

propensity of peptides to self-associate is connected with their physical instability, meaning their likelihood of forming aggregates. Moreover, excipients present in a peptide formulation can chemically degrade, interact with various surfaces during manufacturing, interact with the container or closure, or interact with the peptide itself, thereby negatively affecting critical properties of the preparation (Lars Hovgaard, and Sven Frokjaer, "Pharmaceutical Formulation Development of Peptides and Proteins, 2nd Ed., CRC Press (2012) pp. 212-213).

[0066] The described invention provides effective formulations comprising a cell-penetrating peptide fused to a peptide-based inhibitor of MK2.

SUMMARY

[0067] According to one embodiment, the disclosure provides a pharmaceutical formulation comprising a therapeutic amount of a polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA; SEQ ID NO: 1 or a functional equivalent thereof, wherein the formulation is characterized by preservation of stability and bioavailability of the polypeptide.

[0068] According to one embodiment, the pharmaceutical formulation is a particulate pharmaceutical formulation. According to another embodiment, the pharmaceutical formulation is an aerosolized pharmaceutical formulation. According to another embodiment, the formulation is prepared by a process of spray drying. According to another embodiment, the pharmaceutical formulation comprises 1% w/w solids. According to another embodiment, the pharmaceutical formulation comprises 5% w/w solids. According to another embodiment, the pharmaceutical formulation further comprises trehalose. According to another embodiment, the polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA; SEQ ID NO: 1 or the functional equivalent thereof and the trehalose are in a ratio of 80/20 respectively. According to another embodiment, the MMI-0100 (YARAAARQARAKALARQLGVAA; SEQ ID NO: 1) or the functional equivalent thereof and the trehalose are in a ratio of 92.5/7.5 respectively. According to another embodiment, the pharmaceutical formulation is delivered to a subject via a dry powder inhalation device (DPI).

[0069] According to one embodiment, the pharmaceutical formulation further comprises saline. According to another embodiment, the saline is NaCl. According to another

embodiment, the polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA; SEQ ID NO: 1 or the functional equivalent thereof is at a concentration of 0.7 mg/mL.

According to another embodiment, the polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA; SEQ ID NO: 1 or the functional equivalent thereof is at a concentration of 7.0 mg/mL. According to another embodiment, the pharmaceutical formulation is delivered to a subject via a nebulizer.

[0070] According to one embodiment, the pharmaceutical formulation comprises an ionic complex of a polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA; SEQ ID NO: 1 or a functional equivalent thereof and a nano-polyplex polymer, the ionic complex being characterized by dissociation of the ionic complex in intracellular compartments selected by intracellular pH conditions such that bioactivity and stability of the peptide is preserved.

[0070a] In one aspect, there is provided a pharmaceutical formulation for delivery by inhalation comprising a therapeutic amount of an MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof selected from FAKLAARLYRKALARQLGVAA (SEQ ID NO: 3) and KAFKLAARLYRKALARQLGVAA (SEQ ID NO: 4), up to 5% w/w solids before drying, up to 20% trehalose, and a pharmaceutically acceptable carrier, wherein the formulation is effective to preserve at least about 96% of physical, chemical, microbiological, therapeutic, and toxicological specifications of the MK2i polypeptide for at least 4 weeks at 25°C and bioavailability of the polypeptide.

[0070b] In another aspect, there is provided a pharmaceutical formulation for delivery by inhalation comprising a therapeutic amount of an MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof selected from FAKLAARLYRKALARQLGVAA (SEQ ID NO: 3) and KAFKLAARLYRKALARQLGVAA (SEQ ID NO: 4), up to 5% w/w solids before drying, up to 7.3% glycerin, and a pharmaceutically acceptable carrier, wherein the formulation:

- (i) is isosmotic and non-buffered;
- (ii) has a stable pH of about 7.0 for at least 2 weeks at 60°C; and
- (iii) is effective to preserve at least about 93% stability of physical, chemical, microbiological, therapeutic, and toxicological specifications of the MK2i polypeptide for at least 2 weeks at 60°C, and bioavailability of the MK2i polypeptide.

