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(72) Inventeurs/Inventors:
SPIRA, JACK, SE;
WIDLUND, LARS, SE;
OSTERBERG, THOMAS, SE;
SJOSTROM, BRITA, SE;
MIKAELSSON, MARIANNE, SE

(73) Propriétaire/Owner:
BIOVITRUM AB, SE

(74) Agent: FETHERSTONHAUGH & CO.

(54) Titre : FORMULATION PHARMACEUTIQUE SERVANT A EFFECTUER L'ADMINISTRATION SOUS-CUTANEE,
INTRAMUSCULAIRE OU INTRADERMIQUE DU FACTEUR VIII OU DU FACTEUR IX
(54) Title: PHARMACEUTICAL FORMULATION FOR SUBCUTANEOUS, INTRAMUSCULAR OR INTRADERMAL
ADMINISTRATION OF FACTOR VIII OR FACTOR IX

(57) **Abrégé/Abstract:**

The present invention relates to a pharmaceutical formulation for subcutaneous, intramuscular or intradermal administration comprising coagulation factor VIII or factor IX and use thereof for manufacture of a medicament for treating haemophilia A or B. The formulation comprises coagulation factor VIII or factor IX with an activity of at least 200 IU/ml and an additive increasing the bio-availability of factor VIII or factor IX. Tests with factor VIII gives a therapeutic level of active factor VIII in the blood stream for a surprisingly long period of time after subcutaneous, intramuscular or intradermal administration. The factor VIII is suitably a highly purified recombinant factor VIII, and preferably a deletion derivative thereof, which can be used for the manufacture of a medicament for subcutaneous administration.





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(21) International Application Number: PCT/SE95/00348 (22) International Filing Date: 31 March 1995 (31.03.95) (30) Priority Data: 9401105-3 31 March 1994 (31.03.94) SE 9500036-0 5 January 1995 (05.01.95) SE (71) Applicant (for all designated States except US): PHARMACIA AB [SE/SE]; S-171 97 Stockholm (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): SPIRA, Jack [SE/SE]; Tottvägen 6, S-171 35 Stockholm (SE). WIDLUND, Lars [SE/SE]; Stadingeplan 1, S-163 63 Spånga (SE). ÖSTERBERG, Thomas [SE/SE]; Folkungagatan 88 B, S-116 22 Stockholm (SE). SJÖSTRÖM, Brita [SE/SE]; Skogvaktarvägen 4, S-131 50 Saltsjö-Duvnäs (SE). MIKAELSSON, Marianne [SE/SE]; Smedjevägen 9, S-161 35 Bromma (SE). (74) Agents: WAHLSTRÖM, Christer et al.; Pharmacia AB, S-112 87 Stockholm (SE).			(81) Designated States: AU, BG, BR, BY, CA, CN, CZ, EE, FI, HU, IS, JP, KR, LT, LV, MX, NO, NZ, PL, RO, RU, SI, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). 2186126 Published <i>With international search report.</i>
(54) Title: PHARMACEUTICAL FORMULATION FOR SUBCUTANEOUS, INTRAMUSCULAR OR INTRADERMAL ADMINISTRATION OF FACTOR VIII OR FACTOR IX (57) Abstract The present invention relates to a pharmaceutical formulation for subcutaneous, intramuscular or intradermal administration comprising coagulation factor VIII or factor IX and use thereof for manufacture of a medicament for treating haemophilia A or B. The formulation comprises coagulation factor VIII or factor IX with an activity of at least 200 IU/ml and an additive increasing the bio-availability of factor VIII or factor IX. Tests with factor VIII gives a therapeutic level of active factor VIII in the blood stream for a surprisingly long period of time after subcutaneous, intramuscular or intradermal administration. The factor VIII is suitably a highly purified recombinant factor VIII, and preferably a deletion derivative thereof, which can be used for the manufacture of a medicament for subcutaneous administration.			

Pharmaceutical formulation for subcutaneous,
intramuscular or intradermal administration of
factor VIII or factor IX

FIELD OF THE INVENTION

5 The present invention relates to a pharmaceutical formulation for
subcutaneous, intramuscular or intradermal administration com-
prising coagulation factor VIII or factor IX and use thereof for
manufacture of a medicament for treating haemophilia A or B.
The formulation comprises coagulation factor VIII or factor IX
10 with an activity of at least 200 IU/ml and an additive increasing
the bioavailability of factor VIII or factor IX. Tests with factor
VIII gives a therapeutic level of active factor VIII in the blood
stream for a surprisingly long period of time after subcutaneous,
intramuscular or intradermal administration. The factor VIII is
15 suitably a highly purified recombinant factor VIII, and prefer-
ably a deletion derivative thereof, which can be used for the
manufacture of a medicament for subcutaneous administration.

BACKGROUND OF THE INVENTION

20 Haemophilia is an inherited disease which has been known for
centuries but it is only within the last four decades that it has
been possible to differentiate between the various forms; hae-
mophilia A, haemophilia B and haemophilia C. Haemophilia A is
25 the most frequent form. It affects only males with an incidence
of one or two individuals per 10,000 live-born males. The disea-
se is caused by strongly decreased level or absence of biological-
ly active coagulation factor VIII (antihæmophilic factor), which
is a protein normally present in plasma. The clinical manifesta-
30 tion of haemophilia A is a strong bleeding tendency and before
treatment with factor VIII concentrates was introduced, the
mean age of those patients was less than 20 years. Concentrates
of factor VIII obtained from plasma have been available for
about three decades. This has improved the situation for treat-
35 ment of haemophilia patients considerably and given them possi-
bility to live a normal life.

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It is commonly recognized that the severity of haemophilia A and B, can be divided into three categories: severe, moderate and mild. In severe haemophilia A, the plasma level of factor VIII activity in the blood is less than 1% of the normal plasma level.

