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(54) Title: STABILIZED FACTOR VIII PREPARATIONS

(57) **Abrégé/Abstract:**

The invention relates to stabilized solutions with F VIII coagulation activity, to a process for the preparation thereof and to the use thereof.



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Abstract of the disclosure

Stabilized factor VIII preparations

The invention relates to stabilized solutions with F VIII coagulation activity, to a process for the preparation thereof and to the use thereof.

Stabilized factor VIII preparations

5 The invention relates to stabilized solutions with F VIII coagulation activity, to a process for the preparation thereof and to the use thereof.

10 Coagulation factor VIII:C (F VIII:C) is a plasma protein and essential for the process of the intrinsic pathway of blood coagulation. A deficiency or a defect in blood coagulation factor VIII:C results in a life-threatening disturbance of blood coagulation, hemophilia A. Concentrates of F VIII:C from human plasma or genetically engineered F VIII:C are employed for the therapy of hemophilia A.

15 These F VIII products differ in respect of their purity, i.e. the presence of proteins which do not have coagulation activity in addition to the active substance F VIII:C. A F VIII which has more than 1000 U/mg before stabilization with albumin is called very high purity
20 F VIII (VHP F VIII:C) (WHO, Expert Committee on Biological Standardization).

Such VHP F VIII:C have potential advantages in the treatment of hemophilia. These are the freedom from viruses and a very small content of foreign protein,
25 which means less stress on the immune system of the patients after administration of these concentrates. The advantage which is possible per se, of less stress on the immune system of a hemophiliac patient by administration of a F VIII preparation with high specific activity, is,
30 however, cancelled out by addition of high albumin concentrations to the highly purified product in order to stabilize the VHP F VIII. This addition of albumin means that the highly purified F VIII concentrates reach specific activities of only 3 - 10 U/mg in the final

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formulation thereof.

5 Although addition of albumin entails only a slight risk with respect to virus safety, it has to be borne in mind, however, that with albumin whose purity averages 95% once again unwanted concomitant proteins are administered to the patient and may stress his immune system.

10 High purity F VIII products which dispense with addition of albumin for stabilization of F VIII are known (Schwinn, Smith & Wolter, Drug. Res. 39 (1989), 1302). These products reach specific activities of about 100 U/mg of protein. Based on a maximum achievable F VIII activity of about 5000 U/mg, this means that only about 2% of the protein content of these preparations comprises F VIII:C protein. It is to be assumed in this case that 15 this 2% F VIII:C is stabilized by the 98% concomitant proteins, because a large part of these concomitant proteins is likely to comprise von Willebrand Factor (vWF). It is known that von Willebrand Factor has a stabilizing action on F VIII:C.

20 The situation is different with very high purity products which have specific activities which, before albumin stabilization, are usually more than 25 times higher than for high purity products, and the vWF content thereof is very low. This low vWF content is no longer able to 25 ensure adequate F VIII stabilization so that the F VIII activity in solutions which are not stabilized with albumin rapidly decreases.

30 The object of the present invention was therefore to provide a process which makes it possible to prepare a highly concentrated, physiologically tolerated solution of a VHP F VIII:C product, which solution requires no addition of proteins for stabilization.

This object is achieved according to the invention by adding an amino acid or one of its salts, derivatives or

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homologs to a VHP F VIII:C preparation. It is possible to add L- and/or D-amino acids. Particular suitable are arginine, lysine, ornithine, guanidinoacetic acid or others whose common feature is a basic group in the form of an amino and/or guanidino group.

The invention therefore relates to a solution with factor VIII:C activity containing an amino acid or one of its salts or derivatives and, where appropriate, a detergent or an organic polymer.

10 Preferred embodiments are:

a solution wherein the amino acid is a natural amino acid;

a solution wherein the amino acid is a basic amino acid;

a solution which contains arginine and glycine;

15 a solution wherein the concentration of the amino acid is 0.001 to 1 mol/l;

a solution which additionally contains an organic polymer or a nonionic detergent;

20 a solution wherein the F VIII:C activity derives from human factor VIII in its form which occurs in plasma or from a genetically engineered factor VIII:C or a derivative of these;

and a solution wherein the specific F VIII:C activity is at least 1000 IU/mg.