[0071] According to another embodiment, the disclosure provides a method for treating a vascular graft-induced intimal hyperplasia in a subject in need of such treatment, the method comprising administering the pharmaceutical formulation comprising an ionic complex of a polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA; SEQ ID NO: 1 or a functional equivalent thereof and a nano-polyplex polymer, the ionic complex being characterized by dissociation of the ionic complex in intracellular compartments selected by intracellular pH conditions such that bioactivity and stability of the peptide is preserved, comprising a therapeutic amount of a polypeptide of amino sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof, and a nano-polyplex polymer, wherein the therapeutic amount is effective to inhibit MK2; and to treat a vascular graft-induced intimal hyperplasia.

[0072] According to one embodiment, the nano-polyplex polymer is anionic and endosomolytic. According to another embodiment, the nano-polyplex polymer is poly(propylacrylic acid) (PPAA). According to another embodiment, the nano-polyplex polymer is poly(acrylic acid) (PAA). According to another embodiment, the pharmaceutical formulation comprises a charge ratio (CR) of the polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA; SEQ ID NO: 1 or a functional equivalent thereof to PPAA selected from the group consisting of 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:1.5, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 and 1:10. According to another embodiment, the the charge ratio (CR) is 1:3. According to another embodiment, the pharmaceutical

formulation is delivered to a subject via an implantation device. According to another embodiment, the pharmaceutical formulation is delivered to a subject topically. According to another embodiment, the pharmaceutical formulation is delivered to a subject parenterally.

[0073] According to one embodiment, the functional equivalent is made from a fusion between a first polypeptide that is a protein transduction domain (PTD) and a second polypeptide that is a therapeutic domain (TD). According to another embodiment, the protein transduction domain (PTD) is selected from the group consisting of a polypeptide of amino acid sequence YARAAARQARA (SEQ ID NO: 11), FAKLAARLYR (SEQ ID NO: 16), and KAFAKLAARLYR (SEQ ID NO: 17), and a second polypeptide that is a therapeutic domain (TD) of amino acid sequence KALARQLGVAA (SEQ ID NO: 2).

[0073a] According to another aspect, there is provided use of the pharmaceutical formulation as described herein comprising a therapeutic amount of a polypeptide of amino sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof selected from FAKLAARLYRKALARQLGVAA (SEQ ID NO: 3) and KAFAKLAARLYRKALARQLGVAA (SEQ ID NO: 4), and a polymer in form of a nanoparticle, in the manufacture of a medicament for treating a vascular graft-induced intimal hyperplasia in a subject, wherein the medicament
inhibits MK2; and
treats a vascular graft-induced intimal hyperplasia; and wherein the medicament is to be administered to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0074] FIGURE 1 shows the technical characteristics of the blister lidding –push through.

[0075] FIGURE 2 shows the technical characteristics of the Formpack® - 4PLY.

[0076] FIGURE 3 shows a dynamic vapor sorption isotherm for a MMI-0100 5% solids formulation.

[0077] FIGURE 4 shows a chromatogram of an MMI-0100 working standard.

[0078] FIGURE 5 shows an EPIC inhaler device. On the left is an assembled device (base unit with attached flow channel). The inhaler is tethered to an external drive box (pictured on the right) which contains the electronics.

What is claimed is:

1. A pharmaceutical formulation for delivery by inhalation comprising a therapeutic amount of an MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof selected from FAKLAARLYRKALARQLGVAA (SEQ ID NO: 3) and KAFKLAARLYRKALARQLGVAA (SEQ ID NO: 4), up to 5% w/w solids before drying, up to 20% trehalose, and a pharmaceutically acceptable carrier, wherein the formulation is effective to preserve at least about 96% of physical, chemical, microbiological, therapeutic, and toxicological specifications of the MK2i polypeptide for at least 4 weeks at 25°C and bioavailability of the polypeptide.
2. The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation is a particulate pharmaceutical formulation.
3. The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation is an aerosolized pharmaceutical formulation.
4. The pharmaceutical formulation according to claim 1, wherein the formulation is prepared by a process of spray drying.
5. The pharmaceutical formulation according to claim 2, wherein the pharmaceutical formulation comprises 1% w/w solids before drying.
6. The pharmaceutical formulation according to claim 2, wherein the pharmaceutical formulation comprises 5% w/w solids before drying.
7. The pharmaceutical formulation according to claim 1, wherein the MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or the functional equivalent thereof and the trehalose are in a ratio of 80/20 (% w/w) respectively.
8. The pharmaceutical formulation according to claim 1, wherein the MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or the functional equivalent thereof and the trehalose are in a ratio of 92.5/7.5 (% w/w) respectively.
9. The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation is to be delivered to a subject via a dry powder inhalation device (DPI).
10. The pharmaceutical formulation according to claim 3 further comprising saline before drying.
11. The pharmaceutical formulation according to claim 10, wherein the saline is NaCl.
12. The pharmaceutical formulation according to claim 10, wherein the MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or the functional equivalent thereof is at a concentration of 0.7 mg/mL.

