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(54) Title: METHOD AND COMPOSITIONS FOR THE TREATMENT OF EDEMATOUS-FIRBRSCLEROTIC PANNICULOPATHY

(57) Abstract: The invention relates to the use of angiogenic agents for the manufacture compositions for the treatment of cellulite, better defined by the term "edematousfibrosclerotic panniculopathy" to elicit the endothelial repair and/or the neovascularization of the cellulite-affected area and tissues. The invention also claims a method for treating cellulite which comprises the administration of an angiogenic agent, preferably selected from angiogenic phospholipids, hyaluronan oligosaccharides, peptide growth factors and combination thereof and, optionally, secondary angiogenic agents.

"METHOD AND COMPOSITIONS FOR THE TREATMENT OF EDEMATOUS-FIBROSCLEROTIC PANNICULOPATHY"

FIELD OF THE INVENTION

The present invention relates to the use of angiogenic agents for the manufacture compositions for the treatment of cellulite, better defined by the term "edematous-fibrosclerotic panniculopathy", i.e. compositions which are suitable to elicit the endothelial repair and/or the neovascularization of the cellulite-affected area and tissues.

Also, the invention relates to a method for treating cellulite which encompasses the administration of a dermatological or cosmetic composition comprising an angiogenic agent, preferably selected from angiogenic phospholipids, hyaluronan oligosaccharides, peptide growth factors and combination thereof and, optionally, secondary angiogenic agents.

BACKGROUND OF THE INVENTION

Edematous-fibrosclerotic panniculopathy (EFSP), popularly known as "cellulite", develops on genetic, endocrine and dietary basis in 80% of the Caucasian women after puberty. Nonetheless, there is a controversial attitude toward EFSP, which is sometimes classified among the diseases or just as a widespread female unaesthetism, despite the fact that the EFSP aetiology seems to be deeply rooted at circulatory level.

Actually, EFSP is prompted by venous stasis and insufficiency in the lower limbs, with an abnormal capillary-venous permeability progressively leading to microaneurisms, endothelial damage, plasma transudation and diffusion of lipoedema. The decreased microcirculation thereby induce hyperplastic-hypertrophic changes in pericapillary and periadipocyte reticular fibrils in EFSP tissue. Then the further differentiation of collagen from reticular fibrils provokes the neoformation of connective bundles (lipo-fibrosclerosis) around adipocytes with the further microcirculation impairment.

Most EFSP treatments are handled by the cosmetic industry, aestheticians and beauty centres. These EFSP treatments contain a combination of lipolytic substances (e.g. caffeine) to trigger the fatty acid release from adipocytes and phytochemicals

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and vitamines with draining and vasokinetic activities (e.g. nicotinic derivatives).

The cosmetic products generally offer poor results in EFSP as its main issue, i.e. the circulatory burden, remains untreated.

Phospholipids such lysophosphatidic acid and sphingosine 1-phosphate - beside evoking diverse physiological responses including platelet aggregation, smooth muscle contraction and fibroblasts proliferation by the Ca^{++}-mobilization of mitogen-activated protein kinase - play a key role in angiogenesis and in stabilizing the vascular integrity.

On the other side, tissues may promote angiogenesis under both normal and pathological conditions by peptide growth factors, primarily vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).

Hyaluronan can both promote and inhibit neovascularization depending on its molecular mass. In this work we show that a brain capillary endothelial cell line forms tubes in a collagen gel after stimulation by hyaluronan oligosaccharides (Rahmanian M, et al., Exp Cell Res. 1997 25;237(1):223-30).

The oligosaccharides of hyaluronan are effective angiogenic agents inducing multiple signalling pathways which affect the vascular endothelial cell mitogenesis and wound healing responses (J Biol Chem. 2002 25;277(43):41046-59).

Also, hyaluronan oligosaccharides and a peptide growth factor such as VEGF actually display a synergic action (Montesano R et al., Lab Invest; 75(2):249-62 1996).

The angiogenic agents differ in cell specificity and in the mechanisms, for example they may induce the migration and proliferation of endothelial cells, stabilize the endothelial barrier or stimulate the production of collagenase (Klagsbrun & D'Amore, Ann. Rev. Physiol. 53:217-39, 1991).

Angiogenesis is involved in wound healing as well as in the pathogenesis of a large number of clinical diseases including tissue inflammation, arthritis, asthma, tumor growth and diabetic retinopathy.

For these reasons, a variety of endogenous or exogenous angiogenic agents are studied for the tissue wound healing and post-ischemic treatment whilst, at the same time, several anti-angiogenic agents are under development with the main aim

of limiting the metastatic process in cancer.

The various clinical manifestations associated with angiogenesis referred to the angiogenic diseases have been listed by different authors (e.g. Folkman, J and Klagsbrun M in Science, 235:442-7, 1987), however EFSP have not been comprised nor mentioned among such target disorders.

A treatment of EFSP by the administration of angiogenic agents to elicit the endothelial repair and neovascularization has not been conceived so far.

SUMMARY OF THE INVENTION

We have first envisaged a new treatment of the edematous-fibrosclerotic panniculopathy (EFSP) in human beings, especially in female subjects.

In this frame Applicant has found out that the angiogenic agents may be administered on EFSP to induce endothelial cell responses, including the endothelial cells proliferation or the liberation from established monolayers, chemotactic migration, adherens junction assembly and morphogenesis into capillary structures on EFSP tissues.

This invention therefore relates to the endothelial repair and neovascularization effects of angiogenic agents in EFSP, and specifically relates to the vessel repair and growth effects of angiogenic agents used alone, in combination or in conjuction with other substances for the treatment of EFSP.

The present invention also provides methods of improving the vascularization condition in EFSP tissues through the activation of the angiogenic response in a subject.

In addition, the present invention relates to dermatological and/or cosmetic compositions comprising a one or more angiogenic agents along with a dermatologically and/or cosmetically acceptable carrier suitable for the long-term management of the EFSP condition.

DETAILED DESCRIPTION OF THE INVENTION

Therefore, according to one of its aspects, the present invention provides the use of angiogenic agents for the manufacture of a dermatological and/or cosmetic composition for the treatment of edematous-fibrosclerotic panniculopathy (EFSP).

