Since DFXIs inhibit both free and prothrombinase bound coagulation FXa (European Medicines Agency, 2008. CHMP assessment report for Xarelto, Procedure No. EMEA/H/C/000944. Doc.Ref.: EMEA/543519/2008), effective restoration of normal hemostasis would therefore require either full replacement of circulating coagulation FXa or effective removal of inhibitory compounds from blood.

Currently, there are no specific reversal strategies to prevent and stop potential life-threatening bleeding complications associated with DFXI therapy available. Next to life-supporting and surgical therapies, non-specific reversal therapy using 3- and 4-factor PCC may be considered based on limited evidence (Siegal et al., 2014. Blood 123: 1152-1158; Levi et al., 2014. J Thrombosis Haemostatis, Published online 8 May 2014; doi: 10.1111/jth.12599). A reversal strategy specific for DFXI-associated bleeding is in development, which is based on a catalytically inactive form of recombinant FXa (andexanet alpha) that serves as a decoy for DFXIs by binding and thereby trapping circulating DFXIs, thereby enabling endogenous coagulation FXa to normally participate in coagulation (Lu et al., 2013. Nature Medicine 19: 446). A downside to this approach is that high doses of andexanet alpha need to be administered since stoichiometric concentrations are required to attain inhibition (400mg IV bolus in phase III trial; Portola News Release March 19, 2014). Furthermore, since the half life of DFXI partially depends on renal clearance, the amount of decoy FXa required to trap all circulating inhibitory molecules may even be higher in case of renal failure. This reversal strategy does not provide a fast and direct procoagulant response, as the response is dependent on the generation of free, endogenous coagulation FXa.

At this moment, a direct, adequate reversal strategy to prevent and stop potential life-threatening bleeding complications associated with DFXI anticoagulant therapy is not available.

In one aspect, there is provided a recombinant protein comprising a coagulation Factor Xa polypeptide, said polypeptide having an alteration in a region of amino

acid residues corresponding to the region of amino acid residues between Gly-289 and Asp-320, preferably between His-311 and Asp-320 of SEQ ID NO: 1; wherein the alteration is an insertion of at least one amino acid residue, which altered protein has a decreased sensitivity to inhibition by a direct Factor Xa inhibitor.

In another aspect, there is provided a nucleic acid molecule comprising a DNA sequence that encodes the protein as described herein.

In yet another aspect, there is provided an expression vector comprising the nucleic acid molecule as described herein.

In yet another aspect, there is provided a host cell comprising the nucleic acid molecule as described herein or the expression vector as described herein.

In yet another aspect, there is provided a pharmaceutical composition comprising the protein as described herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

Also disclosed is a method of completely or partially reversing an anti-coagulant effect of a coagulation inhibitor in a subject, said method comprising administering to said subject a therapeutically effective amount of the protein as described herein or the pharmaceutical composition as described herein.

In yet another aspect, there is provided use of the protein as described herein, for the manufacture of a medicament for completely or partially reversing an anti-coagulant effect of a direct Factor Xa inhibitor in a subject.

In yet another aspect, the present invention provides use of the altered recombinant protein described herein, wherein the alteration is an insertion of 1-50 amino acid residues, or of the pharmaceutical composition described herein, comprising an altered recombinant protein having an insertion of 1-50 amino acid residues, for the manufacture of a medicament for completely or partially reversing an anti-coagulant effect of a direct Factor Xa inhibitor in a subject.

Detailed Description

The present invention solves this problem by providing, as an adequate reversal strategy to prevent and stop potential life-threatening bleeding complications

Claims

1. A recombinant protein comprising a coagulation Factor Xa polypeptide, said polypeptide having an alteration in a region of amino acid residues corresponding to the region of amino acid residues between Gly-289 and Asp-320, preferably between His-311 and Asp-320 of SEQ ID NO: 1; wherein the alteration is an insertion of at least one amino acid residue, which altered protein has a decreased sensitivity to inhibition by a direct Factor Xa inhibitor.

2. The protein according to claim 1, wherein the insertion is 1-50 amino acid residues.

3. The protein according to claim 1 or claim 2, wherein the insertion is 1-20 amino acid residues.

4. The protein according to any one of claim 1-3, wherein said insertion is between two amino acid residues corresponding to Lys-316 and Glu-317 of SEQ ID NO: 1.

5. The protein according to any one of claims 1-4, wherein the insertion of at least one amino acid residue is combined with a replacement of between 1-8 amino acid residues in the region of amino acid residues between Gly-289 and Asp-320, preferably between His-311 and Asp-320 of SEQ ID NO: 1.

6. The protein according to any one of claims 1-5, wherein said insertion is 9-13 amino acid residues.

7. The protein according to any one of claims 1-6, wherein the region of amino acid residues corresponding to the region of amino acid residues between His-311 and Asp-320 of SEQ ID NO: 1 has the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11.

8. A nucleic acid molecule comprising a DNA sequence that encodes the protein according to any one of claims 1-7.

9. An expression vector comprising the nucleic acid molecule according to claim 8.

10. A host cell comprising the nucleic acid molecule according to claim 8 or the expression vector according to claim 9.

11. A pharmaceutical composition comprising the protein according to any of claims 1-7, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

12. The altered recombinant protein according to any one of claims 1-7, wherein the alteration is an insertion of 1-50 amino acid residues, or the pharmaceutical composition according to claim 11, comprising an altered recombinant protein having an insertion of 1-50 amino acid residues, for use as a medicament.

13. The altered recombinant protein according to any one of claims 1-7, wherein the alteration is an insertion of 1-50 amino acid residues, or the pharmaceutical composition according to claim 11, comprising an altered recombinant protein having an insertion of 1-50 amino acid residues, for use in a method of completely or partially reversing an anti-coagulant effect of a direct Factor Xa inhibitor in a subject.

14. Use of the altered recombinant protein of any one of claims 1-7, wherein the alteration is an insertion of 1-50 amino acid residues, or of the pharmaceutical composition according to claim 11, comprising an altered recombinant protein having an insertion of 1-50 amino acid residues, for the manufacture of a medicament for completely or partially reversing an anti-coagulant effect of a direct Factor Xa inhibitor in a subject.

15. The use according to claim 14, wherein the direct Factor Xa inhibitor, is rivaroxaban (5-chloro-N-[[(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-5-oxazolidinyl]methyl]-2-thiophenecarboxamide), apixaban (1-(4methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide), edoxaban (N'-(5-chloropyridin-2-yl)-N-[(1S,2R,4S)-4-(dimethylcarbamoyl)-2-[(5-methyl-6,7-dihydro-4H-[1,3]thiazolo[5,4-c]pyridine-2-carbonyl)amino]cyclohexyl]oxamide;4-methylbenzenesulfonic acid), or betrixaban (N-(5-chloropyridin-2-yl)-2-[[4-(N,N-dimethylcarbamimidoyl)benzoyl]amino]-5-methoxybenzamide).