperature.
- Polysiloxanes (also known as silicones) are suitable for use in these bases.

In other embodiments, compositions herein comprise a hydrogel carrier, such as a Pluronic gel (i.e., a nonionic polyoxyethylene-polyoxypropylene copolymer gel) or gels based on HPMC, CMC and other cellulose-based ingredients. Gels may be moldable to conform to

the size and shape of the wound, and can be used to confine the release of therapeutic agents to the site of application or immediately adjacent that site, and additionally provide delayed and/or sustained release of therapeutic agents. Suitable hydrogel materials include natural polymers such as polysaccharides (e.g., starch, dextran, pectin, alginate, cellulose chitosan, 5 hyaluronic acid, gellan, and the like), and polypeptides (e.g., collagen). The natural polymer may be further processed, for example by chemical derivation such as the formation of esters and ethers or pharmaceutically acceptable salts. Alternatively, hydrogels may also be based on synthetic polymers, non-limiting examples of which include polyalkylene oxide-based polymers, poly(eth)acrylate-based and poly(meth)acrylate-based polymers, 10 polyalkyl(meth)acrylate-based polymers, vinyl polymers, polylactide- and polyglycolide-based polymers, polycaprolactam- and polycaprolactone-based polymers, polyurethane-based polymers, and polyurea-based polymers.

Controlled release formulations

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Controlled or sustained release can be achieved by the addition of time-release additives, such as polymeric structures or matrices which are known in the art. Suitable carriers include a mixture or coating of polymers that provide release of the active agents at a constant rate over a prolonged period of time.

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The matrix material is selected based on the period over which release is desired, generally in the range of at least one week to one month, although longer periods may be desirable. In some cases, linear release may be most useful, although in others a pulse release or “bulk release” may provide more effective results.

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In some embodiments, the carrier comprises one or more biodegradable polymers, and the therapeutic agents are delivered mainly by matrix degradation. Examples of biodegradable polymers include synthetic polymers such as hydroxyacid polymers, for example, polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, 30 poly(butyric acid), poly(valeric acid), and poly(lactide-co-caprolactone), natural polymers such as collagen, albumin and other hydrophilic proteins, zein and other prolamines, and combinations thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion.

The carrier may also include one or more non-degradable hydrophilic polymers, such as polyethylene glycol (PEG), polypropylene glycol, poloxamers, hydroxypropylcellulose, polyvinyl alcohol and other water-soluble excipients. The carrier may also include one or more hydrophobic polymers. Non-limiting examples of such polymers are ethylcellulose,
5 acrylic resins, co-polymers of methacrylic acid and acrylic acid ethyl ester, polyethylene vinyl acetate copolymer, polystyrene-butadiene copolymer, and silicone rubber.

In some embodiments, the polymeric matrix is in the form of microparticles or nanoparticles. Microparticles can be in the form of microspheres, where the therapeutic agents are
10 dispersed within a solid polymeric matrix, or microcapsules, where the core is of a different material than the polymeric shell, and the therapeutic agents are dispersed or suspended in the core, which may be liquid or solid in nature. Particles may also be in the form of liposomes (comprising a lipid bilayer separating an aqueous internal compartment from the bulk aqueous phase) or micelles (comprising closed lipid monolayers with a hydrophobic
15 core and polar surface, or polar core and hydrophobic surface). The therapeutic agent may be dispersed in the aqueous or hydrophobic phase of liposomes or micelles. Unless specifically defined herein, microparticles, microspheres, microcapsules, nanoparticles, nanospheres, and nanocapsules are used interchangeably.

20 Alternatively, the polymer matrix may be cast as a thin slab or film, ranging from nanometres to centimetres; a powder produced by grinding or other standard techniques; or a gel such as a hydrogel. Such matrices can be formed by solvent evaporation, spray drying, solvent extraction and other methods known to those skilled in the art.

25 In other embodiments, therapeutic agents are incorporated or encapsulated in a solid or semi-solid bulk matrix (such as a cross-linked hydrogel, patch or dressing) for implantation at the site of the wound.

Wound treatment

30 Compositions, combinations and methods herein are useful for treating wounds, in particular chronic wounds.

Non-limiting examples of chronic wounds include ulcers, such as pressure ulcers (also known as decubitus ulcers), diabetic ulcers, diabetic foot ulcers, arterial ulcers, venous ulcers, venous stasis ulcers, vasculitic ulcers, burn ulcers, trauma-induced ulcers, infectious ulcers, and pyoderma gangrenosum ulcers. Chronic wounds include recurrent wounds
5 caused by chronic skin or epithelial conditions such as acne, psoriasis, atopic dermatitis and keratitis. Chronic wounds also include non-healing or slow-healing surgical wounds, including dehiscent wounds, which are wounds, usually stitched or stapled surgical incisions, that have ruptured or split open.