25 Improved stabilization is achieved by combination of amino acids or their derivatives or with a nonionic detergent such as ^RPolysorbate 20 or ^RPolysorbate 80 or an organic polymer such as polyethylene glycol 1500.

30 A combination of the amino acids arginine and glycine, preferably 0.01 to 1 mol/l, with the nonionic detergent ^RTween 80, preferably 0.001 to 0.5% (v/v), and with a neutral sugar such as sucrose, preferably 0.1 to 10%, has proven particularly suitable for the preparation of a stable, albumin-free VHP F VIII:C solution.

The pH of a solution of this type is adjusted to between pH 5.5 and 8.5, preferably between pH 6.5 and 7.5, by means of an organic acid, preferably 10% strength acetic acid.

- 5 The invention also relates to a pharmaceutical containing a solution of this type. Besides a solution of this type, this pharmaceutical can contain customary, pharmaceutically compatible, stabilizing and/or buffering substances, especially a carbohydrate.
- 10 The invention likewise relates to a process for the preparation of a solution of this type, wherein an amino acid or one of its salts or derivatives and, where appropriate, an organic polymer or a detergent is added to a solution with factor VIII:C activity.
- 15 The advantageous effect of the process according to the invention can be shown, for example, for a F VIII:C preparation which has been purified by chromatography on monoclonal anti-F VIII:C antibodies, it being possible for the F VIII:C to be both obtained from plasma and
- 20 genetically engineered, for example in CHO (Chinese Hamster Ovary) cells. This entails, for example, equal parts of a solution of the abovementioned substances being added to the eluate from the monoclonal antibody column, and subsequently the latter being dialyzed
- 25 against this solution. The stabilized F VIII:C preparation obtained in this way can be sterilized by filtration and bottled with low method-related losses. A lyophilizate of this preparation obtained in this way has unchanged high F VIII:C activities after dissolution.
- 30 It is possible with the process according to the invention to prepare a VHP F VIII:C preparation whose specific volume-based activity is at least 200 IU/ml, with a specific activity of more than 2000 IU/mg. This concentration ensures that there are no problems with
- 35 manipulation owing to the need to administer small

volumes.

5 A preparation of this type does not need further stabilization by proteins, which avoids the risk of virus contamination. At the same time, the reduction in the high protein load means a considerable reduction in the stress on the immune system of the patient due to the addition of the albumin, which is unnecessary for the medicinal action, and of the unwanted impurities contained therein.

10 Since physiologically tolerated substances are added for the stabilization, no intolerance reactions occur on administration of the solution according to the invention.

Example 1:

15 Two VHP F VIII:C preparations were prepared, both by means of affinity chromatography on monoclonal anti-vWF Ig (method of Fulcher & Zimmermann PNAS (1982), 79, 1649) and dissociation of the vWF/F VIII:C complex by solution with a CaCl_2 concentration of 300 mM in 0.1 M acetate, 0.1 M lysine, pH 6.8 (eluate I), and by means of chromatography on monoclonal anti-F VIII:C Ig and elution of the F VIII:C by 50% ethylene glycol in 0.1 M acetate, 0.1 M lysine, pH 6.8 (eluate II). The specific F VIII:C activity determined in eluate I was 2500 IU/mg and 419 IU/ml, and in eluate II was 3280 IU/mg and 454 IU/ml. The two eluates were divided in each case. To one portion in each case was added in the ratio 1:1 by volume a 1% strength human albumin solution in 0.75% sucrose, 3% glycine and 0.1 mol/l NaCl (eluate I_{HSA}, eluate II_{HSA}). The stabilization buffer (0.75% sucrose, 3% glycine, 3% arginine, 0.05% ^RTween 80, pH 6.8) was likewise added 1:1 to the other half in each case (eluate I_s, eluate II_s). The albumin-containing samples were dialyzed against 0.75% sucrose, 3% glycine, 0.1 mol/l NaCl, pH 6.8, and the others against stabilization buffer. Dialysis was

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carried out at 4°C for 16 hours with 1000-fold volume change. The F VIII:C activities were measured before and after the dialysis. Table 1 shows the F VIII:C activity in % relative to the total F VIII:C activity in the particular sample before dialysis.

