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WO 2008073603 A2
US 20130280234 A1
WO 2014005183 A1
MC KELVEY K et al., "Activated Protein C: A regulator of numan epidermal keratinocyte function", World J Biol Chem, (2014-05-26), vol. 5, no. 2, pages 169 - 179
WO 2005007820 A2
WILLIAMS PD et al., "Preclinical Safety and Pharmacokinetic Profile of 3K3-APC, a Novel, Modified Activated Protein C for Ischemic Stroke", Curr Pharm Des., (2012), vol. 18, no. 27, pages 4215 - 4222
WALKER CT et al., "Activated protein C with reduced anticoagulant activity improves functional recovery and reduces bleeding risk following controlled cortical impact", Brain Res, (2010), vol. 1347, ISSN 0006-8993, pages 125 - 131

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(54) Title: USE OF APC ANALOGUE FOR WOUND HEALING

(57) Abstract: The present aplication relates to wound repair and wound healing by the application of a therapeutic amount of Activated Protein C-3K3A ('APC-3K3A'). Specifically, this application is directed to a method of using APC-3K3A for the treatment of dermal or cutaneous wounds, including but not limited to, acute and chronic wounds, burns and ulcers.

Use of APC analogue for wound healing

Field of the invention

The invention relates to wound repair and healing, particularly to dermal or cutaneous wounds including but not limited to acute and chronic wounds, burns and ulcers.

Background of the invention

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

In the adult, the normal response to injury is generally wound repair. Wound repair has been classically described as following three distinct phases consisting of an initial phase in which a fibrin clot is formed, an intermediate phase in which the fibrin clot is lysed and a temporary matrix consisting of proteoglycan, glycoprotein and type III collagen is laid down, and a final phase in which the temporary phase is digested and replaced with a matrix rich in collagen type I.

Local factors such as the type, size and location of the wound, the vascular supply to the wound, the presence of infection, local movement, and exposure to radiation and UV light influence wound repair, as do systemic factors including status of cardiovascular performance, infection, metabolic status and hormones.

It is generally accepted that a normal physiological response to injury is a wound repair process that is complete with evidence of collagen type I deposition by about 3 to 4 weeks from injury. A protraction of the wound repair process beyond this time increases the likelihood of formation of a chronic wound.

While the role of inflammation in wound healing is under debate, it is generally recognised that it is important to contain inflammation during wound healing in terms of the severity and duration of inflammation. In particular it has been observed that an absence of inflammation in wound healing is linked with regeneration, whereas

presence of inflammation results in some degree of fibrosis and scar formation. Further, extended inflammation can lead to chronic inflammation, and insofar as cutaneous wounds are concerned, a failure for a wound to properly close, and ulceration.

Topical application of activated protein C (APC) has been shown to provide improvements in cutaneous wounds including dermal ulcers, burns, oral wounds, bone and cartilage damage, eye wounds and warfarin-related skin necrosis. According to WO2002/100445, it is the anti-coagulation and anti-inflammatory functions of APC that strongly indicate that APC is useful for the treatment of dermal wounds, and in particular for treatment of slow healing wounds. WO2002/100445 also discusses that APC-mediated gelatinase A –activating functions are important for APC –mediated wound repair.

Further to anti-coagulation and anti-inflammatory functions, APC is also known to have an anti-apoptotic function. The anti-apoptotic function is known to be independent of the anti-coagulation function, and in particular it is known that at least 3 lysine residues on the 37-loop of APC are essential for APC –mediated cleavage of factor Va, whereas these residues are not required for the anti-apoptotic, cyto-protective activity that arises by APC-mediated activation of PAR-1 Mosnier and Griffin 2006 Frontiers in Bioscience 11 2381-2399.

While the anti-inflammatory function of APC is thought to arise from APC -mediated activation of at least EPCR and possibly PAR-1, possibly leading to reduced leukocyte activation, dampened release of inflammatory cytokines, reduced extravasation of inflammatory cells at inflammatory sites, the mechanism(s) of the APC-mediated interaction(s), and in particular the relevant conformation site(s) of APC required for the anti-inflammatory function are not known. Mosnier supra. Further to APC –receptor mediated interactions, it has also been thought that APC's anti-inflammatory activity could well be explained by reason of its ability to down regulate generation of the proteases of the coagulation pathway, potentially linking anti-inflammatory activity and anti-coagulation activity.

APC -3K3A is an analogue of APC in which the 3 lysine residues on the 37-loop of APC relevant for Factor Va cleavage have been removed, enabling the analogue to provide anti-apoptotic function whilst eliminating anti-coagulation function. This analogue was prepared for the sole purpose of providing full cyto-protective activity in

conditions involving apoptosis (including stroke and neurodegenerative disorders) while reducing risk of bleeding. Mosnier supra.

There remains a need for improvements in dermal wound repair, in particular for improving the time to wound repair, for improving the rate of wound repair in particular by accelerating wound repair, or for improving the quality of a tissue arising from wound repair.

Summary of the invention

The invention seeks to address one or more of the above mentioned needs and in one embodiment provides a method for the treatment of a dermal wound including the step of contacting a dermal wound with an effective amount of APC-3K3A, thereby treating the dermal wound.

In another embodiment there is provided a method of decreasing the wound area or volume of a dermal wound including the step of contacting a dermal wound with an effective amount of APC-3K3A, thereby decreasing the wound area or volume of the dermal wound.

In another embodiment there is provided a method of accelerating the rate of wound healing, or decreasing the time to completion of wound healing or wound closure including the step of contacting a dermal wound with an effective amount of APC-3K3A, thereby accelerating the rate of wound healing, or decreasing the time to completion of wound healing or wound closure.

In another embodiment there is provided a method of accelerating the rate of wound healing during the first 3 to 7 days, preferably the first 3 to 5 days, preferably the first 3 days following wound formation, including the step of contacting a dermal wound after wound formation with an effective amount of APC-3K3A, thereby accelerating the rate of wound healing. The effective amount of APC-3K3A may be contacted with the dermal wound within 48 hours, preferably within 24 hours or within 12 hours from wound formation. Typically the rate of wound healing is accelerated relative to an untreated wound, or relative to a wound which has been treated with an equivalent amount of APC.

In another embodiment there is provided a method of inducing or promoting or initiating a wound repair mechanism in a dermal wound including the step of contacting a dermal wound with an effective amount of APC-3K3A, thereby inducing or promoting or initiating a wound repair mechanism in a dermal wound. In this embodiment, the wound may be a chronic wound which is devoid of, or which has minimal active wound repair mechanisms.

In another embodiment there is provided a method of minimising inflammation associated with wound repair including the step contacting a dermal wound with an effective amount of APC-3K3A, thereby minimising inflammation associated with wound repair.