10 In one embodiment, the wound is an epithelial wound. An “epithelial wound” refers to any injury or disruption to epithelial tissue, such as the skin or the lining of various organs and body cavities.

In one embodiment, the wound is a cutaneous wound. A “cutaneous wound” refers to any
15 injury or disruption to the epidermal and/or dermal layers of the skin.

Treatment of a wound may comprise one or more of the following: increasing the rate of re-epithelialisation of the wound; increasing the extent of re-epithelialisation of the wound; increasing the rate of wound closure; and/or increasing the extent of wound closure.
20

In some embodiments, the method herein promotes re-epithelialisation of the wound. As used herein, the term “re-epithelialisation” refers to a process in which epithelial cells (e.g., fibroblasts, keratinocytes) at the wound edge differentiate, proliferate and migrate to cover the wound. The method may increase the rate and/or extent of wound re-epithelialisation.
25 Re-epithelialisation may be determined using methods known in the art, such as by visual assessment, by histological analysis by means of cellular or tissue staining of a biopsy sample, or by imaging techniques such as optical coherence tomography.

In some embodiments, the subject has compromised wound healing ability. The subject may
30 be suffering from a disease or condition that delays wound healing, such as diabetes mellitus, chronic skin or epithelial disorders, vascular diseases which result in chronic vascular insufficiency (e.g., atherosclerosis or peripheral artery disease), cancer, immunodeficiency disorders, autoimmune disorders or malnutrition. Alternatively or in addition, the subject may be undergoing treatment which results in impaired wound healing. Subjects with

compromised wound healing ability may have chronic wounds which can be advantageously addressed with the methods of this disclosure.

5 The disclosed compositions may be administered at or near the site of a wound. For cutaneous wounds, the compositions are preferably administered topically. Topical administration may be in any suitable form, such as liquids, creams, ointments, gels, emulsions, lotions, pastes, jellies, hydrogels, soaps, sprays, foams, powders, paints or patches, as described herein. The compositions may also be incorporated into inserts, wound dressings, hydrogels or other materials that come into contact with the wound.

10

The caspase-3 inhibitor and/or polyamines may be formulated for sustained release, such as using polymeric delivery systems, liposomal delivery systems, and hydrogels, as described herein. These can be loaded with the therapeutic agents, injected or implanted at or near the wound site, where the caspase-3 inhibitor and/or polyamines are released over a
15 therapeutically effective time period.

The caspase-3 inhibitor and any partners in combination therapy may be administered in a single dose, in multiple doses, in a continuous or intermittent manner (e.g., at regular intervals), depending, for example, upon the subject's clinical condition or on the condition
20 of the wound. Administration of the compositions may be essentially continuous over an indeterminate period of time, for example, until wound closure. Alternatively, the compositions may be administered continuously for a pre-selected period of time or in a series of spaced doses.

25 Wound detection

Also provided herein are methods of detecting wounds in a subject using caspase-3 as a biomarker. The methods may be advantageous for detecting wounds which are difficult to assess visually, e.g., closed wounds. The methods may be used to distinguish acute from
30 chronic wounds, since chronic wounds can exhibit sustained higher levels of caspase-3 or caspase-3 activity over a period of time than acute wounds. The methods may also be used to identify wounds that are likely to be responsive to treatment with a caspase-3 inhibitor.

Disclosed herein is a method of detecting a wound in a subject, the method comprising

detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3 activity, relative to a reference, indicates that the subject has a wound.

- 5 In some embodiments, the method distinguishes a chronic wound from an acute wound based on the increase in the level of cleaved caspase-3 and/or caspase-3 activity. The method may distinguish a chronic wound from an acute wound based on the extent and/or duration of increase in the level of cleaved caspase-3 and/or caspase-3 activity. For example, acute wounds generally lead to a smaller increase in cleaved caspase-3 level and/or caspase-3
10 activity compared to chronic wounds, and the increase is generally more transient in acute wounds compared to chronic wounds.

- Disclosed herein is a method of detecting a chronic wound in a subject, the method comprising detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample
15 from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3 activity, relative to a reference, indicates that the subject has a chronic wound.

