PROLIPOSOME POWDERS FOR INHALATION STABILISED BY TOCOPHEROL

Field of the invention

The present invention relates to proliposome powders, particularly for inhalation, a process for producing the proliposome powders, compositions containing the proliposome powders and methods for their use.

Technical background

Liposomes are membrane-like vesicles consisting of series of concentric lipid bilayers alternating with hydrophilic compartments. They can be made from a variety of natural and synthetic lipids such as natural and synthetic phosphoglycerolipids, sphingolipids, and digalactosylglycerolipids. One of the main uses for liposomes has been as carriers for different kinds of pharmaceutically active components, in order to improve drug delivery and to minimise side-effects of some treatments. The pharmaceutically active components can be incorporated into liposomes either by encapsulation in hydrophilic compartments of the liposome (when the active component is water-soluble), or by encapsulation into the lipid bilayers, when the active component is lipophilic.

One of the major problems associated with pharmaceutical liposomal formulations is the long-term stability. Aqueous liposome dispersions have a limited stability due to aggregation, loss of the encapsulated active component to the external phase, chemical degradation of the active component or the lipid material, etc.

These problems can to a large extent be overcome if a solid composition is used. Such a solid composition can comprise a liposome powder, i.e. a dried liposome dispersion or a proliposome powder.

WO 96/19199 discusses a variety of liposome and proliposome literature and describes a proliposome powder. The powder contains, in a single phase, discrete particles that contain

a biologically active component and a lipid or mixture of lipids having a phase transition temperature (Tc) of below 37° C.

It has now been found that the stability of the proliposome powder of WO 96/19199 can be increased to a considerable extent.

Disclosure of the invention

The present invention provides a proliposome powder, said powder comprising discrete particles each comprising in a single phase (1) a biologically active component, (2) a stabilising proportion of tocopherol, and (3) a lipid or mixture of lipids having a phase transition temperature of below 37° C.

Preferably the tocopherol is α-tocopherol, and more preferably racemic α-tocopherol.

The powder is particularly suitable for administration by inhalation.

The single phase powder may alternately be described as comprising a homogeneous molecular mixture of a biologically active component, a lipid or mixture of lipids having a phase transition temperature of below 37°C, and tocopherol.

It will be understood from the terms "single phase" and "homogeneous molecular mixture" that there is no separate crystalline phase of the active component, the lipid or the tocopherol in the powder of the present invention.

The single phase powder can be inhaled directly, and in situ, for example in the upper or lower respiratory system, will form liposomes in which the biologically active component is incorporated.

In general, any amphipathic lipid or mixture of lipids known to be suitable for preparing liposomes by known methods could be used in the present invention. The lipid or lipid mixture must have a phase-transition temperature below body temperature (37°C) in order for the product proliposome powder to be capable of hydration under physiological conditions (i.e. in order to be able to form liposomes in the respiratory system). Phase-transition temperatures for different lipid mixtures may be estimated easily, using well-established methods, for example DSC methods - see for example J. Suurkuusk et al., Biochemistry, vol. 15, no.7, p. 1393 (1976). In general any natural or synthetic lipid or mixture of lipids having a phase transition temperature below 37°C is useful in the present invention.

As examples of potentially useful lipids may be mentioned natural and synthetic lipids such as natural and synthetic phosphoglycerolipids, sphingolipids, and digalactosylglycerolipids. Amongst natural lipids may be mentioned sphingolipids (SL) such as sphingomyelin (SM), ceramide and cerebroside; galactosylglycerolipids such as digalactosyldiacylglycerol (DGalDG); phosphoglycerolipids such as egg-yolk phosphatidylcholine (e-PC) and soya-bean phosphatidylcholine (s-PC); and lecithins such as egg-yolk lecithin (e-lecithin) and soya-bean lecithin (s-lecithin). Amongst synthetic lipids may be mentioned dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC), dilauryl phosphatidylcholine (DLPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl -2-myristoyl phosphatidylcholine (PMPC), and dioleoyl phosphatidycholine (DOPC). Amongst mixtures of lipids may be mentioned the following: SM/PC, SM/Cholesterol, ePC/Cholesterol, sPC/Cholesterol, PC/PS/Cholesterol, DMPC/DPPC, DMPC/DPPC/CH, DMPC/CH, DPPC/DOPC, DPPC/DOPC/CH, DLPC/DPPC, DLPC/DPPC/CH, DLPC/DMPC, DLPC/DMPC/CH, DOPC/DSPC, DPSM/DMSM, e-lecithin/Cholesterol and s-lecithin/Cholesterol. In addition to any of the above there may be included a charged lipid such as dimyristoyl phosphatidylglycerol (DMPG), diphospalmitoyl phosphatidylglycerol (DPPG), dimyristoyl phosphatidic acid (DMPA), dipalmitoyl phosphatidic acid (DPPA) or stearylamine (SA).

