PHARMACEUTICAL COMPOSITIONS OF FIBRINOLYTIC AGENT

Field of the Invention

The present invention relates to novel pharmaceutical compositions of a fibrinolytic agent. More specifically, the present invention relates to frozen liquid and lyophilized compositions of fibrolase and, separately, of "novel acting thrombolytic" (NAT), as well as methods for the production and use thereof.

Background of the Invention

In general, polypeptides are marginally stable in the aqueous state and undergo chemical and physical degradation resulting in a loss of biological activity during processing and storage. Another problem encountered in aqueous solution in particular is hydrolysis, such as deamidation and peptide bond cleavage. These effects represent a serious problem for therapeutically active polypeptides which are intended to be administered to humans within a defined dosage range based on biological activity.

To reduce the degradation of polypeptides, water-based pharmaceutical compositions are generally kept refrigerated or frozen until ready for use. As an alternative, the process of freeze-drying is often employed to stabilize polypeptides for long-term storage, particularly when the polypeptide is relatively unstable in liquid compositions. A lyophilization cycle is usually composed of three steps: freezing, primary drying, and secondary drying; Williams and Polli, Journal of Parenteral Science and Technology, Volume 38, Number 2, pages 48-59 (1984).

In the freezing step, the solution is cooled until it is adequately frozen. Bulk water in the solution forms ice at this stage. The ice sublimes in the primary drying stage, which is conducted by reducing chamber pressure below the vapor pressure of the ice, using a vacuum. Finally, sorbed or bound water is removed at the secondary drying stage under reduced chamber pressure and an elevated shelf temperature. The process produces a material known as a lyophilized cake. Thereafter the cake can be reconstituted prior to use.

The standard reconstitution practice for lyophilized material is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization), although dilute solutions of antibacterial agents are sometimes used in the production of pharmaceuticals for parenteral administration; Chen, Drug Development and Industrial Pharmacy, Volume 18, Numbers 11 and 12, pages 1311-1354 (1992).

Lyophilization is considered one of the best ways to remove excess water from polypeptide solutions. The freeze-drying process may yield products that are stable and amenable to handling for long-term storage. Lyophilized products can be stored at room temperature and are therefore easier to handle and distribute to a wider geographic market, such as foreign markets where refrigeration may not be available.

Excipients have been noted in some cases to act as stabilizers for freeze-dried products; Carpenter et al., Developments in Biological Standardization, Volume 74, pages 225-239 (1991). For example, known excipients include polyols (including mannitol,

sorbitol and glycerol); sugars (including glucose and sucrose); and amino acids (including alanine, glycine and glutamic acid).

In addition, polyols and sugars are also often used to protect polypeptides from freezing and drying-induced damage and to enhance the stability during storage in the dried state. In general, sugars, in particular disaccharides, are effective in both the freeze-drying process and during storage. Other classes of molecules, including mono- and di-saccharides and polymers such as PVP, have also been reported as stabilizers of lyophilized products.

Summary of the Invention

The present invention relates to stable pharmaceutical compositions of fibrolase and "novel acting thrombolytic" (NAT), some of which are liquid compositions suitable for storage in the frozen state, and others of which are suitable for lyophilization.

Because of the fibrinolytic properties of fibrolase and NAT, the compositions of this invention are useful to lyse blood clots *in vivo* and may be administered therapeutically for such a purpose.

For purposes of this invention, the term "NAT" refers to the metalloproteinase having fibrinolytic activity which is characterized by SEQ ID NO: 1. The NAT polypeptide is encoded by the cDNA molecule of SEQ ID NO: 2, although any DNA molecule of variant sequence encoding the same polypeptide may be used for expression and manufacture in accordance with methods which are referred to hereinbelow.

Fibrolase is a known metalloproteinase which has been described in the scientific and patent literature; see Randolph et al., Protein Science, Cambridge University Press (1992), pages 590-600, and European patent Application No. 0 323 722 (Valenzuela et al.), published July 12, 1989. Typically, the fibrolase employed in the compositions of this invention will be of SEQ ID NO: 3, which is encoded by the cDNA molecule of SEQ ID NO: 4 (or variants thereof encoding the same amino acid sequence).

Fibrolase and NAT are to be distinguished from other therapeutic agents for the treatment of blood clots *in vivo*, such as urokinase, streptokinase and tPA which are plasminogen activators. Unlike these other agents, fibrolase and NAT act directly on the clot to degrade both fibrin and fibrinogen.