5 In moderate haemophilia A, the plasma level of factor VIII activity in the blood is in the range of from 1 up to 4% of the normal plasma level. In mild haemophilia A, the plasma level of factor VIII activity in the blood is in the range of from 5 up to 25% of the normal plasma level. The normal plasma level of

10 factor VIII activity in the blood is defined as 1 IU/ml of blood. Severe, moderate and mild haemophilia B, are defined by the same plasma levels as those given for factor VIII above. The normal plasma level of factor IX activity in the blood is defined as 1 IU/ml of blood. Reference is here made to Inga Marie Nilsson

15 in Hemophilia, Pharmacia Plasma Products, Stockholm, Sweden, p. 2-3, 1994.

A medicament with a very large and labile molecule, such as coagulation factor VIII with a molecular mass of 170 to 300 kDa, is normally

20 given intravenously since these medicaments normally exhibit a very low bioavailability due to insufficient absorption and severe degradation, if given subcutaneously, intramuscularly or intradermally. Thus, a factor VIII concentrate dissolved in sodium citrate and injected intramuscularly yielded a maximum circulating level of only 1.4% of

25 the normal plasma level (Pool et al, New England J. Medicine, vol. 275, no. 10, p. 547-548, 1966). The studies further revealed that there was no significant difference in the activity recovered in the circulation regardless of whether such citrate was added to the preparation. In a later study, a high-purity factor VIII was administered intramuscu-

30 larly to haemophilic dogs and human volunteers (Johnson et al, Br. J. Hematology, vol. 21, p. 21-41, 1971). Although, the doses were much larger than used by Pool et al, neither the dogs nor the human volunteers showed a significant rise in plasma factor VIII levels. In fact, the plasma factor VIII concentration in the haemophilic human volunteers

35 remained below 1% of the normal plasma level, i.e. the severe haemophilia A prevailed even after administration in the absence of additives increasing the bioavailability.

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Various additives have been proposed for enhancing the absorption of a great number of pharmaceuticals, including small molecules such as insulin and peptide fractions of factor VIII. Thus, WO-A-92/01440
5 relates to use of dimethyl- β -cyclodextrin for enhancing transmucosal administration of insulin with a molecular mass of about 5,800 Da. Information pertaining to transport of small molecules by non-invasive routes, cannot be transferred to transport of large molecules to the intravenous circulation by subcutaneous, intramuscular or intradermal
10 administration. This is especially true, since the aim of WO-A-92/-01440 is to make superfluous injections of (poly)peptides or proteins. Furthermore, before intramuscular administration saline is used for dissolving peptide fractions of factor VIII with a molecular mass of from 1,000 to 20,000 Da (Conte et al, *Arzneim.-Forsch./Drug Res.*, vol.
15 39 (I), no. 4, p. 463-466, 1989). Here, also, the experiments relate to small molecules yielding an unknown bioavailability since the activity of the peptide fractions of factor VIII has not been determined.

EP-A-0 522 491 relates to use of a water-soluble species of hyaluronic
20 acid in combination with a water-soluble protein showing no substantial pharmacological activity for enhancing administration of a pharmacologically active polypeptide, preferably with a molecular mass below about 20 kDa. Blood clotting factors are mentioned amongst a great number of polypeptides. Furthermore, there are no examples
25 showing the possible effect of hyaluronic acids upon administration of any blood clotting factor e.g. by injection.

JP-A-63-063624 relates to use of collagen for providing slow release
30 of an unspecified drug upon e.g. subcutaneous administration.

JP-A-56-127308 relates to emulsions containing blood-coagulation
factor VIII, soybean oil and phospholipids. The object of these emulsions is to eliminate the problems encountered with conventional parenteral administration. The emulsions are therefore administered
35 orally, which is shown in several of the examples.

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Factor VIII can also be used for other therapeutic purposes than for treating haemophilia A. Thus, WO-A-93/24137 relates to formulations containing FVIII intended for intradermal or topical administration as an anti-inflammatory agent. The formulations are intended for local administration to non-haemophiliac human bodies and not to the intravenous circulation of humans suffering from haemophilia A. The therapeutic effect is thus local and not systemic, the latter being required to alleviate haemophilia A.

- Subcutaneous administration of factor IX without additives is known from Berrettini et al, Am. J. Hematology, vol. 47, no. 1, p. 61-62, 1994. Factor IX is a smaller molecule than factor VIII, having a molecular mass of about 56 kDa. The human factor IX was poorly and very slowly transported into the circulation, when injected into the subcutaneous tissue of hemophilia B patients. This is in agreement with the results obtained with factor VIII.

All presently available factor VIII and factor IX preparations on the market are made as a formulation for intravenous administration and the majority of the factor VIII preparations are stabilized with human serum albumin.

Large and labile proteins are thus normally given intravenously so that the medicament is directly available in the blood stream. It would however be advantageous if a medicament could be given subcutaneously, intramuscularly or intradermally as these administration forms are much easier to handle for the patient. Especially if the medicament must be taken regularly during the whole life and treatment is to start early, already during the patients first year of life.

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DESCRIPTION OF THE INVENTION

To our great surprise we have found that factor VIII, which is a very sensitive protein, can be given subcutaneously and, in contrast to all earlier experience we obtain a remarkable absorption and a surprisingly high level of active factor VIII protein in the blood.

We have thus developed a formulation which makes it possible to administer factor VIII subcutaneously, intramuscularly or intradermally and which gives a great advantage for all patients in need of factor VIII.

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Recombinant factor VIII SQ is indicated for treatment of classical haemophilia. The half-life for r-VIII SQ is approximately 12 hours for humans when injected intravenously. For prophylactic treatment 15-40 IU/kg bodyweight is given of factor VIII three times a week.