- Disclosed herein is a method of treating a chronic wound in a subject, the method comprising: (a) detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample
20 from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3 activity, relative to a reference, indicates that the subject has a chronic wound; and (b) administering a therapeutically effective amount of a caspase-3 inhibitor to the subject with a chronic wound.

- 25 Disclosed herein is a method of treating a wound in a subject, the method comprising: (a) detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3 activity, relative to a reference, indicates that the wound is likely to be responsive to caspase-3 inhibitor therapy; and (b) administering a therapeutically effective amount of a caspase-3
30 inhibitor to the subject with a wound likely to be responsive to caspase-3 inhibitor therapy.

As used herein, "cleaved caspase-3" refers to either or both of the 17 kDa and 12 kDa polypeptide products of caspase-3 cleavage. Caspase-3 is produced natively as a pro-caspase

and undergoes enzymatic cleavage to generate two protein cleavage products. Heterotetramerisation of the cleavage products forms the active enzyme.

A “sample” as used herein includes any biological specimen that may be extracted,
5 untreated, treated, diluted or concentrated from a subject. A sample includes within its scope a collection of similar fluids, cells, or tissues (e.g., surgically resected tissue, biopsies, including fine needle aspiration), isolated from a subject, as well as fluids, cells, or tissues present within a subject. Any suitable methods for obtaining a biological sample can be employed; exemplary methods include, e.g., phlebotomy, swab (e.g., buccal swab) and
10 surgical biopsy. The sample may be pooled from multiple aliquots.

In one embodiment, the sample is a wound sample. The wound sample may be taken from a wound exudate, a wound edge or a wound centre.

15 In some embodiments, the sample is taken at least 24 hr after the occurrence of the wound. The sample may be taken at least 24 hr after, at least 48 hr after, at least 3 days after, at least 4 days after, at least 5 days after, at least 6 days after, at least 7 days after, at least 8 days after, at least 9 days after, at least 10 days after, at least 11 days after, at least 12 days after, at least 13 days after, or at least 14 days after the occurrence of the wound. The sample may
20 be taken about 24 hr after the occurrence of the wound, about 48 hr after, about 3 days after, about 4 days after, about 5 days after, about 6 days after, about 7 days after, about 8 days after, about 9 days after, about 10 days after, about 11 days after, about 12 days after, about 13 days after, or about 14 days after the occurrence of the wound.

25 In some embodiments, the method comprises detecting the level of cleaved caspase-3 and/or caspase-3 activity over a period of time, wherein a sustained increase in the level of cleaved caspase-3 and/or caspase-3 activity over the period of time, relative to a reference, indicates that the subject has a chronic wound. The time period for detection may depend on the expected healing time for the wound, for instance, detection may be over a shorter period of
30 time for a wound which is expected to heal quickly.

In some embodiments, the time period for detection of cleaved caspase-3 and/or caspase-3 activity is at least 5 days. The time period for detection may be at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least

12 days, at least 13 days, or at least 14 days. The time period for detection may be about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, or longer than 14 days.

- 5 Two or more samples may be taken within this time period to determine if there is a sustained increase in cleaved caspase-3 and/or caspase-3 activity. For instance, samples may be taken at the start and at the end of the time period. Additional samples may also be taken at any point within the time period, such as daily, every other day, weekly, etc. The skilled person may determine an appropriate sampling frequency to assess for sustained increase in cleaved
- 10 caspase-3 and/or caspase-3 activity based on, e.g., the time period for detection, the condition and/or healing progression of the wound, the clinical status of the subject, etc.

A “reference”, “control”, “reference sample”, or “control sample”, as used herein, refers to a sample, cell, tissue, standard, or level that is used for comparison purposes. In one

15 embodiment, a reference is obtained from a healthy and/or non-wounded part of the body (e.g., tissue or cells) of the same subject or individual. For example, healthy and/or non-wounded cells or tissue adjacent to the wound site. In another embodiment, a reference is obtained from an untreated tissue and/or cell of the body of the same subject or individual. In yet another embodiment, a reference is obtained from a healthy and/or non-wounded part

20 of the body (e.g., tissues or cells) of an individual who is not the subject or individual. In another embodiment, a reference is obtained from an untreated tissue and/or cell of the body of an individual who is not the subject or individual. The reference may be population-average levels for a biomarker (e.g., cleaved caspase-3) in healthy cells or tissues. In yet another embodiment where the method is for detecting chronic wounds, the reference may

25 be obtained from an acute wound from the same or a different subject. The reference may also be population-average levels of caspase-3 in acute wound samples.