In another embodiment there is provided a use of APC-3K3A in the manufacture of a medicament for treating a dermal wound.

In another embodiment there is provided use of APC-3K3A for treating a dermal wound.

In another embodiment there is provided APC-3K3A or formulation containing same including an effective amount of APC-3K3A for use in treatment of a dermal wound.

In the above described embodiments the wound is typically not associated with significant apoptosis. Typically, the dermal wound is not characterised by significant apoptosis, or otherwise does not arise from apoptosis

In the above described embodiments, the effective amount of APC-3K3A may be topically administered to the wound thereby contacting the wound with the APC-3K3A.

In another embodiment there is provided a formulation for treatment of a dermal wound, wherein the formulation includes APC-3K3A. In this embodiment, the formulation is adapted for topical application to a dermal wound. The formulation may be in the form of a gel, ointment, lotion or spray.

In another embodiment there is provided a device, personal care article or dressing formulated or adapted for treatment or management of a dermal wound including an effective amount of APC-3K3A for treatment or management of a dermal

wound. In this embodiment the APC-3K3A may be provided in the form of a gauze, mesh, sponge or bandage.

In the above described embodiments, the dermal wound may be chronic or acute wound and may arise from laceration, burn, incision, maceration, crushing, puncture abrasion or like injury.

Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

Brief description of the drawings

Figure 1. Decreases in wound area as a percentage of original wound in APC, APC-3K3A treated mice.

Figure 2. Improvements in wound repair in APC and APC-3K3A treated mice.

Figure 3. APC-3K3A has a greater potency for decreasing wound area than APC.

Figure 4. APC-3K3A increases rate of wound healing compared with APC.

Figure 5. APC-3K3A and APC increase rate of wound healing compared with placebo.

Figure 6. Amino acid sequences of APC-3K3A.

Detailed description of the embodiments

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

As discussed above, APC -3K3A is an analogue of APC in which the 3 lysine residues on the 37-loop of APC relevant for Factor Va cleavage have been removed, enabling

the analogue to provide anti-apoptotic function whilst eliminating anti-coagulation function. The sequence of APC -3K3A is shown in Figure 6.

APC -3K3A was prepared for the sole purpose of providing full cyto-protective activity in conditions involving apoptosis (including stroke and neurodegenerative disorders) while reducing risk of bleeding. Mosnier supra. According to WO2005/007820, APC-3K3A is designed to enable it to be used for preventing or alleviating damage associated at least in part with apoptosis including in subjects at risk of damage to blood vessels or tissue in various organs caused at least in part by apoptosis. See also WO2008/055145; WO2008/073603; Wang et al. 2012 Stroke 43:2444-2449; and Guo et al. 2009 Eur. J. Neurosci. 29:1119-1130.

The inventors have found that APC-3K3A can be used to improve dermal wound repair. The finding is particularly surprising given that apoptosis is not generally considered to be a cell mechanism that underpins repair of dermal wounds or that is involved in wound pathology including formation of a chronic wound.

This finding has been made in circumstances where prior to the invention, at least the anti-coagulant activity of APC was understood to be relevant to dermal wound repair, and there was a paucity of knowledge regarding whether the elimination of anti-coagulation function would impact on the Gelatinase A activity and anti-inflammatory function of APC necessary for repair of a dermal wound.

One particularly surprising finding of the invention is that APC-3K3A can be utilised to provide a dermal wound repair response that is improved over that observed with APC anti-coagulant function. Specifically, as shown in the examples herein, the inventors have found that APC-3K3A can be used to accelerate wound healing rate and to reduce the time to wound healing in *in vivo* excision models. Thus in one embodiment there is provided a method for the treatment of a dermal wound including the step of contacting a dermal wound with an effective amount of APC-3K3A, thereby treating the dermal wound.

In the above described method, the individual may be one at risk for impaired wound repair or impaired wound healing. In particular, the individual may be one having systemic or local risk factors for protracted wound repair. Systemic risk factors include systemic infection, metabolic syndrome, diabetes or glucose intolerance, impaired cardiovascular function. Local risk factors include those pertaining to the injury including

the nature of the injury itself (for example, a trauma or burn), abnormal inflammation, repeated physical stress by movement, or exposure to UV radiation.

The invention may include the step of assessing an individual to determine whether the individual or injury site has one or more systemic or local risk factors described above for an impaired wound repair process. Typically, the individual is assessed for one or more systemic or local risk factors applicable to formation of a chronic wound such as those described herein.

Where the individual is assessed as having one or more local or systemic risk factors for an impaired wound repair process, the method may include the further step of selecting the individual for treatment with APC-3K3A to minimise the likelihood of onset of an impaired wound repair process.

Typically the injury is one arising from insult to dermal, cutaneous or skin tissue. The insult may impact on all layers of dermal tissue, for example on stratum basale (stratum germinativum), stratum spinosum, stratum granulosum, stratum lucidum. Examples of particular injury include laceration, abrasion, rupture, burn, contusion, compression.

The injury may be a burn, including a 1st, 2nd or 3rd degree burn.

Typically the injury is an acute injury.

In one embodiment, the injury may not be associated with chronic inflammation.

Typically the injury is not associated with fibrosis.

Typically the injury is not an inflammatory disorder, an allergic disorder, or an idiopathic disorder or disease.

The APC-3K3A may be applied to the site of tissue injury before the wound repair process has formed a fibrin clot. In another embodiment the APC-3K3A may be applied to the site of tissue injury before the wound repair process has formed a temporary matrix. In another embodiment the APC-3K3A may be applied to the site of tissue injury before the wound repair process has formed a final matrix. Typically the APC-3K3A is applied at about the time of, or shortly after, formation of the temporary matrix.

Typically the individual is treated with APC-3K3A so as to provide for completion of wound repair within about 3 to 4 weeks of tissue injury.

Notwithstanding the foregoing, it is understood by those skilled in the art that the dosage amount of the APC-3K3A will vary with the disease or condition to be treated,

the severity of the disease or condition, the type(s) of local administration, the rate of excretion of the compound, the duration of the treatment, the identify of any other drugs being administered to the animal, the age, size and species of the animal, and like factors known in the medical arts. In general, a suitable daily dose of a compound or combination of compounds will be that amount which is the lowest dose effective to produce a therapeutic effect. The dosage amount, dosage form and mode of administration will be determined by an attending physician within the scope of sound medical judgment. Effective dosage amounts, dosage forms, and modes of administration for the various compounds and combination(s) of compounds can be determined empirically and making such determinations is within the skill of the art.