The pharmaceutical compositions of this invention will contain, in addition to a therapeutically effective amount of fibrolase or NAT, a zinc stabilizer and, optionally, a bulking agent with or without other excipients in a pharmaceutically-acceptable buffer which, in combination, provide a stable, frozen or lyophilized product that can be stored for an extended period of time.

In one of its aspects, the present invention provides a freezable liquid medicinal composition comprising fibrolase or NAT, a water soluble zinc salt, a citric acid buffer, optionally an additional stabilizer selected from the group consisting of water soluble calcium salts, and optionally a bulking agent (for example, mannitol). A surfactant, such as Tween 80 (BASF, Gurnee, Illinois), may also be added to increase freeze-thaw stability. Tris buffer (Sigma,

St. Louis, Missouri) or another buffer with a buffer capacity above pH 7.0 may be added to stabilize the pH at or above pH 7.4.

In another aspect of the present invention, the pharmaceutical composition can be a lyophilizable or lyophilized pharmaceutical composition comprising fibrolase or NAT, a zinc stabilizer (e.g., water soluble zinc salt), and a citric acid buffer, with or without other excipients (e.g., bulking agent such as mannitol, glycine, or the like). The lyophilized composition may also contain a disaccharide sugar, such as sucrose or trehalose, as a lyoprotectant. A surfactant, such as Tween 80, may be added to protect against lyophilization stresses on the metalloproteinase (fibrolase or NAT). The pH will ideally be maintained at pH 8.0 ± 0.5, using a suitable buffer with a pK_a in this range (for example, Tris).

The invention also comprises a method for preparing a lyophilized composition, comprising the steps of (i) mixing fibrolase or NAT with a buffer and a water soluble zinc salt, as well as any desired optional ingredients, and (ii) lyophilizing this mixture.

In addition, the invention provides a kit for preparing an aqueous pharmaceutical composition, comprising a first container having the aforementioned lyophilized composition and a second container having a physiologically acceptable solvent therefor.

Still another aspect of this invention comprises a method comprising the steps of reconstituting the lyophilized composition and

administering the reconstituted composition to a patient in need of blood clot lysis.

Detailed Description of the Invention

A variety of host-vector systems may be utilized to express the coding sequence for fibrolase or NAT polypeptide in accordance with standard methods for recombinant expression which are well known to those skilled in the art, and thereby obtain the fibrinolytically active polypeptide for the compositions. Such systems include, but are not limited to, eukaryotic cell systems such as mammalian cell systems infected with virus (for example, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (for example, baculovirus); microorganisms such as yeast containing yeast vectors; or prokaryotic cell systems such as bacteria (e.g., E. coli) transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA. The expression elements of these vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

Preferably, a yeast expression system (e.g., *Pichia pastoris*) is employed for recombinant expression because of its greater efficiency. A detailed description of such a system may be found in United States Patent No. 4,855,231 (Stroman et al.), United States Patent No. 4,812,405 (Lair et al.), United States Patent No. 4,818,700 (Cregg et al.), United States Patent No. 4,885,242 (Cregg), and United States Patent No. 4,837,148 (Cregg), the disclosures of which are hereby incorporated by reference. Expression of fibrolase in such a system will typically involve a DNA

molecule of SEQ ID NO: 5, which encodes "prepro" sequence (nucleotides 1-783) in addition to the "mature" polypeptide (nucleotides 784-1392). Expression of NAT in such a system will typically involve a DNA molecule of SEQ ID NO: 6, which encodes "prepro" sequence (nucleotides 1-783) in addition to the "mature" polypeptide (nucleotides 784-1386).

Further details regarding NAT and methods for its preparation may be found in commonly assigned copending patent application Serial No._____(attorney reference A-596), filed concurrently herewith, which is hereby incorporated by reference.

Once the polypeptide (fibrolase or NAT) has been prepared, purified, and then assayed for activity (using procedures for fibrinolytic agents known to those skilled in the art), it may be formulated into pharmaceutical compositions in accordance with this invention.

In the present compositions (whether frozen or lyophilized), a stabilizer (which can also be referred to as a "glass-forming additive") is added to prevent or reduce precipitation and chemical degradation of fibrolase or NAT, whichever the case may be. A hazy or turbid solution at room temperature indicates that the polypeptide has precipitated. The term "stabilizer" means an excipient capable of preventing aggregation or other physical degradation, as well as chemical degradation (for example, autolysis, deamidation, oxidation, etc.) of fibrolase or NAT in an aqueous medium.