Lipids of particular interest in the present invention are DPPC and / or DMPC. A mixture of DPPC and DMPC containing at least 10% (w/w) DMPC is preferred, for example 10-50% DMPC. Especially preferred is a mixture of DPPC and DMPC containing in addition at least one charged lipid such as DMPG, DPPG, DMPA or SA, for example in an amount of up to 5% (w/w). Other preferred mixtures include DPSM and DMSM optionally containing at least one charged lipid, and mixtures of cholesterol with either e-lecithin or s-lecithin, optionally containing at least one charged lipid, and having a T_c of less than 37°C. Other mixtures can be selected easily by a person skilled in the art with reference for example to Gregor Cevc, Phospholipids Handbook, Marcel Dekker, New York (1993) pp 427-435.

The tocopherol is preferably present in a proportion of 0.05 to 1.0%, more preferably 0.1 to 0.6%, by weight of the total single phase containing the lipid(s) and the biologically active component.

The active component preferably has a molecular structure which can be incorporated into the lipid bilayers, to aid encapsulation in the liposomes during hydration. An example of such a molecular structure is a fatty acid ester having a long hydrocarbon chain sufficient to act as hydrophobic anchor.

Suitable active components can be identified readily by a person skilled in the art and may include, for example, antiinflammatory and bronchorelaxing drugs, antihistamines, cyclooxygenase inhibitors, leukotriene synthesis inhibitors, leukotriene antagonists, phospholipase-A2 (PLA2) inhibitors, platelet aggregating factor (PAF) antagonists and prophylactics of asthma. Antiarrhythmic drugs, tranquilisers, cardiac glycosides, hormones, anti-hypertensive drugs, antidiabetic, antiparisitic and anticancer drugs, sedatives, analgesic drugs, antibiotics, antirheumatic drugs, immunotherapies, antifungal drugs, antihypotension drugs, vaccines, antiviral drugs, proteins, peptides and vitamins, may also be of interest.

Specifically, glucocorticosteroids such as budesonide, fluticasone propionate, ciclesonide, rofleponide, e.g. as its palmitate, momethasone, e.g. as its furoate, tipredane, RPR 106541, dexamethasone, betamethasone, fluocinolone, flumethasone, triamcinolone acetonide, flunisolide, beclomethasone and 16, 17-acetals of pregnane derivatives and compounds derived therefrom and β-2 agonists such as terbutaline, salmeterol, salbutamol, formoterol, fenoterol, clenbuterol, procaterol, bitolterol, and broxaterol may be useful in the present invention. The active component may also be a mixture of pharmaceutically active substances; for example a mixture of a glucocortico-steroid with a bronchodilator such as formoterol, salmeterol, terbutaline or salbutamol, may be useful. For the avoidance of doubt, where a reference to any active component is made herein, said reference is intended to include a reference to pharmaceutically acceptable esters, salts, and hydrates thereof.

Where the active component is a steroid it is preferably a steroid ester.

The active component is preferably a steroid, preferably a steroid which is esterified in the 21-position with a saturated or unsaturated fatty acid of at least 8, for example at least 10 or at least 12 carbon atoms. The fatty acid may have, for example, up to 24 carbon atoms, for example up to 20 carbon atoms or up to 18 carbon atoms. More preferably, the active component is a steroid-21-palmitate, myristate, laurate, caprate, caprylate or stearate. The most preferred active component according to the invention is the compound (22R)-16α,17α-butylidenedioxy-6α,9α-difluoro-11β-hydroxy-21-palmitoyloxypregn-4-ene-3,20-dione, i.e. rofleponide palmitate.