As used herein, the term “increase” or “increased” with reference to a biomarker such as caspase-3 may refer to a statistically significant and measurable increase in the biomarker as

30 compared to a reference. The increase may be an increase of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, or more.

- Cleaved caspase-3 may be detected using any method known in the art for detecting a protein. Non-limiting examples of such methods include immunological methods using labelled antibodies or antibody fragments that bind to one or more cleaved caspase-3 products (e.g., using western blotting, immunofluorescent imaging, or immunohistochemical staining of samples); protein function or activity assays; protein binding assays using non-immunological means such as using labelled aptamers, lectins or other binding agents; and mass spectrometry.
- Caspase-3 activity may be detected using any method known in the art for detecting enzyme activity of caspase-3, such as the use of a labelled substrate which generates a signal (e.g., a visible, luminescent or fluorescent signal) upon proteolytic cleavage by caspase-3.

Kits

- The present disclosure also extends to kits for detecting cleaved caspase-3 or caspase-3 activity in a sample obtained from a subject. The kit may allow the detection of a wound in a subject when the level of cleaved caspase-3 or caspase-3 activity in the sample is increased compared to a reference.
- The kit may comprise one or more reagents or materials for detecting cleaved caspase-3 or caspase-3 activity in the sample from the subject. For example, the kit may comprise labelled antigen-binding molecules (e.g., antibodies or aptamers) for binding to and detecting cleaved caspase-3. Alternatively or additionally, the kit may comprise labelled caspase-3 substrates for generating a signal upon proteolytic cleavage by caspase-3.
- The kit may also include appropriate reagents for detection of labels, positive and negative controls, washing solutions, blotting membranes, microtitre plates, dilution buffers and the like. For example, a protein detection kit may include (i) at least one native cleaved caspase-3 polypeptide (which may be used as a positive control), and (ii) one or more antigen-binding molecules that bind specifically to a cleaved caspase-3 polypeptide. The antigen-binding molecules are suitably detectably labelled. The kit can also feature various devices (e.g., one or more) and reagents (e.g., one or more) for performing a detection assay herein, and/or printed instructional material for using the kit to quantify the level of cleaved caspase-3 or

caspase-3 activity. The reagents described herein, which may be optionally associated with detectable labels, can be presented in the format of a microfluidics card, a chip or chamber, or a microarray. The kit may also comprise instructions for selecting an appropriate caspase-3 inhibitor based on the level of cleaved caspase-3 or caspase-3 activity.

5

Also provided herein is a composition comprising (a) a sample obtained from a subject; and (b) an antigen-binding molecule for detecting cleaved caspase-3 in the sample. The sample may be a wound sample.

10 As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

As used in this application, the singular form "a," "an," and "the" include plural references
15 unless the context clearly dictates otherwise. For example, the term "an agent" includes a plurality of agents, including mixtures thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will
20 be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially
25 of" will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

30

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from

it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications, which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

10

Unless otherwise defined, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

15 Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit the scope of the generality hereinbefore described.

EXAMPLES

20

Methods

In order to generate the most clinically relevant data, the profile of caspase-3 activity during wound healing was determined using a human *ex vivo* wounded skin model, a monolayer keratinocyte scratch model, and acute and perturbed rat wound models.

25

1) Human *ex vivo* wound model: Surgical waste skin tissue was collected from a clinician. To analyse polyamine levels in human *ex vivo* wounded skin tissue, 6 mm punch biopsies were made and an excisional wound created within each biopsy. Excisional wounds made in human *ex vivo* skin were cultured at the air-liquid interface with William E medium plus 10% FBS and harvested at 0-, 2-, 4-, 8-, 16-, 24-, 36- and 48-hours post wounding. The wound biopsies were snap frozen and lysed for the measurement of caspase-3 activity.