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An intravenous injection is normally 5-20 ml. An injection given subcutaneously is between 0.05 to 1 ml and the concentration of factor VIII must therefore be very high in such a formulation. This is possible to obtain e.g. with our highly purified recombinant factor VIII in a formulation with an additive such as a hydrolyzed gelatin, hyaluronic acid and/or soybean oil emulsion. Thus, with the present invention it may be possible to obtain a bioavailability of at least about 15%, suitably at least about 30%, after subcutaneous, intramuscular or intradermal administration, compared to the bioavailability obtained after intravenous administration. With an especially careful selection of the constituents of the formulation of the present invention, it is possible to obtain a bioavailability of at least 50% and even 70% after subcutaneous, intramuscular or intradermal administration, compared to the bioavailability obtained after intravenous administration.

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The inventive idea is thus a combination of the finding that factor VIII can be absorbed into the blood-stream when given as a subcutaneous, intramuscular or intradermal pharmaceutical formulation and that it is possible to produce a special formulation giving the required increase in bioavailability of factor VIII for this purpose.

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The present invention thus relates to a pharmaceutical formulation for subcutaneous, intramuscular or intradermal administration comprising coagulation factor VIII or factor IX with an activity of at least 200 IU/ml and an additive increasing the bioavailability of factor VIII or factor IX, which formulation gives a

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therapeutic level of factor VIII or factor IX activity of at least 1.5% of the normal plasma level in the blood for at least 6 hours after administration.

According to one aspect of the present invention,
5 there is provided a pharmaceutical composition for subcutaneous, intramuscular or intradermal administration comprising (i) at least 500 IU/ml of coagulation Factor VIII, or an active derivative thereof, having a molecular mass of at least 170 kDa; and (ii) an organic
10 additive which provides a plasma level of Factor VIII which is at least 1.5% of the normal plasma level in the blood for at least 6 hours after administration, the organic additive being selected from amino acids, peptides, proteins, polysaccharides, emulsions, dispersions of polar lipids, and
15 combinations thereof.

With the formulations of the present invention, it is possible to maintain a therapeutically acceptable level of VIII:C or IX:C for at least 6 hours, suitably more than 12 hours, preferably more than 24 hours, and more
20 preferably more than 48 hours. With optimised formulations it is probably possible to maintain a therapeutically acceptable level of VIII:C or IX:C for at least 96 hours, and even more than 168 hours. In this way, the frequency of administration can be kept to a minimum, while still giving
25 the intended therapeutic effect. The formulations according to the invention may also be suitable for on-demand treatment.

The present invention relates to treatment of haemophilia A or B, diseases which, if present, prevails in
30 the entire blood system. Thus, a prerequisite for a successful treatment is administration in such a way, that a

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systemic effect is obtained. Such a systemic effect is obtained by the present invention, as opposed to other techniques, where local, e.g. anti-inflammatory effects are desired.

- 5 The formulation containing factor VIII or IX are preferably given subcutaneously.

 The factor VIII activity in the formulation is at least 200 IU/ml, suitably more than 500 and preferably more than 1,000 IU/ml. It is particularly preferred that the
10 factor VIII activity is more than 1,500 IU/ml in the formulation and most particularly preferred that the activity is from 5,000 to 100,000 IU/ml.

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The volume given can be more than 0.01 ml, suitably 0.1 to 2 ml, preferably 0.25 to 1.5 ml, and more preferably 0.5 to 1 ml. The volume can also be 0.1 to 1 ml.

- 5 The activities and volumes given above, are equally applicable to factor IX.

Factor VIII and factor IX can either be plasma derived or produced by recombinant DNA techniques.

10

While the invention is applicable to both coagulation factor VIII and factor IX, it will in the following be described in more detail with reference to factor VIII.

- 15 Therapeutic factor VIII concentrates have until now been prepared by fractionation of plasma. However, there are now methods available for production of factor VIII in cell culture using recombinant DNA techniques as reported in e.g. W. Wood et al., Nature 312, p. 330-37, 1984 and EP-A-160 457.

20

- Factor VIII concentrates derived from human plasma contain several fragmented fully active factor VIII forms (Andersson et al, Proc. Natl. Acad. Sci. USA, Vol 83, p. 2979-83, May 1986). The smallest active form of human factor VIII has a molecular mass
25 of 170 kDa and consists of two chains of 90 kDa and 80 kDa held together by a metal ion bridge. Reference is here made to EP-A-197 901. Pharmacia AB of Sweden has developed a recombinant factor VIII product which corresponds to the 170 kDa plasma factor VIII form in therapeutic factor VIII concentrates. The
30 truncated recombinant factor VIII molecule is termed r-VIII SQ and is produced by Chinese Hamster Ovary (CHO) cells in a cell culture process in serum-free medium.

- The structure and biochemistry of recombinant factor VIII products in general have been described by Kaufman in Trends in Biotechnology, Vol 9, 1991 and Hematology, 63, p. 155-65, 1991. The struc-
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ture and biochemistry of r-VIII SQ have been described in WO-A-91/09122.

When factor VIII is recombinant, it can be either in its full-length form or preferably a deletion derivative thereof. More preferably the deletion derivative is recombinant factor VIII SQ (r-VIII SQ). By deletion derivative is here meant coagulation factor VIII, in which the whole or part of the B-domain is missing. Additionally, the factor VIII molecule, and in particular the r-VIII SQ molecule, can be chemically modified, e.g. by pegylation, covalently linked carbohydrates or polypeptides, in order to improve the stability of the molecule in vivo.

Our used factor VIII is highly purified, i.e. has a specific activity of more than 5,000 IU/mg protein, even more than 12,000 IU/mg and is preferably stabilized without the addition of albumin.