13. The pharmaceutical formulation according to claim 10, wherein the MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or the functional equivalent thereof is at a concentration of 7.0 mg/mL.
14. The pharmaceutical formulation according to claim 3, wherein the pharmaceutical formulation is to be delivered to a subject via a nebulizer.
15. The pharmaceutical formulation according to claim 1, comprising an ionic complex of the MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof and a polymer in form of a nanoparticle, wherein the ionic complex dissociates in intracellular compartments selected by intracellular pH conditions such that bioactivity and stability of the peptide is preserved.
16. The pharmaceutical formulation according to claim 15, wherein the polymer in form of a nanoparticle is anionic and endosomolytic.
17. The pharmaceutical formulation according to claim 15, wherein the polymer in form of a nanoparticle is poly(propylacrylic acid) (PPAA).
18. The pharmaceutical formulation according to claim 15, wherein the pharmaceutical formulation comprises a charge ratio (CR) of the MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof to PPAA selected from the group consisting of 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:1.5, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 and 1:10.
19. The pharmaceutical formulation according to claim 18, wherein the charge ratio (CR) is 1:3.
20. The pharmaceutical formulation according to claim 15, wherein the polymer in form of a nanoparticle is poly(acrylic acid) (PAA).
21. The pharmaceutical formulation according to claim 15, wherein the pharmaceutical formulation is to be delivered to a subject via an implantation device.
22. The pharmaceutical formulation according to claim 15, wherein the pharmaceutical formulation is to be delivered to a subject topically.
23. The pharmaceutical formulation according to claim 15, wherein the pharmaceutical formulation is to be delivered to a subject parenterally.
24. Use of a pharmaceutical formulation of claim 15 comprising a therapeutic amount of a polypeptide of amino sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof selected from FAKLAARLYRKALARQLGVAA (SEQ ID NO: 3) and KAFAKLAARLYRKALARQLGVAA (SEQ ID NO: 4), and a polymer in form of a nanoparticle, in the manufacture of a medicament for treating vascular graft-induced intimal hyperplasia in a subject, wherein the medicament

inhibits MK2; and

treats a vascular graft-induced intimal hyperplasia; and wherein the medicament is to be administered to the subject.

25. The use according to claim 24, wherein the polymer in form of a nanoparticle is anionic and endosomolytic.

26. The use according to claim 25, wherein the polymer in form of a nanoparticle is poly(propylacrylic acid) (PPAA).

27. The use according to claim 24, wherein the pharmaceutical formulation comprises a charge ratio (CR) of the polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof to PPAA selected from the group consisting of 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:1.5, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 and 1:10.

28. The use according to claim 27, wherein the charge ratio (CR) is 1:3.

29. The use according to claim 25, wherein the polymer in form of a nanoparticle is poly(acrylic acid) (PAA).

30. The use according to claim 24, wherein the administering is by an implantation device.

31. The use according to claim 24, wherein the administering is by topical administration.

32. The use according to claim 24, wherein the administration is by parenteral administration.

33. A pharmaceutical formulation for delivery by inhalation comprising a therapeutic amount of an MK2i polypeptide of amino acid sequence YARAAARQARAKALARQLGVAA (SEQ ID NO: 1) or a functional equivalent thereof selected from FAKLAARLYRKALARQLGVAA (SEQ ID NO: 3) and KAFKLAARLYRKALARQLGVAA (SEQ ID NO: 4), up to 5% w/w solids before drying, up to 7.3% glycerin, and a pharmaceutically acceptable carrier, wherein the formulation:

(i) is isosmotic and non-buffered;

(ii) has a stable pH of about 7.0 for at least 2 weeks at 60°C; and

(iii) is effective to preserve at least about 93% stability of physical, chemical, microbiological, therapeutic, and toxicological specifications of the MK2i polypeptide for at least 2 weeks at 60°C, and bioavailability of the MK2i polypeptide.