Where the active component is an ester it must be capable of being hydrolysed to the active principal. Surprisingly, the single phase proliposome powder of the present invention facilitates the necessary hydrolysis in situ, whereas such esters in the crystalline state will not generally be hydrolysed.

Where delivery by inhalation is desired, as much as possible of the proliposome powder of the present invention should consist of particles having a diameter of less than 10 microns,

for example 0.01-10 microns or 0.1-6 microns, for example 0.1-5 microns, or agglomerates of said particles. Preferably at least 50% of the powder consists of particles within the desired size range. For example at least 60%, preferably at least 70%, more preferably at least 80% and most preferably at least 90% of the powder consists either of particles within the desired size range or of agglomerates of said particles.

The proliposome powders of the present invention need not contain other ingredients. However pharmaceutical compositions containing the powders of the present invention may also include other pharmaceutically acceptable additives such as a pharmaceutically acceptable adjuvent, diluent or carrier. These may be added to the proliposome composition after any micronisation, or before any micronisation provided that the solvent has been completely removed. Any carrier is preferably a crystalline, hydrophilic substance. A preferred carrier is crystalline lactose monohydrate. Other suitable carriers include glucose, fructose, galactose, trehalose, sucrose, maltose, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, starch, xylitol, mannitol, myoinositol, and the like, and hydrates thereof, and amino acids, for example alanine, and betaine.

The amount of additives present in the formulation may vary over a very wide range. In some circumstances little or no additive may be required, whereas for example it is often preferable to dilute a powder with additive, in order to improve the powder properties for use in an inhaler. In the latter case, for example, at least 50%, for example at least 70% or at least 80% of the formulation may be made up of additives, the remainder being the proliposome powder. The percentage of additives may also be dependant on the potency of the biologically active compound and the optimal amount of powder for inhalation.

Where an additive, for example a carrier is present, the entire composition may be in the form of particles of a size within the respirable particle size range. Alternatively the carrier may comprise coarser particles, of for example mass median diameter greater than 20 microns, or it may comprise agglomerates of the smaller particles, the agglomerates having

a mass median diameter of for example greater than 20 microns, so that in either case an ordered mixture of proliposome and carrier is formed.

A further object of the present invention is the provision of a process for the preparation of the proliposome powder of the present invention, i.e. a process which yields the proliposome powder in a single phase.

Accordingly, the present invention also provides a process for the preparation of a proliposome powder for inhalation, comprising dissolving a lipid or mixture of lipids, tocopherol and a lipophilic biologically active component in a solvent, said lipid or mixture of lipids having a phase transition temperature below 37°C; obtaining a crystalline solvent matrix and a single lipid phase in its glassy state by freezing the solution, said freezing being carried out at a temperature below the phase transition temperature of the lipid phase; and evaporating the frozen solvent at a temperature below the phase transition temperature of the lipid phase.

Freezing of the solution and solvent evaporation may be effected by conventional methods, for example in a conventional freeze-drier. For example the solution of lipid(s), tocopherol and biologically active component may be poured onto the shelves of a freeze-drier and the temperature lowered to freeze the solution. Solvent evaporation may then be achieved for example by lowering the pressure in the freeze-drying chamber; the resulting powder may be scraped from the shelves of the chamber and optionally passed through a sieve.

The freeze-dried powder may if necessary be subjected to further processing in order to obtain particles within the respirable particle size range; for example the freeze-dried powder may be micronised to give respirable particles, for example using an air jet mill.

The freezing of the solution of biologically active component, tocopherol and lipid(s) is carried out in a manner which produces a single lipid phase in the frozen solvent matrix. The production of a single lipid phase is controlled by the final temperature and the rate of freezing of the solution; the optimum rate of freezing of any particular solution will be

somewhere between the time necessary for crystallisation of the solvent in question and the time necessary for crystallisation of the lipid(s), tocopherol and active component and may be determined by a person skilled in the art, simply by trial and error. The optimal final temperature should be 10-20°C below the glass transition temperature of the lipid phase. For example a powder X-ray method may be used to monitor crystallinity and a differential scanning calorimeter may be used for monitoring the degree of incorporation of biologically active component into the liposomes after hydration.

