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(54) Title: LIPOSOMES CONTAINING PROSTAGLANDIN E1 (PGE1) AND A PLANT OESTROGEN, FORMULATIONS FOR TOPICAL USE CONTAINING THEM AND THEIR USE

(57) Abstract: Described herein are liposomes comprising PGE1 in combination with a plant oestrogen and possibly a compound capable of increasing the cationic power of the liposome and their use in the treatment of baldness and hair loss.

LIPOSOMES CONTAINING PROSTAGLANDIN E1 (PGE1) AND A PLANT OESTROGEN,  
FORMULATIONS FOR TOPICAL USE CONTAINING THEM AND THEIR USE

Field of the invention

The present invention relates to the field of pharmaceutical and cosmetic formulations for topical use containing liposomes, in particular for the treatment of alopecia and for hair regrowth in general.

Background of the invention

Hair loss is an extremely widespread phenomenon affecting both men and women, caused by various factors such as stress, diet, seasonal changes, hereditary factors, etc.

The phenomenon often has repercussions on the patient such as to make him believe that he has a true pathology and there is, in any case, no doubt as to its impact from an aesthetic point of view.

Many methods are described for the treatment and prevention of baldness, from specific anti-hair loss shampoos, possibly combined with lotion or other products to be applied at more or less regular intervals, to specific pharmacological products (such as Minoxidil or Finasteride).

Nevertheless, despite the many remedies available on the market, the problem is far from being said as satisfactorily resolved.

International patent application WO 2011/095938, in the name of the same Applicant, describes unilamellar liposomes encapsulating PGE1 and/or PGE1- $\alpha$ -cyclodextrin in combination with L-propionyl carnitine, the outer surface of which is coated by hydrophilic polymers useful for the treatment, following systemic administration, of vascular pathologies in diabetic subjects and for the local treatment (topical administration) of skin ulcers and of diabetic retinopathies.

Summary of the invention

Described herein are liposomes encapsulating PGE1 in combination with a plant oestrogen and possibly a compound capable of increasing the cationic power of the liposome

Detailed description of the invention

This invention makes available new pharmacological and/or cosmetic products for the treatment of baldness and hair loss comprising liposomes, encapsulating

Prostaglandin E1 (PGE1) in combination with a plant oestrogen and possibly a compound capable of increasing the cationic power of the liposome.

The liposomes according to the invention are constituted by a phospholipid vesicle containing a core of aqueous solution.

The phospholipids that constitute the wall of the vesicle are natural or synthetic phospholipids, given their high biocompatibility and the absence of toxicity.

Phospholipids that can be used according to the invention are for example: phosphatidylcholine (lecithin), phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), palmitoyl-stearoylphosphatidylcholine, sphingomyelin, and the like.

Plant oestrogens shall for example mean: equol and isoflavones in general, such as for example: genistein, daidzein, glycitein.

The function of the compound capable of increasing the cationic power of the liposome is to increase adhesiveness and to also facilitate the endothelial cell metabolism of the micro capillaries; compounds useful for this purpose are, for example, essential amino acids or carnitine, which is also a fatty acid carrier and allows the mitochondria to use them for the production of ATP.

The liposomes can further comprise additives that serve as stabilisers or as modifiers of the surface charge, such as for example cholesterol, cholesterol sulphate and the like.

The liposomes according to the present invention can be prepared by simply mixing solutions of the various components in organic solvent and water, sonicating the mixture thus obtained for the required time.

The organic solvent as indicated above is preferably ethanol.

Alternatively, the mixtures constituted by the solutions of the various components can be sonicated in the organic solvent alone (for example, ethanol) with the organic solvent then being evaporated and re-suspended with an aqueous solution.

In addition, to increase the adhesion of the liposomes to cells of the dermis while avoiding the traumatic removal thereof, the outer surface of the liposomes can be coated with hydrophilic polymers such as for example polylysine, polyornithine,

fibronectin and mixtures thereof; preferred is polylysine, which also has bactericidal properties, thus preventing possible infections.

Said coating is obtained by treating the liposomes as described above with an aqueous solution of the hydrophilic polymer as defined above, for example by "dropping" the liposomes themselves, drop by drop, into the polymer solution, under constant stirring.

The formulations for topical use according to the invention will thus comprise the liposomes as described above and will normally be in a form suitable for topical application, such as for example: aqueous solutions, ointments, creams, gels, lotions or polymer films for topical application wherein the liposomes are dispersed using excipients normally used in pharmacopoeia or in cosmetics for the preparation of said formulations.

In particular, the polymeric films as mentioned above are known and are prepared using organic polymers such as for example: sodium hyaluronate, hydroxypropyl cellulose (HPMC), polyethylene glycol 400 (PEG 400) and water, in appropriate ratios and are characterised in terms of viscoelastic properties, thickness and bioadhesion in vitro respectively using a rheometer, a micrometer and a tensiometer.

The films are then used for the preparation of medications to be applied topically and consisting for example of strips of various sizes for application onto the skin.

The encapsulation efficiency (E%) of PGE<sub>1</sub>, carnitine and plant oestrogen in the liposomes was determined by means of HPLC (clearly after having broken down the liposomes with a suitable membrane lysing means, for example with Triton X-100.)

The liposomes are characterised in terms of size, polydispersity index (PI) and zeta potential, while their structural morphological characteristics were studied through transmission electron microscopy (TEM) and polarised light optical microscopy.

The invention will now be better illustrated in the light of the following examples.

