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(54) **PHARMACEUTICAL COMPOSITION WITH HEALING ACTIVITY COMPRISING AT LEAST ONE SOLUBLE DEXTRAN DERIVATIVE AND AT LEAST ONE PLATELET-DERIVED GROWTH FACTOR**

(75) Inventors: **Guy Dubreucq**, Lille (FR); **Latifa Dahri-correia**, Saint Bonnet de Mure (FR); **Jose Correia**, Saint Bonnet de Mure (FR)

Correspondence Address:
OLIFF & BERRIDGE, PLC
P.O. BOX 19928
ALEXANDRIA, VA 22320 (US)

(73) Assignee: **BIODEX**, SAINT AMAND LES EAUX (FR)

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(57) **ABSTRACT**

Pharmaceutical composition with healing activity comprising at least one soluble dextran derivative and at least one platelet-derived growth factor. The present invention relates to a pharmaceutical composition with healing activity comprising at least one soluble dextran derivative and at least one platelet-derived growth factor, and also to the use thereof for the preparation of a medicament with healing action, in particular for use in the treatment of ulcers.

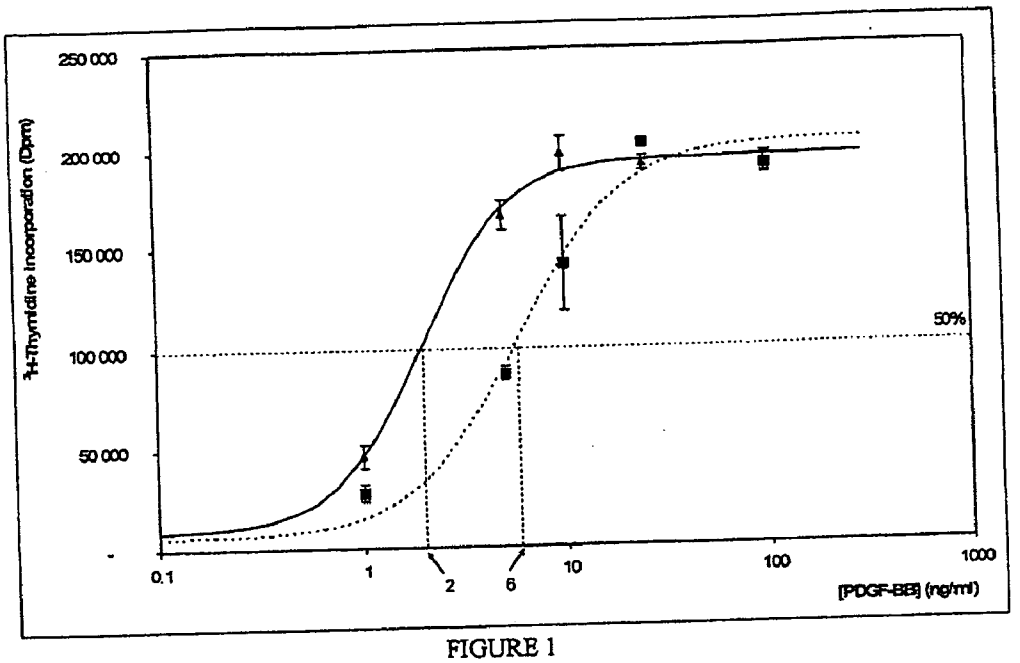

FIGURE 1

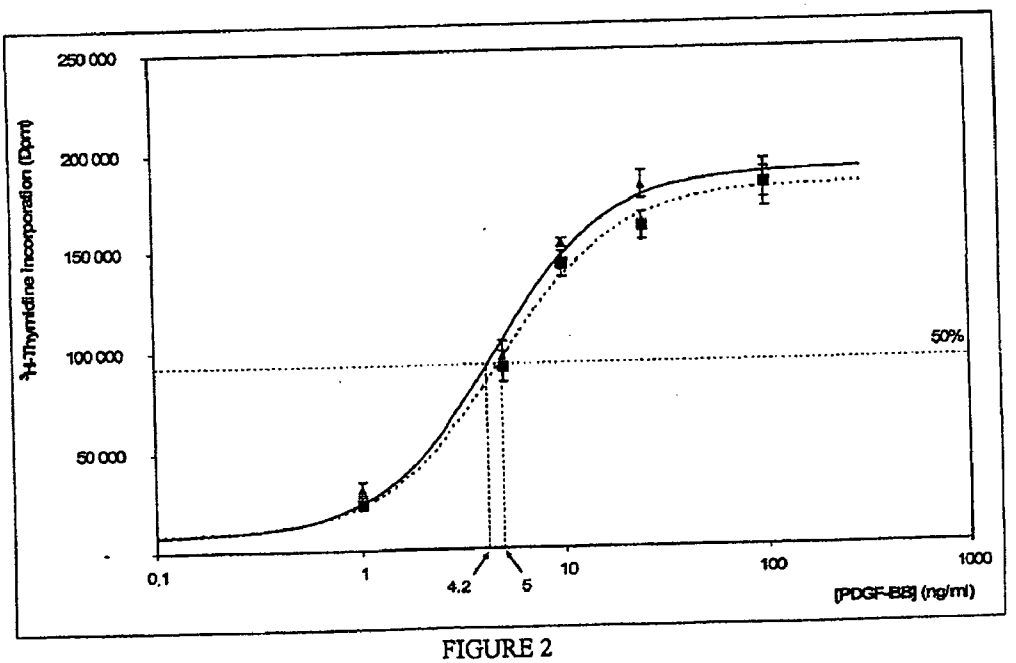

FIGURE 2

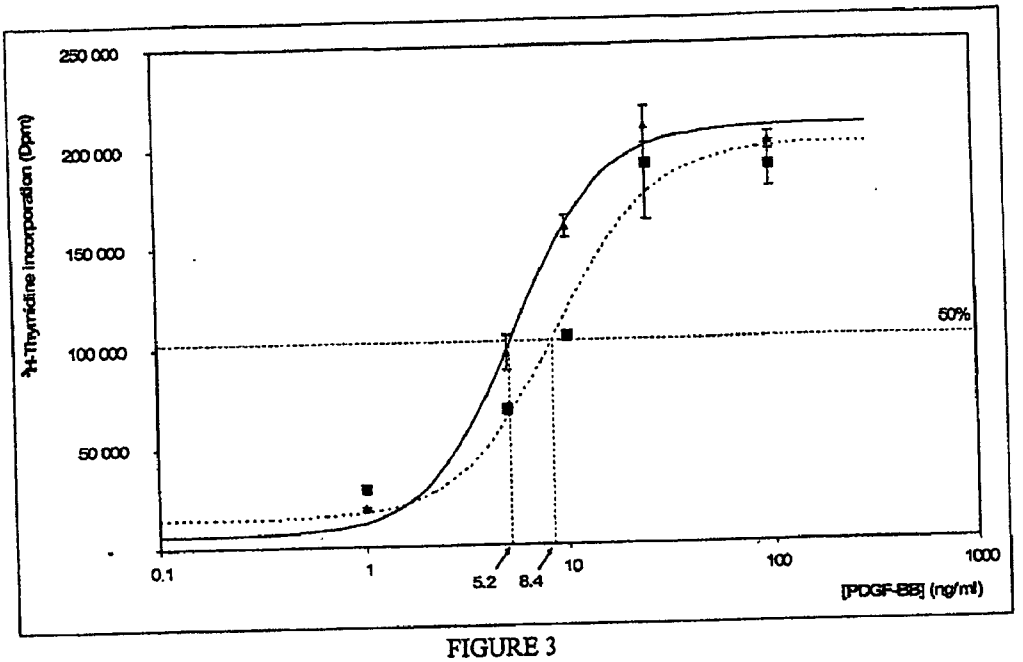
250 000
200 000
150 000
100 000
50 000
-
^{3}H-Thymidine incorporation (Dpm)
50%
0.1
1
5.2
8.4
10
100
1000
[PDGF-BB] (ng/ml)