30

- 2) Monolayer keratinocyte scratch model: N/TERT, an immortalised keratinocyte cell line, was used for this model. Scratching on confluent monolayer of N/TERT keratinocytes made a gap to mimic a wound. The keratinocytes were collected at different time points after scratching.
- 5 3) Acute rat wound model: Six-week-old Sprague Dawley rats were anaesthetised with 4% isoflurane, 20% oxygen, and 10% nitrous oxide, and maintained with 1.5% isoflurane. After injected subcutaneously with 0.03 mg/ml buprenorphine (Vetergesic), rat backs were shaved and full-thickness excisional 6 mm biopsy punch wounds were made, two on each side of the dorsal midline. The wounds were collected at indicated time points
- 10 after wounding.
- 4) Perturbed rat wound model: A perturbed rat wound model was used as an animal model for chronic non-healing wounds. A perturbed rat wound was made by placing an oversize scaffold containing 20% collagen and senescence-inducing FK866 into an excisional rat wound, which generates a foreign body reaction over 10 days and
- 15 inflammation which does not resolve for at least 15 days after scaffold removal. Perturbed rat wounds were treated with 200 μ M Z-DEVD-FMK (a caspase-3 specific inhibitor) delivered in a Pluronic gel, and treated wounds were harvested at 0-, 1-, 2- and 5-days post wounding to determine the expression of migration and proliferation markers at the wound edge (n = 6 animals per time point). Photographs of rats were
- 20 taken daily for documentation, scoring and analysis to measure multiple parameters including size and depth of wound. Pictures were assessed using size and depth with larger size and depth resulting in a higher severity score. If two wounds were of similar size and depth, active inflammation (degree of redness) would take the higher severity score. Standard H&E histology analysis for re-epithelialisation, granulation tissue
- 25 formation and angiogenesis in order to score the wounds was performed.

The wound edge tissues and scratched keratinocytes were collected at multiple time points after wounding until wound closure as described above. The skin samples and keratinocytes were lysed with lysis buffer (10 mM Tris HCl pH 7.5, 10% glycerol, 0.2 mM EDTA, 150

30 mM NaCl, protease inhibitor cocktail). Caspase-3 activity was measured with caspase-3 assay kit (#39383, Abcam, UK). The efficiency of cell migration was determined by staining and immunoblotting EMT markers with anti-E-cadherin (1:200; #3195; Cell Signaling, USA) and anti-SLUG (1:200; ab27568; Abcam, UK). The housekeeping proteins, actin and

tubulin, were determined by immunoblot using anti-beta actin antibody (#sc-47776, Santa Cruz, USA) and anti-beta tubulin antibody (#sc-5274, Santa Cruz, USA).

EXAMPLE 1: Caspase-3 activity is transiently inhibited on wounding

5

Human *ex vivo* wounded skin

Excisional wounds made in human *ex vivo* skin were cultured at the air-liquid interface with rich medium and collected at different time points post wounding (Fig. 2A). Caspase-3 activity in human *ex vivo* wounds exhibited a dynamic profile and reached its lowest level at 16 hr after wounding and then increased gradually until it recovered to a similar level as immediately after wounding. Caspase-3 activity showed little change in non-wounded skin samples (Fig. 2B).

Monolayer keratinocyte scratch model

The profile of caspase-3 activity was determined using a monolayer keratinocyte scratch model. Caspase-3 activity in scratched keratinocytes was found to show a similar dynamic pattern with that of human *ex vivo* wounds. Caspase-3 activity was gradually suppressed until 4 hr, after which it recovered to the level as observed immediately upon scratching (Fig. 3). These data further confirm that caspase-3 activity is dynamic and transiently suppressed during wound healing.

Acute and perturbed rat wound models

The role of caspase-3 in wound healing was explored using rat wound models (Fig. 4A). In the acute rat wound model, caspase-3 activity showed a dynamic profile and reached its lowest level at 48 hr post-wounding, while it was dysregulated in perturbed rat wounds and maintained significantly higher levels at all time points (Fig. 4B). These data demonstrate that transient inhibition of caspase-3 occurs on wounding and is misregulated in a perturbed wound rat model.

EXAMPLE 2: Topical addition of caspase-3 specific inhibitor Z-DEVD-fmk promotes wound closure in perturbed rat wounds

To determine the effect of caspase-3 inhibition on the healing of perturbed wounds, caspase-3-specific inhibitor Z-DEVD-fmk in Pluronic gel was topically applied to the perturbed rat

wounds. It was found that Z-DEVD-fmk treatment significantly promoted healing efficiency compared with the untreated control (Fig. 5A, B). Spermine has been reported to be able to promote cell migration in acute wound models and it is also shown that spermine promoted improved healing in the perturbed wounds. Treatment with caspase-3 inhibitor significantly
5 promoted cell migration and re-epithelialisation, as shown in H&E staining (Fig. 5C). Caspase-3 activity was significantly suppressed by Z-DEVD-fmk treatment (Fig. 5D). In perturbed wounds the levels of cell migration factors Slug, Snail, uPA, uPAR and TGF- β 1 were low. However, their expression levels were significantly upregulated in perturbed rat wounds under Z-DEVD-fmk treatment (Fig. 5E). This data demonstrates that caspase-3 is a
10 potential therapeutic target for chronic wounds.

