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(54) Title: SUSTAINED RELEASE OF MICROCRYSTALLINE PEPTIDE SUSPENSIONS

(57) Abstract: The invention relates to a method of preventing gel formation of a hydrophobic peptides by contacting the hydrophobic peptide with a counter-ion in an amount and at a molar ratio sufficient to provide a fluid, milky microcrystalline aqueous suspension of the peptide without formation of a gel. The invention also relates to a fluid, milky microcrystalline aqueous suspension of a hydrophobic peptide and a counter-ion in water, wherein the peptide and counter-ion are present in amounts and at a molar ratio sufficient to form, upon mixing, the suspension without formation of a gel.

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SUSTAINED RELEASE OF MICROCRYSTALLINE PEPTIDE SUSPENSIONS

BACKGROUND OF THE INVENTION

There is frequently a need to deliver biologically active peptides to animals and humans in formulations providing a sustained release of the active principle. Such formulations may be provided by incorporating the active principle in biodegradable and biocompatible polymers in form of microcapsules, microgranules or implantable rods, or alternatively using mechanical devices such as micropumps or non-biodegradable containers. If the peptide is highly soluble in aqueous media, it can be formulated as a complex with non-degradable polymers such as cellulose derivatives, or mixed with polymer solutions, which form a gel upon parenteral injection, from which the active peptide is slowly released.

All the above-mentioned formulations have drawbacks and limitations, such as the large volume of suspending fluids or the need to remove the non-degradable device. In the case of gel forming peptides, there is frequently a problem of bioavailability, which interferes with the desired sustained action of the active principle.

Some of the problems due to physico-chemical aspects of peptides have been described in article by R. Deghenghi "Antarelix" in Treatment with GnRH Analogs: Controversies and Perspectives", edited by M. Filicori and C. Flamigni, The Parthenon Publishing Group, New York and London 1996, pages 89-91. Additional problems were illustrated by J. Rivier "GnRH analogues towards the next millennium" in GnRH Analogues, edited by B. Lunenfeld, The Parthenon Publishing Group, New York and London 1999, pages 31-45 and by other workers such as M.F. Powell et al. "Parenteral Peptide Formulations: Chemical and Physical Properties of Native LHRH and Hydrophobic Analogues in Aqueous Solution" in Pharmaceutical Research, Vol. 8, 1258-1263 (1991).

Accordingly, there is a need for new formulations and methods of administration that avoid these problems, and this need is addressed by the present invention.

SUMMARY OF THE INVENTION

A first aspect of the present invention provides a method of preventing gel formation of a hydrophobic peptide which comprises contacting the hydrophobic peptide with a counter-ion derived from a strong acid in an amount and at a molar ratio sufficient to provide a fluid, milky microcrystalline aqueous suspension of the peptide without formation of a gel.

A second aspect of the present invention also provides a fluid, milky microcrystalline aqueous suspension of a hydrophobic peptide and a counter-ion derived from a strong acid in water, wherein the peptide and counter-ion are present in amounts and at a molar ratio sufficient to form, upon mixing, the suspension without formation of a gel.

In an embodiment, the avoidance of a gel enables an injectable suspension to be formulated. These aqueous suspensions can be injected parenterally (i.e., subcutaneously or intramuscularly) into a mammal, such as a human, so that a sustained release of the hydrophobic peptide over time can be obtained.

Preferably, the counter-ion is a salt of a strong acid, such as trifluoroacetic acid or sulfuric acid. Also, the hydrophobic peptide may be a GnRH analogue, and preferably is a GnRH antagonist. More preferred GnRH antagonists are selected from the groups of Azaline B, Abarelix, Antide, Ganirelix, Cetrorelix, or FE200486 in the form of their strong acid salts, e.g., trifluoroacetate or sulfate salts. Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 trifluoroacetate and Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 sulfate are the most preferred compounds.

The hydrophobic peptide salt is preferably suspended in the aqueous medium at a concentration of equal to or higher than 25 mg/ml and has a molar ratio of at least 1.6:1 of acid:peptide. The peptide salt is at least partially in the form of needles having a particle size of between about 5 and 150 µm.

If desired, the aqueous suspension can contain an isotonic agent, such as mannitol. Also, the aqueous suspension may contain a pharmaceutically acceptable excipient. Advantageously, the suspension can be dried to a lyophilized state which can be reconstituted by mixing with water or a buffer solution. Lyophilized compositions comprising these dried suspensions, as well as the methods for making the dried suspensions, represent additional embodiments of the invention.

