



(86) Date de dépôt PCT/PCT Filing Date: 1999/11/15  
(87) Date publication PCT/PCT Publication Date: 2000/06/02  
(85) Entrée phase nationale/National Entry: 2001/05/18  
(86) N° demande PCT/PCT Application No.: US 99/27074  
(87) N° publication PCT/PCT Publication No.: WO 00/30677  
(30) Priorité/Priority: 1998/11/20 (60/109,153) US

(51) Cl.Int.<sup>7</sup>/Int.Cl.<sup>7</sup> A61K 38/48, A61P 7/02  
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(54) Titre : PROCEDE DE TRAITEMENT DES FIEVRES VIRALES HEMORRAGIQUES  
(54) Title: METHOD OF TREATING VIRAL HEMORRHAGIC FEVER

(57) **Abrégé/Abstract:**

The present invention provides a method of treatment of viral hemorrhagic fever with protein C. The claimed invention provides a needed therapy for a serious and debilitating disorder while avoiding complications such as bleeding tendency, toxicity and general side effects of currently available anti-coagulant agents.



**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>A61K 38/48, A61P 7/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/30677</b> <b>(43) International Publication Date:</b> 2 June 2000 (02.06.00)
<b>(21) International Application Number:</b> PCT/US99/27074 <b>(22) International Filing Date:</b> 15 November 1999 (15.11.99) <b>(30) Priority Data:</b> 60/109,153      20 November 1998 (20.11.98)      US <b>(71) Applicant (for all designated States except US):</b> ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> FISHER, Charles, Jack [US/US]; 12538 Pebblepointe Pass, Carmel, IN 46033 (US). YAN, Sau-Chi, Betty [US/US]; 8131 Menlo Court East Drive, Indianapolis, IN 46240 (US). <b>(74) Agents:</b> BARRETT, Brian, P. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> METHOD OF TREATING VIRAL HEMORRHAGIC FEVER  <b>(57) Abstract</b>  The present invention provides a method of treatment of viral hemorrhagic fever with protein C. The claimed invention provides a needed therapy for a serious and debilitating disorder while avoiding complications such as bleeding tendency, toxicity and general side effects of currently available anti-coagulant agents.		

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**Method of treating viral hemorrhagic fever**

This application claims priority of Provisional Application Serial No. 60/109,153 filed November 20, 1998.

5 This invention relates to medical science particularly the treatment of viral hemorrhagic fever with protein C.

Protein C is a vitamin K dependent serine protease and naturally occurring anticoagulant that plays a role in the regulation of hemostasis by inactivating Factors Va and  
10 VIIIIa in the coagulation cascade. Human protein C circulates as a 2-chain zymogen, but functions at the endothelial and platelet surface following conversion to activated protein C (aPC) by limited proteolysis with thrombin in complex with the cell surface membrane protein,  
15 thrombomodulin.

In conjunction with other proteins, aPC functions as perhaps the most important down-regulator of blood coagulation resulting in protection against thrombosis. In addition to its anti-coagulation functions, aPC has anti-  
20 inflammatory effects through its inhibition of cytokine generation (e.g. TNF and IL-1) and also exerts profibrinolytic properties that facilitate clot lysis. Thus, the protein C enzyme system represents a major physiological mechanism of anti-coagulation, anti-  
25 inflammation, and fibrinolysis.

Viral hemorrhagic fever is a clinical syndrome associated with significant mortality. Without exception, hemorrhagic fever viruses are enveloped RNA viruses that belong to four viral families: Arenaviridae [Junin, Machupo,  
30 Lassa fever], Bunyaviridae [Crimean-Congo hemorrhagic fever,



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Rift Valley fever, Hantaan and related viruses], Filoviridae [Ebola, Marburg] and Flaviviridae [Dengue, Yellow fever, Omsk hemorrhagic fever, Kyasanur Forest disease], [Cosgriff, T.M., Reviews of Infectious Diseases 11(4): S672-S688, 5 1989]. These agents produce a wide spectrum of disease severity, but the most extreme manifestations include circulatory instability, increased vascular permeability, and diffuse hemorrhage [Lacy, et al. Advances in Pediatric Infectious Diseases, 12:21-53, 1997].