FIGURE 3

PHARMACEUTICAL COMPOSITION WITH HEALING ACTIVITY COMPRISING AT LEAST ONE SOLUBLE DEXTRAN DERIVATIVE AND AT LEAST ONE PLATELET-DERIVED GROWTH FACTOR

[0001] Pharmaceutical composition with healing activity comprising at least one soluble dextran derivative and at least one platelet-derived growth factor

BACKGROUND

[0002] The present invention relates to a pharmaceutical composition with healing activity comprising at least one soluble dextran derivative and at least one platelet-derived growth factor, and also to the use thereof for the preparation of a medicament with healing action, in particular for the treatment of ulcers.

[0003] Growth factors are a category of polypeptides having properties that regulate numerous parameters of cell life (such as proliferation, differentiation, survival). These factors are secreted by many types of cells. The growth factors exert their various effects by binding and activating a distinct subfamily of surface cellular receptors that have an intrinsic tyrosine kinase activity.

[0004] PDGFs are growth factors released by platelets during blood clotting, capable of promoting the growth of various types of cells (Ross R. et al., Proc. Natl. Acad. Sci. USA, 1974, 71, 1207; Kohler N. & Lipton A., Exp. Cell Res., 1974, 87, 297). It is now known that PDGF is produced by a certain number of cells other than platelets and that it is mitogenic for most of the cells derived from the mesenchyma, i.e. blood, muscle, bone and cartilaginous cells, and also connective tissue cells (Raines E. W., in "*Biology of Platelet-Derivated Growth Factor*", 1993, Westermark, B. and C. Sorg, Pub. Basle, Kerger, p. 74). Numerous articles also tend to demonstrate that macrophage-derived PDGF behaves as a chemotactic and mitogenic agent with respect to smooth muscle cells, and that it contributes to the myointimal thickening of artery walls that is characteristic of arteriosclerosis (Ross R. et al., Science, 1990, 248, 1009). The activities of PDGF also and in particular include stimulation of granule release by neutrophilic monocytes (Tzeng D. Y. et al., Blood, 1985, 66, 179), facilitation of steroid synthesis by Leydig cells (Risbridger G. P., Mol. Cell. Endocrinol., 1993, 97, 125), stimulation of neutrophile phagocytosis (Wilson E. et al., Proc. Natl. Acad. Sci. USA, 1987, 84, 2213), modulation of thrombospondin expression and secretion (Majak R. A. et al., J. Biol. Chem., 1987, 262, 8821) and post-regulation of the ICAM-1 gene in vascular smooth muscle cells (Morisaki N. et al., Biochem. Biophys. Res. Commun., 1994, 200, 612).

[0005] Given its various properties, the use of recombinant PDGFs in the pharmaceutical field has already been envisaged.

[0006] Ulcer healing, just like healing in general, is a process that takes place mainly in three major phases. The healing process begins with a first inflammatory phase during which blood flow and blood streaming in increase around the site of ulceration. If bleeding from damaged blood vessels occurs, platelets invade the site of ulceration and allow clotting in order to stop the bleeding. The platelets also release PDGFs which will send signals to the neighbouring cells triggering the proliferation thereof, i.e. the second phase of the healing process. The third, final, phase of the healing process is the remodelling phase. Now, diabetic ulcers have the particularity of healing very slowly and sometimes incompletely because the healing process does not occur normally, certainly because blood flow in the skin is reduced in diabetics. This can lead to serious bacterial infections in the ulcers, that can spread and sometimes require amputation of the foot or of the leg.

[0007] For this reason, there currently exists on the market, in particular on the American market, a recombinant human PDGF-BB-based medicament corresponding to the international non-proprietary name "becaplermin", sold under the trade name Regranex®. This medicament is indicated for the treatment of lower limb ulcers in diabetics. It is in the form of a gel for topical application and makes it possible to promote ulcer healing. It makes it possible in particular, just like kendogenous PDGF, to promote cell proliferation and therefore the formation of new tissues.

