Summary

After extensive and thorough experimentation, the inventors have surprisingly discovered that maleimidobutyric acid N-hydroxysuccinimide ester (hereinafter, SM), a heterobifunctional crosslinking agent, in which each molecule thereof binds a peptide to the transport protein, used as a crosslinking agent for preparing CysAβ(33-40) peptide (SEQ ID NO: 1) and KLH conjugates, produces conjugates that allow generating an improved immune response in comparison to the immune response produced by conjugates generated with other homo- or heterobifunctional crosslinking agents of the prior art such as SPDP which also allow the binding of a peptide to the transport protein. Such improvement is clearly shown in examples 1 to 4 of the present specification. In addition, said examples show that said new conjugates or the pharmaceutical compositions comprising said conjugates produce:

- an immune response that is as specific as possible, for the purpose of minimizing the side effects associated with the (preventive or therapeutic) vaccine treatment;

- the highest possible immune response for the purpose of assuring an effective immunization of patients and reducing the required doses of immunogenic conjugate.

In one aspect, the present invention refers to a composition comprising a conjugate of at least one CysAβ(33-40) peptide (SEQ ID NO: 1) linked to the keyhole limpet hemocyanin (KLH), wherein the crosslinking agent connecting each CysAβ(33-40) peptide to the keyhole limpet hemocyanin (KLH) of the conjugate is the maleimidobutyric acid N-hydroxysuccinimide ester (SM), wherein the composition further comprises aluminum hydroxide gel.

In another aspect, the present invention refers to a pharmaceutical composition comprising i) a conjugate comprising at least one CysAβ(33-40) peptide (SEQ ID NO: 1) linked to the keyhole limpet hemocyanin (KLH) and ii) aluminum hydroxide gel, wherein the crosslinking agent connecting each CysAβ(33-40) peptide to the keyhole limpet hemocyanin (KLH) of the conjugate is the maleimidobutyric acid N-hydroxysuccinimide ester (SM), and wherein the pH of the composition ranges from 5.8 to 7.0.

In yet another aspect, the present invention refers to a glass ampoule comprising the pharmaceutical composition of the present invention.

In yet another aspect, the present invention refers to a method to manufacture a pharmaceutical composition, which comprises the following steps:

a. Adding maleimidobutyric acid N-hydroxysuccinimide ester (SM) to a solution comprising keyhole limpet hemocyanin (KLH) in a buffer at a pH between 7.0 to 9;
b. Eliminating the excess of maleimidobutyric acid N-hydroxysuccinimide ester from the solution of step a);
c. Adding CysAβ(33-40) peptides (SEQ ID NO: 1) in DMSO to the solution of step b) at a pH of between 6.6 to 7.0 to produce the conjugates;
d. Eliminating the free peptide from the solution from step c);
e. Optionally filtrating the solution of step d);
f. Adjusting the pH of the solution of step d) or e) to a pH range of between 5.8 to 6.2; and

g. Adding aluminum hydroxide gel to the solution of step f) once the pH has been adjusted.

In yet another aspect, the present invention refers to a use of the pharmaceutical composition as disclosed herein or the glass ampoule as disclosed herein in the manufacture of a medicament for treating or preventing an amyloid disease.

Thus in a first example, the present disclosure relates to a conjugate characterized in that the crosslinking agent is SM.

In another example, the present disclosure relates to a composition comprising the conjugate of the present disclosure.

In additional examples of the present disclosure, the use of said conjugate for preparing a medicinal product, more specifically, a medicinal product intended for the treatment or prevention of amyloid diseases, is contemplated.

Another example to which the present disclosure relates is to compositions comprising the conjugate of the present disclosure for use as a medicinal product, more specifically for use in the treatment or prevention of amyloid diseases.

Additionally, the present disclosure also relates to a method of treatment or prevention of an amyloid disease by means of the delivery of a composition comprising the conjugate of the present disclosure.

In yet another example, the present disclosure relates to a method of manufacturing antibodies based on the use of the conjugate of the present disclosure.

