useful for the detection and purification of human FVIII and/or FVIII-like polypeptides from solutions such as blood or conditioned media.

In US Pat. No. 7,033,590 FIX/FIXa activating antibodies and antibody derivatives are used for increasing the amidolytic activity of FIXa, and for treating blood coagulation disorders such as hemophilia A and hemorrhagic diathesis.

In US Pat. No. 7,084,109 FVIIa antagonists are disclosed. These antagonists are peptides that inhibit FVIIa activity and are said to be useful for prevention of arterial thrombosis in combination with thrombolytic therapy.

The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

There remains a great need in the art for a low molecular weight peptide with procoagulant activity for treatment of patients with hemophilia A (FVIII deficiency). The present invention provides novel low molecular weight peptides with procoagulant activity which can be used for the non-intravenous treatment of hemophilia A. The present prevention also provides these novel peptides for the treatment of a deficiency in FV, FVII, FX and/or FXI.

SUMMARY

In a first aspect, there is provided a peptide or peptide derivative comprising: (i) an amino acid sequence comprising imfwydcye; or (ii) a variant amino acid sequence comprising one, two, three, four, five or six amino acid substitutions in imfwydcye, wherein said peptide or peptide derivative has procoagulant activity.

In a second aspect, there is provided a dual peptide comprising a peptide or peptide derivative as defined in the first aspect conjugated to a further peptide or peptide derivative as disclosed herein, wherein the peptide or peptide derivative may be the same as or different from the further peptide or peptide derivative, and wherein the dual peptide has procoagulant activity.

In a third aspect, there is provided a pharmaceutical composition comprising the peptide or peptide derivative of the first aspect and one or more pharmaceutically acceptable excipients, carriers and/or diluents.

In a fourth aspect, there is provided the peptide or peptide derivative of the first aspect for use in medicine.

In a fifth aspect, there is provided the peptide or peptide derivative of the first aspect for treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI.

In a sixth aspect, there is provided use of a peptide or peptide derivative of the first aspect in the manufacture of a medicament for the treatment of a deficiency in FV, FVII, FVIII, FX and/or FXI.

In a seventh aspect, there is provided use of a therapeutically effective amount of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI.

In an eighth aspect, there is provided a method of making the peptide or peptide derivative of the first aspect by solid phase synthesis.

In a first embodiment, disclosed is a peptide or peptide derivative comprising:
(i) WDLYFEIVW (SEQ ID NO: 1); or
(ii) a variant amino acid sequence comprising one, two, three or four L-amino acid substitutions in WDLYFEIVW (SEQ ID NO: 1); or
(Hi) the retro-inverso variant of the peptide or peptide derivative of either one of parts (i) and (ii),
wherein said peptide or peptide derivative has procoagulant activity.

For the avoidance of doubt, the sequence WDLYFEIVW (SEQ ID NO: 1) may be represented as the L-amino acids Trp-Asp-Leu-Tyr-Phe-Glu-Ile-Val-Trp using the three letter code for amino acids. The retro-inverso variant of WDLYFEIVW (SEQ ID NO: 1) is wviefyldw and comprises D-amino acids.

In a second embodiment, disclosed is a peptide or peptide derivative comprising:
(i) an amino acid sequence comprising imfwydcye; or
(ii) a variant amino acid sequence comprising one, two, three, four, five or six amino acid substitutions in imfwydcye,
wherein said peptide or peptide derivative has procoagulant activity.

For the avoidance of doubt, the sequence cimfwydcye may be represented as D-amino acids ile-met-phe-trp-tyr-asp-cys-tyr-glu using the three letter code for amino acids.

In a third embodiment, disclosed is a dual peptide comprising a peptide or peptide derivative of the first or second embodiments of the disclosure conjugated to a further peptide or peptide derivative of the first or second embodiments of the disclosure, wherein the two peptides/derivatives may be the same as or different from each other and wherein the dual peptide has procoagul ant activity.

In a fourth embodiment, disclosed is a pharmaceutical composition comprising the peptide or peptide derivative of the first or second embodiments of the disclosure or the dual peptide of the third embodiment of the disclosure.

In a fifth embodiment, disclosed is a peptide or peptide derivative of the first or second embodiments or a dual peptide of the third embodiment of the disclosure for treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI.

In a sixth embodiment, disclosed is a use of a peptide or peptide derivative of the first or second embodiments or a dual peptide of the third embodiment of the disclosure in the manufacture of a medicament for the treatment of a deficiency in FV, FVII, FVIII, FX and/or FXI in a patient.

In a seventh embodiment, disclosed is a method of treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI comprising administering a therapeutically effective amount of the pharmaceutical composition of the fourth embodiment of the disclosure.