[0008] The human recombinant PDGF-BB or becaplermin is obtained according to a method of preparation using recombinant DNA technology by insertion of the gene encoding the B chain of PDGF-BB into a yeast, *Saccharomyces cerevisiae*. This technique is tricky, long and expensive and results in the production of a medicament which is itself expensive for the patients. Regranex® is available as a gel sold in tubes of 2, 7.5 or 15 grams, each gram of gel containing 100 μg of becaplermin. The amount of gel to be applied depends, of course, on the surface area of the ulcer; however, the average cost of a treatment lasting twenty weeks is excessively high (of the order of 1400 American dollars).

SUMMARY

[0009] The inventors have therefore given themselves the aim of finding an excipient for increasing the activity of PDGF-BB and reducing the doses administered and, consequently, the toxicological risks and thus obtaining treatments that are less expensive for diabetic patients suffering from ulcers of the lower limbs.

[0010] They have solved this problem, as is demonstrated hereinafter in the examples illustrating the present application, by using certain suitably selected soluble dextran derivatives.

[0011] Specifically, the latter make it possible to increase the activity of PDGF-BB, in particular in the context of the treatment of ulcers of the lower limbs in diabetic patients.

[0012] This result is obtained through the stabilization of the PDGF with respect to chemical or physical degradations that may occur at physiological pH in vitro and in vivo, by developing a complex between a soluble dextran derivative and PDGF.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. **1** represents the amount of tritiated thymidine incorporated by human dermal fibroblasts after stimulation of proliferation by PDGF-BB alone at a concentration ranging from 0.1 to 100 μg/ml or in the presence of a dextran derivative of formula (I) at a concentration of 1 μg/ml in Example 1.

[0014] FIG. 2 represents the amount of tritiated thymidine incorporated by human dermal fibroblasts after stimulation of proliferation by PDGF-BB alone at a concentration ranging from 0.1 to 100 μg/ml or in the presence of a dextran derivative of formula (I) at a concentration of 1 μg/ml in Example 2.

[0015] FIG. 3 represents the amount of tritiated thymidine incorporated by human dermal fibroblasts after stimulation of proliferation by PDGF-BB alone at a concentration ranging from 0.1 to 100 μg/ml or in the presence of a dextran derivative of formula (I) at a concentration of 1 μg/ml in Example 3.

DETAILED DESCRIPTION OF EMBODIMENTS

[0016] A subject of the present invention is therefore a pharmaceutical composition, characterized in that it comprises, in a pharmaceutically acceptable support:

[0017] at least one platelet-derived growth factor (PDGF), and

[0018] at least one soluble dextran derivative corresponding to formula (I) below:

$$DMC_aB_bSU_c \quad (I)$$

in which:

[0019] D represents a polysaccharide chain, preferably consisting of series of glucoside units,

[0020] MC represents methylcarboxylic groups,

[0021] B represents N-benzylmethylenecarboxamide groups,

[0022] Su represents sulphate groups (sulphation of the free hydroxyl functions borne by the glucoside units),

[0023] a, b and c represent the degree of substitution (ds), respectively of the MC, B and Su groups, with

[0024] i) a strictly greater than 0;

[0025] $ii)_b$ is such that:

[0026] either b is greater than or equal to 0.3 and c is between 0.1 and 0.5;

[0027] or b is strictly less than 0.3 and c corresponds to equation (1) below:

$$c \geq 8.5b^2 - 5.41b + 0.86 \quad (1).$$

[0028] These dextran derivatives of formula (I), and also the method for preparing them, are described more generally in patent application WO 99/29734. These dextran derivatives of formula (I) are trivially called DMCBSu and are considered to be copolymers consisting of R—OH and R—OX subunits, it being possible for X to be a methylcarboxylic (MC), benzylamide (B) or sulphate (Su) group. Thus, a methylcarboxylic dextran (DMC) with a degree of substitution (ds) of 0.6, in terms of methylcarboxylic groups, contains 0.6 substituted group (R—MC) and 2.4 hydroxyl groups (R—OH), per unit.

[0029] According to the invention, D preferably has a molar mass of between 1000 and 2 000 000 Da, and even more particularly less than 70 000 Da.