In certain embodiments, it is important that the APC-3K3A is provided so as to enable contact of APC-3K3A with skin cells as described herein at the site of tissue injury, as, while not wanting to be bound by hypothesis, it is believed that it is by this contact that the APC-3K3A provides for improvements in wound healing. Generally, those cells that have been contacted with APC-3K3A can be recognised by having the following characteristics: increased proliferation and decreased apoptosis; decreased caspase-3; activation of protease-activated receptors 1, 2 or 3; reduced NF-kB activation; reduced activation of signalling molecule, p38; reduced TNF secretion; increased matrix metalloproteinase (MMP)-2 protein and activation; reduced MMP-9; increased sphingoisine-1-phosphate; increased Angiopoietin (Ang)1 and decreased Ang 2; increased Tie2 activation; activation of signalling molecule Akt. Therefore, contact of cells with APC-3K3A, and therefore, therapeutic efficacy of treatment can be established by assessing for these cell phenotypes.

In one embodiment, a therapeutically effective amount of APC-3K3A may prevent or inhibit the formation of a pathologic scar in an individual. This outcome can be assessed by the qualitative or quantitative measures discussed below.

In certain embodiments, the therapeutically effective amount of APC-3K3A is from 0.1 µg to 5000µg of APC-3K3A per cm^2 of the region of skin to which the APC-3K3A is applied, or from 1µg to 2000µg of APC-3K3A per cm^2 of the region of skin to which the APC-3K3A is applied, or from 10µg to 1000µg of APC-3K3A per cm^2 2of the region of skin to which the APC-3K3A is applied, or from 10µg to 200µg, or from 10 µ g to 400 µ g, or from 10 µ g to 800 µ g of APC-3K3A per cm^2 of the region of skin to which the APC-3K3A is applied

The APC-3K3A may be administered once per week up to twice daily, depending on the nature of the tissue injury. It is generally provided for no more than 20 weeks of consecutive days, or from no more than 6 weeks of consecutive days.

Topical treatment methods, for example, using a paste, gel, cream, oil, lotion, foam, ointment or like substance are particularly useful where the relevant skin region is one that contains a ruptured skin surface, as this permits penetration of the APC-3K3A to the relevant strata of the skin tissue where the fibroblasts reside.

In one embodiment, the therapeutically effective amount of APC-3K3A may be from 0.1 to 2000 μ g, preferably from 20 to 200 μ g of APC-3K3A per cm^2 of the region of skin. A higher amount is generally preferred where the skin is more severely affected, or where the individual is at particular risk because of presence of local or systemic factors for impaired wound repair, as described above. Lower amounts may be preferred where the skin is not severely affected.

The concentration of APC-3K3A in the formulation may be between about 10 g/ml and 1mg/ml and the volume of composition applied to the skin region is about 100 l to 10ml.

In one embodiment, a formulation including APC-3K3A as the active component for wound healing or repair includes about 200 to 600μg of APC-3K3A preferably about 200 to 250μg of APC-3K3A, more preferably 250μg of APC-3K3A or 500μg of APC-3K3A. It is believed that this amount of APC-3K3A provides for acceleration of wound healing that is generally greater than that observed with APC alone, particularly where the wound area is less than $20cm^2$. Thus in another embodiment there is provided a method for the treatment of a dermal wound including the step of contacting a dermal wound with about 200 to 600μg of APC-3K3A, preferably about 200 to 250μg of APC-3K3A, more preferably 250μg of APC-3K3A, or 500μg of APC-3K3A, thereby treating the dermal wound. Where the wound area is greater than $20cm^2$ larger amounts of APC-3K3A are required, particularly from about 600 to 1000μg of APC-3K3A, preferably about 800 to 1000μg of APC-3K3A. Thus in another embodiment there is provided a method for the treatment of a dermal wound including the step of contacting a dermal wound with about 600 to 1000μg of APC-3K3A, preferably about 800 to 1000μg of APC-3K3A. In these embodiments, the formulation may be provided in a form suitable for topical or parenteral administration.

The composition may be provided to the skin generally with a sterile surface, such as a finger or spatula in a layer of no more than about 10 mm thickness, preferably about 3 mm thickness. It may then be rubbed or massaged into the skin region and surrounding area. The application is generally from once per day to once per week, and generally no longer than 20 weeks, or no longer than 12 weeks.

In one embodiment, the APC-3K3A containing composition may be applied to a solid substrate i.e. a bandage, dressing or the like, and the substrate then fixed to the relevant skin region.

In certain embodiments, the above outcomes are obtained by establishing a local concentration of APC-3K3A at least 2 times higher than basal line of APC. This amount of APC-3K3A and APC can be measured by measuring APC-3K3A and APC activity of skin biopsy using ELISA and chromogenic substrate Spectrozyme PCa assay as mentioned above. Intradermal or subcutaneous injection is generally preferred as an administration route when the stratum corneum is intact and of such nature that there is limited penetration of APC-3K3A across the skin layer. Generally a fine gauge needle on a (~28-34G) needle on a 0.3 to 1 ml syringe may be used. Multiple injections may be given to cover the surface area of the skin, with ~1 injection per cm^2. The amount per injection will vary from 10 µl to 1 ml, with typical amount being 50 µl. Generally the administration is given from once per day to once per week, and generally no longer than 20 weeks. Intradermal or sub cutaneous injection can be used concurrently with topical application of APC-3K3A.

APC-3K3A for use in a method or product described herein may be produced by a process as described in WO2005/007820. Further, APC-3K3A may be obtained from ZZ Biotech. The amino acid sequence of APC-3K3A is shown in Figure 6.

In certain embodiments, APC-3K3A may incorporate modifications (eg amino acid substitutions, deletions, and additions of heterologous amino acid sequences), thereby forming APC-3K3A analogues which may, for example, enhance biological activity or expression of the respective protein. For example an APC-AK3A analogue may contain the RR229/230AA mutation corresponding to the calcium loop of APC, the RR306/312AA mutation corresponding to the autolysis loop of APC, or the RKRR306/314AAAA corresponding to the autolysis loop of APC. Each of these examples of APC analogues have reduced anticoagulant activity as compared with

activity of native APC. However, each of them has related APC function in terms of binding to EPCR and PAR-1 or PAR-3.

APC-3K3A analogues generally have a sequence that is homologous to human protein C sequence. Percentage identity between a pair of sequences may be calculated by the algorithm implemented in the BESTFIT computer program (Smith & Waterman. J. Mol. Biol. 147:195-197, 1981 ; Pearson, Genomics 11 :635-650, 1991). Another algorithm that calculates sequence divergence has been adapted for rapid database searching and implemented in the BLAST computer program (Altschul et al., Nucl. Acids Res. 25:3389-3402, 1997). In comparison to the human sequence, the protein C polynucleotide or polypeptide may be only about 60% identical at the amino acid level, 70% or more identical, 80% or more identical, 90% or more identical, 95% or more identical, 97% or more identical, or greater than 99% identical.