The invention also provides dermatological and/or cosmetic compositions for

the treatment of edematous-fibrosclerotic panniculopathy (EFSP) which comprise one or more angiogenic agents.

According to another aspect, the invention provides a method for the for the treatment of the EFSP condition which comprises administering to a mammal in need thereof, an effective amount of one or more angiogenic agents.

According to a preferred aspect of the invention, the angiogenic agents particularly useful in the method and compositions of invention are selected from the following:

a) angiogenic phospholipids;

b) hyaluronan oligosaccharides;

c) peptide growth factors, and/or their functionally equivalent;

and their mixture,

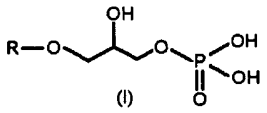
optionally in combination with

d) secondary angiogenic agents.

According to the present invention, the expression "angiogenic phospholipids (a)" relates to phospholipidic which have high affinity agonists for G-protein coupled EDG (endothelial differentiation gene) receptors, which include lysophosphatidic acid and analogues thereof (also referred as "LPA"), phosphatidic acid and analogues thereof (also referred as "PA"), and sphingosine-1-phosphate (also referred as "S1P").

The LPA useful in the methods and compositions of the subject invention include the compounds (i, ii, iii) as further detailed.

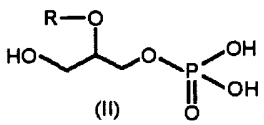
i) 1-acyl-glycero-3-phosphates (also referred as "LPA1") of formula (I) :



(I)

wherein R is a C_2-C_{24} acyl, preferably a C_{16}-C_{18} fatty acid residue, most preferred C_{16}-C_{18} fatty acid residue is oleoyl.

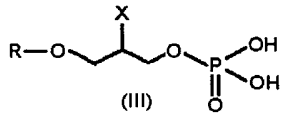
ii) 2-acyl-glycero-3-phosphates (also referred as "LPA2") of formula (II) :



(II)

wherein R is as define above.

iii) 2-deoxy-lysophosphatidic acid and analogues and 2-deoxy-2-halo-lysophosphatidic acid (also referred as "LPA analogues") of formula (III) :

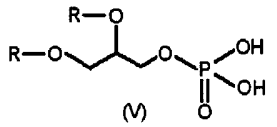


(III)

wherein R is as define above; X is hydrogen or halogen, preferably fluorine.

The PA useful in the methods and compositions of the subject invention include the compounds (j, jj, jjj) as further detailed.

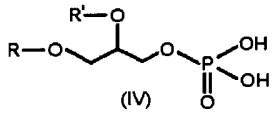
j) 1,2-diacyl-glycerol-3-phosphate (also referred as "pure PA") of formula (IV) :



(V)

wherein the two R are identical, being defined as above.

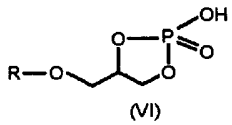
jj) 1-acyl-2-acyl'-glycerol-3-phosphates (also referred as "mixed acyl PA") of formula (V) :



(IV)

wherein R and R', each independently, are different, being defined as above.

jjj) 1-acyl-glycerol-2,3-cyclic monophosphate (also referred as "cyclic PA") of formula (VI) :



(VI)

wherein R is as defined above.

Compounds of formulae I-VI may be present in the form of D-, L- or DL-isomers and usually their L- or DL-isomers are used.

Preferably the acyl group (R and/or R') are naturally occurring fatty acid residues, most preferably selected in the group consisting of oleyl (C18:1), stearoyl (C18:0), palmitoyl (C16:0), palmitoleoyl (C16:1), myrystoyl (C14:0), lauroyl (C12:0), linoleoyl (C18:2 ω6), α-linoleoyl (C18:2 ω3), γ-linolenoyl (C18:3 ω6), α-

linolenoyl (C18:3 ω3), eicosapentaenoyl (C20:5 ω3), docosahexaenoyl (C22:6 ω3), dihomolinolenoyl acid (C20:3 ω6), arachidonoyl (C20:4 ω6), and mixture thereof

The lysosphingolipids useful in the methods and compositions of the subject invention is intended the product generated by the metabolism of sphingomyelin (Spiegel & Merrill, FASEB J, 10:1388-1397, 1996), called sphingosine-1-phosphate (S1P), which together with sphingenine-1-phosphate, are collectively called sphingosine-1-phosphate (SPN-1-P).

S1P may be obtained by full chemical synthesis, e.g. as described in Example I of U.S. Pat. N. 5,391,800 or, alternatively, by enzymatic degradation of sphingosyl-phosphocholine by PLD, as described by Veldhoven et al. in J. Lipid Res., 1989, 30:611.

LPA and PA to be used in the present invention are known compounds. They can be obtained may be obtained with a variety of approaches including: total chemical or enzymatic synthesis from C3-precursors; chemical or enzymatic semi-synthesis from preformed glycerides or phospholipids, extraction and purification from natural sources, and combination thereof (Paltauf & Hermetter in Prog. Lipid Res., 33, 239-328, 1994).

Pure PA may be prepared by chemical acylation of glycerol-3-phosphate, as described by Gupta (Proc. Nat. Acad.Sci., 74:4315-4319, 1977) and Schena in EP473435.

Mixed acyl-PA may be obtained by introducing a fatty acid to the 1-position of glycerol or a C3-synthone and then introducing an acyl group to 2- and 3-position, followed by phosphorylation of glycerol at the 3-position. In each step of the synthesis, a protecting group may be introduced as may be required. Synthetic methods of that kind to afford LPA and LPA-analogues are described by Erickson in U. S. Pat. N. 6,380,177.

The chemical synthesis of PA and LPA may be also carried out by the phoshorylation of di- and monoglycerides, as described by Perie et al. in FR2636331.

The enzymatic synthesis of PA and LPA may be achieved by phospholipase D (PLD) for the direct hydrolysis of the polar head of a natural phospholipids (PC) or synthetic PC to afford mixed acyl or pure PA, respectively. Phospholipase A2

(PLA2) in free or immobilised form can hydrolyze the *sn-2* fatty acid to convert PA into LPA.