Table 1

Eluate I _{HSA}	Eluate I _s	Eluate II _{HSA}	Eluate II _s
92	94	94	93

The results show that stabilization of the VHP F VIII:C eluates by means of the stabilization solution according to the invention is achieved irrespective of the preparation method and to the same extent as by addition of albumin.

Example 2:

An F VIII:C eluate with a specific F VIII:C activity of 3860 IU/mg of protein and 462 IU/ml was obtained after immunoaffinity chromatography on monoclonal anti-F VIII:C antibodies. Various stabilization solutions were added to this in the ratio 1:1 by volume, and it was dialyzed against the relevant stabilization solution as described in Example 1. A pH of 6.8 was adjusted in all solutions where appropriate with 10% acetic acid.

The following stabilization solutions were employed:

- I. 0.75% sucrose, 0.4 M glycine, 0.15 M sodium chloride
- II. 0.01 M sodium citrate, 0.08 M glycine, 0.016 M lysine, 0.0025 M calcium chloride, 0.4 M sodium chloride
- III. 1% sucrose, 0.14 M arginine, 0.1 M sodium chloride
- IV. 1% sucrose, 0.4 M glycine, 0.14 M arginine, 0.1 M sodium chloride, 0.05% Tween 80

The F VIII:C activity was determined before and after the

dialysis. In Table 2 the F VIII:C activity after dialysis is plotted in % relative to the relevant activity before dialysis.

Table 2

5	Mixture	I	II	III	IV
	F VIII:C activity				
	after dialysis for	39.3%	35.1%	82.4%	96.2%
	16 hours				

10 The solutions employed under I and II can be employed for the stabilization of albumin-free HP F VIII products with specific F VIII:C activities of 100 - 200 IU/mg, dispensing with addition of albumin. Solutions III and IV are suitable for stabilization of VHP F VIII preparations with specific F VIII:C activities greater than

15 1000 IU/mg.

We claim:

1. A solution with factor VIII:C activity containing an amino acid or one its salts or derivatives.
- 5 2. A solution with factor VIII:C activity as claimed in claim 1 further comprising a detergent or an organic polymer.
- 10 3. A solution as claimed in claims 1 or 2, wherein the amino acid is a natural amino acid.
4. A solution as claimed in any one of claims 1-3, wherein the amino acid is a basic amino acid.
- 15 5. A solution as claimed in any one of claims 1-4, which contains arginine and glycine.
6. A solution as claimed in any one of claims 1-5, wherein the concentration of the amino acid is 0.001 to 1 mol/l.
- 20 7. A solution as claimed in claim 2, which contains a nonionic detergent.
8. A solution as claimed in any one of claims 1-7, wherein factor VIII:C activity derives from human factor VIII in its form which occurs in plasma or from a genetically engineered factor VIII:C or from a derivative of these.
- 25 9. A solution as claimed in any one of claims 1-8, wherein the specific factor VIII:C activity is at least 1000 IU/mg.
- 30 10. A pharmaceutical containing a solution as claimed in any one of claims 1-9.
11. A pharmaceutical as claimed in claim 10, which contains customary, pharmaceutically compatible, stabilizing and/or buffering substances.

12. A pharmaceutical as claimed in claim 11, which contains a carbohydrate.

13. A process for the preparation of a solution as claimed in claim 1, which
comprises adding an amino acid or one of its salts or derivatives to a solution
5 with factor VIII:C activity.

14. The process as claimed in claim 12, further comprising adding an organic
polymer or a detergent to a solution with factor VIII:C activity.