Additives increasing the bioavailability of factor VIII are suitably organic compounds *per se*, salts thereof, emulsions or dispersions containing organic compounds *per se* or salts thereof, e.g. dispersions of polar lipids, or any combination or sequence of addition thereof. Organic compounds *per se* useful in the present invention are e.g. amino acids, peptides, proteins, and polysaccharides. Peptides include dipeptides, tripeptides, oligopeptides and polypeptides, such as collagen and gelatin. The collagen and gelatin are preferably hydrolyzed. Polysaccharides include e.g. chitosans, cyclodextrins, starch, hyaluronic acids, dextrans, cellulose, and any derivatives, combinations and/or sequence of addition thereof. The starch is preferably hydrolyzed. The emulsions include oil-in-water emulsions with oil as the dispersed phase and water-in-oil emulsions with oil as the continuous phase. The oil can be of vegetable or animal origin or synthetically produced. Suitably, the vegetable oil of the emulsions is soybean oil or safflower oil, or any combination thereof. A preferred emulsion is Intralipid® sold by Pharmacia AB of Stockholm, Sweden, which further contains egg lecithin. Suitably, the polar lipids are one or

more phospholipids or glycolipids or any combination thereof. Preferably, the hyaluronic acid is Healon[®] sold by Pharmacia AB of Uppsala, Sweden. Preferably, the dextran is Promiten[®] sold by Pharmacia AB of Uppsala, Sweden.

5

The additives increasing the bioavailability of factor VIII could be added to the formulation before drying or upon reconstitution, or it could be added to a stable solution or dispersion containing factor VIII. A combination of at least two of the additives mentioned could also be added. Furthermore, two or more of the additives mentioned could be added in a sequence.

10

One or more anticoagulants can also be added to the formulation, in a non-therapeutic amount. The anticoagulants can be e.g. glucosaminoglycans such as heparins, heparin fragments, heparin derivatives, or heparan sulphate. A preferred group of glucosaminoglycans is the low molecular weight heparins (LMWH) with an average molecular mass of up to about 10,000 Da, more preferably from 2,000 up to 8,000 Da.

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Before administration, one or more aqueous solutions or dispersions could be added, in any mixture or sequence, to a formulation according to the present invention, which is a stable aqueous solution, a dispersion or in dried form.

25

The formulation can also comprise sodium or potassium chloride, preferably in an amount of more than 0.1 M.

30

The association of the heavy and light chains of factor VIII, is dependent on the presence of calcium (or other divalent metal ions). Here, calcium was added as calcium chloride (CaCl₂) but other salts such as calcium gluconate, calcium glubionate or calcium gluceptate can also be used, preferably in an amount of more than 0.5 mM.

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An amino acid is preferably used to buffer the system and it also

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protects the protein in the amorphous phase if the formulation is freeze-dried. A suitable buffer could be L-histidine, lysine and/or arginine. L-Histidine has primarily been chosen because of the good buffer capacity of L-histidine around pH 7.

5

A non-ionic surfactant can also be present in the formulation and is then preferably chosen from block co-polymers, such as a poloxamer or polyoxyethylene sorbitan fatty acid ester, such as polyoxyethylene-(20)-sorbitan monolaurate or polyoxyethylene-(20)-sorbitan monooleate. The non-ionic surfactant, if used, should preferably be present in an amount above the critical micelle concentration (CMC). See Wan and Lee, Journal of Pharm Sci, 63, p. 136, 1974.

10

The polyoxyethylene sorbitan fatty acid ester is preferably used in an amount of at least 0.01 mg/ml.

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To this formulation monosaccharide, disaccharides or sugar alcohols, preferably sucrose, could be added. Also antioxidants such as glutathione, acetylcystein, tocopherol, methionine, butyl hydroxy toluene and/or butyl hydroxy anisole could be added.

20

Complexing agents, such as EDTA and citric acid, can also be present in small concentrations for stabilizing the factor VIII molecules, if they exhibit a stronger affinity for destabilizing metal ions than for calcium or other divalent metal ions associating the chains of factor VIII. Furthermore, preservatives such as benzyl alcohol, phenol, sorbic acid, parabens and chlorocresol could be added.

25

The formulation comprises preferably L-histidine and sucrose. The ratio of sodium chloride to L-histidine and sucrose in the composition for freeze-drying is suitably more than 1:1 (w:w), preferably more than 2:1 (w:w).

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The formulation could comprise
i) at least 200 IU/ml of factor VIII, preferably at least 1,500 IU/ml of a deletion derivative of recombinant factor VIII

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- ii) at least 0.01 mg/ml of a polyoxyethylene sorbitan fatty acid ester
 - iii) sodium chloride, preferably in an amount of more than 0.1 M.
 - iv) calcium salt, such as calcium chloride or calcium gluconate,
5 preferably in an amount of more than 0.5 mM.
 - v) an amino acid such as L-histidine in an amount of more than 1 mM.
 - vi) an additive increasing the bioavailability of factor VIII.
- 10 The formulation could be in a dried form, preferably freeze-dried. Before administration, the dried product can be reconstituted with an aqueous solution or a dispersion, e.g. a suspension, a liposomal formulation or an emulsion.
- 15 The claimed formulation can also be a stable aqueous solution ready for administration. It can also be a dispersion, e.g. a suspension, a liposomal formulation or an emulsion.
- 20 The formulation could be stored in an oxygen-reduced environment as disclosed in the copending patent application WO-A-94/26286.
- 25 The invention also relates to the use of the claimed formulation for the manufacture of a medicament for subcutaneous, intramuscular or intradermal administration for treating haemophilia, preferably for the use of a deletion derivative of recombinant factor VIII for the manufacture of a medicament for subcutaneous administration. The medicament can be in a stable aqueous solution or dispersion, or dried. It also relates to a method for
30 treatment of haemophilia by subcutaneous, intramuscular or intradermal administration of the claimed formulation.
- 35 The data presented in the examples indicate that factor VIII, and in particular r-VIII SQ can be injected subcutaneously and recovered in an active form intravenously *in-vivo*. This is a very surprising finding, as no such formulation has been known earlier.

