

[0006] There remains a need to develop materials and methods for conjugating water soluble polymers to proteins that improves the protein's pharmacodynamic and/or pharmacokinetic properties while minimizing the costs associated with the various reagents and minimizing the health risks to the patient recipient.

SUMMARY

[0007] In one aspect, the present invention refers to a modified Factor VIII (FVIII) comprising a modification that increases FVIII half-life and reduces binding of said modified FVIII to a ligand selected from the group consisting of von Willebrand Factor (VWF) and low density lipoprotein (LDL)-receptor-related protein 1 (LRP1).

[0007a] In another aspect, the present invention refers to a pharmaceutical composition comprising the modified FVIII as disclosed herein and a pharmaceutically acceptable carrier, diluent, salt, buffer, or excipient.

[0007b] In yet another aspect, the present invention refers to a modified, recombinant FVIII comprising a modification that increases FVIII half-life and reduces binding of said modified FVIII to a ligand selected from the group consisting VWF and LRP1, wherein said modification comprises a PSA with an aminooxy linker, and wherein said aminooxy linker is attached to an oxidized carbohydrate of said modified FVIII;

wherein the half-life of said modified, recombinant FVIII is longer than an unmodified, recombinant FVIII and/or a PEGylated, recombinant FVIII; and

wherein the half-life of said modified, recombinant FVIII is longer than an unmodified, recombinant FVIII and/or a PEGylated, recombinant FVIII; and

wherein the binding to VWF or LRP1 by said modified, recombinant FVIII is lower as compared to binding of VWF or LRP1 by an unmodified, recombinant FVIII and/or a PEGylated, recombinant FVIII.

[0007c] In yet another example, the present invention refers to the use of the modified FVIII as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a hemorrhagic defect in a mammal.

[0007d] The present disclosure provides materials and methods for conjugating polymers to proteins to improve the protein's *in vivo* half-life, pharmacodynamic and/or pharmacokinetic properties.

[0008] In one example, a modified Factor VIII (FVIII) is provided. The modified FVIII comprises a modification increasing FVIII half-life and reducing binding of the modified FVIII to a ligand binding FVIII. Exemplary ligands are selected from von Willebrand Factor (VWF) and low density lipoprotein (LDL)-receptor-related protein 1 (LRP1). Exemplary modifications are discussed herein and include, for example, chemical modifications such as attachment of water-soluble polymers.

[0009] In an exemplary example, the modified FVIII is plasma-derived. In an exemplary example, the FVIII is recombinantly produced from an engineered host cell. In various examples, the modified FVIII includes an intact B domain. In various examples, the FVIII is a full-length FVIII and includes an intact B domain.

[0010] In an exemplary example, the modified FVIII binds to VWF and LRP1 with a lower affinity (KD) compared to an unmodified FVIII.

[0011] In an exemplary example, the modified FVIII comprises a polysialic acid (PSA) conjugated thereto. Exemplary PSA moieties of use in this example have a mean molecular weight of about 20 kDa. In exemplary examples, the PSA has a mean molecular weight selected from about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, or about 50 kDa. In an exemplary example, the PSA has a low polydispersity. Exemplary PSA conjugates include an aminooxy linker between the PSA and the FVIII molecule. An exemplary aminooxy linker is attached to an oxidized carbohydrate of the modified FVIII.

[0012] The disclosure also provides a pharmaceutical composition. An exemplary pharmaceutical formulation includes an aforementioned modified FVIII and a pharmaceutically acceptable carrier, diluent, salt, buffer, and/or excipient.

[0013] In various examples, an aforementioned modified FVIII has an *in vivo* half-life that is longer than a PEGylated FVIII. In various examples, an aforementioned modified FVIII has an *in vivo* half-life that is longer than a PEGylated FVIII, which is conjugated to a PEG moiety of about the same mean molecular weight as the mean molecular weight of the PSA of the conjugated FVIII with the longer *in vivo* half-life. In an exemplary example, the PSA modified FVIII is conjugated to a PSA moiety having a mean molecular weight of about 20 kDa and has an *in vivo* half-life that is longer than a PEGylated FVIII, which is conjugated to a PEG moiety having a mean molecular weight of about 20 kDa. In various examples, the half-life is longer by a factor of about 1-, about 2- or about 3-fold. In still other various examples, the half-life is longer by a factor of about 1-, about 2-, about 3-, about 4-, about 5-, about 6-, about 7-, about 8-, about 9-, or about 10-fold.