Conversely, the use of 1,3-specific lipase for the direct esterification of glycerophosphate and fatty acid in the 1-position provide LPA1, as described by Virto et al. in Enz. Microb. Tech., 24:1, 651-657, 1999. However, spontaneous acyl migration, i.e. intramolecular transfer of the fatty acid from the 1 to the *sn*-2 position results in the formation of LPA2, which can be re-acylated by the enzyme to give PA. The water activity is a key factor to orient the activity on synthetic or reverse hydrolytic reactions.

Of course, the chemical and enzymatic will afford optically pure PA and LPA whenever the starting material are chirally pure, either from being chiral C3-building block or being natural phospholipids or enzymatically obtained mono- and di-glycerides which retain the L-α-configuration at the glycerol moiety.

Enriched fraction of PA and LPA may be obtained from vegetable by-products, e.g. form degummed oil as described by Dijkstra in U. S. Pat. Nos. 4,698,185; 5,214,171.

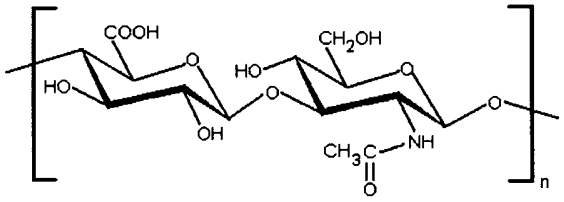
Further purification of PA and LPA from natural source or from the synthetic by-products can be achieved by several means, including chromatography, ion exchange and the like, or by selective precipitation, for examples as calcium salts in cold acetone.

The angiogenic phospholipids may in the form of dermatologically or cosmetically acceptable salts, thereby including alkali metal salts (e.g. Na, K); alkaline earth metal salts (e.g. Ca, Mg); non-toxic heavy metal salts; ammonium salts; and trialkylammonium salts (e.g. methylammonium and triethylammonium).

Preferred angiogenic phospholipids are "LPA1" and "mixed acyl PA" as defined above, wherein acyl groups derive from the enzymatic hydrolysis of a natural lecithin such as soybean, rapeseed and egg yolk lecithin.

According to the present invention, the expression "hyaluronan oligosaccharides (b)" relates to low molecular weight fragment of hyaluronan, which are the ubiquitous glycosaminoglycan that acts as a structural component of extracellular matrices and mediates cell adhesion.

The hyaluronan oligosaccharides useful in the methods and compositions of the subject invention are the oligopolymer of D-glucuronic acid and N-acetyl-glucosamine (GlcA/GlcNAc) of formula (VII) :



wherein n is an integer from 3 to 40; and salt and solvates thereof.

Preferably n is comprised between 3 and 30, even more preferably n is comprised between 3 and 10.

The hyaluronan oligosaccharides to be used in the present invention can be obtained may be obtained by known purification procedure as the fraction of hyaluronan, e.g. following the depolymerization of intact, high molecular weight hyaluronan.

Preferred hyaluronan oligosaccharides for the use on the present invention are obtainable by partial digestion of hyaluronan with testicular hyaluronidase, e.g. as described by Tawada A, et al. (Glycobiology. 2002;12(7):421-6). Many different hyaluronidases obtained from animal, microbial and plants are suitable for our purposes, e.g. cited by Hoving & Linker (Comp Biochem Physiol, Part B, 124, 319-326, 1999).

Further preferred hyaluronan oligosaccharides for the use on the present invention are obtainable by depolymerization of hyaluronan in the presence of inorganic ions (e.g. as in U. S. Pat. No. 4,973,580), as well as by heat, strong acid, strong alkali and other chemicals methods (J. Pat Nos. 62-79790, 63-57602) and by shear forces (WO91-04279) and combination thereof. The purification of hyaluronan oligosaccharides in fraction of defined length and purity may be carried out by known techniques, e.g. those described by Mahoney DJ et al. (Glycobiology. 2001;11(12):1025-33).

As used herein, the expression "peptide growth factor" refers to a peptide that, either directly or indirectly, enhances endothelial cell growth, promotes growth of new blood vessels from existing vasculature ("angiogenesis") and/or the

proliferation of endothelial cells at nano- and picomolar concentrations.

Suitable peptide growth factors for use according to the invention include, for example vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), transforming growth factor α and β (TGF-α,β), platelet-derived endothelial cell growth factor (PD-ECGF), platelet-derived growth factor (PDGF), tumor necrosis factor a (TNF-a), hepatocyte growth factor (HGF), colony stimulating factor (CSF), macrophage-CSF (M-CSF), granulocyte/macrophage CSF (GM-CSF) and nitric oxide synthase (NOS).

Other peptide growth factors are peptide or cytokines such as insulin-like growth factor (IGF), interleukin-8 (IL-8), angiopoietins 1- and 2-, cadherins, vitronectin, fibronectin, heparin binding growth factors (HBGFs), corticotropin-releasing hormone (CRH), angiogenin (ribonuclease A homologue), and αL-, αM-, β2-integrin monomers.

The term "peptide growth factors" also refers to functional analogues of the abovementioned factors. Such functional analogues include, for example, functional peptides or portions of the factors. Exemplary "functionally equivalent variants" of the include fragments of peptide growth factors, muteins and peptide analogues containing conservative amino acid substitutions, provided that these peptide variants and analogues are capable of provided they exert the same function, i.e. increasing the vascularity of EFSP tissues.

Functional analogues include anti-idiotypic antibodies binding to the factor receptors and mimic their angiogenetic activity, e.g. WO 97/23510 and fusion peptides as disclosed in U. S. Pat. No. 6,387,663.

The preferred peptide growth factors of the invention are the human VEGF isoforms and the with human bFGF, administered alone or in combination. Peptide growth factors further embrace functionally equivalent variants, and analogues of peptide growth factors, provided that the fragments, variants, and analogues alleviate a symptom of EFSP. The invention also embraces proteins and peptides coded for by any of the foregoing peptide growth factor nucleic acids. The invention also embraces agents that upregulate expression of a peptide growth factor in vivo.