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The protection is not limited to a composition under these examples.

EXPERIMENTAL

5 Material and methods

The production of recombinant factor VIII SQ (r-VIII SQ) was essentially performed as described in patent WO-A-91/09122, example 1-3. A DHFR deficient CHO celline (DG44N.Y.) was electroporated with an expression vector containing the r-VIII SQ gene and an expression vector containing the dihydrofolate-reductase gene. Following selection on selective media surviving colonies were amplified through growth in stepwise increasing amounts of methotrexate. Supernatant from the resulting colonies were individually screened for factor VIII activity. A production clone was chosen and this was subsequently adapted to serum free suspension growth in a defined medium and finally a large scale fermentation process was developed. Supernatant is collected after certain time periods and further purified as described below.

The clarified conditioned medium was pH adjusted and applied to a S-SepharoseTM FF column. After washing, factor VIII was eluted with a salt buffer containing 5 mM CaCl₂.

Immunoadsorption was carried out on an immunoaffinity resin where the ligand was a monoclonal antibody (8A4) directed towards the heavy chain of factor VIII. Before loading to the column the S-eluate was treated with 0.3 % TNBP and 1 % Octoxynol 9.

The column was equilibrated, washed and factor VIII was eluted with a buffer containing 0.05 M CaCl₂ and 50 % ethylene glycol.

The mAb-eluate was loaded on a Q-SepharoseTM FF column, equilibrated with the elution buffer in the immunoaffinity step. After washing, factor VIII was eluted with 0.05 M L-Histidine, 0.6 M Sodium chloride, 4 mM calcium chloride and pH 6.8.

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The Q-eluate was applied to a gel filtration column (Superdex 200 p.g.). Equilibration and elution was carried out with a buffer containing L-histidine, sodium chloride and calcium chloride.

5 The protein peak was collected and the solution was formulated before freeze-drying.

10 This material of r-VIII SQ was received from the final purification step. The factor VIII activity and the concentration of the inactive components were adjusted by diluting with an appropriate buffer containing polyethylene glycol (PEG). The solution was then sterile filtered (0.22 μ m), dispensed and freeze-dried.

EXAMPLE 1

15 Recombinant factor VIII was prepared according to the method described under Experimental.

The freeze-dried composition containing r-VIII SQ was the following per vial, which was reconstituted in 4 ml sterile water for injection:

20 Composition per vial:

	L-Histidine, mg	31.0
	Sodium chloride, mg	70.1
	Calcium chloride $\cdot$ (2 H ₂ O), mg	2.35
25	Polyethylene glycol (PEG 4000), mg	4.0
	Polyoxyethylene-(20)-sorbitan monooleate (Tween 80®), mg	1
	VIII:C charged, IU/vial	4,400
	VIII:C in reconstituted solution, IU/ml	1,060

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Male albino mice weighing about 30 g and of strain NMRI, SPF, were injected subcutaneously in the neck with the reconstituted r-VIII SQ solution.

35 The volume injected at the dose level of 10,00 IU/kg was 9.4 ml/kg and at the higher dose level 50,000 IU/kg, 5 times larger, 47 ml/kg. In the placebo treatment saline was used, 9.4 ml/kg.

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3-5 minutes before the blood sampling the mice were anaesthetized intra peritoneally with Mebumal[®] (pentobarbital) vet. 60 mg/ml. The volume injected was 9.4 ml/kg i.e. about 0.3 ml/- mouse. Under the anaesthesia 0.45 ml blood was collected from
5 the vena cava in plastic syringes containing 0.05 ml 0.13 M sodium citrate. Plasma was then prepared from the collected blood by centrifugation (8,800 g for 7 minutes) and kept frozen in plastic Cryoflex[™] tubes at -70°C until the time of determination of factor VIII activity.

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Results:

TABLE 1

VIII:C in plasma from mice receiving r-VIII SQ 10,000 IU/kg
5 body weight subcutaneously.

	Time after administration (hours)	VIII:C (IU / ml) X $\pm$ Sd	n number of observations
10	0	0.82 $\pm$ 0.43	12
	0.33	1.23 $\pm$ 0.41	3
	1.0	1.38 $\pm$ 0.56	4
	1.5	1.83 $\pm$ 0.60	12
	2.0	1.43 $\pm$ 0.91	8
15	4.0	1.34 $\pm$ 0.72	6
	6.0	1.27 $\pm$ 0.42	4
	8.0	1.55 $\pm$ 0.80	4
	16.0	0.62 $\pm$ 0.28	4
	20.0	0.58 $\pm$ 0.12	3
20	24.0	0.76 $\pm$ 0.53	4

TABLE 2

VIII:C in plasma from mice receiving r-VIII SQ 10,000 IU/kg
body weight intravenously. The number of observations were 6
25 throughout the tests.

	Time after administration (min)	VIII:C (IU / ml) X
30	0	1.6
	5	66.7
	15	43.7
	30	32.1
	45	27.4
35	60	22.5
	120	15.7
	240	2.3

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TABLE 3.

Dose - Response in mice receiving r-VIII SQ subcutaneously.

Dose: Dose r-VIII SQ administrated (IU/kg).

5 Response: VIII:C in plasma (IU/ml) 1.5 hours after subcutaneous administration.

	Dose (IU/kg)	Response (IU/ml)	Response (IU/ml) (baseline adjusted)	n number of obs.
10	Blank (baseline)	0.82 ± 0.43	-	12
15	Saline	1.21 ± 0.34	0.39	4
	10,000	1.83 ± 0.60	1.01	12
	50,000	2.47 ± 0.60	1.65	6

Results

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1. The change of VIII:C in plasma with time after a subcutaneous dose of r-VIII SQ, 10,000 IU/kg, shows the typical pattern for a drug being absorbed from a subcutaneous depot (see Table 1). The maximum VIII:C level in plasma is seen at about 1.5 hours after administration (see Table 1). The extremely high dosis used in the above tests, were chosen to get an effect that was statistically significant.