Accordingly, a third aspect of the present invention provides a lyophilized composition comprising the dried suspension as defined above.

A fourth aspect of the present invention provides a method of preparing a sustained release formulation of a hydrophobic peptide which comprises contacting the hydrophobic peptide with a counter-ion derived from a strong acid, wherein the peptide and counter-ion are present in amounts and at a molar ratio sufficient to form, upon mixing, the suspension without formation of a gel.

A fifth aspect of the present invention provides a method of preparing a lyophilized composition as defined in the third aspect which comprises a method as defined above followed by freeze-drying or spray-drying to obtain the composition.

A sixth aspect of the present invention provides a method of preparing an injectable fluid, milky, microcrystalline aqueous suspension of a hydrophobic peptide which comprises reconstituting with water or a buffer solution the lyophilized composition as defined in the third aspect.

A seventh aspect of the present invention provides a microcrystalline aqueous suspension of Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 trifluoroacetate.

An eighth aspect of the present invention provides a microcrystalline aqueous suspension of Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 sulfate.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph which illustrates the pharmacodynamic effect (testosterone suppression) obtained by subcutaneous injection in rats of a suspension of teverelix® trifluoroacetate according to the invention; and

Figure 2 is graph which illustrates the sustained release of the peptide teverelix® for several weeks in rats injected with the suspension of teverelix® trifluoroacetate according to the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

We made the unexpected discovery that a highly concentrated aqueous suspension of the peptide of the formula Ac-D-Nal-D-pClPhe-D-Pal-Ser-Tyr-D-Hci-Leu-Lys(iPr)-Pro-D-Ala-NH_2 (teverelix®, a GnRH antagonist) as a trifluoroacetate (TFA) or sulfate salt does not, as might be expected by its hydrophobic character, form a gel but instead forms a microcrystalline milky suspension which is easy to inject parenterally in animals or humans, and which releases the active principle over several weeks (see Figures 1 and 2). Such

behavior is not elicited by other salts such as the acetate, which result in the expected, but unwanted, formation of gels with poor bioavailability *in vivo*.

Our invention represents a simple and elegant solution of the problem of how to suppress gelation of hydrophobic peptides while obtaining a prolonged sustained delivery of such peptides in form of microcrystalline highly concentrated suspensions.

An additional advantage of our discovery is the small volume of such suspensions, allowing parenteral injections through a fine needle and thus improving the local tolerance of the injected material. In such injections, the amount of peptide ranges from about 0.1 to 5mg per kg body weight of the mammal to which the suspension is to be administered.

The amount of counter-ion is preferably that which is in excess of what is necessary to form the salt. This amount is typically at least 1.6 mol acid/mole peptide and preferably 2mol/mol or greater. In addition, the injectable suspension should be concentrated to obtained the most desirable release profiles. By concentrated, we mean that the amount of peptide should be above 2.5% by weight of the overall formulation.

The suspensions can be dried by freeze-drying or spray drying to form lyophilized compositions that can be stored as is and reconstituted with water or buffer solutions when an injectable formulation is to be prepared.

EXAMPLES

Example 1

200µL of 5% mannitol were added to approximately 15mg of the LHRH antagonist teverelix® trifluoroacetate. The mixture was stirred using vortex during one minute and a flowing milky pearly suspension was obtained. The suspension is made of microcrystals of about 10µm length. Microcrystals may clump together to form urchin like structures. The suspension was injected in rats

(1mg) sub-cutaneously and provided the pharmacodynamic effect of testosterone suppression for more than 45 days (Figure 1). The pharmacokinetic analysis showed a sustained release of the peptide for several weeks (Figure 2).

Example 2

200µL of water were added to approximately 15mg of the LHRH antagonist teverelix® trifluoroacetate. The mixture was stirred using vortex during one minute and a flowing milky pearly suspension was obtained.

Example 3

200µL of water were added to approximately 15mg of the LHRH antagonist teverelix® acetate. The mixture was stirred using vortex during one minute and a transparent gel was obtained. The addition of 20µL of TFA (3mols/mol) to the gel resulted in the formation of a fluid, flowing milky pearly suspension.