Detailed description of the invention

Definitions

As used herein, “amyloid disease” and the plural thereof refer to diseases associated with the β-amyloid

CLAIMS

1. A composition comprising a conjugate of at least one CysAβ(33-40) peptide (SEQ ID NO: 1) linked to the keyhole limpet hemocyanin (KLH), wherein the crosslinking agent connecting each CysAβ(33-40) peptide to the keyhole limpet hemocyanin (KLH) of the conjugate is the maleimidobutyric acid N-hydroxysuccinimide ester (SM), wherein the composition further comprises aluminum hydroxide gel.

2. A pharmaceutical composition comprising i) a conjugate comprising at least one CysAβ(33-40) peptide (SEQ ID NO: 1) linked to the keyhole limpet hemocyanin (KLH) and ii) aluminum hydroxide gel, wherein the crosslinking agent connecting each CysAβ(33-40) peptide to the keyhole limpet hemocyanin (KLH) of the conjugate is the maleimidobutyric acid N-hydroxysuccinimide ester (SM), and wherein the pH of the composition ranges from 5.8 to 7.0.

3. The pharmaceutical composition according to claim 2, wherein the pH of the composition ranges from 6.2 to 7.0.

4. The pharmaceutical composition according to claim 2, wherein the pH of the composition ranges from 5.8 to 6.2.

5. The pharmaceutical composition according to any of claims 2 to 4, wherein the amount of CysAβ(33-40) peptides (SEQ ID NO: 1) in the pharmaceutical composition is at least 100µg.

6. The pharmaceutical composition according to any of claims 2 to 4, wherein the amount of CysAβ(33-40) peptides (SEQ ID NO: 1) in the pharmaceutical composition is at least 150µg.

7. The pharmaceutical composition according to any of claims 2 to 4, wherein the amount of CysAβ(33-40) peptides (SEQ ID NO: 1) in the pharmaceutical composition is from 150µg to 400µg.

8. The pharmaceutical composition according to any of claims 2 to 4, wherein the amount of CysAβ(33-40) peptides (SEQ ID NO: 1) in the pharmaceutical composition is from 160µg to 240µg.

9. The pharmaceutical composition according to any of claims 2 to 8, wherein the conjugates in turn comprise a ratio of at least 45 CysAβ(33-40) peptides (SEQ ID NO: 1) linked to each keyhole limpet hemocyanin (KLH).

10. A glass ampoule comprising the pharmaceutical composition of any of claims 2 to 9.

11. A method to manufacture a pharmaceutical composition according to any of claims 2 to 9, which comprises the following steps:

a. Adding maleimidobutyric acid N-hydroxysuccinimide ester (SM) to a solution comprising keyhole limpet hemocyanin (KLH) in a buffer at a pH between 7.0 to 9;
b. Eliminating the excess of maleimidobutyric acid N-hydroxysuccinimide ester from the solution of

step a);

c. Adding CysAβ(33-40) peptides (SEQ ID NO: 1) in DMSO to the solution of step b) at a pH of between 6.6 to 7.0 to produce the conjugates;
d. Eliminating the free peptide from the solution from step c);
e. Optionally filtrating the solution of step d);
f. Adjusting the pH of the solution of step d) or e) to a pH range of between 5.8 to 6.2; and
g. Adding aluminum hydroxide gel to the solution of step f) once the pH has been adjusted.

12. The method of claim 11, wherein the excess of step b) is eliminated by using 0.02 M Na-Phosphate-buffer at a pH of 6.6 to 7.0.

13. The method of any of claims 11 or 12, wherein the excess of step d) is eliminated by using 0,01 M PBS-buffer at a pH of 6.6 to 7.0.

14. The method of any of claims 11 to 13, wherein the solution is filtrated in step e) by using a filter of 0.2 μm.

15. The method of any of claims 11 to 14, wherein the pH of step f) is adjusted to a pH range of 6.0.

16. Use of the pharmaceutical composition of any of claims 2 to 9 or the glass ampoule of claim 10in the manufacture of a medicament for treating or preventing an amyloid disease.

17. The use of claim 16, wherein the amyloid disease is selected from the list consisting of Alzheimer's disease, Parkinson's disease, cerebral amyloid angiopathy, vascular dementia of an amyloid origin, inclusion-body myositis and dementia with Lewy bodies.

18. The use of claim 17, wherein the amyloid disease is Alzheimer's disease.