Conservative amino acid substitutions (e.g., Glu/Asp, Val/Ile, Ser/Thr, Arg/Lys, Gln/Asn) may also be considered when making comparisons because the chemical similarity of these pairs of amino acid residues are expected to result in functional equivalency in many cases. Amino acid substitutions that are expected to conserve the biological function of the polypeptide would conserve chemical attributes of the substituted amino acid residues such as hydrophobicity, hydrophilicity, side-chain charge, or size. In comparison to the human sequence, the protein C polypeptide may be only about 80% or more similar, 90% or more similar, 95% or more similar, 97% or more similar, 99% or more similar, or about 100% similar. Functional equivalency or conservation of biological function may be evaluated by methods for structural determination and bioassay.

The codons used may also be adapted for translation in a heterologous host by adopting the codon preferences of the host. This would accommodate the translational machinery of the heterologous host without a substantial change in chemical structure of the polypeptide.

APC-3K3A may also be glycosylated by methods well known in the art and which may comprise enzymatic and non-enzymatic means.

Suitable APC-3K3A mimetic compounds (ie compounds which mimic the function of APC-3K3A) may be designed using any of the methods well known in the art for designing mimetics of peptides based upon peptide sequences in the absence of secondary and tertiary structural information. For example, peptide mimetic compounds

may be produced by modifying amino acid side chains to increase the hydrophobicity of defined regions of the peptide (eg substituting hydrogens with methyl groups on aromatic residues of the peptides), substituting amino acid side chains with non-amino acid side chains (eg substituting aromatic residues of the peptides with other aryl groups), and substituting amino- and /or carboxy-termini with various substituents (eg substituting aliphatic groups to increase hydrophobicity).

Alternatively, the mimetic compounds may be so-called peptoids (ie non-peptides) which include modification of the peptide backbone (ie by introducing amide bond surrogates by, for example, replacing the nitrogen atoms in the backbone with carbon atoms), or include N-substituted glycine residues, one or more D-amino acids (in place of L-amino acid(s)) and /or one or more α-amino acids (in place of β-amino acids or γ-amino acids). Further mimetic compound alternatives include "retro-inverso peptides" where the peptide bonds are reversed and D-amino acids assembled in reverse order to the order of the L-amino acids in the peptide sequence upon which they are based, and other non-peptide frameworks such as steroids, saccharides, benzazepinel,3,4-trisubstituted pyrrolidinone, pyridones and pyridopyrazines. Suitable mimetic compounds may also be designed /identified by structural modelling/ determination, by screening of natural products, the production of phage display libraries, minimised proteins, SELEX (Aptamer) selection, combinatorial libraries and focussed combinatorial libraries, virtual screening/ database searching, and rational drug design techniques well known in the art.

Suitable pharmaceutical compositions of APC-3K3A comprise APC-3K3A and a pharmaceutically-acceptable carrier. An APC-3K3A -containing composition may generally be one that is a stable lyophilized product of high purity comprising a bulking agent (such as sucrose, mannitol, trehalose, and raffinose), a salt (such as sodium chloride and potassium chloride), a buffer (such as sodium citrate, Tris-acetate, and sodium phosphate), and APC-3K3A. For example, a stable lyophilized composition may comprise a weight ratio of about 1 part APC-3K3A, between about 7-8 parts salt, and between about 5-7 parts bulking agent. An example of such a stable lyophilized composition is: 5.0 mg APC, 30 mg sucrose, 38 mg NaCl, and 7.56 mg citrate, pH 6.0, per vial.

The various recombinant and synthetic forms of APC-3K3A and APC-3K3A analogues can be tested for use in the treatment of a pathologic scar by screening for the relevant efficacy in an established animal model, examples of which are described below.

In one particularly preferred embodiment, the APC-3K3A is provided in the form of a composition or formulation that is adapted for topical administration to a relevant site of tissue injury, according to a method described herein. Examples of such formulations include those that can be applied directly to the relevant surface enabling local administration of the APC-3K3A to the relevant site. These formulations include gels, oils, sprays, roll on formulations, ointments, lotions, foams and the like. In one embodiment, the APC-3K3A is provided in the form of a methyl-cellulose gel and may contained stabilisers such as carbohydrates and salts.

Skin ointment may be a combination of organic, health, beauty or medicinal ingredients, usually in a petroleum oil base. This gives skin ointment a thicker, less water-soluble formula that stays on the surface of the body longer so that the ingredients can work more effectively to treat a wide variety of problems. There are many natural and organic skin ointments which can be ordered from companies (such as Therapex).

Clobetasol propionate (CP) foam (0.05%) may also be used. This is an emulsion aerosol foam that has been used for the treatment of inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses in the United States and for inflammatory and pruritic manifestations of moderate to severe atopic dermatitis in Canada (Olux-E (clobetasol propionate) foam, 0.05% Stiefel Laboratories Inc, Research Triangle Park, NC (2011).

Where the formulation is a gel, it may contain APC-3K3A in an amount of 10-5000 μ g/g gel.

An injectable formulation of APC-3K3A may be supplied as a sterile, lyophilized powder for intravenous infusion including APC-3K3A, sucrose, NaCl, and sodium citrate. The vials may be reconstituted with Sterile Water for Injection, USP, to give a concentration of about 2 mg/ml APC-3K3A, and this diluted APC-3K3A may then be added to 0.9% Sodium Chloride Injection to give a concentration of from about 100 to about 500 μg/ml APC-3K3A for administration to a patient. This is a particularly preferred formulation for administration of APC-3K3A by subcutaneous injection.

Examples

Example 1 APC-3K3A promotes wound repair in an in vivo excisional model

Materials & methods

C57BL/6J mice were at 7-8 weeks of age when starting wounding protocol. Mice were obtained from and housed at Kearns Facility, Kolling Medical Research Institute, under a 12 h light/12 h dark cycle at 22°C.

Full-thickness skin wounds were made using an iris scissor and a sterile 6-mm punch biopsy tool was used to outline a pattern on the dorsum of the mice.

Recombinant (r)3K3A-APC, rAPC or phosphate buffered saline (PBS) control was injected into the skin at 4 points, each receiving 10 µl, around the internal periphery of the wounds, and 10 µl added topically to the wound, once a day for the first three consecutive days.

The animals were then kept under anaesthesia for 15 minutes to allow absorption of the solution. The wounds were left open and the animals were housed in individual cages.