Peptide growth factors and analogue thereof can be obtained from either biotechnology or natural sources. For examples the ovalbumin from egg whites contains a combination of peptide growth factors such as EGF, TGF-α, aFGF and bFGF which are suitable for our purposes, e.g. following.purification from the non-peptide growth factors in ovalbumin. Alternatively, a "functionally equivalent variants" of the peptide growth factors may be produced by recombinant DNA technologies in E. coli or insect cells, for example the oligopeptide NH_2-GKKEKPEKK (Growth Factors 10(2):89-98, 1994).

The purification from animal and vegetal tissues can be also applied to the different source as peptide growth factors by applying know techniques, e.g. those described by Mant CT in "HPLC of Peptides and Proteins: Separation, Analysis, and Conformation" CRC Press, 1991, as well as purchased by specialized vendors, e.g. Biopeptide Co., LLC (San Diego, CA, USA) or Sigma-Aldrich Co.

Also included in the peptide growth factors and/or their functionally equivalent are the "peptide growth factor nucleic acid", i.e. any nucleic acid sequence (DNA, cDNA and mRNA) which encode for a peptide growth factor or portions thereof which can be used to express it (Folkman, et al., Science, 235: 442-447, 1987). Complete coding sequence for representative human peptide growth factor cDNA and predicted amino acid sequence are available in public databank and literature, e.g. as GenBank Accession Nos. NM 003376, 003377, 005429, 004469, AF024710, or as U. S. Patent Nos. 6,013,780, 5,935,820, 5,607,918, and 5,219,739.

The angiogenic agents are herein classified as either primary or secondary angiogenic factors. The exemplary primary angiogenic agents are the compounds (a), (b) and (c) as disclosed above, with not only induce the angiogenic response or the endothelial stabilization, but also stimulate the individual components of vascular growth.

In a further preferred embodiment of the present invention, the methods and compositions of the present invention also comprises a secondary angiogenic agents (d).

The term "secondary angiogenic agents " as used herein means any low molecular weight agents that can help angiogenesis and/or the proliferation of

endothelial cells by the induction the vascular growth, but do not generally act through the direct stimulation of endothelial proliferation, migration, and protease production.

Exemplary secondary angiogenic agents include, but are not limited to, prostaglandin E1 and E2 (J. Natl. Cancer Inst. 69, 475-482, 1982); lipid amides such as erucamide (Biochem. Biophys. Res. Commun., 30;168(2):423-9, 1990); lipid metabolites such as 12(R)-hydroxyeicosatrienoic acid (J Biol Chem., 30;269(39):24321-7, 1994); saponins such as the Ginseng radix rubra saponins (Br J Pharmacol, 115(7):1188-93, 1995); phytosterols such as β-sitoserol form Aloe vera gel (Planta Med., 68(4):330-5, 2002); plant polyphenols such as the cinnamic and benzoic acids derivatives form Populus nigra (Acta Pol Pharm., 54(2):151-4, 1997). Further secondary angiogenic agents may be found as plant extract and plant derivatives such as Salvia miltiorrhiza and salvianolic acid B, triterpenoids of Centella asiatica (e.g. asiatic acid, madecassic acid, asiaticoside, madecaside), phytic acid, Ginseng and gingsenosides, giberellic acid and gibberellin hormone-like molecules, ruscogenin, escin, esculin, troxerutin, anthocyanes, proanthocyanosides. Secondary angiogenic agents also include other substances such as adenosine, inosine, hypoxanthine, nicotine, and nicotinamide.

The mode of action and types of secondary angiogenic agents are cited by Terrell & Swain (Matrix, 11(2):108-14, 1991) and Timar et al., (Pathol Oncol Res 2001;7(2):85-94). The secondary angiogenic agents (c) may be used in combination with the angiogenic agents (a), (b) and (c) to enhance the angiogenic response in EFSP.

According a preferred embodiment of the present invention the angiogenic agents are most preferably administered in the form of appropriate "local treatment".

The expression "local treatment" as used herein, means the administration of a composition which delivers the angiogenic agent by topical, transdermal or intradermal route on EFSP area.

The term "topical" as employed herein relates to the use of the active ingredient incorporated in a suitable cosmetic or dermatological carrier, and administered at the site of the disease for exerting local action. Accordingly, such

topical composition includes conventional such as ointments, lotions, pastes, jellies, sprays, aerosols, bath oils and the like, water-soluble and emulsion-type bases, e.g., petrolatum, lanolin, polyethylene glycols, as well as mixtures thereof. Chemical penetration enhancers that augment the delivery of the active ingredients to the subdermal layers, e.g those listed in our co-pending application PCT/IB03/00856, are recommended in topical transdermal routes.

For the transdermal route several aids can be used, including physical percutaneous penetration enhancers that augment the delivery of the active ingredient to the subdermal layers, such as those discussed by Smith EW & Maibach HI, eds., in "Percutaneous penetration enhancers", CRC Press, NY, 1995. Transdermal route of administration include those capable to enhance skin porosity and/or hydration, such as occlusion devices, hydrocolloid patches, delipidization as well as physically assisted techniques such as electroporation, iontophoresis, ultrasound, sonophoresis, and the like.

Intradermal route include local injection, e.g. by syringe or dermojet, in that angiogenic agents will conveniently be suspended in a sterilized liquid formulation. Intradermal injection refers to the administration of the composition in the deep dermis by needle injection, or by high pressure air injection.

Compositions suitable for local injection conveniently comprise a sterile aqueous, preferably isotonic, formulation of the angiogenic agents, which may be formulated according to known methods using dispersing or wetting agents and suspending agents in a non-toxic solvent, e.g. 1,3-butane diol, Ringer's solution, or isotonic NaCl solution.

The angiogenic agents of the invention are administered in effective amounts to delay the onset, or inhibit the progression, or reverse the impaired microcirculatory disorder in EFSP tissues. As noted above, the effective amount will vary with the subject's age and condition, as well as the extent and the subtype of the disorder, all of which can be determined by one of ordinary skill in the art.