It has been found that the incorporation of a zinc stabilizer, and more specifically a water soluble

zinc salt, increases the stability of the metalloproteinase (fibrolase or NAT) in each type of composition, as compared to formulations in which inorganic or other types of organic compounds are used to prevent aggregation and/or polypeptide decomposition. Specifically, zinc concentrations above 0.01 millimolar (mM) will stabilize the metalloproteinase, with the proviso that zinc concentrations above 1 mM significantly limit the solubility of fibrolase or NAT. Thus, a range from about 0.01 mM to about 1 mM is advised. Examples of suitable zinc salts are zinc acetate, zinc sulfate and zinc chloride.

Frozen liquid compositions in accordance with this invention, in particular, may optionally (but not necessarily) also include a water soluble calcium salt as an additional stabilizer. Examples are calcium acetate, calcium sulfate or calcium chloride, which are preferably present in a concentration from about 0.001 to about 0.02 mM, and more preferably at a concentration of about 0.01 ± 0.002 mM.

If desired, other stabilizers that are conventionally employed in pharmaceutical compositions, such sucrose, trehalose or glycine, may be used in addition to the above mentioned. Typically, such stabilizers will be added in minor amounts ranging from, for example, about 0.1% to about 0.5% (w/v). Surfactant stabilizers, such as Tween 20 or Tween 80 (BASF), may also be added in conventional amounts.

If desired, the frozen liquid and lyophilized compositions can also include a bulking/osmolarity regulating agent. Preferably, mannitol is incorporated in a concentration of about 2% to about 8% weight by

volume (w/v), and usually at a concentration of about 5% (w/v).

The choice of a pharmaceutically-acceptable buffer and pH has also been found to affect the stability of the present compositions. Fibrolase or NAT is most stable above a neutral pH (7.0). Significant precipitation of either metalloproteinase occurs at a pH below 7.0 when the frozen composition is thawed or the lyophilized composition is reconstituted. The buffer system present in the compositions is selected to be physiologically compatible and to maintain a desired pH in the reconstituted solution as well as in the solution before lyophilization. Preferably, the buffers have a pH buffering capacity in the range of from about pH 7.0 to about pH 8.5.

Specifically, citric acid buffers (i.e., citric acid or a citric acid salt) are preferably incorporated in a concentration of about 20 mM to about 110 mM, and most preferably at about 100 mM in the frozen liquid composition and about 20 mM in the lyophilized composition. Citric acid salts are used as both buffering agents and stabilizing agents in the compositions of this invention. Whether an acid form itself or a salt thereof is used, the citric acid buffer will be chosen to adjust the pH of the composition to a value within the desired range as indicated above (in the case of the lyophilized composition, after reconstitution). Additional buffering agents, such as Tris, may be added in suitably effective amounts to maintain an adequate buffering capacity above pH 7.0.

A preferred liquid composition to be frozen will contain, in addition to solubilized fibrolase or

NAT, zinc acetate in a concentration of about 0.08 mM to about 0.12 mM, calcium acetate in a concentration of about 0.008 mM to about 0.012 mM, and citric acid (or sodium citrate) in a concentration of about 95 mM to about 105 mM, at about pH 7.4. Another preferred liquid composition will contain fibrolase or NAT, zinc acetate in a concentration of about 0.08 mM to about 0.12 mM, citric acid (or sodium citrate) in a concentration of about 18 mM to about 22 mM, Tris in a concentration of about 0.02 mM to about 0.06 mM, mannitol in a concentration of about 3% to about 6% (w/v), and Tween 80 in a concentration of about 0.008% to about 0.012% (w/v), at a pH of about 8.0.

A preferred lyophilizable composition for will contain, in addition to fibrolase or NAT, zinc sulfate in a concentration of about 0.08 mM to about 0.12 mM, citric acid (or sodium citrate) in a concentration of about 18 mM to about 22 mM, Tris in a concentration of about 3 mM to about 6 mM, mannitol in a concentration of about 3% to about 6% (w/v), and Tween 80 in a concentration of about 0.008% to about 0.012% (w/v), at a pH of about 8.0.

For all compositions in accordance with this invention, fibrolase or NAT is present in a concentration of about 0.1 mg/ml to about 50 mg/ml, preferably, with a concentration of about 5 mg/ml to about 40 mg/ml being more preferred, and a concentration of about 10 mg/ml to about 15 mg/ml being the most preferred.