Wound healing was monitored by taking digital photographs and blindly measuring the wound area by tracing the wound perimeter with a thin tipped marker onto sterile Visitrak Grid 6. Tracings were then scanned to obtain a digital reading of the wound area.

All procedures were performed according to the guidelines of the local animal care and ethics committee.

Results & discussion

Figure 1 and Figure 2: results are shown as mean ± SE. For PBS, 10 µg r3K3A-APC and 10 µg rAPC: number of wounds = 12, number of mice = 6. For other concentrations: wounds = 6, mice = 3. $P < 0.01$, PBS vs r3K3A-APC 10 µg. $P < 0.01$, r3K3A-APC 10 µg vs rAPC 10µg (Repeated measures ANOVA). In summary, the optimal dose in this study for rAPC-3K3A is 10µg. The data shows that rAPC-3K3A enhances wound healing compared to PBS control. Further, rAPC-3K3A enhances wound healing compared to rAPC.

There was a significant difference between 10ug 3K3A and PBS at early timepoints, with $p<0.01$ on days 2, 3 and 4. Similar differences were seen between 10ug APC and 10ug 3K3A. The % improvement of 10ug 3K3A over PBS was, 28%, 23% and 30% on days 2, 3 and 4 respectively. The % improvement of 10ug 3K3A over 10ug APC was

24%, 29% and 36% on days 2, 3 and 4 respectively. The faster a wound heals, especially at early stages, the greater the likelihood of tissue regeneration occurring, rather than just repair. Regeneration usually refers to new tissue that is the same as the original tissue whereas repair is associated with scarring *(Min, Su; Wang, Song W.; Orr, William (2006). "Graphic general pathology: 2.2 complete regeneration:". Pathology. pathol.med.stu.edu.cn. Retrieved 2012-12-07)*. The first few days is the critical time period for regeneration during wound healing - the slower the healing, the more likelihood that scarring will occur (Ferguson MW1, O'Kane S; Philos Trans R Soc Lond B Biol Sci. 2004 May 29;359(1445):839-50. Scar-free healing: from embryonic mechanisms to adult therapeutic intervention). (Profyris C, Tziotzios C and Do Vale I. Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics Part I. The molecular basis of scar formation. J Am Acad Dermatol 2012; 66: 1-10). Thus, the marked improvement induced by 10 ug 3K3A at early timepoints more likely leads to the desired scarless healing, compared to treatment with 10ug APC or PBS. This early thrust provides momentum for accelerated angiogenesis, granulation tissue formation, epithelialization and matrix reorganisation. This provides a tangible advantage over normal healing (Control) and treatment with 10ug APC.

Treatment with 30 ug 3K3A displayed slower healing than 10 ug 3K3A. This type of response is not uncommon in biological drug studies. One explanation is that the 3K3A receptors become saturated and a signalling feedback mechanism occurs, where intracellular messengers send biochemical signals to inhibit the action of 3K3A possibly by affecting its receptors. Precedent for this effect is seen by APC's effects on keratinocyte migration and wound closure in vitro (Xue M, Thompson P, Kelso I, Jackson C. Exp Cell Res. 2004 Sep 10;299(1):119-27. Activated protein C stimulates proliferation, migration and wound closure, inhibits apoptosis and upregulates MMP-2 activity in cultured human keratinocytes.) Such a feedback mechanism was not observed for APC, as 30 ug APC had a greater effect than 10 ug APC ($p<0.01$). These data suggest that APC at 10 ug is yet to reach its peak whereas 10 ug 3K3A has peaked, implying that 3K3A is more potent than APC as a therapeutic drug for wound healing. Overall, the data above show that 3K3A is different from and has a clear advantage over APC.

Figure 3: *P<0.05, ** P<0.01, when compared to control (ANOVA, Bnferonni's). On Days 8 and 9, r3K3A-APC at both 10 and 30 µg enhanced wound healing whereas for

rAPC only the higher concentration, 30 µg, was effective. On day 9, there was a significant difference between 10 µg r3K3A-APC and 10 µg rAPC.

Figure 4: P<0.01, between 3K3A-APC (n=12) 10 µg Vs PBS (n=12). P<0.05, between 3K3A-APC10 µg (n=12) Vs rAPC10 µg (n=12). No difference between rAPC 10 µg and PBS (p=0.064) (Using Kaplan-Meier and log rank analysis). In summary, at 10 µg 3K3A-APC completely heals wounds faster than placebo or rAPC (10 µg).

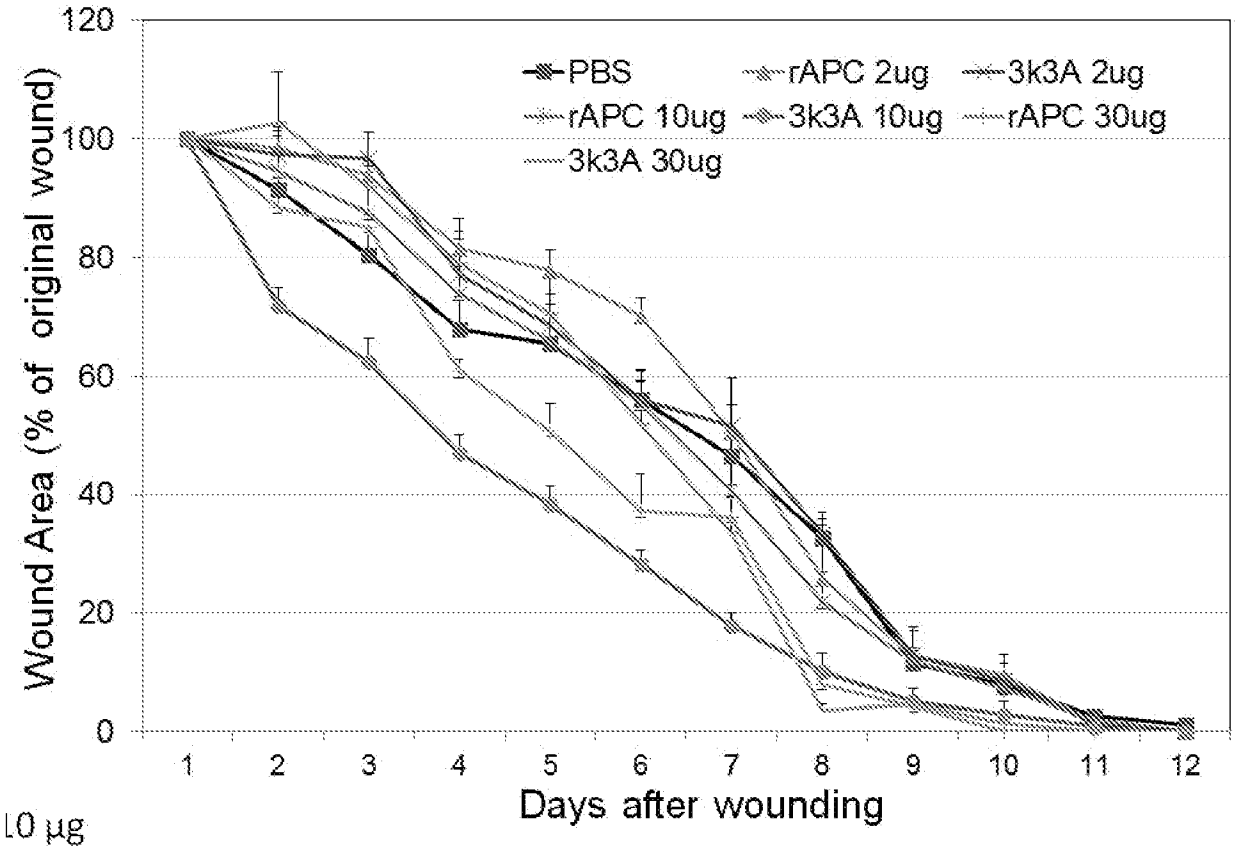
Figure 5: P<0.05, between rAPC 30 µg (n=6) Vs PBS (n=12). P<0.05, between 3k3A-APC 30 µg (n=6) Vs PBS (n=12). No difference between rAPC 30 µg and 3k3A APC 30 µg. (Using Kaplan-Meier and log rank analysis). In summary, at 30 µg, 3K3A-APC and rAPC both completely heal wounds faster than placebo.

