methods of treating subjects with diseases or conditions associated with ADAMTS13 and VWF dysfunction.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0016] ~~In one aspect, t~~The present disclosure~~invention~~ provides formulations of ADAMTS13 (A13) suitable for pharmaceutical administration. Advantageously, in some embodiments, the disclosed~~invention provides~~ A13 formulations ~~that~~ may be stored for extended periods of time without loosing activity or becoming overly aggregated. In certain embodiments, the disclosed formulations ~~of the invention~~ may be stored for at least 6 months at temperatures up to at least about 37°C.

[0017] ~~In one aspect, t~~The present disclosure~~invention~~ provides a stabilized formulation of ADAMTS13 (A13) comprising (a) 0.05 mg/ml to 10.0 mg/ml ADAMTS13; (b) 0 mM to 200 mM of a pharmaceutically acceptable salt; (c) 0.5 mM to 20 mM calcium; (d) a sugar and/or sugar alcohol; (e) a nonionic surfactant; and (f) a buffering agent for maintaining a pH between 6.0 and 8.0.

[0017a] In one aspect, there is provided a stabilized formulation of ADAMTS13, comprising: (a) 0.05 mg/ml to 10.0 mg/ml ADAMTS13; (b) less than 100 mM of a pharmaceutically acceptable salt (c) 0.5 mM to 20 mM calcium; (d) a sugar and/or sugar alcohol; (e) a nonionic surfactant; and (f) a buffering agent for maintaining a pH between 6.0 and 8.0.

[0017b] In another aspect, there is provided a lyophilized formulation of ADAMTS13, wherein the formulation is lyophilized from a liquid formulation as defined above.

[0018] In a related embodiment~~aspect~~, the present disclosure~~invention~~ provides a stabilized lyophilized formulation of ADAMTS13 (A13), wherein the formulation is lyophilized from a liquid formulation comprising (a) 0.05 mg/ml to 10.0 mg/ml ADAMTS13; (b) 0 mM to 200 mM of a pharmaceutically acceptable salt; (c) 0.5 mM to 20 mM calcium; (d) a sugar and/or sugar alcohol; (e) a nonionic surfactant; and (f) a buffering agent for maintaining a pH between 6.0 and 8.0.

[0019] In one embodiment~~aspect of the invention~~, recombinant ADAMTS13 (rA13) formulations suitable for pharmaceutical administration are provided. In some embodiments, the formulations provided herein have high specific activities and are stable upon storage for extended periods of time.

[0020] In another embodiment~~aspect of the invention~~, formulations are provided that have reduced dimerization or aggregation of A13 and rA13. In certain embodiments, the formulations provided herein retard the formation of A13 and rA13 dimers and aggregation when stored for extended periods of time. In some embodiments, these formulations are suitable for pharmaceutical administration.

[0021] In one embodiment~~aspect~~, the present disclosure~~invention~~ relates to a method of treating or preventing a disorder associated with the formation and/or presence of one or more thrombus and to a method of disintegrating one or more thrombus in a patient in need thereof. Examples of

disorders associated with the formation and/or the presence of one or more thrombus are hereditary thrombotic thrombocytopenic purpura (TTP), acquired TTP, arterial thrombosis, acute myocardial infarction (AMI), stroke, sepsis, and disseminated intravascular coagulation (DIC). In one embodiment, the methods of treating or preventing include the administration of an A13 or rA13 formulation provided herein.

[0022] In another embodimentaspect, the present disclosureinvention provides methods of treating or preventing an infarction in a patient in need thereof. In certain embodiments, methods are provided for treating or preventing a disease or condition associated with an infarction, including without limitation, myocardial infarction (heart attack), pulmonary embolism, cerebrovascular events such as stroke, peripheral artery occlusive disease (such as gangrene), antiphospholipid syndrome, sepsis, giant-cell arteritis (GCA), hernia, and volvulus. In one embodiment, the methods of treating or preventing include the administration of an A13 or rA13 formulation provided herein.

[0023] In one embodimentaspect, the present disclosureinvention provides methods of formulating A13 or rA13 with high stability and/or high specific activities. In certain embodiments, the methods are useful for formulating solutions and lyophilisates that can be stored for extended periods of time at temperatures up to at least about 37°C without loosing high specific activities desirable for pharmaceutical administration.

[0024] In another embodimentaspect, kits for the pharmaceutical administration of A13 or rA13 are provided herein. The kits of the invention, in certain embodiments, may comprise liquid or lyophilized formulations that may be stored for extended periods of time.

[0024a] In another aspect, there is provided a method for manufacturing a stabilized ADAMTS13 formulation, the method comprising the steps of: (a) expressing an ADAMTS13 protein, or a biologically active derivative thereof, in a cell cultured in a medium comprising zinc at a concentration from 2 μM to 12 μM and calcium at a concentration from 0.5 mM to 1.5 mM; (b) purifying the ADAMTS13 protein; and (c) preparing a formulation as defined above.

[0024b] In another aspect, there is provided a kit comprising a stabilized ADAMTS13 formulation as defined above.

[0024c] In yet another aspect, there is provided the use of an ADAMTS13 formulation as defined above in the manufacture of a medicament for treating or preventing a disease or condition associated with an ADAMTS13 or VWF dysfunction in a subject.

[0024d] In still another aspect, there is provided the use of an ADAMTS13 formulation as defined above in the manufacture of a medicament for treating or preventing a disease or condition associated with the formation and/or presence of a thrombus in a subject.

[0024e] In still another aspect, there is provided the use of an ADAMTS13 formulation as defined above in the manufacture of a medicament for treating or preventing an infarction in a subject.

[0024f] In still another aspect, there is provided an ADAMTS13 formulation as defined above for use in therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **Figure 1.** Recombinant ADAMTS13 was formulated in buffer containing 150 mM NaCl, 2% sucrose, 0.05% polysorbate 80, at a pH of 7.0 with 20 mM of a buffering agent selected from (1) histidine, (2) phosphate buffer, or (3) sodium citrate. Solutions were stored at 4°C or 37°C for up to 6 months. **(A)** FRETS-VWF73 activity and **(B)** ADAMTS13 protein concentration as measured by ELISA, were determined at the indicated time points.

[0026] **Figure 2.** Recombinant ADAMTS13 was formulated in buffer containing 150 mM NaCl, 2% sucrose, 0.05% polysorbate 80, at a pH of 7.0 with 20 mM of a buffering agent selected from (1) histidine, (2) phosphate buffer, or (3) sodium citrate and lyophilized. Lyophilized formulations were stored at 4°C or 37°C for up to 6 months. ADAMTS13 formulations were reconstituted with sterile water and **(A)** FRETS-VWF73 activity and **(B)**