The compositions of invention will contain a cosmetically and/or dermatologically acceptable solid or liquid carrier and secondary ingredients suited for topical, transdermal or intradermal administration. Example of such carriers and

secondary ingredients include fillers, binders, bulking and coloring agents, antioxidants, anionic, nonionic, cationic and zwitterionic surfactants detergents, oils, fats, waxes, humectants, thickening agents, antioxidants, viscosity stabilizers, chelating agents, buffers, preservatives, perfumes, dyestuffs, water and organic solvents, humectants, thickeners, preservatives, stabilizers, antibacterials, antifungals, vitamins, sunscreens, anti-acne agents, antibiotics, retinoids, etc.

In some particular embodiments, a preferred vehicle is a biocompatible micro particle or implant that is suitable for implantation into the subject, e.g. as described in WO95/24929 and PCT/US/03307. Examples of non-biodegradable polymers include ethylene vinyl acetate, poly(meth)acrylic acid, and polyamides. Examples of biodegradable polymers include polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butirric acid), poly(valeric acid), and poly(lactide-cocaprolactone), natural polymers such as alginate and polysaccharides including dextran and cellulose, collagen, albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof.

In another preferred embodiment of the present invention, angiogenic phospholipids preferably in combination with angiogenic phospholipids are composed in liposomes entrapping a peptide growth factor and/ or hyaluronan oligosaccharides for synergistic activity on EFSP.

The method according to the invention shall contain a suitable effective amount of one or more angiogenic agent to be locally administered to the EFSP area.

A suitable effective amount of the primary and secondary angiogenic will vary throughout a broad dosage range. It shall be adjusted by one skilled in the art based on the potency of each angiogenic agent as well as the route of administration thereof, subject's age and condition, the nature and extent of the EFSP in the subject, all of which can be determined by one of ordinary skill in the art. The dosage may be adjusted by the individual physician, particularly in the event of any complication.

A locally administered compositions may comprise different combination of the angiogenic agents, each of those trigger the angiogenic response and/or endothelial repair in EFSP tissues at different concentrations. A suitable effective

dose to elicit the angiogenic response in EFSP tissues shall provide a local concentration of LPA and/or PA in the range from 1 μM to 100 μM, of hyaluronan oligosaccharides in the range from 0.1 nM to 10 nM, and for peptide growth factors in the range from about 1 to 100 pM.

Accordingly, the composition according to the invention may contain an amount of angiogenic phospholipids from 0.5 mg to 50 mg per g of the composition, preferably from 3 mg to 30 mg per g of a composition for topical and transdermal routes of administration, and from 0.6 mg to 6 mg per g of a composition for intradermal route.

The composition according to the invention may contain an amount of the hyaluronan oligosaccharide fraction from 0.5 mg to 50 mg per g of the composition, preferably from 0.3 mg to 30 mg per g of a composition for topical/transdermal routes, and from 60 μg to 600 μg per g of a composition for intradermal route.

The composition according to the invention may contain an amount of a peptide growth factor from 0.5 μg to 500 μg per g of the composition, preferably from 6 μg to 60 μg per g of the composition for any of the routes of administration.

According a preferred embodiment of the present invention, the angiogenic agents are applied in tandem to effectively treat EFSP, as they display divergent and potent effects on angiogenic properties and differentiation of endothelial cells. Again, PA itself have complementary action effect than LPA, from PA exerts the induction of endothelial monolayer permeability (English et al., J. Cell Biochem., 75:105-117, 1999), the early event of neovascolarization, whilst LPA induce endothelial growth and stabilization. If the combination of different angiogenic agents shall be utilized whenever possible, their simultaneous presence may produce either synergistic activity or just an additive effect. Strikingly, the co-addition of hyaluronan oligosaccharides and VEGF, but not co-addition of hyaluronan oligosaccharides and bFGF, induces an angiogenic response that is greater than the sum of the effects elicited by either agent separately.

Noteworthy, the methods and compositions of the present invention substantially differ from those which occasionally contain trace amount of angiogenic phospholipid (e.g. as trace component of natural lecithin used as

emulsifier), which not only are unsuited to elicit the angiogenic response but, instead, can provoke angiogenic inhibition when in nanomolar amount (Tigyi et al., Proc Nat Acad USA, 91, 1908-1912, 1994).

In the transdermal administration is particularly preferred the association of angogenic phospholipids with peptide growth factors and/or hyaluronan oligosaccharides. Liposomes are well known in the art and have closed structures made up of a lipid bilayer containing an encapsulated aqueous volume (i.e. unilamellar liposomes), or concentric lipid bilayer (i.e. multilamellar liposomes). It is within the aqueous phase of the angiogenic phospholipid-containing liposomes that peptide growth factors or hyaluronan oligosaccharides are entrapped, thus with enhanced delivery onto the subdermal layer when topically administered to EFSP subjects.

The liposome combination is also desirable for topical formulation, such as cream, oil, pastes and so on, as well as for transdermal administration such as hydrocolloid patches, delipidization, electroporation, ultrasound, and the like methods. In intradermal administration such as iontophoresis and local injection, the angiogenic agents (b) and (c) may be easily administered alone or in combination, with or without the presence of (a).

The composition shall be administered in one or more doses daily, for at least one week, preferably for a months, even more preferably the angiogenic agents are administered over a period of few months to years, particularly in the case of advanced EFSP (cellulite grade IV).

The concentration of the secondary angiogenic agents shall be those which sustain and enhance the activity of the angiogenic agents (a), (b) and (c).

Particular attention may be paid on the lower part of the legs of treated subjects to avoid the formation of teleangectasias, which may be evoked by the angiogenic agents.