In an eighth embodiment, disclosed is a peptide or peptide derivative which has procoagulant activity, wherein the peptide or peptide derivative is not FVIII or a fragment thereof and, wherein the procoagulant activity is a thrombin generation time of 25, 50 or 100 µM of peptide, peptide derivative or dual peptide equivalent to that of at least 100 mU/mL Factor Eight Inhibitor Bypassing Activity (FEIBA), preferably at least 300 mU/mL FEIBA, more preferably at least 900 mU/mL FEIBA, most preferably at least 1200 mU/mL FEIBA in the Defined Intrinsic Thrombin Generation Assay.

In a ninth embodiment, disclosed is a peptide or peptide derivative which has procoagulant activity, wherein the peptide or peptide derivative is not FVIII or a fragment thereof and, wherein the procoagulant activity is a thrombin generation time of 25, 50 or 100 µM of peptide, peptide derivative or dual peptide in a Defined Intrinsic Thrombin Generation Assay peaking within 30 minutes, preferably within 15 minutes and most preferably within 10 minutes.

In a tenth embodiment, disclosed is a peptide or peptide derivative which has procoagulant activity, wherein the peptide or peptide derivative is not FVIII or a fragment thereof and, wherein the peptide or peptide derivative can at least partially compensate for the absence of biologically active FVIII when administered in an animal model of severe human hemophilia A.

DESCRIPTION OF FIGURES

Figure 1: Effect of therapeutics approved for treatment of hemophilia on peak thrombin generation and thrombin peak time in a defined Dual-pathway thrombin generation assay

Figure 2: Effect of A01 on FVIII -/- mouse bleeding model - blood loss

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

The term "amino acid" within the scope of the present invention is intended to include all naturally occurring L α-amino acids. The one and three letter abbreviations for naturally occurring amino acids are used herein (Lehninger, Biochemistry, 2d ed., Worth Publishers, New York, 1995: 71-92). The term

CLAIMS

1. A peptide or peptide derivative comprising:

(i) an amino acid sequence comprising imfwydcye; or

(ii) a variant amino acid sequence comprising one, two, three, four, five or six amino acid substitutions in imfwydcye,

wherein said peptide or peptide derivative has procoagulant activity.

2. The peptide or peptide derivative of Claim 1 comprising:

(i) an amino acid sequence comprising cimfwydcye; or

(ii) a variant amino acid sequence comprising one, two, three, four, five, six or seven amino acid substitutions in cimfwydcye.

3. The peptide or peptide derivative of Claim 1, wherein the variant amino acid sequence comprises an amino acid sequence comprising $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, wherein X_1 ,where present, is c, s, y, i D-Pen, C, t, D-Nva, D-Nle or k, X_2 is i, y, w or d, X_3 is c or m, X_4 is f, t, v or c, X_5 is w or c, X_6 is y or c, X_7 is d, e or f, X_8 is c, e, f, y or d, X_9 is y or w and X_{10} is e or i.

4. The peptide or peptide derivative of Claim 3, wherein the variant amino acid sequence comprises an amino acid sequence comprising $X_1X_2X_3X_4$wydX_8ye, wherein X_1 is c, C, D-Pen or s, X_2 is i, y or w, X_3 is c or m, X_4 is f, t, or v and X_8 is c or e.

5. The peptide or peptide derivative of Claim 4, wherein the variant amino acid sequence comprises an amino acid sequence comprising X_1X_2mX_4wydX_8ye, wherein X_1 is c, C or D-Pen, X_2 is i or y, X_4 is f, t, or v and X_8 is c or e.

6. The peptide derivative of Claim 1 which is acetylated at the N-terminus, amidated at the C-terminus and/or PEGylated at either terminus.

7. The peptide derivative of Claim 2 which is acetylated at the N-terminus, amidated at the C-terminus and/or PEGylated at either terminus.