The solvent must have the capacity to dissolve the lipid(s), tocopherol and the biologically active component completely since it is essential that all the components are in solution prior to freezing in order to avoid precipitation or phase-separation which will give rise to a powder with more than one phase. In addition the solvent should be toxicologically acceptable, have an appropriate freezing point and preferably a high vapour pressure. The solvent may be for example an organic solvent, for example an alcohol, or a mixture of aqueous and organic solvents. The preferred solvent for use in the present invention is tertiary butanol.

The powder may optionally be agglomerated into small spheres, in order to control the cohesiveness of the powder. The spheres should preferably be not larger than 1 mm in diameter; spheres larger than this may be removed for example by sieving. Any agglomerates should be friable, so that they may easily be deagglomerated for example in the air stream generated in a powder inhaler.

The proliposome powder of the present invention is useful for the local or systemic treatment of diseases and may be administered for example via the upper and lower respiratory tract, including by the nasal route. As such the present invention also provides said proliposome powder for use in therapy; the use of the proliposome powder in the manufacture of a medicament for the treatment of diseases via the respiratory tract; and a method for the treatment of a patient in need of therapy, comprising administering to said

patient a therapeutically effective amount of the proliposome powder of the present invention.

For example the proliposome powder of the present invention may be used in the treatment of inflammmatory diseases in the respiratory tract, for example asthma, rhinitis, alveolitis, bronchiolitis and bronchitis.

Administration to the respiratory tract may be effected for example using a dry powder inhaler or a pressurised aerosol inhaler.

Suitable dry powder inhalers include dose inhalers, for example the single dose inhaler known by the trade mark Monohaler ® and multi-dose inhalers, for example a multi-dose, breath-actuated dry powder inhaler such as the inhaler known by the trade mark Turbuhaler®.

While the proliposome powder of the present invention is particularly adapted for administration by inhalation, it may also be included in formulations adapted for other forms of delivery. For example oral, topical and injectable formulations may be prepared, for use in the treatment of for example inflammatory joint diseases, for example arthritis, skin diseases, and intestinal bowel diseases.

The following Examples are intended to illustrate, but not limit, the scope of the invention. The parts are by weight.

Example 1

Rofleponide palmitate (10 parts), DPPC (63 parts), DMPC (24 parts), NaDPPG (3 parts), and racemic α-tocopherol (0.1 part) were dissolved in tertiary butanol (1300 parts) at 80°C. The solution was poured onto the shelves of a freeze-dryer cooled to -35°C. The solution had reached this temperature after about 30 minutes; the pressure in the freeze-dryer was then reduced in order to induce sublimation of the solvent. While the rate of sublimation

could be adjusted by decreasing the pressure and increasing the temperature, the temperature throughout the process was not allowed to exceed -10°C. Freeze-drying was continued until all the solvent had been removed. The resulting powder was scraped from the shelves of the freeze-dryer and passed through a sieve.

This powder was micronised in an air jet mill to a powder particle size of less than 5 μm. The micronised powder was mixed with lactose monohydrate (20 parts powder: 80 parts lactose monohydrate) by a sieving process and the mixture further homogenised by micronising at low pressure, in a jet mill.

The powder mixture was agglomerated into spheres no larger than 1 mm, using standard techniques. Larger spheres were removed by sieving. The agglomerated powder was filled into Turbuhaler® dry powder inhaler.

In separate experiments the amount of α-tocopherol in the above formulation was changed to 0.06 parts and 0.6 parts respectively.

Surprisingly it was found that the proliposome formulations of Example 1 are more stable than equivalent formulations containing other antioxidants.

Powder analysis

X -ray powder diffraction carried out on the powder mixture of Example 1 showed that no crystalline state was present in the powder.