The relative proportions of the excipients in these compositions will depend on several factors. For example, the amount of the metalloproteinase and bulking agent (e.g., mannitol) has an effect on the

amount of zinc (and calcium, if present) needed to stabilize the composition. The amount of stabilizer used in the compositions will depend on the amount needed to maintain the structural integrity of fibrolase or NAT during lyophilization or other processing or upon storage.

Still other excipients known in the art can also be included in the composition, provided they are physiologically compatible and are in no way detrimental to fibrolase or NAT. For example, the composition may contain minor amounts of additives, such as preservatives, tonicity-adjusting agents, anti-oxidants, or other polymers (for example, viscosity adjusting agents or extenders). Those skilled in the art can readily determine appropriate reagents that would be pharmaceutically useful, based on knowledge of and experience with other pharmaceutical compositions. See, for example, Remington's Pharmaceutical Sciences (latest edition), Mack Publishing Company, Easton, PA.

The compositions are expected to be stable for at least two years at -30°C for the frozen composition, and two years at 2°C to 8°C for the lyophilized composition. This long-term stability is beneficial for extending the shelf life of the pharmaceutical product and for long distance shipments.

In another aspect, the present invention also provides a method for preparing a lyophilized composition comprising the steps of:

(a) adjusting the pH of a mixture containing the composition ingredients without fibrolase or NAT to between pH 7.6 and pH 8.2,

(b) buffer exchanging a fibrolase or NAT containing solution into the composition solution of step (a) and then adding an effective amount of surfactant, and

(c) lyophilizing the mixture of step (b).

Fibrolase or NAT and effective amounts of the excipients are admixed under conditions effective to reduce aggregation of the dried fibrolase or NAT polypeptide upon reconstitution with the reconstitution medium, e.g., a solvent which is compatible with the selected administration route and does not negatively interfere with the metalloproteinase, such as sterile water, physiological saline solution, glucose solution or other aqueous solvents (e.g., alcohols such as ethyl, n-propyl or isopropyl, butyl alcohol or mixtures thereof) and, optionally, other components such as antibacterial agents.

The excipients may be admixed with the metalloproteinase at a suitable time before lyophilization. The time taken to mix the excipients and metalloproteinase should be for a sufficient period to prepare a suitable admixture; preferably, mixing will be carried out from about one to about thirty minutes.

Thereafter, the formulated metalloproteinase may be lyophilized, stored and reconstituted using standard methods; see Pikal, *supra*. The specific conditions under which fibrolase or NAT is freeze-dried and reconstituted are not particularly critical, provided that the conditions selected do not degrade the metalloproteinase and not be deleterious to the stabilizer. A preferred lyophilization cycle comprises

freezing the composition at -40°C, annealing the frozen sample at -12°C, and conducting the primary drying at -30°C to -35°C for twenty to fifty hours and secondary drying at 20°C for twenty to forty hours. Generally, the reconstituted composition will be used soon after reconstitution.

Both NAT and fibrolase are best delivered locally to the site of the clot for most effective treatment. Like fibrolase, NAT is covalently bound by α_2 macroglobulin in the general circulation. While complexed with α_1 macroglobulin, neither fibrolase nor NAT can access the target substrate (i.e., fibrin or fibrinogen) and are largely ineffective unless and until the maximum innate levels of α_2 macroglobulin are exceeded. Thus, it is preferred that the compositions of this invention be administered directly to the blood clot via intraarterial or intravenous catheterization.

Description of Specific Embodiments

The following examples further illustrate of the present invention.

The recombinant NAT (SEQ ID NO: 1) used in Examples 1-3 was produced by expression in *P. pastoris* Details regarding a suitable expression system and method may be found in the Stroman et al., Lair et al., Cregg et al. and Cregg patents referred to above. All chemicals were either analytical or USP grade.

Example 1

Preparation of Frozen Liquid Composition

An aqueous solution containing 100 mM of citric acid, 0.01 mM of calcium acetate and 0.1 mM of zinc sulfate is prepared by admixture of the ingredients, with the pH adjusted to 7.4. An NAT-containing solution is buffer exchanged into the solution by dialysis (alternatively, ultrafiltration can be used). The resulting NAT solution is concentrated to 10 mg/ml and stored frozen at a temperature of -30°C until ready for use.