[0014] In an exemplary example, an aforementioned PSA modified FVIII binds to VWF or LRP1 with a lower binding affinity as compared to the binding of a PEGylated FVIII to VWF or LRP1 when the binding of both species is measured under comparable conditions. In an exemplary example, an aforementioned PSA modified FVIII binds to VWF or LRP1 with a lower binding affinity as compared to the binding of a PEGylated FVIII to VWF or LRP1 when the binding of both species is measured under comparable conditions, and the PEG and PSA are of about the same mean molecular weight. In various examples, binding to VWF or LRP1 is lower by a factor of about 0.5-fold as compared to binding of a PEGylated FVIII to VWF or LRP1. In still other examples, binding to VWF or LRP1 is lower by a factor of about 0.1-, about 0.2-, about 0.3-, about 0.4-, about 0.5-, about 0.6-, about 0.7-, about 0.8-, about 0.9-, about 1-, about 2-, about 3-, about 4-, about 5-, about 6-, about 7-, about 8-, about 9-, or about 10- as compared to binding of a PEGylated FVIII to VWF or LRP1.

[0015] In one example, a modified, recombinant FVIII is provided comprising a modification that increases FVIII half-life and reduces binding of said modified FVIII to a ligand selected from the group consisting VWF and LRP1, wherein said modification comprises a PSA attached to a FVIII through an aminooxy linker. In an exemplary example, the aminooxy linker is attached to an oxidized carbohydrate of the modified FVIII, wherein the *in vivo* half-life of the modified, recombinant FVIII is longer than an unmodified, recombinant FVIII and/or a PEGylated, recombinant FVIII and wherein the binding affinity to VWF or LRP1 of the modified, recombinant FVIII is lower as compared to binding of VWF or LRP1 by an unmodified, recombinant FVIII and/or a PEGylated,

recombinant FVIII. In an exemplary example, the comparative species include a PSA and a PEG moiety of about the same mean molecular weight.

[0016] The disclosure further provides a method of treating a subject in need of such treatment with a modified FVIII of the disclosure. In an exemplary example, a pharmaceutical composition of the disclosure, including the disclosed modified FVIII is administered to a mammal diagnosed with disease or disorder associated with FVIII deficiency, or deficiency of another factor (e.g., FVII, FIX).

[0017] In an exemplary example, the disclosure provides a method of treating a hemorrhagic defect in a mammalian subject. An exemplary method includes administering an aforementioned modified FVIII or a pharmaceutical composition thereof to the subject, in need of such treatment, in an amount effective to reduce or eliminate one or more symptoms of the hemorrhagic defect. In an exemplary example, the administration of the pharmaceutical formulation achieves an amount effective to reduce or eliminate one or more symptoms of the hemorrhagic defect when administered to the subject not more than once every 4 days, not more than once every 5 days, not more than once every 6 days, not more than once every 7 days, not more than once every 8 days, not more than once every 9 days, or not more than once every 10 days.

FIGURES

[0018] **Figure 1** shows binding signals expressed as $R_{\max}$, which is the calculated maximum binding at saturation, for PSA-rFVIII groups and rebuffered rFVIII at the three different densities of the sensor-chip-immobilized VWF.

[0019] **Figure 2** shows results for FVIII protein-concentration-dependent binding to LRP1 for PSA-rFVIII and for re-buffered rFVIII.

[0020] **Figure 3** shows that rFVIII binds strongly to LRP1, PEG-rFVIII displayed residual association with LRP1, and PEG-rFVIII was virtually unable to associate with LRP1.

[0021] **Figure 4** shows FXa generation over time after activation of FVIII by thrombin.

[0022] **Figure 5** shows the results of the evaluation of the peak thrombin generation curves for rebuffered rFVIII and PSA-rFVIII.

[0023] **Figure 6** shows total thrombin generation curves for rebuffered rFVIII and PSA-rFVIII.

wherein the half-life of said modified, recombinant FVIII is longer than an unmodified, recombinant FVIII and/or a PEGylated, recombinant FVIII; and

wherein the binding to VWF or LRP1 by said modified, recombinant FVIII is lower as compared to binding of VWF or LRP1 by an unmodified, recombinant FVIII and/or a PEGylated, recombinant FVIII.

17. The modified FVIII of any one of claims 1-10 and 12-16, wherein said modified FVIII is administered to a mammal diagnosed with disease or disorder associated with FVIII deficiency.

18. Use of the modified FVIII of any one of claims 1-10 and 12-16, or the pharmaceutical composition of claim 11 in the manufacture of a medicament for treating a hemorrhagic defect in a mammal.