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WO 94/27640 A1 **WO 93/19770 A1** **WO 92/11861 A1**
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(54) Pharmaceutical compositions containing cytokines

(57) The present invention provides compositions for promoting the healing of wounds and fibrotic disorders with reduced scarring, comprising anti-inflammatory cytokines or fragments or analogues thereof, and methods of using same. Preferred cytokines are IL-4 and IL-10.

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Pharmaceutical Composition

The present invention concerns pharmaceutical compositions for promoting the healing of wounds or fibrotic disorders, in particular for promoting the healing of wounds or fibrotic disorders with reduced scarring.

By "wounds or fibrotic disorders" is meant any condition which may result in the formation of scar tissue. In particular, this includes the healing of skin wounds, the repair of tendon damage, the healing of crush injuries, the healing of central nervous system (CNS) injuries, conditions which result in the formation of scar tissue in the CNS, scar tissue formation resulting from strokes, and tissue adhesion, for example, as a result of injury or surgery (this may apply to e.g. tendon healing and abdominal strictures and adhesions). Examples of fibrotic disorders include pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, and proliferative vitreoretinopathy.

By "reduced scarring" is meant reduced level of scarring relative to an untreated wound or fibrotic disorder.

In particular, there is a lack of compositions for promoting the healing of wounds or fibrotic disorders with reduced scarring. Scar tissue formation, although providing mechanical strength to a healed wound, can be unsightly and may impair the function of the tissue.

This is particularly the case in wounds which result in scar tissue formation in the CNS, the scar tissue inhibiting the reconnection of severed or re-growing nerve ends, so significantly affecting their function.

There is also a lack of compositions for treating and promoting the healing of chronic wounds, for example venous ulcers, diabetic ulcers and bed sores (decubitus ulcers), especially in the elderly and wheel chair bound patients. Such compositions may be extremely useful in patients where wound healing is either slow or in whom the wound healing process has not yet started. Such compositions may be used to "kick-start" wound healing and may then be used in combination with compositions for promoting healing with reduced scarring. Hence not only may a chronic wound be healed, but it may be healed with reduced scarring.

IL-10 (Interleukin-10) was originally identified as a product of Th2 cells (Fiorentino, D.F. and Moddman, T.R., 1989, J. Exp. Med., 170: 2081-2095) but was also independently identified (O'Garra, A. *et al.*, 1990, Internal Immunol., 2: 821-823) as a product of B - cell lymphomas that prolonged the survival of mast cells and enhanced proliferation of thymocytes.

Molecular characterisation of human and murine IL-10 by Moore, K.W. *et al.* (1990, Science, 248: 1230-1234) and Vieira, P. *et al.* (1991, Proc. Natl. Acad. Sci. USA, 88: 1172-1176) showed that there was an 80% homology of their nucleotide sequences. Mouse IL-10 (mIL-10) protein consists of 157 amino acids with two potential N- glycosylation sites although glycosylation is not essential for the biological activities of mIL-10. Human IL-10 (hIL-10) protein consists of 160 amino acids with one potential

N-glycosylation site which is not used (Vieira *et al.*, 1991). Both mIL-10 and hIL-10 contain four cysteine residues that form two intramolecular disulfide bonds generating biologically active homodimers with molecular weights of 32kDa and 39kDa respectively, and it is not clear whether monomeric forms of IL-10 are biologically active. Although there is 80% homology between hIL-10 and mIL-10, only hIL-10 acts on both human and mouse cells, whereas mIL-10 has species specificity activity (Vieira *et al.*, 1991; Kim, J.M. *et al.*, 1992, J. Immunol., 148: 3618-3623).

There are many cellular sources and major biological activities of IL-10, all of which may play some role in the wound microenvironment. It has been shown that IL-10 possesses many stimulatory and inhibitory effects - van Vlasselar *et al.* (1994, J. Cell Biol., 124: 569-577) showed that IL-10 inhibited TGF-β synthesis required for osteogenic commitment of mouse bone marrow cells, and hence the resulting mineralised matrix, whereas Go *et al* (1990, J. Exp. Med., 172: 1625-1631) showed IL-10 to be a novel B-cell stimulatory factor. IL-10 has also been shown by Bogdan *et al.* (1991, J. Exp. Med., 174: 1549-1555) to directly act on macrophages and inhibit their subsequent activation and hence release of pro-inflammatory cytokines.