2015246656 21 May 2020

CLAIMS

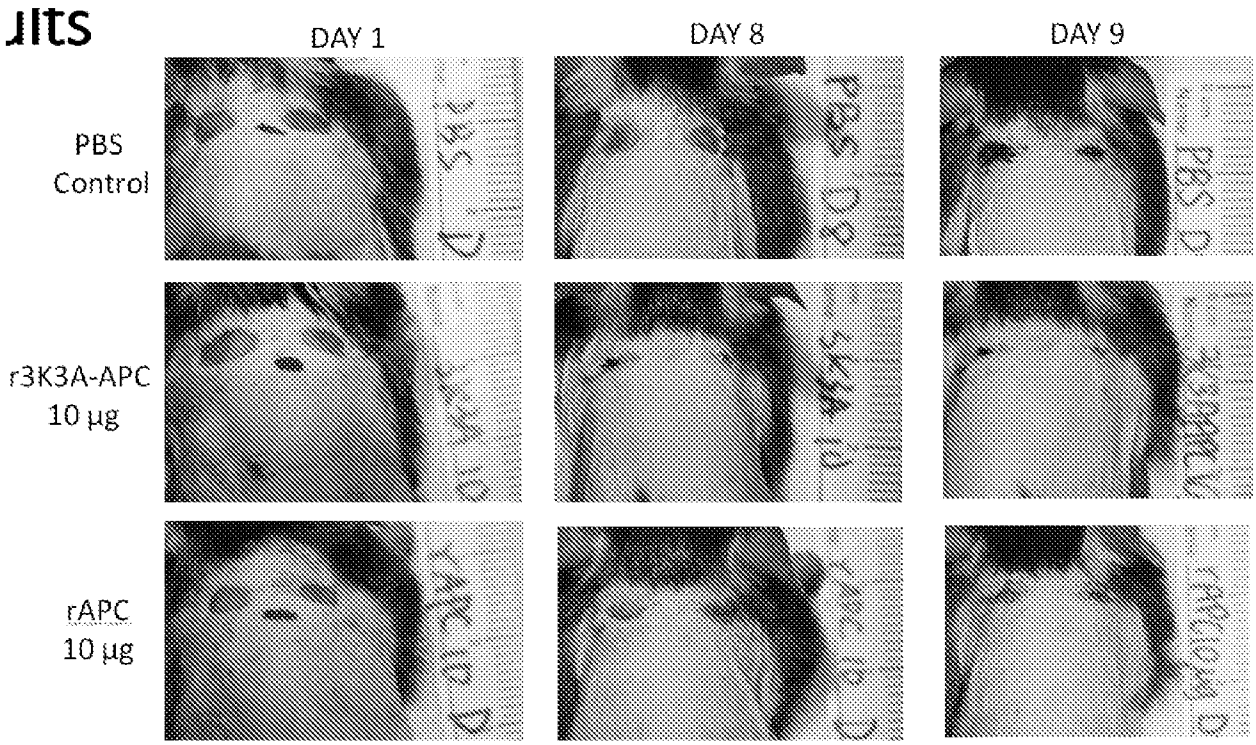
1. A method for accelerating dermal wound repair, comprising contacting a dermal wound with an effective amount of 3K3A-APC, thereby accelerating repair of the dermal wound.
2. The method of claim 1 comprising the step of topically administering the 3K3A-APC to the wound, thereby contacting the dermal wound with the effective amount of 3K3A-APC.
3. The method of claim 2 wherein 3K3A-APC is topically administered to the wound in the form of a formulation selected from the group consisting of a gel, an ointment, a lotion and a spray.
4. The method of claim 1 wherein the 3K3A-APC is administered to the wound by injection, thereby contacting the wound with an effective amount of 3K3A-APC.
5. Use of 3K3A-APC in the manufacture of a medicament for accelerating dermal wound repair, wherein the medicament is formulated for contact with a dermal wound.
6. The use of claim 5, wherein the medicament is in the form of a gel, an ointment, a lotion, a spray or an injectable formulation.
7. The method or use of any one of the preceding claims wherein the dermal wound is not characterised by significant apoptosis, or otherwise does not arise from apoptosis.
8. The method or use of any one of the preceding claims wherein the wound is a chronic or an acute wound.
9. The method or use of any one of the preceding claims wherein the wound is a burn, incision, laceration, abrasion, puncture or ulcer.
10. The method of any one of the preceding claims wherein the 3K3A-APC is provided in an amount of 250μg or 500μg.

