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(54) Title: LIPOSOMES CONTAINING PROSTAGLANDIN E1 (PGE1), FORMULATIONS CONTAINING THEM AND THEIR USE

(57) Abstract: Unilamellar liposomes comprising PGE1 and/or PGE1 - $\alpha$ -cyclodextrin combined with carnitine, in which there are hydrophilic polymers on the external surface of the liposomes are described.

**Liposomes containing prostaglandin E1 (PGE1), formulations containing them and their use**

Field of the invention

The present invention relates to the field of pharmaceutical formulations and particularly those comprising liposomes.

State of the art

The purpose of the present invention is to make available efficient and safe liposomes encapsulating prostaglandin E1 (PGE1) for the treatment (following systemic administration) of vascular disorders in diabetic patients, and for the local treatment (following topical administration) of skin ulcers and diabetic retinopathies.

When administered systemically, prostaglandin E1 (PGE1) is known to be rapidly metabolised in the lung so the majority of its efficacy is obviously lost. To overcome the above-mentioned problem, liposomes of phosphatidylcholine or other phospholipids have been studied to ascertain the feasibility of obtaining the release of the PGE1- $\alpha$ -cyclodextrin complex for the treatment of pathological conditions associated with diabetes (see, for instance, Toyota T. et al.: Lipo-PGE1. A new lipid-encapsulation of prostaglandin E1: placebo and prostaglandin E1 controlled trails in patients with diabetic neuropathy and leg ulcers. Prostaglandins 1993, 46, 453-468.

In previous research (see Golub M. et al.: Metabolism of prostaglandins A1 and E1. J. Clin. Invest. 1975; 56, 1404-1410), liposomes of phosphatidylcholine were used as the drug carrier to enable the PGE1 to pass beyond the pulmonary filter and avoid it being metabolised by 15-hydroxy dehydrogenase.

Given the importance of the use of PGE1- $\alpha$ -cyclodextrin in treating the vascular diseases of diabetics, and for the local treatment of skin ulcers and diabetic retinopathies, there is an evident need to develop new formulations capable of overcoming the above-mentioned problems, ensuring an effective

extension of the half-life of the PGE1 administered and facilitating the drug's release at the required site.

#### Summary of the invention

Unilamellar liposomes encapsulating PGE1 and/or PGE1- $\alpha$ -cyclodextrin, in combination with L-propionyl carnitine, the external surface of which is coated with hydrophilic polymers are described.

#### Detailed description of the invention

The present invention allows to overcome the abovedescribed problems thanks to the combination of unilamellar liposomes comprising prostaglandin E1 with L-propionyl-carnitine, on the external surface of which hydrophilic polymers are present.

In fact, it has surprisingly been found that said polymer coating not only enables a better adhesion to the cells of the endothelial tissue, but also improves the stability of the liposome in the plasma and consequently prolongs its circulation time with a consequent increase in the drug's half-life and an improved capacity of the delivery system to bind to a specific site.

The unilamellar liposomes according to the invention consist of a phospholipid vesicle containing a nucleus of aqueous solution.

The phospholipids constituting the wall of the vesicle are natural or synthetic phospholipids, given their high biocompatibility and non-toxicity.

The phospholipids suitable for use according to the invention include, for instance: phosphatidylcholine (lecithin), phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, dimyristoylphosphatidylcholine (DPMC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), palmitoyl-stearoylphosphatidylcholine, sphingomyelin, and the like.

The liposomes may also include additives that serve as stabilisers or surface charge modifiers, such as cholesterol, cholesterol sulphate and the like.

The aqueous solution contained in the vesicle consists of phosphate buffer or physiological solution.

The liposomes as described above encapsulate the active ingredient (be it in free form [PGE1] or in the form of an inclusion compound [PGE1- $\alpha$ -cyclodextrin]) and L-propionyl-carnitine, the purpose of which is to facilitate the beta-oxidation of the lipid chains, increasing the metabolism of the endothelial cells.

The external surface of the liposomes is then coated with hydrophilic polymers, such as poly-L-lysine (PLL), polyornithine and fibronectin, or mixtures thereof.

Poly-L lysine is preferred to ensure adhesion to the cells in culture plates, since it has been demonstrated that poly-L lysine makes the liposome adhere to the endothelial cells.