Incorporation of active component into the liposomes

The proliposome powders of Example 1 were hydrated and the degree of incorporation of the active component was measured using differential scanning calorimerty methods. The DSC showed that the active component was fully incorporated into the liposomes.

Claims

1. A proliposome powder, said powder comprising discrete particles each comprising in a single phase (1) a biologically active component, (2) a stabilising proportion of tocopherol, and (3) a lipid or mixture of lipids having a phase transition temperature of below 37° C.

2. A powder according to claim 1, wherein the tocopherol is α-tocopherol.

3. A powder according to claim 2, wherein the tocopherol is racemic α-tocopherol.

4. A powder according to any one of the preceding claims, wherein the tocopherol is present in a proportion of 0.05 to 1.0% by weight of the single phase.

5. A powder according to claim 4, wherein the tocopherol is present in a proportion of from 0.1 to 0.6% by weight.

6. A powder according to any one of the preceding claims, comprising one or more lipids selected from the group consisting of natural and synthetic phosphoglycerolipids, sphingolipids and digalactosylglycerolipids.

7. A powder according to any one of the preceding claims, comprising a mixture of lipids selected from the group consisting of SM/PC, SM/Cholesterol, ePC/Cholesterol, sPC/Cholesterol, PC/PS/Cholesterol, DMPC/DPPC, DMPC/DPPC/CH, DMPC/CH, DPPC/DOPC, DPPC/DOPC/CH, DLPC/DPPC, DLPC/DPPC/CH, DLPC/DMPC, DLPC/DMPC/CH, DOPC/DSPC, DPSM/DMSM, e-lecithin/Cholesterol and s-lecithin/Cholesterol.

8. A powder according to any one of the preceding claims, comprising DPPC or DMPC, or a mixture of DPPC and DMPC.

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9. A proliposome powder according to claim 8, wherein the mixture comprises at least 10% DMPC.

10. A powder according to any one of the preceding claims, additionally including a charged lipid.

11. A powder according to claim 10, wherein the charged lipid is selected from the group consisting of dimyristoyl phosphatidylglycerol (DMPG), diphospalmitoyl phosphatidylglycerol (DPPG), dimyristoyl phosphatidic acid (DPPA), and stearylamine (SA).

12. A powder according to any one of the preceding claims, wherein the active component comprises a glucocorticosteroid.

13. A powder according to claim 12, wherein the active component comprises a glucocorticosteroid which is esterified in the 21 position with a fatty acid of at least 8 carbon atoms.

14. A powder according to claim 13, wherein the active component comprises a glucocorticosteroid which is esterified in the 21 position with a fatty acid of at least 10 carbon atoms.

15. A powder according to claim 14, wherein the active component comprises a glucocorticosteroid-21-palmitate.

16. A powder according to claim 15, wherein the active component comprises rofleponide palmitate.

17. A powder according to any one of the preceding claims, wherein at least 50% of the powder consists of particles having a diameter of less than 10 microns.

18. A powder according to claim 17, wherein at least 50 % of the powder consists of particles having a diameter of 0.01-10 microns.

19. A powder according to claim 18, wherein at least 50% of the powder consists of particles having a diameter of 0.1-6 microns.

20. A powder according to any one of the claims 17-19, comprising agglomerated particles.

21. A pharmaceutical composition comprising a powder according to any one of the preceding claims and a pharmaceutically acceptable carrier.

22. A pharmaceutical composition according to claim 21, wherein the carrier is crystalline lactose monohydrate.

23. A pharmaceutical composition according to either of claims 21 or 22, wherein the carrier comprises particles of mass median diameter less than 10 microns or agglomerates of said particles.

24. A dry powder inhaler device containing a proliposome powder according to any one of the preceding claims.

25. A dry powder inhaler device according to claim 24, wherein the inhaler is a single dose inhaler.



26. A method of treating a patient in need of therapy with a given biologically active compound, comprising administering to said patient a therapeutically effective amount of a powder according to any one of claims 1 to 20 or a composition according to any one of claims 21 to 23.

27. Use of a powder according to any one of claims 1-20 in the preparation of a formulation for use in therapy.

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