Figure 1



L0 µg

Figure 2



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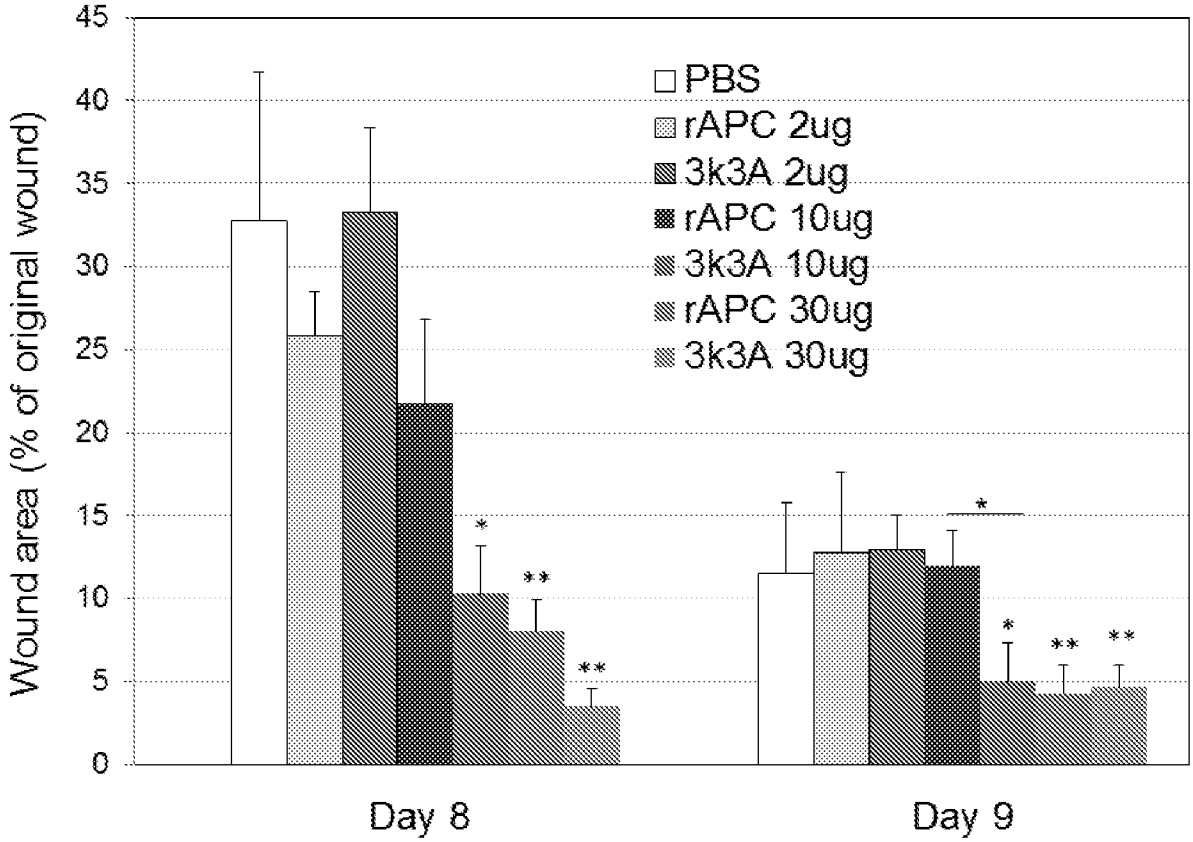
Wound area (% of original wound)
45
40
35
30
25
20
15
10
5
0
PBS
rAPC 2ug
3k3A 2ug
rAPC 10ug
3k3A 10ug
rAPC 30ug
3k3A 30ug
*
**
**
*
*
**
**
Day 8
Day 9

Figure 4

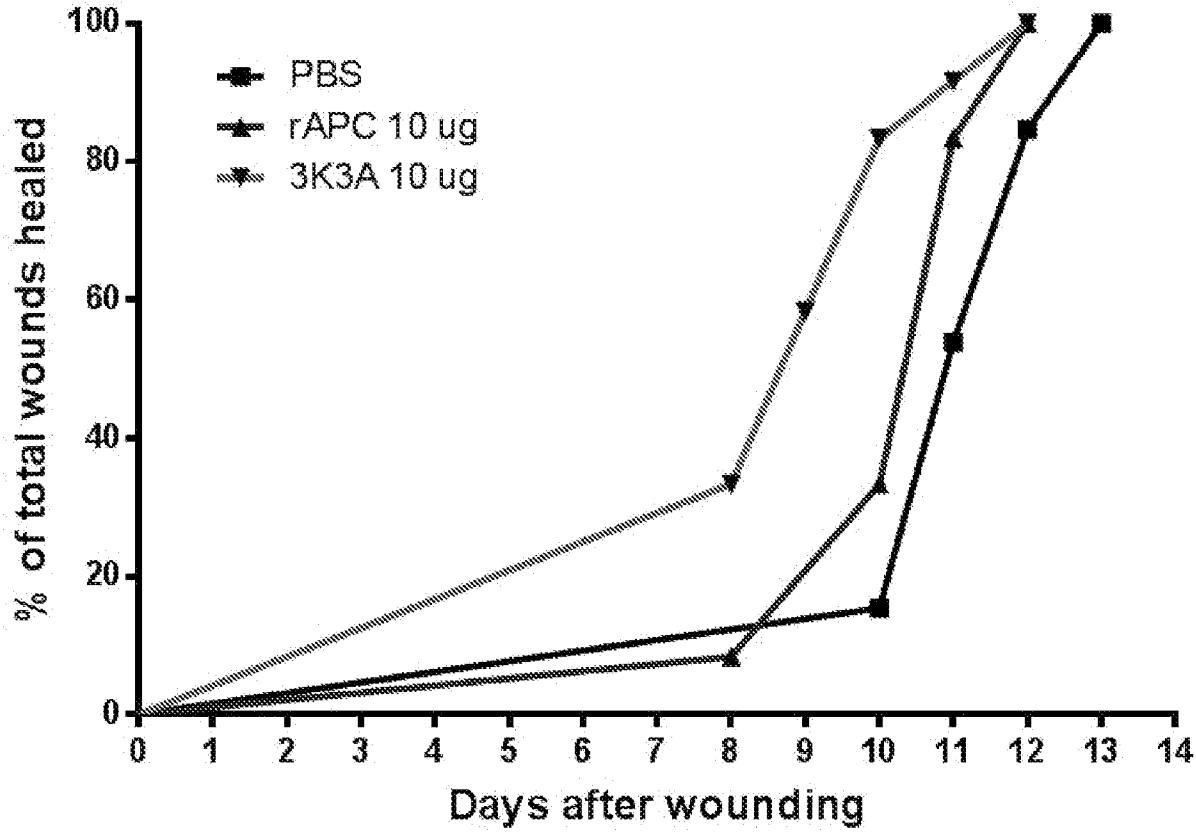
100
80
60
40
20
0
% of total wounds healed
PBS
rAPC 10 ug
3K3A 10 ug
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after wounding