8. The peptide or peptide derivative of Claim 1 which is cyclic.

9. The peptide or peptide derivative of Claim 1 comprising or consisting of: Ac-cimfwydeye-NH_2, Disulphide-Dimer(Ac-cimfwydeye-NH_2)$_2$, Ac-TTDS-(cymfwydc)-ye-NH_2, K-TTDS-(cymfwydc)-ye-NH_2, Ac-cimtwydcye-NH_2, Ac-cimvwydcye-NH_2, cymfwydcye, Ac-(cymfwydc)-yeG-NH_2, Ac-(D-Pen)imfwydeye-NH_2, O(CH_2-CH_2-O-CH_2-CO-imfwydeye-NH_2)$_2$, Pyridine-3,5-(CO-imfwydeye-NH_2)$_2$, H_2N-E-TTDS-(cymfwydc)-ye-NH_2, Ac-(cymfwydc)-yeK, Ac-(cymfwydc)-ye-TTDS-K, Ac-simfwydeye-NH_2, Ac-simfwydeye-NH_2, Ac-ydmcwcefyi-NH_2, Ac-idmccyfywe-NH_2, Ac-cimfwyddye-NH_2, Ac-(cymfwydc)-ye, Ac-(cymfwydc)-ye-TTDS-NH_2, Ac-TTDS-(cymfwydc)-ye-TTDS-NH_2, K-(cymfwydc)-ye-NH_2, Ac-K-(cymfwydc)-ye-NH_2, E-(cymfwydc)-ye-NH_2, Ac-K-TTDS-(cymfwydc)-ye-NH_2, Ac-(cymfwydc)-yeK-NH_2, Ac-(cymfwydc)-ye-TTDS-K-NH_2, Ac-(cymfwydc)-ye-TTDS-E-NH_2, Ac-timfwydeye-NH_2, Ac-(cimfwydc)-ye-NH_2, Ac-(cymfwydc)-ye-NH_2, Ac-(cwmfwydc)-ye-NH_2, Ac-cicfwydcye-NH_2, Ac-(D-Nva)imfwydeye-NH_2, Ac-(D-Nle)imfwydeye-NH_2, Ac-(Cys)imfwydeye-NH_2, (cymfwydc)-ye-NH_2, TTDS-(cymfwydc)-ye-TTDS-NH_2, Ac-kimfwydeye-NH_2,
wherein –TTDS- is 4,7,10-trioxa-1,13-tridecanediamine, (D-Pen) is D-penicillamine, (D-Nva) is D-norvaline, (D-Nle) is D-norleucine.

10. A dual peptide comprising a peptide or peptide derivative as defined in Claim 1 conjugated to a further peptide or peptide derivative as defined in any preceding claim, wherein the peptide or peptide derivative may be the same as or different from the further peptide or peptide derivative, and wherein the dual peptide has procoagulant activity.

11. The peptide or peptide derivative of Claim 1 which has a molecular weight of between 0.5 and 3.5kD.

12. The peptide or peptide of Claim 1, wherein the procoagulant activity is a thrombin generation time of 25, 50 or 100 µM of peptide, peptide derivative or dual peptide equivalent to that of at least 100 mU/mL Factor Eight Inhibitor Bypassing Activity (FEIBA), preferably at least 300 mU/mL FEIBA, more preferably at least 900 mU/mL FEIBA, most preferably at least 1200 mU/mL FEIBA in the Defined Intrinsic Thrombin Generation Assay.

13. The peptide or peptide derivative of Claim 1, wherein the procoagulant activity is a thrombin generation time of 25, 50 or 100 µM of peptide, peptide derivative or dual peptide in a Defined

Intrinsic Thrombin Generation Assay peaking within 30 minutes, preferably within 15 minutes and most preferably within 10 minutes.

14. The peptide or peptide derivative of Claim 1 which can at least partially compensate for the absence of biologically active FVIII when administered in an animal model of severe human hemophilia A.

15. The peptide or peptide derivative of Claim 1 which has a stability in human plasma at 30 minutes of at least 50%, preferably at least 70%, more preferably at least 80% and most preferably at least 90%.

16. The peptide or peptide derivative of Claim 1 which has an aqueous solubility in phosphate buffered saline pH 7.4 of at least 25 µM, preferably at least 60 µM and most preferably at least 100 µM.

17. A pharmaceutical composition comprising the peptide or peptide derivative of Claim 1 and one or more pharmaceutically acceptable excipients, carriers and/or diluents.

18. The pharmaceutical composition of Claim 17 suitable for subcutaneous, nasal, buccal, oral or pulmonary administration.

19. The pharmaceutical composition of Claim 18 suitable for intravenous administration.

20. The peptide or peptide derivative of Claim 1 for use in medicine.

21. The peptide or peptide derivative of Claim 1 for treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI.

22. Use of a peptide or peptide derivative of Claim 1 in the manufacture of a medicament for the treatment of a deficiency in FV, FVII, FVIII, FX and/or FXI.

23. Use of a therapeutically effective amount of the pharmaceutical composition of Claim 17 in the manufacture of a medicament for treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI.

24. Use of a therapeutically effective amount of the pharmaceutical composition of Claim 18 in the manufacture of a medicament for treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI.

25. Use of a therapeutically effective amount of the pharmaceutical composition of Claim 19 in the manufacture of a medicament for treating a patient having a deficiency in FV, FVII, FVIII, FX and/or FXI.

26. A method of making the peptide or peptide derivative of Claim 1 by solid phase synthesis.