Despite the aforementioned studies of cytokines, the present inventor has found that, surprisingly, IL-10 may be used to promote the healing of wounds or fibrotic disorders with reduced scarring. It appears that by inhibiting inflammation at a wound site or site of a fibrotic disorder, in particular at an early stage after wounding/onset, there is a "knock-on" effect upon the resulting collagen matrix, resulting in an improved architecture and reduced scarring. This result is particularly surprising since in the short-term, there was no inhibition of re-epithelialisation or early wound repair, whilst in the

longer-term, it improved the quality of later scar formation and reduced scarring.

According to the present invention there is provided a composition for promoting the healing of wounds or fibrotic disorders with reduced scarring comprising an anti-inflammatory cytokine or a fragment or an analogue thereof. The cytokine may be IL-10 or IL-4.

By 'fragment or analogue thereof' is meant a fragment or analogue of a cytokine which retains the anti-inflammatory healing functionality of the cytokine from which it is derived, although it may of course have additional functionality.

The composition may be used in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

The composition may be used in conjunction with a composition for promoting the healing of wounds with reduced scarring.

The composition may be used in conjunction with a composition for promoting the healing of chronic wounds.

Also provided according to the present invention is a method of promoting the healing of wounds or fibrotic disorders comprising the use of an anti-inflammatory cytokine or a fragment or an analogue thereof. The cytokine may be IL-4 or IL-10.

The cytokine may be administered to a wound site or site of a fibrotic disorder.

The cytokine may be administered at a concentration of between about 1μM and about 10μM. It may be administered at a concentration of between about 2.5μM and about 5μM.

The cytokine may be administered immediately prior to wound healing, but may be effective if administered within about 7 days of wounding. The cytokine could be administered on at least two occasions.

The cytokine may be administered in the form of a composition also comprising a pharmaceutically acceptable carrier, diluent or excipient.

The method may be used in conjunction with a method or composition for promoting the healing of wounds or fibrotic disorders with reduced scarring.

The method may be used in conjunction with a method or composition for promoting the healing of chronic wounds.

The invention will be further apparent from the following example which shows, by way of example only, compositions and methods of promoting the healing of wounds or fibrotic disorders with reduced scarring.

EXPERIMENTAL

Rats were wounded and treated with various compositions and then harvested and the wounds analysed in order to analyse the effects of anti-inflammatory cytokines upon wound healing. Results show that in the short-term, there was no inhibition of re-epithelialisation or early wound repair, whilst in the longer-term, it improved the quality of later scar formation and reduced scarring.

Material and Methods

Male Sprague Dawley rats age and weight matched at 200-250g were anaesthetised using equal parts halothane, oxygen and nitrous oxide. 1cm full thickness (through the panniculus carnosus) linear incisions were made 5 and 8 cm from the base of the skull and 1cm either side of the dorsal midline. The wounds were treated by intradermal injection with either 100µl of IL-10 (2.5µg/ml) or phosphate buffered saline (PBS control). Animals were split into groups: group A were injected with IL-10 or PBS on day 0 prior to wounding, group B were injected with IL-10 or PBS on day 0 prior to wounding and day 7 post wounding. A third group, C had the same injection regime as group B although they were treated with double the dose of IL-10 (5µg/ml). Animals were killed on day 3 (group A only), 7, 14 and 84 post wounding. Wounds and approximately 0.5cm of normal skin either side, were excised and bisected for routine wax histology and immunocytochemistry.

7µm-thick wax sections were cut and stained with Haemotoxylin and eosin, Mallory's and Masson's collagen trichrome stain for the assessment of cellular infiltrate

and collagen architecture respectively. 7μm-thick cryosections were cut and stained with antibodies to assess inflammation (ED1; Serotec), angiogenesis (von Willebrand factor) and extracellular matrix deposition (fibronectin and collagen I). Wound sections were analysed in detail using a Joyce Lobel image analysis Magiscan. Six areas, within the wound margins below the epidermal/dermal junction and above the dermal/panniculus junction, were viewed through a x10 objective and images were captured and using the analysis package GENIAS 25 (Joyce Lobel) the area stained within the field was obtained.