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2. The absorption of r-VIII SQ from a subcutaneous depot is further verified by the increase in maximum concentration seen when the dose is increased five-fold. No significant effect was observed on the obtained plasma level of VIII:C when the subcutaneous injection volume was changed from 9.4 to 47 ml/kg while the dose of r-VIII SQ was kept constant. Furthermore, the VIII:C obtained in plasma was essentially not dependent on the osmolality of the administration solution.

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3. The bioavailability of r-VIII SQ after subcutaneous (s.c.) administration in mouse was about 10% of the bioavailability after intravenous (i.v.) administration, the latter being 100% by definition. In the present tests, the dose level s.c. was the same as the dose level i.v. The bioavailability was calculated from the area under the activity (VIII:C) - time curve (AUC). Thus, the bioavailability after subcutaneous administration, can be calculated according to the following equation (for data see Tables 1 and 2):

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$$\text{Bioavailability}_{\text{s.c.}} = \text{AUC}_{\text{s.c.}} / \text{AUC}_{\text{i.v.}} \cdot \text{dose}_{\text{i.v.}} / \text{dose}_{\text{s.c.}} \quad (1)$$

EXAMPLE 2

15 Recombinant factor VIII was prepared according to the method described under Experimental with the following exceptions: (i) the material of r-VIII SQ that was received from the final purification step was diluted with a buffer not containing PEG, (ii) the r-VIII SQ solution was not freeze-dried, it was stored at -70°C.

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The r-VIII SQ-solution had the following composition:

Composition per vial:

	L-Histidine, mg	7.5
25	Sucrose, mg	158
	Sodium chloride, mg	45
	Calcium chloride · (2 H ₂ O), mg	1.25
	Polyoxyethylene-(20)-sorbitan monooleate (Tween 80®), mg	0.50
	VIII:C charged, IU/vial	6,070
30	VIII:C, IU/ml *	1,130

* Diluted r-VIII SQ-solution (1 part of r-VIII SQ-solution + 1 part of water, v:v)

The r-VIII SQ-solution was diluted with sterile water for injection (1 part of r-VIII SQ-solution + 1 part of water, v:v) prior to administration when the dose of VIII:C was below 3,000 IU/kg body-weight.

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Female cynomolgus monkey (*Macaca fascicularis*) weighing about 3-3.5 kg were injected with the r-VIII SQ-solution subcutaneously in the dorsal region. Depending on the dose, the volume of injection was varied between approximately 0.2 to 2.0 ml/kg body-weight. Subcutaneous injections with single doses of 250, 2,500 and 5,000 IU/kg were administered. On each sampling occasion 1.8 ml of blood was collected into tubes containing citrate as anticoagulant (0.2 ml). After centrifugation plasma was separated and frozen in aliquots (< -60°C).

Results:

TABLE 4

VIII:C in plasma from monkeys receiving r-VIII SQ, 250 IU/kg, subcutaneously. The VIII:C in the administration solution was about 1,130 IU/ml.

Monkey No	1	2	3
Sampling after inj. (Hours)	VIII:C (IU/ml)	VIII:C (IU/ml)	VIII:C (IU/ml)
0	2.15	1.49	1.76
1	2.32	1.75	1.92
4	2.43	1.52	1.89
8	2.48	1.67	1.92
10	2.41	1.76	2.03
12	2.29	1.70	2.01
14	2.20	1.69	2.00
24	1.76	1.32	1.98
30	2.31	1.46	1.90
48	2.18	1.64	1.96

TABLE 5

VIII:C in plasma from monkeys receiving r-VIII SQ, 2,500 IU/kg, subcutaneously. The VIII:C in the administration solution was about 1,130 IU/ml.

5	Monkey No	4a	5a	6a
	Sampling after inj. (Hours)	VIII:C (IU/ml)	VIII:C (IU/ml)	VIII:C (IU/ml)
10	0	1.85	1.43	2.18
	1	1.96	1.82	2.72
	4	2.70	2.22	3.14
	8	3.19	2.60	3.37
15	10	3.19	2.81	3.46
	12	3.06	1.56	3.02
	14	2.56	2.42	2.85
	24	2.34	2.12	2.75
	30	2.20	2.01	2.55
20	48	2.02	1.83	2.38
	Monkey No	4b	5b	6b
25	Sampling after inj. (Hours)	VIII:C (IU/ml)	VIII:C (IU/ml)	VIII:C (IU/ml)
	0	1.98	1.35	2.30
	2	2.43	1.88	3.05
	6	3.51	2.45	3.59
30	9	3.91	2.47	4.16
	11	3.47	2.24	3.68
	13	3.11	2.06	3.34
	22	2.38	1.55	2.79
	40	2.01	1.49	2.38
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TABLE 6

VIII:C in plasma from monkeys receiving r-VIII SQ, 5,000 IU/kg, subcutaneously. The VIII:C in the administration solution was about 2,470 IU/ml.

5	Monkey No	7	8	9
	Sampling after inj.	VIII:C (IU/ml)	VIII:C (IU/ml)	VIII:C (IU/ml)
10	(Hours)			
	0	2.04	1.78	1.54
	2	2.99	2.37	2.99
	6	4.56	3.33	5.32
	9	4.75	3.89	5.70
15	11	4.72	3.32	5.46
	13	4.23	3.06	4.63
	22	3.03	2.33	3.46
	40	2.76	1.77	2.60

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TABLE 7

Dose - Response in monkey receiving r-VIII SQ subcutaneously.
Dose: Dose r-VIII SQ administrated (IU/kg bodyweight).
Response: VIII:C in plasma (IU/ml) about 9 hours after admini-
stration.