10       The underlying mechanism of the bleeding in the hemorrhagic fevers is complex. Possible factors include thrombocytopenia alone, or thrombocytopenia associated with disseminated intravascular coagulation (DIC). Central to the mechanism may well be endothelial cell dysfunction, 15 which has profound implications for both platelets and coagulation. Another possible factor is a decrease in levels of coagulation factors in plasma as the result of either increased consumption or impaired synthesis. Increased consumption occurs in DIC, while impaired 20 synthesis is the likely consequence of liver injury. Liver involvement is a universal occurrence in viral hemorrhagic fever. For example, in Yellow fever, Rift Valley fever and Crimean-Congo hemorrhagic fever, the temporal association of hemorrhage with severe hepatic dysfunction is evident.

25       Viruses alter hemostasis in two general ways. The first is through direct effect on cellular functions, and the second is through activation of immune and inflammatory pathways. Both mechanisms may lead to variable degrees of cellular injury, including cell death. Activation of 30 coagulation pathways is an important part of immune and

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inflammatory reactions and accounts for the fibrin deposition that sometimes is observed in these reactions.

Thrombocytopenia is a universal occurrence in viral hemorrhagic fevers. For example, in dengue hemorrhagic fever both changes suggestive of decreased thrombopoiesis and of increased platelet consumption has been determined. This is also the case in hemorrhagic fever with renal syndrome (HFRS) caused by Hantaan and related viruses. Other mechanisms for increased platelet destruction in viral infections include direct interaction of platelets with viruses, DIC, and endothelial injury.

In Ebola and Marburg hemorrhagic fevers, generalized hemorrhages are found in most organs. Focal necrosis without significant inflammation is also widely seen, especially in the lungs, liver, kidneys, and lymphoid organs. DIC is common.

Currently, there is no effective therapy to treat viral hemorrhagic fever. In the absence of viral-specific chemotherapy, management is primarily supportive. Therefore, a need exists for a safe, effective therapy of patients with viral hemorrhagic fever.

The present invention is the first to describe the treatment of viral hemorrhagic fever with protein C. Protein C, with its anticoagulant, anti-inflammatory, and profibrinolytic activities, is useful for the treatment of the hypercoagulable state or protein C deficiency that occurs in viral hemorrhagic fever patients.

The present invention provides a method of treating a patient suffering from viral hemorrhagic fever which



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comprises, administering to said patient a pharmaceutically effective amount of protein C.

The present invention further provides a method of treating viral hemorrhagic fever in a patient in need thereof, which comprises administering to said patient a pharmaceutically effective amount of activated protein C such that an activated protein C plasma level of about 2 ng/ml to about 300 ng/ml is achieved.

For purposes of the present invention, as disclosed and claimed herein, the following terms are as defined below.

Protein C refers to a vitamin K dependent serine protease with anticoagulant, anti-inflammatory, and profibrinolytic properties which includes, but is not limited to, plasma derived and recombinant produced protein C. Protein C includes and is preferably human protein C although protein C may also include other species or derivatives having protein C proteolytic, amidolytic, esterolytic, and biological (anticoagulant, profibrinolytic, and anti-inflammatory) activities. Examples of protein C derivatives are described by Gerlitz, et al., U.S. Patent No. 5,453,373, and Foster, et al., U.S. Patent No. 5,516,650, the entire teachings of which are hereby included by reference.

Zymogen - an enzymatically inactive precursor of a proteolytic enzyme. Protein C zymogen, as used herein, refers to secreted, inactive forms, whether one chain or two chains, of protein C.

Activated protein C or aPC refers to protein C zymogen which has been converted by limited proteolysis to its activated form. aPC includes and is preferably human

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protein C although aPC may also include other species or derivatives having protein C proteolytic, amidolytic, esterolytic, and biological (anticoagulant or pro-fibrinolytic) activities. Examples of protein C derivatives  
5 are noted above in the description of protein C.

HPC - human protein C zymogen.

r-hPC - recombinant human protein C zymogen.

r-aPC - recombinant human activated protein C produced by activating r-hPC *in vitro* or by direct secretion of the  
10 activated form of protein C from procaryotic cells, eukaryotic cells, and transgenic animals or plants, including, for example, secretion from human kidney 293 cells as a zymogen then purified and activated by techniques well known to the skilled artisan and demonstrated in Yan,  
15 U.S. Patent No. 4,981,952, and Cottingham, WO97/20043, the entire teachings of which are herein incorporated by reference.