Example 2

Preparation of Lyophilized Composition

Preparation of lyophilizable composition. An aqueous solution containing 5 mM of Tris, 20 mM of citric acid, 5% (w/v) of mannitol, 0.5% (w/v) of sucrose and 0.1 mM of zinc sulfate was prepared by admixture of the ingredients, with the pH adjusted to 8.0. A NAT containing solution was buffer exchanged into the composition solution by dialysis (ultrafiltration can be used instead). The resulting NAT solution was concentrated to 10 to 12 mg/ml. Tween 80 was added to a final concentration of 0.01% (w/v). The solution was stored at a temperature of 2-8°C until ready for lyophilization.

Freeze-drying cycle for lyophilized product. The above-prepared composition was first frozen at a temperature of -40°C in the lyophilizer. The annealing temperature was set at -12°C; the primary drying temperature was set at -30°C; and the secondary drying temperature was set at 20°C. The resulting freeze-dried cake showed good morphology and contained less than 3% water, as detected by the Karl Fischer titration method; see Fischer, *Angew Chemie*, Volume 48,

page 394 (1935). After the freeze-drying process was finished, the lyophilized cake was put into vials and rubber stoppers were sealed completely under vacuum by pressing down the upper metal shelves in the lyophilizer. The vials were then crimped with 13-mm flip-off aluminum seals and placed in incubators set at different temperatures.

Example 3

Analyses of Reconstituted Lyophilized Samples

Sample time points analysis. Sample vials were withdrawn from incubators at predetermined time intervals for the time points analysis. The lyophilized sample cake was first reconstituted by 0.9 ml of sterile water, i.e., "water-for-injection" (McGaw Inc., Irvine, CA). Clarity of the reconstituted sample solutions was visually examined. The filtered solution was analyzed by HPLC, UV-Vis spectroscopy and enzyme activity in order to quantify the remaining soluble NAT in these lyophilized samples.

Based on the above analyses, greater than 90% of NAT was recovered after reconstitution of the lyophilized product.

UV/Vis absorbence. 150-200 µl of NAT solution was loaded into a quartz glass suprasil 1-cm path length ultra-microcell. UV/Vis absorbence was measured on an HP 8452A diode-array spectrophotometer (Hewlett-Packard Co., Wilmington, DE). NAT concentrations were determined using $A^{0.1\%} = 1.05$ at 280 nm, based on calculation from the amino acid composition; for reference, see Edelhoch, Biochemistry, Volume 6, pages 1948-1954 (1967). After rehydration of the lyophilized

product, no detectable turbidity was observed when measuring the absorbence at 350 nanometers (nm).

High performance liquid chromatography. HPLC analyses of NAT samples were performed using an HP 1050 liquid chromatography system equipped with an HP 3D Chemstation for data acquisition (Hewlett-Packard Co.). NAT species were detected by absorbence at 280 nm and 214 nm using an HP diode-array detector.

For reversed-phase HPLC (RP-HPLC), samples were injected onto a Zorbax 300SB -C8 column (4.6 X 250 mm) (Hewlett-Packard Co.) in a mobile phase consisting of 51.5% buffer A (2% isopropanol, 0.1% TFA) and 48.5% buffer B (90% acetonitrile, 2% isopropanol, 0.1% TFA) at a flow rate of 0.6 ml/min. Buffer B was held for six minutes and then ramped up to 51% over twenty minutes. This concentration was held for one minute, followed by an eight-minute ramp and five-minute hold at 90%. Finally, buffer B was ramped back to 48.5% over a period of three minutes. Recovery of NAT after lyophilization as detected by this method was greater than 92%.

For ion-exchange HPLC (IEX-HPLC), samples were injected onto a Tosohaas DEAE-5PW column (7.5 X 75 mm) (Tosohaas, Montgomeryville, Alabama) in a mobile phase consisting of 90% buffer A (20 mM Tris, pH 8.5) and 10% buffer B (20 mM Tris, 250 mM NaCl, pH 8.5) at a flow rate of 0.5 ml/min. Then a gradient was applied, increasing from 10% buffer B to 75% buffer B in 20 minutes, then from 75% B to 90% buffer B in one minute. Buffer B was then held for five minutes, followed by a ramp to 10% buffer B in four minutes. Recovery of NAT after lyophilization as detected by this method was greater than 90%.