The small unilamellar vesicles (SUV) according to the invention can be prepared, using known techniques, starting from multilamellar liposome vesicles (MLV) by extrusion of the latter through polycarbonate filters, using a suitable extruder, or by homogenisation.

The SUV thus obtained are then coated with the hydrophilic polymers by "dropping" the liposomes drop by drop into the polymer solution under constant stirring.

The multilamellar liposome vesicles (MLV) can also be prepared, according to known methods, by dispersion of all the components (phospholipids, any stabiliser, PEG1 and/or PGE1- $\alpha$ -cyclodextrin, carnitine) in phosphate buffer (pH 7.4) under mechanical stirring at a temperature higher than the transition temperature ( $T_c$ ) of the phospholipid being used. The resulting MLV are used to obtain SUV by extrusion through a polycarbonate membrane or by homogenisation. The SUV thus obtained are then purified by elimination of the unencapsulated active ingredient by dialysis.

The formulations for systemic use therefore consist of the liposomes thus obtained suitably diluted - with saline solution, for instance - to obtain a formulation suitable, for example, for systemic administration by venous injection in a slow infusion over 24 hours.

If formulations for topical use (e.g. for the treatment of ulcers or retinopathies, as mentioned previously) are required, the liposomes are dispersed in suitable polymer films.

Said films are known and are prepared using organic polymers, such as sodium hyaluronate, hydroxypropylcellulose (HPMC), polyethylene glycol 400 (PEG 400) and water, in appropriate proportions, and are characterised on the basis of their visco-elastic properties, thickness and bio-adhesion in vitro, using a rheometer, a micrometer and a tensiometer, respectively.

The films are then used to prepare the medication for in situ application consisting, for instance, of strips of various sizes to be applied over lesions or on the surface to be treated. They may also be in the form of contact lenses to apply directly to the eye for the treatment of retinopathies.

If required, the formulations can also be lyophilised and subsequently reconstituted at the time of their use.

The efficiency (E%) of encapsulation of the active ingredient and the carnitine in the liposomes was determined by HPLC (obviously after having broken down the liposomes with a suitable membrane lysing agent, such as Triton X-100.)

The liposomes were characterised on the basis of their dimensional, polydispersion index (PI) and z potential, while their morphological and structural characteristics were studied under transmission electron microscope (TEM) and polarised-light optical microscope.

In vitro release studies were performed using Franz diffusion cells.

The coated and uncoated liposomes were then tested in vitro, using endothelial cells to assess the effect of the polymers used on the vesicle's adhesion to said cells.

The distribution of the PGE1 inside the cells was also evaluated.

The cells were treated with the liposomes coated with hydrophilic polymer and containing PGE1 (in free and complex form) . The liposomes were "marked" with hydrophilic and lipophilic dyes to enable them to be located inside the cell. At the end of the experiment, the cells were fixed and studied

under the microscope to ascertain the liposomes' internalisation and their location.

The invention is now further illustrated in the light of the examples given below.

5 Example 1

The liposomes were prepared in aseptic conditions using sterile raw materials. A solution of phosphatidylcholine (50mg/ml), PGE1 (free or compound) (60 $\mu$ g/ml) and L-propionyl-carnitine (10mg/ml) was used. The components were placed in a suitable sterile container with sterile aqueous medium (buffer, pH 7.4.)

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The dispersion was maintained under constant mechanical stirring for 2 hours. A homogeneous colloidal (vesicular) dispersion of multilamellar liposome (MLV) was thus obtained.

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The MLV were then extruded using polycarbonate filters with a pore diameter of 50 nm to obtain small unilamellar vesicles (SUV).

The unilamellar liposomes were then dialysed to remove any unencapsulated drug.

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The resulting liposomes were then "poured drop by drop" into a solution of polylysine (MW 150,000-300,000) at a concentration corresponding to (0.01-1%) and maintained under constant stirring for 2-3 hours to guarantee the electrostatic interaction of the (positively charged) hydrophilic polymer with the (negatively charged) liposomal surface. The interaction between the liposome and the polymer was identified from the variation in the surface charge (the z potential becomes positive) and from the dimensions of the vesicles (from 60 nm to 98 nm).