Plasma derived activated protein C - activated protein C produced by activating plasma HPC as described in Eibl,  
20 U.S. Patent No. 5,478,558, the entire teaching of which is herein incorporated by reference.

Continuous infusion - continuing substantially uninterrupted the introduction of a solution into a vein for a specified period of time.

25 Bolus injection - the injection of a drug in a defined quantity (called a bolus) over a period of time up to about 120 minutes.

Suitable for administration - a lyophilized formulation or solution that is appropriate to be given as a  
30 therapeutic agent.



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Unit dosage form - refers to physically discrete units suitable as unitary dosages for human subjects, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

Pharmaceutically effective amount - represents an amount of a compound of the invention that is capable of inhibiting sepsis in humans. The particular dose of the compound administered according to this invention will, of course, be determined by the attending physician evaluating the particular circumstances surrounding the case.

Viral hemorrhagic fever - refers to hemorrhagic fever caused by enveloped RNA viruses that belong to four viral families: Arenaviridae [Junin, Machupo, Lassa fever], Bunyaviridae [Crimean-Congo hemorrhagic fever, Rift Valley fever, Hantaan and related viruses], Filoviridae [Ebola, Marburg] and Flaviviridae [Dengue, Yellow fever, Omsk hemorrhagic fever, Kyasanur Forest disease]. These agents produce a wide spectrum of disease severity, the most extreme manifestations includes circulatory instability, increased vascular permeability, and diffuse hemorrhage.

The present invention provides for the treatment of viral hemorrhagic fever with protein C. Protein C, with its anticoagulant, anti-inflammatory, and profibrinolytic activities, is useful for the treatment of the hypercoagulable state and/or protein C deficiency that occurs in viral hemorrhagic fever patients.

The protein C administered according to this invention may be generated and/or isolated by any means known in the art or as described in U.S. Patent No. 4,981,952, and U.S.



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Patent No. 5,550,036, herein incorporated by reference. For example, protein C can be produced by secreting full-length, soluble protein C, or biologically active polypeptide variants of protein C from a cell which comprises (a) constructing a vector comprising DNA encoding protein C; (b) transfecting the cell with the vector; and (c) culturing the cell so transfected in culture medium under conditions such that full length soluble protein C or biologically active polypeptide variants of protein C, is secreted. Further, the cell is a eukaryotic cell, e.g. mammalian cell such as Syrian hamster AV12 cell, human embryonic 293 cell, or Baby Hamster Kidney cell.

The protein C used in the treatment of viral hemorrhagic fever can be formulated according to known methods to prepare pharmaceutically useful compositions. For example, a desired formulation would be one that is a stable lyophilized product of high purity comprising a bulking agent such as sucrose, a salt such as sodium chloride, a buffer such as sodium citrate and protein C or aPC.

The protein C will be administered parenterally to ensure its delivery into the bloodstream in an effective form by injecting the appropriate dose as continuous infusion for about 1 hour to about 240 hours.

Those skilled in the art can readily optimize pharmaceutically effective dosages and administration regimens for therapeutic compositions comprising protein C, as determined by good medical practice and the clinical condition of the individual patient. Generally, the amount of protein C administered will be from about 5.0  $\mu\text{g/kg/hr}$  to

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about 250  $\mu\text{g/kg/hr}$ . Preferably, the protein C used in the treatment of viral hemorrhagic fever is activated protein C (aPC). The amount of aPC administered will be from about 1.0  $\mu\text{g/kg/hr}$  to about 96  $\mu\text{g/kg/hr}$ . More preferably the amount of aPC administered will be about 1.0  $\mu\text{g/kg/hr}$  to about 50  $\mu\text{g/kg/hr}$ . While more preferably the amount of aPC administered will be about 1.0  $\mu\text{g/kg/hr}$  to about 35  $\mu\text{g/kg/hr}$ . Even more preferably the amount of aPC administered will be about 5.0  $\mu\text{g/kg/hr}$  to about 30  $\mu\text{g/kg/hr}$ . Yet even more preferably the amount of aPC administered will be about 15  $\mu\text{g/kg/hr}$  to 30  $\mu\text{g/kg/hr}$ . Still even more preferably the amount of aPC administered will be about 20  $\mu\text{g/kg/hr}$  to 30  $\mu\text{g/kg/hr}$ . The preferable amount of aPC administered will be about 24  $\mu\text{g/kg/hr}$ . The most preferable amount of aPC administered will be about 48  $\mu\text{g/kg/hr}$ . The appropriate dose of aPC administered will result in a reduction of the thrombotic complications associated with viral hemorrhagic fever.