For size-exclusion HPLC (SEC-HPLC), samples were loaded into a Tosohaas G-2000SWXLcolumn (300 x 7.8 mm). Isocratic elution was applied at a flow rate of 0.8 ml/min using a buffer containing 15 mM sodium phosphate, pH 7.0, and 0.140 M sodium chloride. Recovery of NAT after lyophilization as detected by this method was greater than 95%.

Bioassay. Samples were screened for activity against fibrin clots. Small aliquots of a serial dilution of NAT ranging from 0.01 to 1.0 mg/ml were loaded onto preformed fibrin clots in 96-well plates. The samples were incubated for eighteen hours, and clot lysis was quantitated by absorbence at 500 nm. A plot of absorbence vs. NAT concentration for various formulations were compared to a prepared NAT standard for relative activity. There was no measurable difference in the fibrinolytic activity of the NAT after lyophilization, relative to the control (non-lyophilized) sample.

Similar test results are obtained with the frozen liquid composition as well, after the latter is thawed at 4°C and tested using these same protocols.

The foregoing invention has been described in some detail for purposes of clarity and understanding. It will also be obvious that various other combinations in form and detail can be made without departing from the scope of the invention as defined in the appended claims.

Example 4

The procedures of Examples 1 and 2 are repeated with recombinant fibrolase in place of NAT to produce similar frozen liquid and lyohilized pharmaceutical compositions.

CLAIMS

1. A pharmaceutical composition comprising a metalloproteinase fibrinolytic agent selected from the group consisting of fibrolase and novel acting thrombolytic (NAT), a zinc stabilizer and, optionally, a bulking agent, in a pharmaceutically-acceptable buffer.

2. The pharmaceutical composition of claim 1 wherein the zinc stabilizer is a water soluble zinc salt selected from the group consisting of zinc sulfate, zinc acetate and zinc chloride.

3. The pharmaceutical composition of claim 1 wherein the buffer is citric acid or a water soluble citric acid salt.

4. The pharmaceutical composition of claim 1 wherein the bulking agent is mannitol.

5. The pharmaceutical composition of claim 1 which has a pH in the range of about 6.5 to about 8.5

6. The pharmaceutical composition of claim 1 which is in the form of a frozen liquid.

7. The pharmaceutical composition of claim 6 which optionally contains a water soluble calcium salt.

8. The pharmaceutical composition of claim 7 in which the water soluble calcium salt is selected from the group consisting of calcium acetate, calcium sulfate and calcium chloride.

9. The pharmaceutical composition of claim 1 which is lyophilized.

10. The pharmaceutical composition of claim 1 wherein the metalloproteinase has the amino acid sequence of SEQ ID NO: 1.

11. An aqueous pharmaceutical composition comprising about 0.1 to about 50 mg/ml of a metalloproteinase fibrinolytic agent selected from the group consisting of fibrolase and novel acting thrombolytic (NAT), about 0.08 to about 0.12 mM of zinc sulfate, about 0.008 mM to about 0.012 mM of calcium acetate, and about 95 to 110 mM of citric acid or sodium citrate, with the pH of said composition being about 7.4.

12. A pharmaceutical composition according to claim 11, comprising 10 mg/ml of the metalloproteinase in an aqueous solution comprising 100 mM of citric acid, 0.01 mM calcium acetate and 0.1 mM zinc sulfate.

13. An aqueous pharmaceutical composition comprising about 0.1 to about 50 mg/ml of a metalloproteinase fibrinolytic agent selected from the group consisting of fibrolase and novel acting thrombolytic (NAT), about 0.08 to about 0.12 mM of zinc acetate, about 18 to about 22 mM of citric acid or sodium citrate, about 0.02 to about 0.06 mM of Tris, about 3 to about 6 percent (w/v) of mannitol, and about 0.008 to about 0.012 percent (w/v) of Tween 80, with the pH of said composition being about 8.0.

14. An aqueous pharmaceutical composition suitable for lyophilization, comprising about 0.1 to about 50 mg/ml of a metalloproteinase fibrinolytic agent selected from the group consisting of fibrolase and novel acting thrombolytic (NAT), about 0.08 to about 0.12 mM of zinc sulfate, about 18 to about 22 mM of citric acid or sodium citrate, about 3 to about 6 mM of Tris, about 3 to about 6 percent (w/v) of mannitol, and about 0.008 to about 0.012 percent (w/v) of Tween 80, and optionally about 0.1 to about 0.5 percent (w/v) of sucrose, with the pH of said composition being about 8.0.