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	Dose (IU/kg)	Response (IU/ml)	Response (IU/ml, baseline adj.)	n number of observations
30	Blank (baseline)	1.8 ± 0.3	-	9
	250		0.37 ± 0.05	3
	2,500		1.70 ± 0.06	3
	2,500		2.05 ± 0.57	3
35	5,000		3.4 ± 1.20	3

TABLE 8

The bioavailability of r-VIII SQ after subcutaneous administration in monkeys. VIII:C in the administration solution was varied between about 1,130 IU/ml to about 2,470 IU/ml. The bioavailability was calculated according to equation 1, from data given in tables 4-6 and corresponding data after intravenous administration.

	Dose (IU/kg bodyweight)	Bioavailability (%)
10	250	11
	250	9
	2,500	7
	2,500	11
15	2,500	6
	2,500	6
	2,500	5
	2,500	5
	5,000	7
20	5,000	3
	5,000	9

Results

- 25 1. According to the results (tables 4-6) the plasma concentration as a function of time follows the typical pattern for a drug being absorbed after subcutaneous administration. The maximum concentration of VIII:C is seen about 9 hours after administration.
- 30 2. The dose-response relationship obtained (table 7), gives a further verification that there is an absorption of VIII:C into the blood stream following a subcutaneous injection.
- 35 3. The bioavailability of r-VIII SQ after subcutaneous administration in monkey was about 5-10 % (table 8). The bioavailability was essentially independent of the dose of r-VIII SQ.

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EXAMPLE 3

Recombinant factor VIII (r-VIII SQ) of both the compositions (examples 1 and 2) mentioned above were used.

- 5 Male albino mice weighing about 30 g and of strain NMRI, SPF, were injected subcutaneously in the neck with the r-VIII SQ formulation.

The dose was 10,000 IU/kg and the injection volume was about 10 ml/kg. 3-5 min before the blood sampling the mice were anaesthetized
 10 intra peritoneally with Mebumal® (pentobarbital) vet. 60 mg/ml. The dose volume was about 0.3 ml/mouse. Under anaesthesia 0.45 ml blood was collected from vena cava in plastic syringes containing 0.05 ml sodium citrate (0.13 M). Plasma was prepared from the blood by centri-
 15 Cryoflex tubes at -70°C until determination of factor VIII activity.

The r-VIII SQ-solution in combination with different additives increasing the bioavailability of factor VIII (table 9) was administered subcu-
 20 taneously in mice.

TABLE 9

The additives that were added to the r-VIII SQ-solution prior to subcu-
 taneous administration in mice.

25	Additive	Commercial name/ Commercial source	Concentration of additive in the administered r-VIII SQ-solution
	Amino acid mixture	Vamin®/Pharmacia AB	4.5 g N/l
30	Dextran	Promiten®/Pharmacia AB	150 mg/ml
	Hyaluronic acid	Healon®/Pharmacia AB	5 mg/ml
	Hydrolyzed collagen	Prionex®/Pentapharm Ltd	150 g/l
	Hydrolyzed gelatin	Haemaccel®/Hoechst	17.5-35 g/l
	Human serum albumin	Albumin/Pharmacia AB	100-200 g/l
35	Poly (0-2-hydroxyethyl) starch	HAES-steril®/Meda	50-100 g/l
	Soybeanoil emulsion	Intralipid®/Pharmacia AB	150-200 mg/ml

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Results:

TABLE 10

VIII:C in plasma from mice receiving r-VIII SQ-solution containing hydro-
 5 lyzed gelatin subcutaneously. The VIII:C in the administration solution
 was about 1,100 IU/ml. The r-VIII SQ dose was 10,000 IU/kg body-
 weight.

	Time after administration (hours)	VIII:C (IU/ml)	n number of observations
	0	0.82 ± 0.43	12
	1.5	1.83 ± 0.37	4
15	6	2.02 ± 0.43	6
	16	1.94 ± 0.44	9
	24	2.00 ± 0.52	5
	30	1.52 ± 0.41	6
	48	1.32 ± 0.41	6
20			

TABLE 11

VIII:C in plasma from mice receiving r-VIII SQ-solution containing
 soybean oil emulsion subcutaneously. The VIII:C in the administration
 solution was about 1,100 IU/ml. The r-VIII SQ dose was 10,000 IU/kg
 25 bodyweight.

	Time after administration (hours)	VIII:C (IU/ml)	n number of observations
30	0	0.82 ± 0.43	12
	1.5	1.41 ± 0.44	4
	3.5	1.46 ± 0.34	5
	6	1.48 ± 0.87	4
	16	1.29 ± 0.25	6
35	16	1.35 ± 0.31	3
	30	1.92 ± 0.51	6
	48	1.97 ± 0.29	5

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TABLE 12

VIII:C in plasma from mice receiving r-VIII SQ-solution containing Poly (0-2-hydroxyethyl) starch subcutaneously. The VIII:C in the administration solution was about 1,100 IU/ml. The r-VIII SQ dose was 10,000 IU/kg bodyweight.

	Time after administration (hours)	VIII:C (IU/ml)	n number of observations
10	0	0.82 ± 0.43	12
	1.5	1.00 ± 0.44	5
	3.5	1.62 ± 0.19	4
	6	1.65 ± 0.69	6
15	16	1.62 ± 0.38	6
	16	1.19 ± 0.40	4
	24	0.73 ± 0.52	4
	30	1.33 ± 0.39	5
	48	1.10 ± 0.38	3
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TABLE 13

VIII:C in plasma from mice receiving r-VIII SQ-solution containing hyaluronic acid subcutaneously. The calculated VIII:C in the administration solution was about 1,100 IU/ml. The r-VIII SQ dose was 10,000 IU/kg bodyweight.