The plasma ranges obtained from the amount of aPC administered will be about 2 ng/ml to about 300 ng/ml. The preferred plasma ranges are from about 2 ng/ml to 200 ng/ml. Most preferably, plasma ranges are from about 30 ng/ml to about 150 ng/ml and still more preferably about 100 ng/ml.

Alternatively, the aPC will be administered by injecting one third of the appropriate dose per hour as a bolus injection followed by the remaining two thirds of the hourly dose as continuous infusion for one hour followed by continuous infusion of the appropriate dose for twenty-three hours which results in the appropriate dose administered over 24 hours. In addition, the bolus injection will be



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administered via an intravenous bag drip pump or syringe pump at 2 times the normal rate for 15 minutes followed by 1.5 times the normal rate for 45 minutes. The normal rate i.e. that rate which has been determined to administer the appropriate dose level of the therapeutic agent per time period, is then continued for up to 240 hours.

The use of protein C in the treatment of viral hemorrhagic fever as presented in the present invention will provides a needed therapy for a serious and debilitating disorder. The use of protein C is efficacious and avoids complications such as bleeding tendency, toxicity, and other general side effects of currently available anti-coagulant agents.

The following examples are provided merely to further illustrate the present invention. The scope of the invention shall not be construed as merely consisting of the following examples.

#### Preparation 1

##### Preparation of Human Protein C

Recombinant human protein C (r-hPC) was produced in Human Kidney 293 cells by techniques well known to the skilled artisan such as those set forth in Yan, U.S. Patent No. 4,981,952, the entire teaching of which is herein incorporated by reference. The gene encoding human protein C is disclosed and claimed in Bang, et al., U.S. Patent No. 4,775,624, the entire teaching of which is incorporated herein by reference. The plasmid used to express human protein C in 293 cells was plasmid pLPC which is disclosed in Bang, et al., U.S. Patent No. 4,992,373, the entire

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teaching of which is incorporated herein by reference. The construction of plasmid pLPC is also described in European Patent Publication No. 0 445 939, and in Grinnell, et al., 1987, Bio/Technology 5:1189-1192, the teachings of which are also incorporated herein by reference. Briefly, the plasmid was transfected into 293 cells, then stable transformants were identified, subcultured and grown in serum-free media. After fermentation, cell-free medium was obtained by microfiltration.

10       The human protein C was separated from the culture fluid by an adaptation of the techniques of Yan, U.S. Patent No. 4,981,952. The clarified medium was made 4 mM in EDTA before it was absorbed to an anion exchange resin (Fast-Flow Q, Pharmacia). After washing with 4 column volumes of 20 mM  
15   Tris, 200 mM NaCl, pH 7.4 and 2 column volumes of 20 mM Tris, 150 mM NaCl, pH 7.4, the bound recombinant human protein C zymogen was eluted with 20 mM Tris, 150 mM NaCl, 10 mM CaCl<sub>2</sub>, pH 7.4. The eluted protein was greater than 95% pure after elution as judged by SDS-polyacrylamide gel  
20   electrophoresis.

      Further purification of the protein was accomplished by making the protein 3 M in NaCl followed by adsorption to a hydrophobic interaction resin (Toyopearl Phenyl 650 M, TosoHaas) equilibrated in 20 mM Tris, 3 M NaCl, 10 mM CaCl<sub>2</sub>,  
25   pH 7.4. After washing with 2 column volumes of equilibration buffer without CaCl<sub>2</sub>, the recombinant human protein C was eluted with 20 mM Tris, pH 7.4.