Figure 5

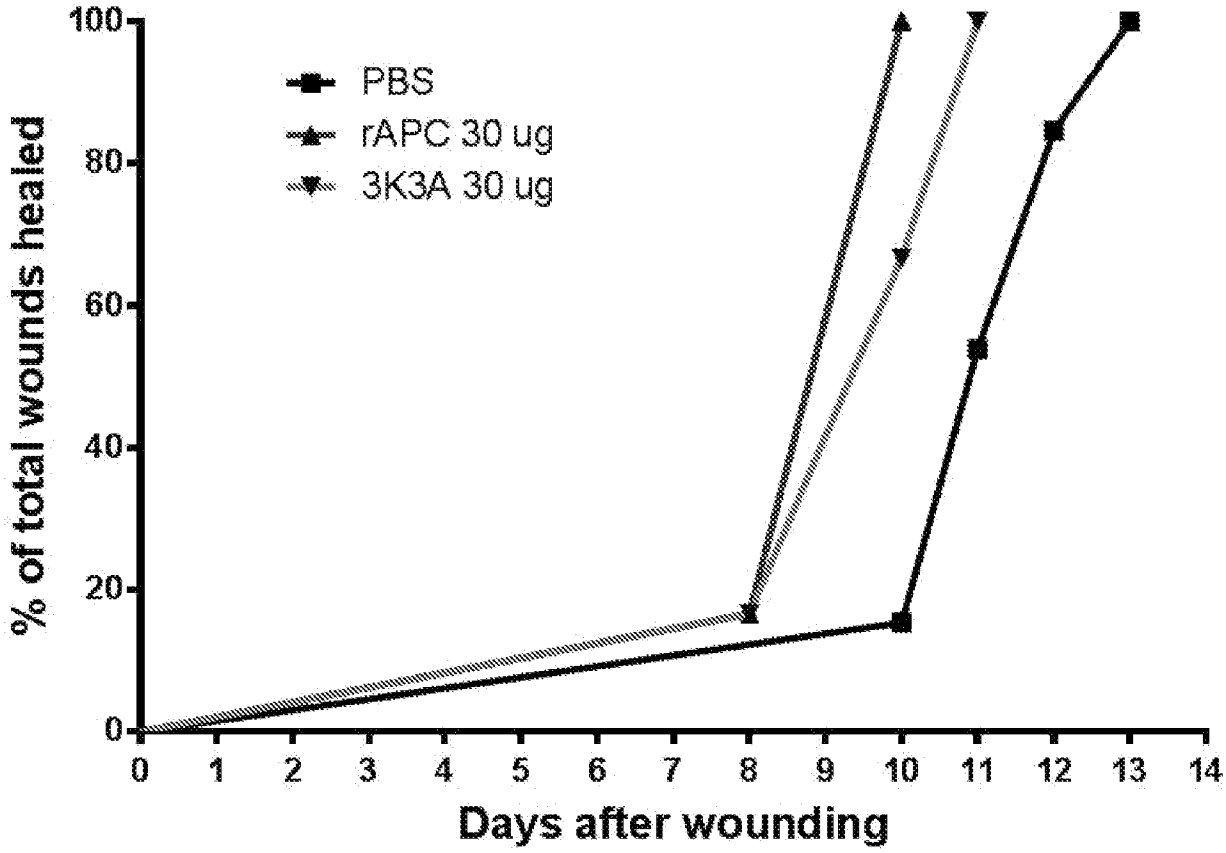
100
80
60
40
20
0
% of total wounds healed
PBS
rAPC 30 ug
3K3A 30 ug
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after wounding

Figure 6

ANSFLEELRHSSLERECIEEICDFEEAKEIFQNVDDTLAFWSKHVDGDQCLVLPLEHPC
ASLCCGHGTCIDGIGSFSCDCRSGWEGRFCQREVSFLNCSLDNGGCTHYCLEEVGW
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            20                  25                  30

Asn Val Asp Asp Thr Leu Ala Phe Trp Ser Lys His Val Asp Gly Asp
        35                  40                  45

Gln Cys Leu Val Leu Pro Leu Glu His Pro Cys Ala Ser Leu Cys Cys
    50                  55                  60

Gly His Gly Thr Cys Ile Asp Gly Ile Gly Ser Phe Ser Cys Asp Cys
65                  70                  75                  80

Arg Ser Gly Trp Glu Gly Arg Phe Cys Gln Arg Glu Val Ser Phe Leu
                85                  90                  95

Asn Cys Ser Leu Asp Asn Gly Gly Cys Thr His Tyr Cys Leu Glu Glu
            100                 105                 110

Val Gly Trp Arg Arg Cys Ser Cys Ala Pro Gly Tyr Lys Leu Gly Asp
        115                 120                 125

Asp Leu Leu Gln Cys His Pro Ala Val Lys Phe Pro Cys Gly Arg Pro
    130                 135                 140

Trp Lys Arg Met Glu Lys Lys Arg Ser His Leu Lys Arg Asp Thr Glu
145                 150                 155                 160

Asp Gln Glu Asp Gln Val Asp Pro Arg Leu Ile Asp Gly Lys Met Thr
                165                 170                 175
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            180                 185                 190

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        195                 200                 205

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    210                 215                 220

Glu Tyr Asp Leu Arg Arg Trp Glu Lys Trp Glu Leu Asp Leu Asp Ile
225                 230                 235                 240

Lys Glu Val Phe Val His Pro Asn Tyr Ser Lys Ser Thr Thr Asp Asn
                245                 250                 255

Asp Ile Ala Leu Leu His Leu Ala Gln Pro Ala Thr Leu Ser Gln Thr
            260                 265                 270

Ile Val Pro Ile Cys Leu Pro Asp Ser Gly Leu Ala Glu Arg Glu Leu
        275                 280                 285

Asn Gln Ala Gly Gln Glu Thr Leu Val Thr Gly Trp Gly Tyr His Ser
    290                 295                 300

Ser Arg Glu Lys Glu Ala Lys Arg Asn Arg Thr Phe Val Leu Asn Phe
305                 310                 315                 320

Ile Lys Ile Pro Val Val Pro His Asn Glu Cys Ser Glu Val Met Ser
                325                 330                 335

Asn Met Val Ser Glu Asn Met Leu Cys Ala Gly Ile Leu Gly Asp Arg
            340                 345                 350

Gln Asp Ala Cys Glu Gly Asp Ser Gly Gly Pro Met Val Ala Ser Phe
        355                 360                 365

His Gly Thr Trp Phe Leu Val Gly Leu Val Ser Trp Gly Glu Gly Cys
    370                 375                 380

Gly Leu Leu His Asn Tyr Gly Val Tyr Thr Lys Val Ser Arg Tyr Leu
385                 390                 395                 400

Asp Trp Ile His Gly His Ile Arg Asp Lys Glu Ala Pro Gln Lys Ser
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Trp Ala Pro
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