In another preferred embodiment of the present invention, the angiogenic agents are combined with complementary ingredients commonly used in the treatment of cellulite, thus including the following:

a) lipolytic agents, i.e. beta-stimulator activity (adrenergic beta-agonists),

adenylate cyclase agonist and/or anti-phosphodiesterase activities such as xanthines (e.g. caffeine, theophylline, theobromine, aminophylline), tyroxine (triiodotyrosine), conjugated linoleic acid, extracts from Ipomea spp., Salvia spp. and Rosmarinus officinalis, yohimbine-type alkaloids and plant extracts containing dimeric flavones such as amentoflavone, bilobetine, sciadopitisine, ginkgonetine, or extracts of Malvaceae (e.g. Malva, Althea, Hibiscus, Hoheria, Sidalcea, Abutilon and Gossypium);

b) anti-inflammatory compounds which include, but are not limited to, rosmarinic acid, glycyrrizinate derivatives, alpha bisabolol, azulene and derivatives thereof, quercetin, rutin, betulinic acid, catechin and derivatives, thereof;

c) vasoactive compounds which include, but are not limited to, papaverine, yohimbine, visnadin, khellin, bebellin, nicotinate derivatives

Also included are further ingredients used in cellulite with detoxifying, draining and diuretic action, e.g. those disclosed in Eur. J Dermatol 2000; 10, 595-603, provided that these complementary active ingredients shall not inhibit the angiogenic activity which is pursued in the present invention.

It is to be understood that the present invention is based on EFSP being considered as both a medical problem and an aesthetic question. The EFSP management is thereby conceived with a phlebologic approach, i.e. to ensure an adequate and well-functioning microcirculation as well as to repair circulatory disarrays and impairments on EFSP areas.

The following examples are given solely for the purpose of illustration and are not to be construed as limitations of the present invention, as many variations thereof are possible without departing from the spirit and scope of the invention.

EXAMPLES

Preparative Example 1 – Enzymatic preparation of a PA-LPA mixture

A stirred solution of 10 g Epikuron 170 (70% phosphatidylcoline, Lucas Meyer, Germany), 100 ml of toluene was added with 150 mg of PL-D (Italfarmaco srl, Cinisello B., Italy) and an aqueous solution (10 ml) containing phospholipase-A2 (Biocatalyst Ltd, Pontypridd, UK). Then 50 ml of 1M calcium chloride solution and

1N sodium acetate trihydrate solution and glacial acetic acid were added until a pH of about 6 was attained. The resulting diphasic system is heated to a temperature of 35 °C and kept under stirring for about 8h. After the completion of the reaction, the mixture was allowed to stand so as to separate, the solvent was distilled off under reduced pressure to give, upon filtration and dried under vacuum at 90°C for 20 minutes for enzyme inactivation. The work-up provided 7 g of a waxy mixture containing LPA, PA and remaining phosphatides ("PL") at an approximate ratio LPA:PA:PL ratio of 3:1:4 according the TLC analysis (Silica gel, $CHCl_3$:MeOH:H_2O at 32:12:2 v/v/v, I_2 vapours for spot visualization).

Preparative Example 2 – Metal-assisted preparation of hyaluronan oligosaccharides(HOS)

A stirred solution of 5 g hyaluronic acid sodium salt from Streptococcus equi (Fluka product code 53747) in 100 ml of water, 10 g of sodium acetate are added. 15 ml of a 0.32M solution of $CuSO_4$ and 50 ml of 5.4% hydrogen peroxide, which is dropped within 40 minutes. The reaction is kept at 60°C for 3 hours while the pH adjusted to 7.5 with NaOH. The reaction mass is cooled, filtered on decalite, the filtrate is added with 0.6 g of disodium ethylenediaminetetraacetate dehydrate (EOTA), then precipitated with 300 ml of methanol. The precipitate is filtered, dissolved again in 300 ml of water and added with 0.6 g of EOTA and 18 g of sodium acetate, then reprecipitated with 600 ml of methanol. The product is filtrated and vacumm dried to afford 4.6 g of hyaluronan with MW around 1 and 7 kDa by gel electrophoresis (Anal Biochem, 2002, 15;311(2):157-65).

Example 1 – Microinjections formulation #1

A sterile solution can be prepared by dissolving 0.1 g of hyaluronan oligosaccharides from the preparative example 2, 10 μg of PD-ECGF (Sigma-Aldrich, product code P 5208) and 15 μg of angiogenin (Sigma-Aldrich, product code A 6955) in 100 ml of sterile water.

The female subject is treated with an cream containing lidocaine (AMLA®), thereby administered to the skin to reduce the pain in view of the subsequent injection. Then the areas to be treated were wiped with alcohol and patted dry. With a fine-gauge needle, 100 μl aliquots of the solution are injected into different sites

into the subdermis on the limbs affected by EFSP.

The treatment can be repeated once or three times a week for 1 to 3 months.

Example 2 – Microinjections formulation #2

A sterile solution can be prepared by dissolving 2 g of Oleyl-LPA (Fluka, product code 75347) and 0.2 g of tocopherol in 20 ml ethanol, then a solution of 5 μg of VEGF (Sigma-Aldrich, product code V 4512) into 80 ml of sterile water are added and sonicated until formation of a translucent suspension.

This composition can be administered to the patient either by microinjection, as in the example 1, or by dermoject injection.

Example 3 – Ionthophoresis formulation

A solution can be prepared by dissolving 0.5 g of of PA-LPA mix from the preparative example 1, 50 mg of hyaluronan oligosaccharides from the preparative example 2 and 5 μg of VEGF (Sigma-Aldrich, product code V 4512) into 100 ml of sterile water, followed by the application through an iontophoresis apparatus.

Example 4 – Transdermal patches

A slow-release transdermal patch to be applied over long period of time may contain as angiogenic agents 2 g of the LPA/PA mix of the preparative example 1 and 1 g of hyaluronan oligosaccharides from the preparative example 2, which are compounded into an adhesive mass for transdermal patches comprising the following ingredients:

lactose	37.1 g
erucamide	2.2 g
polyisobutene	23.7 g
hydrogenated colofonia	18.5 g
polyalkadiene	18.5 g

The transdermal patch is made of vertical strips (60 cm), each composed of three layers, i.e. a basal sheet, an adhesive film containing the angiogenic agents, and a coverage film. The patches can be were customarily cut in strips to match the geometrical feature of legs mark of ESFP and applied thereof during the first session. The actives are is released during 24 hours, thus ensuring a continuous administration. The subject was given a number of strips of the same size and shape

and instructed to substitute the former strips every day at the morning for 3 months, and wear each strips until next day.