      The eluted protein was prepared for activation by removal of residual calcium. The recombinant human protein  
30   C was passed over a metal affinity column (Chelex-100, Bio-



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Rad) to remove calcium and again bound to an anion exchanger (Fast Flow Q, Pharmacia). Both of these columns were arranged in series and equilibrated in 20 mM Tris, 150 mM NaCl, 5 mM EDTA, pH 7.4. Following loading of the protein, 5 the Chelex-100 column was washed with one column volume of the same buffer before disconnecting it from the series. The anion exchange column was washed with 3 column volumes of equilibration buffer before eluting the protein with 0.4 M NaCl, 20 mM Tris-acetate, pH 6.5. Protein concentrations 10 of recombinant human protein C and recombinant activated protein C solutions were measured by UV 280 nm extinction  $E^{0.1\%}=1.81$  or 1.85, respectively.

## Preparation 2

### 15      Activation of Recombinant Human Protein C

Bovine thrombin was coupled to Activated CH-Sepharose 4B (Pharmacia) in the presence of 50 mM HEPES, pH 7.5 at 4°C. The coupling reaction was done on resin already packed into a column using approximately 5000 units thrombin/mL 20 resin. The thrombin solution was circulated through the column for approximately 3 hours before adding 2-amino-ethanol (MEA) to a concentration of 0.6 mL/L of circulating solution. The MEA-containing solution was circulated for an additional 10-12 hours to assure complete blockage of the 25 unreacted amines on the resin. Following blocking, the thrombin-coupled resin was washed with 10 column volumes of 1 M NaCl, 20 mM Tris, pH 6.5 to remove all non-specifically bound protein, and was used in activation reactions after equilibrating in activation buffer.

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Purified r-hPC was made 5 mM in EDTA (to chelate any residual calcium) and diluted to a concentration of 2 mg/mL with 20 mM Tris, pH 7.4 or 20 mM Tris-acetate, pH 6.5. This material was passed through a thrombin column equilibrated at 37°C with 50 mM NaCl and either 20 mM Tris pH 7.4 or 20 mM Tris-acetate pH 6.5. The flow rate was adjusted to allow for approximately 20 min. of contact time between the r-hPC and thrombin resin. The effluent was collected and immediately assayed for amidolytic activity. If the material did not have a specific activity (amidolytic) comparable to an established standard of protein C, it was recycled over the thrombin column to activate the r-hPC to completion. This was followed by 1:1 dilution of the material with 20 mM buffer as above, with a pH of either 7.4 or 6.5 to keep the protein C at lower concentrations while it awaited the next processing step.

Removal of leached thrombin from the protein C material was accomplished by binding the protein C to an anion exchange resin (Fast Flow Q, Pharmacia) equilibrated in activation buffer (either 20 mM Tris, pH 7.4 or 20 mM Tris-acetate, pH 6.5) with 150 mM NaCl. Thrombin does not interact with the anion exchange resin under these conditions, but passes through the column into the sample application effluent. Once the protein C is loaded onto the column, a 2-6 column volume wash with 20 mM equilibration buffer is done before eluting the bound protein C with a step elution using 0.4 M NaCl in either 5 mM Tris-acetate, pH 6.5 or 20 mM Tris, pH 7.4. Higher volume washes of the column facilitated more complete removal of the dodecapeptide. The material eluted from this column was



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stored either in a frozen solution (-20°C) or as a lyophilized powder.

The anticoagulant activity of activated protein C was determined by measuring the prolongation of the clotting time in the activated partial thromboplastin time (APTT) clotting assay. A standard curve was prepared in dilution buffer (1 mg/mL radioimmunoassay grade bovine serum albumin [BSA], 20 mM Tris, pH 7.4, 150 mM NaCl, 0.02% NaN<sub>3</sub>) ranging in protein C concentration from 125-1000 ng/mL, while samples were prepared at several dilutions in this concentration range. To each sample cuvette, 50 µL of cold horse plasma and 50 µL of reconstituted activated partial thromboplastin time reagent (APTT Reagent, Sigma) were added and incubated at 37°C for 5 min. After incubation, 50 µL of the appropriate samples or standards were added to each cuvette. Dilution buffer was used in place of sample or standard to determine basal clotting time. The timer of the fibrometer (CoA Screener Hemostasis Analyzer, American Labor) was started immediately after the addition of 50 µL 37°C 30 mM CaCl<sub>2</sub> to each sample or standard. Activated protein C concentration in samples are calculated from the linear regression equation of the standard curve. Clotting times reported here are the average of a minimum of three replicates, including standard curve samples.