Example 4

200µL of 100mM TFA were added to approximately 15mg of the LHRH antagonist teverelix® acetate (2mols/mol) to obtain a flowing milky suspension. In addition, mixing 200µL of 75mM TFA with approximately 15 mg of the LHRH antagonist teverelix® acetate (1.5mol/mol) resulted in a transparent gel being obtained after mixing. In another study, 100µL of TFA of various concentrations were added to 7.5mg of the LHRH antagonist teverelix® acetate, with the TFA/Teverelix molar ratio ranging from 1 to 3. A flowing milky suspension was obtained with molar ratios of ≥ 1.6, whereas gels were obtained at other molar ratios.

Example 5

200µL of 150mM TFA were added to amounts of the LHRH antagonist teverelix® acetate ranging from 5 to 30mg (concentration ranging from 25 to 150mg/ml). A flowing milky suspension was obtained with concentrations up to 100mg/ml.

Example 6

200µL of 150mM TFA were added to approximately 15mg of the LHRH antagonist teverelix® acetate (3mols/mol) and a flowing milky suspension was obtained after mixing. The suspension was freeze-dried over-night. 200µL of water or 5% mannitol were added to the lyophilisate and a flowing milky suspension was obtained after mixing and reconstitution.

Example 7

1mL of 150mM TFA were added to approximately 75 mg of the LHRH antagonist teverelix® acetate (3mols/mol) and a flowing milky suspension was obtained after mixing. The suspension was freeze-dried over-night. 1mL of water and 0.2M acetate buffer pH 4.0 were added to the lyophilisate and a flowing milky suspension was obtained after mixing and reconstitution. These suspensions were stable for at least 3 days at room temperature.

Example 8

100µL of a 250mM H_2SO_4 were added to 7.5mg of the LHRH antagonist teverelix® acetate (5mols/mol) and a flowing milky suspension was obtained after several hours. The suspension is made of microcrystals of about 100µm length. Microcrystals may assemble together to form urchin like structures. The suspension was freeze-dried over-night. 100µL of water or 5% mannitol were added to the lyophilisate and a flowing milky suspension was obtained after mixing and reconstitution.

CLAIMS

1. A method of preventing gel formation of a hydrophobic peptide which comprises contacting the hydrophobic peptide with a counter-ion derived from a strong acid in an amount and at a molar ratio sufficient to provide a fluid, milky microcrystalline aqueous suspension of the peptide without formation of a gel.

2. The method of claim 1 wherein the counter-ion is derived from trifluoroacetic acid or sulfuric. acid.

3. The method of claim 1 or claim 2 in which the hydrophobic peptide is a GnRH analogue.

4. The method of claim 3 in which the GnRH analogue is a GnRH antagonist.

5. The method of claim 4 in which the GnRH antagonist is Ac-D-Nai-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-llys-Pro-D-Ala-NH_2 and the counter-ion is the trifluoroacetate ion.

6. The method of claim 4 in which the GnRH antagonist is Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-llys-Pro-D-Ala-NH_2 and the counter-ion is the sulfate ion.

7. The method of claim 4 in which the GnRH antagonist is selected from the group of peptides Azaline B, Abarelix, Antide, Ganirelix, Cetrorelix, Teverelix of FE200486 and the counter-ion is the trifluoroacetate ion or the sulfate ion.

8. The method of any one of claims 1 to 7 in which the hydrophobic peptide salt is suspended in the aqueous medium at a concentration of equal to or higher than 25 mg/ml.

9. The method of any one of claims 1 to 8 in which the aqueous suspension contains an isotonic agent.

10. The method of claim 9 in which the isotonic agent is mannitol.

11. The method of any one of claims 1 to 10 in which the aqueous suspension contains a pharmaceutically acceptable excipient.

12. The method of any one of claims 1 to 11 in which the aqueous suspension is obtained extemporaneously from a lyophilized peptide salt.

13. A fluid, milky microcrystalline aqueous suspension of a hydrophobia peptide and a counter-ion derived from a strong acid in water, wherein the peptide and counter-ion are present in amounts arid at a molar ratio sufficient to form, upon mixing, the suspension without formation of a gel.