[0030] According to a preferred embodiment of the invention, the dextran derivatives are chosen from the compounds of formula (I) in which b is greater than or equal to 0.35.

[0031] In this case, and according to a most particularly preferred embodiment of the invention, the dextran derivatives are chosen from the compounds of formula (I) in which a is between 0.5 and 0.8, and c is between 0.1 and 0.5

[0032] Among such dextran derivatives of formula (I), mention may in particular be made of the compounds in which a=0.66; b=0.38 and c is between 0.2 and 0.4.

[0033] The dextran derivatives of formula (I) in which a=0.66; b=0.38 and c=0.29 are particularly preferred.

[0034] The amount of dextran derivatives of formula (I) present in the pharmaceutical composition in accordance with the invention is preferably between 0.5 and 100 mg/g, and even more preferably between 5 and 50 mg/g of composition.

[0035] According to the invention, the PDGFs are preferably chosen from human recombinant PDGFs containing two B chains (rhPDGF-BB). Such PDGFs can be obtained according to the conventional techniques known to those skilled in the art or else can be directly purchased commercially, for example from the company Research Diagnostic Inc. (USA).

[0036] The amount of PDGFs present in the pharmaceutical composition in accordance with the invention is preferably between 1 and 200 μg/g of composition, and even more preferably between 10 and 100 μg/g of composition.

[0037] According to a specific and preferred embodiment of the invention, the dextran derivatives of formula (I)/PDGFs weight ratio is between 100 and 1000, and even more preferably between 300 and 700.

[0038] The pharmaceutical composition in accordance with the invention is preferably a composition for topical application that may be in the form of gels, creams, sprays or patches, the presentation in the form of gels being particularly preferred.

[0039] The nature of the excipients that may be present in the pharmaceutical composition in accordance with the invention is chosen as a function of its presentation form according to the general knowledge of those skilled in the art, i.e. of specialists in galenics.

[0040] Thus, when the composition in accordance with the invention is in the form of a gel, the latter is preferably a cellulose gel, such as, for example, a carboxymethylcellulose (CMC) gel.

[0041] The pharmaceutical composition in accordance with the invention may also contain one or more additives such as those chosen from fillers, preserving agents such as methyl para-hydroxybenzoate, propyl para-hydroxy-benzoate or m-cresol, anti-oxidants, stabilizers such as L-lysine hydrochloride, acidifying and basifying agents, opacifiers, etc.

[0042] When they are administered topically, the pharmaceutical compositions in accordance with the invention are most particularly for use in the treatment of ulcers, preferably for ulcers of the lower limbs in diabetic patients. The inventors have in fact noted that the use of such a compo-

sition makes it possible to improve and accelerate the healing of ulcers, with compositions containing amounts of PDGFs that are lower than in the compositions currently available on the market (such as, for example, the medicament sold under the name Regranex®).

[0043] Thus, another subject of the invention is therefore the use of at least one pharmaceutical composition as described above, i.e. containing at least one dextran derivative of formula (I) as described above and at least one platelet-derived growth factor, for the preparation of a medicament with healing action, for use in the treatment of ulcers by topical application, and in particular in the treatment of ulcers of the lower limbs in diabetic patients.

[0044] In this case, said medicament is preferably intended to be administered as a treatment of 2 to 10 weeks at a rate of 1 or 2 applications a day on the lesioned portion.

[0045] In addition to the above arrangements, the invention also comprises other arrangements that will emerge from the description that follows, which refers to an example of demonstration of the increase in the proliferative effect of a PDGF-BB with a dextran derivative of formula (I) and also to the attached FIG. **1** which represents the amount of tritiated thymidine incorporated by human dermal fibroblasts (in $Dpm \times 10^3$), after stimulation of proliferation by PDGF-BB alone at a concentration ranging from 0.1 to 100 μg/ml (dashed-line curve) or in the presence of a dextran derivative of formula (I) (solid-line curve) at a concentration of 1 μg/ml.

[0046] It should be clearly understood, however, that this example is given only by way of illustration of the subject of the invention, for which it in no way constitutes a limitation.