Example 5 – Cream for domiciliary topical application

100 g of emulsion contain	(g)
LPA/PA from the Preparative Example 1	2.6
HOS from the Preparative Example 1	1.5
Ovalbumin (*)	9.0
Fluid paraffin	5.0
Cetyl alcohol	5.5
Petrolatum	5.5
Glycerine monostearate	33
2-Octyldodecyl ether-EO (20 mole)	3.0
Glycerine	7.0
Dipropylene glycol	20
Perfume, additives, preservatives	qb
Water	qb to 100 g

(*) Containing EGF, TGF-α, aFGF and bFGF.

Follow-up Example – Diagnostic monitoring of the EFSP treatment

Female patients with a diagnoses of EFSP on the trochanteric, upper lateral and medial regions of the thigh and knee can be monitored before, during and after the treatment by the angiogenic agents.

1a - Capillaroscopy

An optic probe video-capillaroscopy (OPVC) examinations of four points on the anteromedial surface of the thigh and one point on the anteromedial surface of the knee will showing pathological alterations and the local microcirculation in the subpapillary cutaneous plexus. For the measurements, patients shall remain supine for at least one hour at a controlled constant temperature. Few drops of microscope immersion oil shall be applied to each examination site and spread over circular areas to render the stratum corneum diaphanous and improve the transparency of the hypodermis.

A portable optic probe fitted with a 50-watt cold halogen lamp and contact

200x and 400x objectives, with a portable videotape recorder (e.g. Sony 8mm) will be attached to the equipment to allow optimal and continuous monitoring of the capillaroscopic images. The images recorded on the videotape can be viewed on the monitor (e.g Scopeman-Moritex MS 504) linked to a fixed video-capillaroscope.

The relevant images on selected zone will be printed (e.g. Color Video Printer VY-SS50, Hitachi, Japan) to compare the capillarity pattern on a selected WEFSP area before, during and after the treatment with the method and compositions of invention, e.g. those described in the examples 1 to 5.

1b - Thermographic analysis

For the thermographic examinations, an IPS instrument with fixed-plate on a metal support with RS28, 29 and 30 ST plates.

Thermographic images can be filmed (e.g. Sony 8mm videocamera) fitted with an additional support-mounted lens. The images can be viewed on a monitor and transferred to a computer for digital image quantification.

As for the case 1a, the treated images can be then transferred back to a monitor and printed to compare the blood flow before, during and after the treatment with the method and compositions of invention, e.g. those described in the examples 1 to 5.

1c - Flowmeter analysis

The laser-Doppler flowmetry examination may use two-channel Periflux 4000 equipment (Perimed, Linkoeping, Sweden) connected to a computer with software (e.g. Perisoft) for the analysis of the colors corresponding to the different skin temperatures.

The Laser-Doppler flowmeter examinations on the upper lateral and upper medial regions of the thigh and on the knee showed serious disturbances to microcirculatory function. These were especially noticeable at site where finger palpation revealed painful subcutaneous nodules.

Before the treatment by the present invention method, the flow signals shall appear irregular and with low flow values (between 8 and 9 U.I), with the high-frequency rhythmic variations (HFRV) ranging from 6.27 minimum to 11.76 maximum.

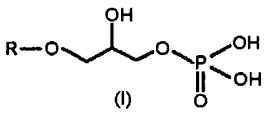
At the end of the treatment by the method of examples 1 to 5, the same area will provide a more regular signal, with average flows from 16 to 22 U.I. and a wider HFRV range.

Further monitoring

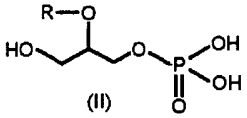
Diagnostic tools which are currently used in phlebology (Curri SB, Phlebologie 1990;43(3):407-30), thus including computerized Laser-Doppler Flowmetry and infra red Photo-Pulse Plethysmography, computerized Telethermography and High Performance Contact Thermography are also helpful to characterize the circulatory improvement prompted by the application of the inventive method and compositions.

CLAIMS

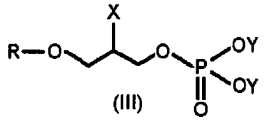
1. Use of angiogenic agents for the manufacture of a dermatological and/or cosmetic composition for the treatment and/or prevention of edematous-fibrosclerotic panniculopathy (EFSP).
2. Use according to claim 1, wherein said angiogenic agent is selected among the following:
   a) angiogenic phospholipids;
   b) hyaluronan oligosaccharides;
   c) peptide growth factors, and/or their functionally equivalent;
   and their mixture,
   optionally in combination with
   d) secondary angiogenic agents.
3. Use according to claim 1 wherein the an angiogenic phospholipid (a) is one or more lysophosphatidic acid of formulae I, II and III :



(I)

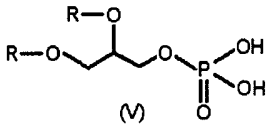


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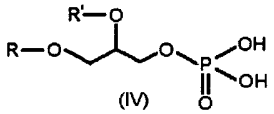


(III)

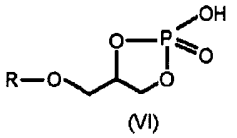
or a phosphatidic acid of formulae IV, V and VI :



(V)



(IV)

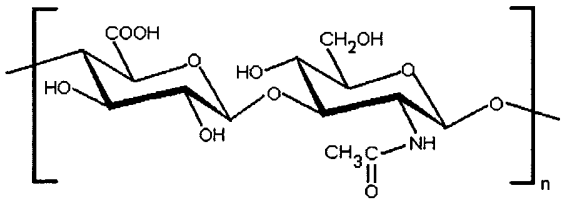


(VI)

wherein R and R', each independently, are a C_2-C_{24} acyl; and X is halogen;
or sphingosine-1-phosphate;
and mixture thereof.