The above descriptions enable one with appropriate skill in the art to prepare protein C for utilization in the treatment of viral hemorrhagic fever.

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Preparation 3Formulation of Activated Protein C

A stable lyophilized formulation of activated protein C was prepared by a process which comprises lyophilizing a solution comprising about 2.5 mg/mL activated protein C, about 15 mg/mL sucrose, about 20 mg/mL NaCl, and a sodium citrate buffer having a pH greater than 5.5 but less than 6.5. Additionally, the stable lyophilized formulation of activated protein C comprises lyophilizing a solution comprising about 5 mg/mL activated protein C, about 30 mg/mL sucrose, about 38 mg/mL NaCl, and a citrate buffer having a pH greater than 5.5 but less than 6.5.

The ratio of protein C:salt:bulking agent (w:w:w) is an important factor in a formulation suitable for the freeze drying process. The ratio varies depending on the concentration of protein C, salt selection and concentration and bulking agent selection and concentration. Particularly, a ratio of about 1 part activated protein C to about 7.6 parts salt to about 6 parts bulking agent is preferred.

A unit dosage formulation of activated protein C suitable for administration by continuous infusion was prepared by mixing activated protein C, NaCl, sucrose, and sodium citrate buffer. After mixing, 4 mL of the solution was transferred to a unit dosage receptacle and lyophilized. The unit dosage receptacle containing about 5 mg to about 20 mg of activated protein C, suitable for administering a dosage of about 0.01 mg/kg/hr to about 0.05 mg/kg/hr to patients in need thereof, was sealed and stored until use.



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Example 1A placebo-controlled double blind trial of recombinant human activated protein C (r-aPC) in patients with viral hemorrhagic fever

5       Studies of viral hemorrhagic fever patients have demonstrated abnormalities of platelet survival and aggregation as well as alterations in clotting factors. The current treatment approach to patients with viral hemorrhagic fever is primarily supportive in the absence of  
10   an effective anti-viral agent.

      This trial aims to show that the infusion of r-aPC results in a statistically significant reduction in mortality associated with viral hemorrhagic fever.

      Inclusion criteria include patients with viral  
15   hemorrhagic fever. These patients are entered into the trial immediately upon diagnosis and entry into the hospital. Patients meeting the inclusion criteria for viral hemorrhagic fever are given the standard supportive care. In addition, the patients receive either placebo or r-aPC  
20   for 96 hours. r-aPC is given in a dose of 24 µg/kg/hr.

      The primary endpoints of the study are: the safety and efficacy of r-aPC as compared to placebo, and; the ability of r-aPC to correct coagulopathy and effect mortality.

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We Claim:

1. A method of treating a patient suffering from viral  
5 hemorrhagic fever which comprises, administering to said  
patient a pharmaceutically effective amount of protein C.

2. The method of Claim 1 wherein the protein C is  
human protein C zymogen.

10

3. The method of Claim 1 wherein the protein C is  
human activated protein C.

4. The method according to Claim 3, wherein the amount  
15 of human activated protein C is about 1  $\mu\text{g/kg/hr}$  to about  
96  $\mu\text{g/kg/hr}$ .

5. The method of Claim 4, wherein the human activated  
protein C is administered by continuous infusion for about 1  
20 to about 240 hours.

6. A method of treating viral hemorrhagic fever in a  
patient in need thereof, which comprises administering to  
said patient a pharmaceutically effective amount of  
25 activated protein C such that an activated protein C plasma  
level of about 2 ng/ml to about 300 ng/ml is achieved.

7. The method of Claim 6 wherein the activated protein  
C is administered in a bolus injection.

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8. The method of Claim 6 wherein the activated protein C is administered by continuous infusion for about 1 to about 240 hours.

5        9. The method of Claim 6 wherein the activated protein C is administered first as a bolus then as a continuous infusion.

10       10. The method of Claim 9 wherein one third of the activated protein C required to achieve activated protein C plasma levels in the range of about 2 ng/ml to about 300 ng/ml is administered in a bolus injection followed by continuous infusion of the remaining two thirds of the activated protein C.