EXAMPLE 1

Demonstration of the Increase in the Proliferative Effect of a PDGF-BB with a Dextran Derivative of Formula (I)

1) Procedure

[0047] A primary culture of human dermal fibroblasts (Human Dermal Fibroblast adult (HDFa), the company Cascade Biologics) was realized at a temperature of 37° C. in αMEM medium (Minimum Essential Medium, the company Gibco) with Glutamax, without ribo/deoxyribo-nucleotides, supplemented with 10% of foetal calf serum (FCS, the company Dutscher) and 1% of penicillin-streptomycin (the company Gibco) in an atmosphere saturated with humidity and enriched in CO_2 (5%). The medium was renewed every 4 days. The human fibroblasts were used between passages 1 and 5. A dilution of the cell suspension in the culture medium was then realized in order to seed culture dishes at a density of 5000 cells/well for 96-well plates (the company Nunc).

[0048] For each batch of cells, the increase in the effect of PDGF-BB by means of a DMCBSu having a molar mass of 52 000 g/mol in which a=0.66; b=0.38 and c=0.29 (prepared according to the method described in International Application WO 99/29734), at various concentrations, was verified by incorporation of tritiated thymidine (5000 cells/well in 100 μl). To do this, after withdrawal for 24 hours, the fibroblasts were stimulated through the addition of PDGF-BB (Research Diagnostics Inc.), at various concentrations ranging from 0.1 to 100 ng/ml, in the absence or presence of a dextran derivative of formula (I) for which a=0.63; b=0.38 and c=0.29, at a concentration of 1 μg/ml. The tritiated thymidine incorporation (at 52 Ci/mmol, the company Amersham Biosciences) was carried out 18 hours after the stimulation with PDGF-BB in the absence or presence of the dextran derivative, by adding a solution at 50 μCi/ml, i.e. 0.5 μCi/well. After incubation for 6 hours (i.e. 24 hours of stimulation), the culture medium was suctioned off, and the cells were fixed for one hour at 4° C. by adding 100 μl/well of 10% trichloroacetic acid (TCA). The wells were then rinsed three times with 100 μl of ultrapure water and the cells were lysed overnight at 4° C. with 100 μl of 100 mM NaOH. The radioactivity was recovered in counting vials, the wells were rinsed with 100 μl of 100 mM NaOH and the radioactivity was counted after the addition of 1 ml of scintillation fluid (Zinsser Analytic), on an automatic counter sold by the company Beckman (LS 6000 TA). For all the experiments, each stimulation condition was carried out in triplicate.

2) Results

[0049] The results obtained are represented in the attached FIG. **1** in which the amount of tritiated thymidine incorporated by the fibroblasts (in $Dpm \times 10^3$) is expressed as a function of the concentration of PDGF-BB in μg/ml.

[0050] The solid-line curve represents the results in the presence of the dextran derivative at a concentration of 1 μg/ml and the dashed-line curve represents the results in the absence of the dextran derivative.

[0051] The ED_{50} corresponds to the concentration of PDGF-BB to obtain 50% proliferation of the human fibroblasts. The ratio R is the ratio of the ED_{50} values, calculated as follows:

$$R=ED_{50}(PDGF\text{-}BB)/ED_{50}(PDGF\text{-}BB+DMCBSu)$$

[0052] These results show that, when the PDGF-BB is used alone, it is necessary to use 6 μg/ml thereof in order to obtain 50% proliferation, whereas, when the PDGF-BB is complexed with a dextran derivative of formula (I), 2 μg/ml are sufficient to attain 50% proliferation. In this case, the ratio R is equal to 3.

EXAMPLE 2

Demonstration of the Absence of Increase in the Proliferative Effect of a PDGF-BB with a Dextran Derivative not Included in Formula I

[0053] According to a protocol identical to that described in Example 1, the increase in the effect of PDGF-BB by means of a DMCBSu of general formula in which a=0.81; b=0.14; c=0.19 (prepared according to the method described in International Application WO 99/29734), at various concentrations, was verified by tritiated thymidine incorporation (5000 cells/well in 100 μl).