14. The suspension of claim 13 wherein the counter-ion is derived from trifluoroacetic acid or sulfuric acid.

15. The suspension of claim 13 or claim 14 in which the hydrophobic peptide is a GnRH analogue.

16. The suspension of claim 15 in which the GnRH analogue is a GnRH antagonist.

17. The suspension of claim 16 in which the GnRH antagonist is Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-llys-Pro-D-Ala-NH_2 and the counter-ion is the trifluoroacetate ion.

18. The suspension of claim 16 in which the GnRH antagonist is Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-llys-Pro-D-Ala-NH_2 and the counter-ion is the sulfate ion.

19. The suspension of claim 16 in which the GnRH antagonist is selected from the group of peptides Azaline B, Abarelix, Antide, Ganirelix, Cetrorelix, or FE200486 and the counter-ion is the trifiuoroacetate ion or sulfate ion.

20. The suspension of any one of claims 13 to 19 in which the hydrophobic peptide salt is suspended in the aqueous medium at a concentration of equal to or higher than 25 mg/ml.

21. The suspension of any one of claims 13 to 20 in which the aqueous suspension contains an isotonic agent.

22. The suspension of claim 21 in which the isotonic agent is mannitol.

23. The suspension of any one of claims 13 to 22 which further comprises a pharmaceutically acceptable excipient.

24. The suspension of any one of claims 13 to 23 wherein the microcrystals are in the form of needles having a particle size of between about 5 and 150 µm.

25. A lyophilized composition comprising the dried suspension of any one of claims 13 to 24.

26. A method of preparing a sustained release formulation of a hydrophobic peptide which comprises contacting the hydrophobic peptide with a counter-ion derived from a strong acid, wherein the peptide and counter-ion are present in amounts and at a molar ratio sufficient to form, upon mixing, the suspension without formation of a gel.

27. The method of claim 26 wherein the counter-ion is derived from trifluoroacetic acid or sulfuric acid.

28. The method of claim *26* or claim 27 in which the hydrophobic peptide is a GnRH analogue.

29. The method of claim 28 in which the GnRH analogue is a GnRH antagonist.

30. The method of claim 29 in which the GnRH antagonists is Ac-D-Nal-D-Cpa-D-

Pal-Ser-Tyr-D-Hci-Leu-llys-Pro-D-Ala-NH_2 and the counter-ion is the trifluoroacetate ion.

31. The method of claim 29 in which the GnRH antagonists is Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-llys-Pro-D-Ala-NH_2 and the counter-ion is the sulfate ion.

32. The method of claim 29 in which the GnRH antagonist is selected from the group of peptides Azaline B, Abarelix, Antide, Ganirelix, Cetrorelix, Treverelix or FE200486 and the counter-ion is the trifluoroacetate ion or sulfate ion.

33. The method of any one of claims 26 to 32 in which the hydrophobic peptide salt is suspended in the aqueous medium at a concentration of equal to or higher than 25 mg/ml.

34. The method of any one of claims 26 to 33 in which the aqueous suspension contains an isotonic agent

35. The method of claim 34 in which the isotonic agent is mannitol.

36. The method of any one of claims 26 to 35 in which the aqueous suspension contains a pharmaceutically acceptable excipient.

37. The method of any one of claims 26 to 36 in which the aqueous suspension is obtained extemporaneously from a lyophilized peptide salt.

38. A method of preparing a lyophilized composition according to claim 25 which comprises a method as claimed in any one of claims 1 to 12 followed by freeze-drying or spray-drying to obtain the composition.

39. A method of preparing an injectable fluid, milky, microcrystalline aqueous suspension of a hydrophobic peptide which comprises reconstituting with water

or a buffer solution the lyophilized composition of claim 25.

40. A microcrystalline aqueous suspension of Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 trifluoroacetate.

41. A microcrystalline aqueous suspension of Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 sulfate.

42. The suspension of Claim 40 or Claim 41, which provides, when administered to a subject, a sustained release of peptide *in vivo.*

43. The suspension of any one of Claims 40 to 42 in which the salt is suspended in the aqueous medium at a concentration of equal to or higher than 25 mg/ml.

44. The suspension of any one of Claims 40 to 43 in which the aqueous suspension contains an isotonic agent.

45. The suspension of Claim 44 in which the isotonic agent is mannitol.

46. The suspension of any one of Claims 40 to 45, which further comprises a pharmaceutically acceptable excipient.

47. The suspension of any one of Claims 40 to 46 wherein the microcrystals are in the form of needles having a particle size of between 5 and 150 µm.