	Time after administration (hours)	VIII:C (IU/ml)	n number of observations
30	0	0.82 ± 0.43	12
	1.5	1.67 ± 0.42	5
	3.5	1.33 ± 0.35	4
	6	1.72 ± 0.61	4
	16	1.36 ± 0.43	5

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TABLE 14

VIII:C in plasma from mice receiving r-VIII SQ-solution containing dextran subcutaneously. The VIII:C in the administration solution was about 1,100 IU/ml. The r-VIII SQ dose was 10,000 IU/kg bodyweight.

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	Time after administration (hours)	VIII:C (IU/ml)	n number of observations
	0	0.82 ± 0.43	12
10	1.5	1.43 ± 0.43	6
	3.5	1.74 ± 0.10	5
	6	1.02 ± 0.51	5
	16	1.54 ± 0.36	6

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TABLE 15

VIII:C in plasma from mice receiving r-VIII SQ-solution containing hydrolyzed collagen subcutaneously. The VIII:C in the administration solution was about 1,100 IU/ml. The r-VIII SQ dose was 10,000 IU/kg bodyweight.

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	Time after administration (hours)	VIII:C (IU/ml)	n number of observations
25	0	0.82±0.43	12
	1.5	1.00±0.54	6
	7	1.36±0.71	4
	16	1.39±0.93	6
	24	1.31±0.51	5
30	30	0.93±0.59	4
	48	1.16±0.51	6

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TABLE 16

VIII:C in plasma from mice receiving r-VIII SQ-solution containing a mixture of amino acids subcutaneously. The VIII:C in the administration solution was about 1,100 IU/ml. The r-VIII SQ dose was 10,000 IU/kg bodyweight.

	Time after administration (hours)	VIII:C (IU/ml)	n number of observations
10	0	0.82±0.43	12
	1.5	1.47±0.79	4
	7	1.66±0.21	4
	16	1.26±0.61	6

TABLE 17

The bioavailability of r-VIII SQ after subcutaneous administration in mice. The subcutaneously administered r-VIII SQ-solution contained varying additives in the concentration given in table 8. The VIII:C in the administration solution was about 1,100 IU/ml and the dose was 10,000 IU/kg. The bioavailability was calculated from the area under the activity (VIII:C) - time curve up to 48 h. The bioavailability after subcutaneous administration of r-VIII SQ is given in percent of the bioavailability obtained upon intravenous administration with the same dose (10,000 IU/kg).

	Additive increasing the bioavailability of factor VIII	Bioavailability (%)	Mode of administration
	None	100	intravenous
30	None	10	subcutaneous
	Dextran	35	subcutaneous
	Hyaluronic acid	45	subcutaneous
	Hydrolyzed collagen	20	subcutaneous
	Hydrolyzed gelatin	50	subcutaneous
35	Poly (0-2-hydroxyethyl) starch	25	subcutaneous
	Soybean oil emulsion	60	subcutaneous

Results

1. The results show that it is possible to increase the bioavailability of r-VIII SQ. It is also shown, that a high plasma concentration of active
5 r-VIII SQ is obtained during a much longer period of time, up to more than 48 h, when an additive increasing the bioavailability according to the present invention is included in the subcutaneously administered formulation. This is compared to the plasma concentration obtained when a r-VIII SQ solution without such an additive is administered
10 subcutaneously. A bioavailability of active r-VIII SQ of at least 50% is reached upon subcutaneous injection of a solution containing r-VIII SQ and hydrolyzed gelatin or soybean oil emulsion.

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CLAIMS

1. A pharmaceutical composition for subcutaneous, intramuscular or intradermal administration comprising (i) at least 500 IU/ml of coagulation Factor VIII, or an active derivative thereof, having a molecular mass of at least 170 kDa; and (ii) an organic additive which provides a plasma level of Factor VIII which is at least 1.5% of the normal plasma level in the blood for at least 6 hours after administration, the organic additive being selected from amino acids, peptides, proteins, polysaccharides, emulsions, dispersions of polar lipids, and combinations thereof.
2. The composition according to claim 1, wherein the organic additive is selected from amino acids, peptides, proteins, and combinations thereof.
3. The composition according to claim 1 or 2, wherein the protein is gelatin.
4. The composition according to claim 1 or 2, wherein the protein is collagen.
5. The composition according to any one of claims 1 to 4, wherein the organic additive is a polysaccharide.
6. The composition according to claim 5, wherein the polysaccharide is selected from chitosans, cyclodextrins, starch, dextrans, cellulose, and derivatives and combinations thereof.
7. The composition according to claim 5, wherein the polysaccharide is hyaluronic acid.
8. The composition according to any one of claims 1 to 4, wherein the organic additive is selected from oil-in-water emulsions with oil as the dispersed phase, water-in-

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oil emulsions with oil as the continuous phase, and dispersions of polar lipids.

9. The composition according to claim 8, wherein the oil is soybean oil.

5 10. The composition according to any one of claims 1 to 9, wherein the coagulation Factor VIII is recombinant Factor VIII.

10 11. The composition according to any one of claims 1 to 9, wherein the active derivative of Factor VIII is a recombinant derivative of Factor VIII having a molecular mass of at least 170 kDa.

12. The composition according to claim 11, wherein the active derivative of Factor VIII is the derivative designated as r-VIII SQ.

15 13. The composition according to any one of claims 1 to 12, further comprising at least 0.01 mg/ml of polyoxyethylene sorbitan fatty acid ester or at least 0.1 M of sodium chloride or at least 0.5 M of a calcium salt or at least 1 mM of L-histidine or any combination thereof.

FETHERSTONHAUGH & CO.

OTTAWA, CANADA

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