4. Use according to claim 3 wherein said acyl is selected in the group consisting of oleyl, stearoyl, palmitoyl, palmitoleoyl, myrystoyl, lauroyl, linoleoyl, α-linoleoyl, γ-linolenoyl, α-linolenoyl, eicosapentaenoyl, docosahexaenoyl, dihomolinolenoyl, arachidonoyl, and mixture thereof.
5. Use according to claim 3 wherein said acyl derives from a lecithin selected from the group consisting of soybean lecithin, rapeseed lecithin and egg yolk lecithin.

6. Use according to claims 2-5 wherein the angiogenic phospholipid is present either as pure phospholipids or a pharmaceutically acceptable salt thereof, or in an percentage of at least 30% by weight of an admixture of natural phospholipids selected in the group consisting of phosphatidyl choline, N-acyl phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl glycerol, diphosphatidyl glycerol, and the lysophospholipids thereof.
7. Use according to claim 2 wherein the hyaluronan oligosaccharide (b) is a fraction of oligosaccharides of formula (VII):



wherein n is an integer from 3 to 40;
or salts or solvates thereof.

8. Use according to claim 7 wherein n is comprised between 3 and 20.
9. Use according to claim 7 wherein n is comprised between 3 and 10.
10. Use according to claim 1 wherein the peptide growth factor (c) is one or more angiogenic peptide growth factors or their functionally equivalent variants.
11. Use according to claim 10 wherein the peptide growth factor is selected from the group consisting of VEGFs, bFGF, aFGF, EGF, TGFs, PD-EGF, PDGF, HNA, HGF, CSFs, M-CSF, G/M-CSF, tK, and angiogenin.
12. Use according to claim 10 wherein the peptide growth factor is a VEGF.
13. Use according to claim 10 wherein the VEGF is selected in the group consisting of VEGF A, VEGF B, VEGF C, VEGF D, phVEGFl2l, phVEGF 45, phVEGFl6s, and phVEGFlg9.
14. Use according to claim 10 wherein the peptide growth factors are those contained in ovalbumin, selected in the group consisting of EGF, TGF-α, aFGF and bFGF
15. Use according to claim 10 wherein the peptide growth factor is angiogenin or bFGF.
16. Use according to claim 10, 11, 12, 13, 14, or 15 wherein the peptide growth factor is a functionally equivalent variant or analogue of the peptide growth

factors, provided that it is capable of inducing neovascularization and/or endothelial repair in EFSP.

17. Use according to claim 1 further comprising a secondary angiogenic agent.

18. Use according to claim 1 wherein (d) is selected from the group consisting of prostaglandins, erucamide, 12(R)-hydroxyeicosatrienoic acid, saponins, and β-sitoserol.

19. se according to claim 1 wherein (d) is selected from the group consisting of salvianolic acid B, asiatic acid, madecassic acid, asiaticoside, madecaside, phytic acid, gingsenosides, giberellic acid, gibberellin hormone-like molecules, ruscogenin, escin, esculin, troxerutin, adenosine, inosine, hypoxanthine, nicotine, and nicotinamide.

20. Method for the treatment of edematous-fibrosclerotic panniculopathy (EFSP) which comprises administering to mammal in need thereof an effective amount of one or more angiogenic agents.

21. Method according to claim 20, wherein said angiogenic agents are selected from the following:

a) angiogenic phospholipids;

b) hyaluronan oligosaccharides;

c) peptide growth factors, and/or their functionally equivalent;

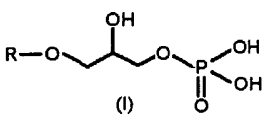
and their mixture,

optionally in combination with

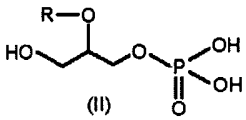
d) secondary angiogenic agents.

22. Method according to claim 21, wherein said angiogenic agents are selected from

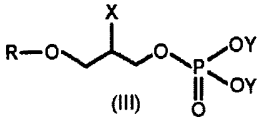
- lysophosphatidic acid of formulae I, II and III :



(I)

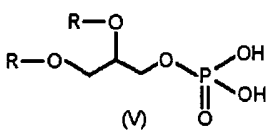


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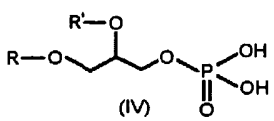


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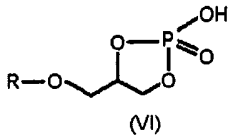
- phosphatidic acid of formulae IV, V and VI :



(V)



(IV)



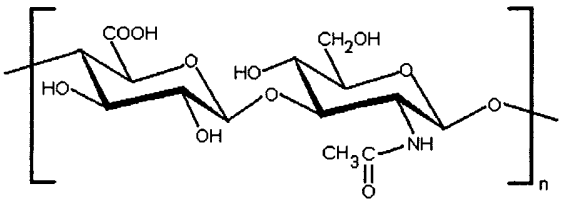
(VI)

wherein R and R', each independently, are a C_2-C_{24} acyl; and X is halogen;

- a sphingosine-1-phosphate;

and mixture thereof.

23. Method according to claim 21, wherein said angiogenic agents are selected from oligosaccharides of formula (VII):



wherein n is an integer from 3 to 40;

and salt and solvates thereof.

24. Method according to claim 21, wherein said angiogenic agents are peptide growth factors or their functionally equivalent variants selected from the group consisting of VEGFs, bFGF, aFGF, EGF, TGFs, PD-EGF, PDGF, HNA, HGF, CSFs, M-CSF, G/M-CSF, tK, and angiogenin.

25. Method according to claims 20-24, which comprises the topical application of the angiogenic agents.

26. Method according to claims 20-24, which comprises the transdermal application of the angiogenic agents.

27. Method according to claims 20-24, which comprises the intradermal application of the angiogenic agents.

28. Cosmetic/dermatolgical composition comprising as the active principle angiogenic agents are selected from the following:

a) angiogenic phospholipids;

b) hyaluronan oligosaccharides;

c) peptide growth factors, and/or their functionally equivalent;

and their mixture,

optionally in combination with

d) secondary angiogenic agents.

29. Use of a composition according to claim 28 for the treatment and prevention of edematous-fibrosclerotic panniculopathy (EFSP).