Results

Qualitative analysis of Haemotoxylin and Eosin-stained wound sections suggests that IL-10 treatment decreases the number of inflammatory cells influxing into the wound at day 3 and 7 post wounding when compared to PBS treatment (control). The degree of scarring was qualitatively assessed by studying Masson's trichrome stained wound sections at 84 days post wounding and grading features of the neodermis such as fibre size, length and density. Wounds treated with IL-10 (2.5μg/ml) on day 0 only showed improved restitution of the dermal architecture when compared with control wounds. The IL-10 treated wounds had larger, less densely packed fibres in a more random orientation compared with control wounds where the collagen fibres are finer, more densely packed and aligned parallel to the epidermis.

Wounds which were treated on day 0 and day 7 postwounding with IL-10 (2.5μg/ml) had a resultant dermal architecture resembling that of normal skin with a

more basketweave configuration of the collagen fibres within the wound. The appearance of the scar was far superior to control wounds and wounds treated with IL-10 on day 0 only. 2.5µg/ml of IL-10 appears to be the maximal dose as wounds treated with the higher dose, 5µg/ml, have a more visible macroscopic scar.

Whilst IL-10 treatment appears to inhibit inflammation and improve the quality of later scar formation, it does not inhibit re-epithelialisation, or early wound repair.

Qualitative histological analysis was further corroborated by quantitative image analysis which showed that IL-10 inhibits the infiltration of monoctyes and macrophages into the wound when compared to controls, although IL-10 has no effect on angiogenesis within the wound when compared to controls. Staining for fibronectin shows that IL-10 treated wounds had less fibronectin present in the wound area at 3 and 7 days postwounding when compared with control wounds.

CLAIMS

1. A composition for promoting the healing of wounds or fibrotic disorders with reduced scarring comprising an anti-inflammatory cytokine or a fragment or an analogue thereof.

2. A composition according to claim 1 wherein the cytokine is selected from either one of the group of IL-10 and IL-4.

3. A composition according to any one of the preceding claims when used in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

4. A composition according to any one of the preceding claims when used in conjunction with a composition for promoting the healing of wounds with redeuced scarring.

5. A composition accroding to any one of the preceding claims when used in conjunction with a composition for promoting the healing of chronic wounds.

6. A method for promoting the healing of wounds or fibrotic disorders with reduced scarring comprising the use of an anti-inflammatory cytokine or a fragment or an analogue thereof.

7. A method according to claim 6 wherein the cytokine is selected from either one of the group of IL-10 and IL-4.

8. A method according to either one of claims 6 or 7 wherein the cytokine is administered to wound site or site of a fibrotic disorder.

9. A method according to claim 8 wherein the cytokine is administered to the site in the form of a composition also comprising a pharmaceutically acceptable carrier, diluent or excipient.

10. A method accoring to either one of claims 8 or 9 wherein the cytokine is administered at a concentration of between about 1μM abd about 10μM.

11. A method according to claim 10 wherein the cytokine is administered at a concentration of between about 2.5μM and about 5μM.

12. A method according to any one of claims 6-11 when used in conjunction with a method or composition for promoting the healing of wounds or fibrotic disorder with reduced scarring.

13. A method according to any one of claims 6-12 when used in conjunction with a method or composition for promoting the healing of chronic wounds.

Patents Act 1977 Examiner's report to the Comptroller under Section 17 (The Search report)	Application number GB 9516287.1
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Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE: WPI, CLAIMS, CHEMICAL ABSTRACTS	Documents considered relevant following a search in respect of Claims :- 1-5

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
X	WO 94/27640 A1	(UNIVERSITY OF MICHIGAN) see Claims and page 9 line 23 to page 12 line 10	1-5
X	WO 93/19770 A1	(ISIS INNOVATION) see eg Claims and Examples	1-5
X	WO 92/11861 A1	(SCHERING CORP)* see eg Claims and Examples	1-5
X	WO 91/07186 A1	(UNIVERSITY OF MELBOURNE) see eg page 4 line 20 to page 8 line 11	1-5
X	WO 90/07932 A1	(UNIVERSITY OF MELBOURNE)* see eg page 5 line 25 to page 10 line 7	1-5

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