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The resulting liposomes can then be lyophilised and preserved at room temperature, and they can be used for the preparation of formulations for systemic administration, e.g. for dilution in a 0.09% saline solution.

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Alternatively, if formulations for topical application need to be prepared, the coated or uncoated liposomes are incorporated in polymer films for the topical administration of the drug. The films are prepared using sodium hyaluronate,

hydroxypropylcellulose (HPMC), polyethylene glycol 400 (PEG 400) and water, in appropriate proportions.

The films are characterised in vitro on the basis of their visco-elastic properties, thickness and bio-adhesion, using a rheometer, a micrometer and a  
5 tensiometer, respectively. Here again, the in vitro release studies are performed using Franz diffusion cells.

#### Liposome characterisation

The mean diameter of the liposomes was found to be 60 nm with a polydispersion index of 0.2.

10 The quantity of PGE 1 contained in the liposomes after purification was in the range of 30-50 µg/ml, while the quantity of carnitine was in the range of 0.05 to 0.2 mg/ml.

The liposomes were characterised on the basis of their dimensions, polydispersion index (PI) and z potential, respectively, by photon correlation  
15 spectroscopy (PCS) (dimensions and PI) and M3-PALS (phase analysis light scattering), which measures the electrophoretic mobility of the particles in a thermostat-controlled cell (z potential), using the Zetasizer Nano (Malvern Instruments, UK).

## CLAIMS

1. Unilamellar liposomes comprising PGE1 in combination with l-carnitine wherein on the external surface of said liposomes hydrofile polymers are present.
- 5 2. Unilamellar liposomes according to Claim 1 comprising natural or synthetic phospholipides chosen among: lecithene, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerole, phosphatidylinositole, dimirystofosfatidylcoline, - DPMC, dipalmitoylphosphatidylcoline DPPC, distearoylphosphatidylcoline DSPC, palmitoyl-stearoylphosphatidylcoline, sphingomyeline possibly in combination with additives acting as stabiliser or  
10 modifier of the superficial charge..
3. Unilamellar liposomes according to Claim 1 wherein said PGE1 is E1 prostaglandine both in free form or as inclusion complex PGE1- $\alpha$ -cyclodextrine.
- 15 4. Unilamellar liposomes according to Claim 1 wherein said hydrofile polymers are chosen among: Poly-L-Lisine - PLL, polyornithine and fibronectine or mixtures thereof.
5. Process for the preparation of liposomes according to Claims 1 -4 wherein said liposomes are obtained by homogenisation or extrusion through  
20 polycarbonate filters of multi-lamellar liposomes.
6. Formulations for systemic administration comprising liposomes according to Claims 1 - 4.
7. Formulations for topic application containing liposomes according to Claims 1 - 4.
- 25 8. Formulations according to Claim 7 wherein said formulations comprise liposomes according to claims 1 - 4 dispersed in polymer films.
9. Formulations according to Claim 8 wherein said polymer films are prepared using organic polymers chosen among: sodium hyaluronate, hydroxypropylcellulose - HPMC, polyethylenglycole400 and water in suitable  
30 ratios.



10: Formulations according to Claim 9 consisting of strips to be applied "in situ".

# INTERNATIONAL SEARCH REPORT

International application No PCT/IB2011/050463
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<b>A. CLASSIFICATION OF SUBJECT MATTER</b> INV. A61K47/48      A61K9/127      A61P9/00      A61P17/00      A61P27/02 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
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 practical, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TOYOTA T ET AL: "Lipo-PGE1, a new lipid-encapsulated preparation of prostaglandin E1: Placebo-and prostaglandin E1-controlled multicenter trials in patients with diabetic neuropathy and leg ulcers", PROSTAGLANDINS, BUTTERWORTH, STONEHAM, MA, US LNKD- DOI:10.1016/0090-6980(93)90081-H, vol. 46, no. 5, 1 November 1993 (1993-11-01), pages 453-468, XP023446007, ISSN: 0090-6980 [retrieved on 1993-11-01] pages 453-454 -----	1-10
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Date of the actual completion of the international search	Date of mailing of the international search report	
18 April 2011	29/04/2011	
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