In sum, transient suppression of caspase-3 activity is essential for efficient cell migration during wound healing. It was shown in a perturbed rat wound model that caspase-3 inhibition led to improved wound closure and increased expression of cell migration factors. Therefore,
15 targeting dysregulated caspase-3 is a potential therapeutic strategy to promote healing in chronic wounds.

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended
20 to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

CLAIMS

1. A method of treating a wound in a subject, the method comprising administering a therapeutically effective amount of a caspase-3 inhibitor to the subject.
2. The method of claim 1, wherein the wound is a chronic wound.
3. The method of claim 1 or 2, wherein the wound is an epithelial wound.
4. The method of any one of claims 1 to 3, wherein the wound is a cutaneous wound.
5. The method of any one of claims 1 to 4, wherein the caspase-3 inhibitor is a small molecule, peptide, polypeptide, nucleic acid, or a combination thereof.
6. The method of claim 5, wherein the caspase-3 inhibitor is flubendazole, L-tryptophan, fenoprofen, diflunisal, pranoprofen, or a pharmaceutically acceptable salt or solvate thereof.
7. The method of any one of claims 1 to 6, wherein the caspase-3 inhibitor is administered at or near the wound site.
8. The method of any one of claims 1 to 7, wherein the subject is a mammal.
9. The method of any one of claims 1 to 8, wherein the subject has compromised wound healing ability.
10. The method of any one of claims 1 to 9, wherein the caspase-3 inhibitor promotes re-epithelialisation of the wound.
11. The method of any one of claims 1 to 10, wherein the caspase-3 inhibitor is administered simultaneously or sequentially with a therapeutically effective amount of spermine and/or spermidine.
12. A caspase-3 inhibitor, for use in treating a wound in a subject, wherein a therapeutically effective amount of the caspase-3 inhibitor is to be administered to the subject.
13. Use of a caspase-3 inhibitor in the manufacture of a medicament for treating a wound

in a subject, wherein a therapeutically effective amount of the caspase-3 inhibitor is to be administered to the subject.

14. A method of promoting re-epithelialisation of a wound in a subject, the method comprising administering a therapeutically effective amount of a caspase-3 inhibitor to the subject.
15. A method of detecting a chronic wound in a subject, the method comprising detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3 activity, relative to a reference, indicates that the subject has a chronic wound.
16. A method of treating a chronic wound in a subject, the method comprising: (a) detecting the level of cleaved caspase-3 and/or caspase-3 activity in a sample from the subject, wherein an increase in the level of cleaved caspase-3 and/or caspase-3 activity, relative to a reference, indicates that the subject has a chronic wound; and (b) administering a therapeutically effective amount of a caspase-3 inhibitor to the subject with a chronic wound.
17. The method of claim 15 or 16, wherein the level of cleaved caspase-3 and/or caspase-3 activity is detected over a period of time, and wherein a sustained increase in the level of cleaved caspase-3 and/or caspase-3 activity over the period of time, relative to a reference, indicates that the subject has a chronic wound.
18. The method of claim 17, wherein the time period is at least 5 days.
19. The method of any one of claims 15 to 18, wherein the sample is a wound sample.
20. A pharmaceutical composition comprising (a) a caspase-3 inhibitor and (b) spermine and/or spermidine.
21. The pharmaceutical composition of claim 20, wherein the pharmaceutical composition is a topical composition.
22. A pharmaceutical combination comprising (a) a caspase-3 inhibitor and (b) spermine and/or spermidine.

23. A pharmaceutical composition of claim 20 or 21, or a pharmaceutical combination of claim 22, for use as a medicament.
24. A method of treating a wound in a subject, the method comprising administering a pharmaceutical composition of claim 20 or 21, or a pharmaceutical combination of claim 22, to the subject.
25. A pharmaceutical composition of claim 20 or 21, or a pharmaceutical combination of claim 22, for use in treating a wound in a subject.
26. Use of a pharmaceutical composition of claim 20 or 21, or a pharmaceutical combination of claim 22, in the manufacture of a medicament for treating a wound in a subject.