#### Example 1

Three solutions are prepared as follows:

1 ml of equal is diluted in 1 ml of ethanol and the solution is brought to 10 ml with water;

1 mg of PGE1 in 1 ml of ethanol brought to 10 ml with water;

2 g of carnitine in 10 ml of water.

The above three solutions are placed in a sonicator together with 10 g of phosphatidylcholine (Lipid S75 Human-grade) and sonicated for a sufficient time to obtain liposomes with diameter of less than 100 nm.

The liposome solution thus obtained is poured into an aqueous solution of polylysine 40 - 100 MW (0.01 mg/10 ml) and constantly stirred for 30 minutes.

The solution thus obtained can be used directly on the scalp at least once daily.

The results of the hair loss cessation can already be observed after just 7 days and the first new hair regrowth results between 45 and 90 days.

#### Example 2

Three solutions are prepared as follows:

1 ml of equol is diluted in 1 ml of ethanol;

1 mg of PGE1 in 1 ml ethanol;

2 g of carnitine in 2 ml of ethanol;

The above three solutions are placed in a sonicator together with 10 g of phosphatidylcholine (Lipid S75 Human-grade) and sonicated for a sufficient time to obtain liposomes with a diameter of less than 100 nm.

The ethanol is completely evaporated and the liposomes obtained are placed in contact with 5 ml of a polylysine 40-100 MW (0.01 / 10 ml) solution. The liposome solution thus obtained is poured into an aqueous solution of polylysine 40 - 100 MW (0.01 mg/10 ml) and constantly stirred for 30 minutes then adding 5 ml of buffered water or of saline solution (NaCl 0:9/100 )

#### Characterisation of liposomes

The diameter of the liposomes was found to be 60 nm on average with polydispersity index equal to 0.2

The amount of PGE 1 in the liposomes following purification is in the range of 30-50 pg/ml while the amount of carnitine is between 0.05 and 0.2 mg/ml.

The liposomes were characterised in terms of size, polydispersity index (PI) and zeta potential respectively by means of Photon Correlation Spectroscopy (PCS)

(dimensions and PI) and M3-PALS (Phase Analysis Light Scattering), which measures the electrophoretic mobility of the particles in a thermostated cell, (zeta potential) using the Zetasizer nano (Malvern Instrument, UK).

## CLAIMS

1. Liposomes comprising prostaglandin E<sub>1</sub> in combination with a plant oestrogen and possibly a compound capable of increasing the cationic power of the liposome.
2. Liposomes according to claim 1 comprising natural or synthetic phospholipids selected from: lecithin, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, dimyristoylphosphatidylcholine - DMPC, dipalmitoylphosphatidylcholine DPPC, distearoylphosphatidylcholine DSPC, palmitoyl-stearoylphosphatidylcholine, sphingomyelin possibly in combination with additives that serve as stabilisers or as modifiers of the surface charge.
3. Liposomes according to claim 1, wherein said plant oestrogen is selected from: equol, isoflavones such as: genistein, daidzein, glycitein.
4. Liposomes according to claim 1, wherein said compound capable of increasing the cationic power of the liposome is selected from: carnitine and essential amino acids.
5. Liposomes according to claims 1 - 4 on the outer surface of which hydrophilic polymers are present.
6. Liposomes according to claim 5, wherein said hydrophilic polymers are selected from: polylysine, polyornithine, fibronectin and mixtures thereof.
7. A process for preparing liposomes according to claims 1 - 6, wherein solutions in ethanol and water of the various components are mixed, the so obtained mixture is sonicated and optionally the so obtained solution is treated with an hydrophobic polymer in aqueous solution.
8. Process for the preparation of liposomes according to claims 1 - 6, wherein solutions in ethanol of the various components are mixed, the so obtained mixture is sonicated, the solvent is evaporated and the remaining solid is re-suspended with an hydrophobic polymer in aqueous solution.
9. Formulations for systemic topical use comprising the liposomes according to claims 1 - 6.
10. Formulations according to claim 9, consisting of: aqueous solutions, ointments, creams, gels, lotions or polymer films for topical application wherein the

liposomes are dispersed.

11. Formulations according to claims 9 and 10 for use in the treatment of baldness or hair loss.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K8/00 A61K9/127 A61K9/00 A61P17/14  
ADD.  
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED  
Minimum documentation searched (classification system followed by classification symbols)  
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/213859 A1 (ZELICKSON BRIAN D [US]) 28 October 2004 (2004-10-28) page 2, paragraph 0013 page 3, paragraph 0036 page 4, paragraph 0048 claims 1,14	1-11
A	----- US 2011/301105 A1 (MILLER FREDA [CA] ET AL) 8 December 2011 (2011-12-08) page 2, paragraph 0014 page 9, paragraph 0123 page 13, paragraph 0157	1-11
A	----- US 2008/275118 A1 (SHAW MARI M [US] ET AL) 6 November 2008 (2008-11-06) page 2, paragraphs 0019,0020,0022 page 4, paragraph 0042 - paragraph 0044 ----- -/--	1-11

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

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- "E" earlier application or patent but published on or after the international filing date
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2011/095938 A1 (BIORICERCA DI GIOVANNI BROTZU & C SNC [IT]; BROTZU GIOVANNI [IT]) 11 August 2011 (2011-08-11) page 2, line 8 - line 26 -----	1-11

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/IB2013/053912
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			EP 2598150 A2	05-06-2013
			US 2011301105 A1	08-12-2011
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			US 2013039975 A1	14-02-2013
			WO 2011095938 A1	11-08-2011
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