[0054] The results obtained are represented in FIG. **2**, the ratio R is equal to 1.2.

[0055] An absence of increase in the effect of PDGF-BB is observed.

EXAMPLE 3

Demonstration of the Increase in the Proliferative Effect of a PDGF-BB with a Dextran Derivative Included in Formula I

[0056] According to a protocol identical to that described in Example 1, the increase in the effect of PDGF-BB by means of a DMCBSu of general formula in which a=0.66; b=0.38; c=0.95 (prepared according to the method described in International Application WO 99/29734), at various concentrations, was verified by tritiated thymidine incorporation (5000 cells/well in 100 μl).

[0057] The results obtained are represented in FIG. **3**, the ratio R is equal to 1.6.

[0058] An increase in the effect of PDGF-BB is observed.

1. Pharmaceutical composition, characterized in that it comprises, in a pharmaceutically acceptable support:

at least one platelet-derived growth factor (PDGF), and

at least one soluble dextran derivative corresponding to formula (I) below:

DMCaBbSuc (I)

in which:

D represents a polysaccharide chain,

MC represents methylcarboxylic groups,

B represents N benzylmethylenecarboxamide groups,

Su represents sulphate groups,

a, b and c represent the degree of substitution (ds), respectively of the MC, B and Su groups, with

i) a strictly greater than 0;

ii)$_b$ is such that:

either b is greater than or equal to 0.3 and c is between 0.1 and 0.5;

or b is strictly less than 0.3 and c corresponds to equation (1) below:

$c \geq 8.5b^2 - 5.41b + 0.86$ (1).

2. Composition according to claim 1, characterized in that D consists of series of glucoside units.

3. Composition according to claim 1, characterized in that D has a molar mass of between 1000 and 2 000 000 Da.

4. Composition according to claim 1, characterized in that the dextran derivatives are chosen from the compounds of formula (I) in which b is greater than or equal to 0.35.

5. Composition according to claim 4, characterized in that the dextran derivatives are chosen from the compounds of formula (I) in which a is between 0.5 and 0.8, and c is between 0.1 and 0.5.

6. Composition according to claim 1, characterized in that the dextran derivatives of formula (I) are chosen from the compounds in which a=0.66; b=0.38 and c is between 0.2 and 0.4.

7. Composition according to claim 6, characterized in that the dextran derivatives of formula (I) in which a=0.66; b=0.38 and c=0.29.

8. Composition according to claim 1, characterized in that the amount of dextran derivatives of formula (I) is between 0.5 and 100 mg/g of composition.

9. Composition according to claim 8, characterized in that the amount of dextran derivatives of formula (I) is between 5 and 50 mg/g of composition.

10. Composition according to claim 1, characterized in that the PDGFs are chosen from human recombinant PDGFs containing two B chains.

11. Composition according to claim 1, characterized in that the amount of PDGF is between 1 and 200 μg/g of composition.

12. Composition according to claim 11, characterized in that the amount of PDGF is between 10 and 100 μg/g of composition.

13. Composition according to claim 1, characterized in that the dextran derivatives of formula (I)/PDGFs weight ratio is between 100 and 1000.

14. Composition according to claim 1, characterized in that it is a composition for topical application in the form of gels, creams, sprays or patches.

15. Composition according to claim 14, characterized in that it is in the form of a cellulose gel.

16. Composition according to claim 1, characterized in that it contains one or more additives chosen from fillers, preserving agents, anti-oxidants, stabilizers, acidifying and basifying agents and opacifiers.

17. Use of at least one pharmaceutical composition as defined in claim 1, for the preparation of a medicament with healing action.

18. Use according to claim 17, characterized in that the medicament is for use in the treatment of ulcers by topical application.

19. Use according to claim 17, characterized in that the medicament is for use in the treatment of ulcers of the lower limbs in diabetic patients.

20. Use according to claim 17, characterized in that said medicament is intended to be administered as a treatment of 2 to 10 weeks at a rate of 1 or 2 applications a day on the lesioned portion.

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