15. A pharmaceutical composition according to claim 14, comprising 12 mg/ml of the metalloproteinase, 5 mM of Tris, 20 mM of citric acid, 5 percent (w/v) of mannitol, 0.5 percent (w/v) of sucrose, 0.01 percent (w/v) of Tween 80, and 0.1 mM of zinc sulfate, with the pH of said composition being about 8.0.

16. A method for preparing a lyophilized composition, comprising the steps of:

(a) forming a mixture of a metalloproteinase fibrinolytic agent selected from the group consisting of fibrolase and novel acting thrombolytic (NAT), a zinc salt, a bulking agent, a stabilizing disaccharide and a surfactant in a buffer, and

(b) lyophilizing the mixture from step (a).

17. The method of claim 16, in which the pH of said composition is adjusted to between about 7.8 and about 8.2 prior to lyophilization.

18. The method of claim 16, comprising the steps of:

(a) adjusting the pH of a solution containing the zinc salt, bulking agent, and stabilizing disaccharide to a pH between 7.6 and 8.2,

(b) buffer exchanging solution containing the metalloproteinase into the solution of step (a), and then adding an effective amount of the surfactant, and

(c) lyophilizing the mixture of step (b).

19. A lyophilized pharmaceutical composition prepared by the method of claim 18.

20. Use of a therapeutically effective amount of a reconstituted aqueous pharmaceutical composition of claim 1 which has been lyophilized in the manufacture of a medicament for treating a patient in need of blood clot lysis.

21. A kit for preparing an aqueous pharmaceutical composition comprising a first container having a lyophilized composition of a metalloproteinase fibrinolytic agent selected from the group consisting of fibrolase and novel acting thrombolytic (NAT) and a second container having a physiogically acceptable solvent for the lyophilized composition.

SEQUENCE LISTING

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Peterson, Brian A.

<120> PHARMACEUTICAL COMPOSITIONS OF FIBRINOLYTIC AGENT

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35 40 45

Asn Ile Gln Phe Thr Leu Val Gly Leu Glu Ile Trp Ser Asn Gln Asp
50 55 60

Leu Ile Thr Val Thr Ser Val Ser His Asp Thr Leu Ala Ser Phe Gly
65 70 75 80

Asn Trp Arg Glu Thr Asp Leu Leu Arg Arg Gln Arg His Asp Asn Ala
85 90 95

Gln Leu Leu Thr Ala Ile Asp Phe Asp Gly Asp Thr Val Gly Leu Ala
100 105 110

Tyr Val Gly Gly Met Cys Gln Leu Lys His Ser Thr Gly Val Ile Gln
115 120 125

Asp His Ser Ala Ile Asn Leu Leu Val Ala Leu Thr Met Ala His Glu
130 135 140

Leu Gly His Asn Leu Gly Met Asn His Asp Gly Asn Gln Cys His Cys
145 150 155 160

Gly Ala Asn Ser Cys Val Met Ala Ala Met Leu Ser Asp Gln Pro Ser
165 170 175

Lys Leu Phe Ser Asp Cys Ser Lys Lys Asp Tyr Gln Thr Phe Leu Thr
180 185 190

Val Asn Asn Pro Gln Cys Ile Leu Asn Lys Pro
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<210> 4
<211> 609
<212> DNA
<213> Agkistrodon contortrix

<220>
<223> Encodes native fibrolase of Agkistrodon Contortrix

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attaacgaaa tctacagacc actgaacatc caattcactt tggttggttt ggaaatctgg 180
tccaaccaag atttgatcac cgttacttct gtatcccacg acactctggc atccttcggt 240
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<210> 5
<211> 1392
<212> DNA
<213> Agkistrodon contortrix

<220>
<223> Native profibrolase of Agkistrodon Contortrix

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tactcagatt tagaagggga tttcgatgtt gctgttttgc cattttccaa cagcacaaat 180
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<210> 6
<211> 1386
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: proNAT (analog
of profibrolase of Agkistrodon Contortrix

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tactcagatt tagaagggga tttcgatgtt gctgttttgc cattttccaa cagcacaaat 180
aacgggttat tgtttataaa tactactatt gccagcattg ctgctaaaga agaaggggta 240
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gcaaactcct gtgttatggc tgctatgctg tccgatcaac catccaaact gttctccgac 1320
tgctctaaga aagactacca gaccttcctg accgttaaca acccgcagtg tatcctgaac 1380
aaaccg 1386