(54) Title: PHARMACEUTICAL PREPARATION CONTAINING A RECEPTOR-ANTAGONIST FOR TREATING BLOOD-CLOTTING DISORDERS

(57) Abstract:
The invention relates to a pharmaceutical preparation for treating blood-clotting disorders which is characterized in that it contains at least one protein selected from the group of pro-proteins of the blood clotting and additionally contains a coagulation-physiologically inert receptor binding competitor. The invention also relates to the medical application of said preparation.
Pharmaceutical preparation comprising a receptor antagonist for the treatment of blood coagulation disorders

ABSTRACT:

A pharmaceutical preparation for the treatment of blood coagulation disorders, characterized in that it contains at least one protein, selected from the group of pro-proteins of blood coagulation, and a coagulation physiologically inert receptor binding competitor, and its medical use are described.
Pharmaceutical preparation comprising a receptor antagonist for the treatment of blood coagulation disorders

The invention relates to a pharmaceutical preparation based on blood coagulation proteins and measures to extend their biological half-lives.

Therapeutic proteins, particularly preparations for the treatment of blood coagulation disorders, which contain blood coagulation proteins, such as e.g. von Willebrand factor (vWF), often have relatively short half-lives in the organism. Therefore, therapeutic administrations of such preparations may become ineffective within a short period of time or, at least, their effectiveness may be greatly reduced.

A lot of what is involved in the physiological degradation pathways of blood coagulation proteins is unknown, just like the exact factors determining their half-lives in the organism.

The only known fact is that activated factor VIII binds to the lipoprotein receptor related protein (LRP) via its A2 domain, said binding being responsible for internalizing factor VIII as well as for degrading it. It is also known that the internalization of the A2 domain may be inhibited by a receptor associated protein (RAP), an LRP inhibitor, and it is assumed that the dissociation of the A2 subunit from the rest of the activated factor VIII molecule is reversible.

The low density lipoprotein related protein is a multifunctionally endocytotically active receptor which binds structurally and functionally different ligands and is capable of endocytosis. RAP (receptor associated protein) inhibits all interactions of ligands with LRP in vitro.

It is assumed that, in principle, this mechanism may be important in regulating factor VIIIa activity (Blood 90 (10), Suppl. 1: 31a (1997)).

It is also known that LRP may act as a clearance receptor for tissue plasminogen activator (tPA), which means that it is responsible for its removal from blood circulation. Moreover, further receptors, such as e.g. the mannose receptor, seem to be of
importance there as well. On the other hand, it is known that LRP may be involved in the clearance of many different ligands, such as proteinases, inhibitors, their complexes with proteinases, and various lipoproteins. LRP may be present both in cell-bound and in soluble form (Quinn K.A. et al., XVIIth Congress of the International Society of Thrombosis and Haemostasis, 1999, abstract).

It has been found that tPA is cleared from blood circulation very quickly and that tPA mutants and clearance inhibitors may be used to reduce the dosage of tPA in thrombolysis therapy (Fibrinolysis and Proteolysis (1997), pp. 173-186).

In RAP deficient mice it has been demonstrated that α2-macroglobulin clearance in the liver is disturbed. It has also been found that the amount of mature processed LRP is reduced in both the liver and the brain (PNAS, 92 (1995), pp. 4537-4541).

EP-0 713 881-A2 describes von Willebrand factor (vWF) concentrates in combination with antithrombolytic agents and/or fibrinolytic agents. The purpose of these preparations is to reduce the risks of hemorrhages in anticoagulation therapy.

US 5 304 383 describes a pharmaceutical preparation containing lys-plasminogen in combination with a serine protease inhibitor, such as aprotinin or α2-macroglobulin, which may be used for the treatment of plasminogen deficiency and thromboses.

The object of the invention is to provide a pharmaceutical preparation based on a blood coagulation protein characterized by an improved half-life in vivo. In addition, the object of the invention is to extend the in vivo half-life of a blood coagulation protein in order to, on the one hand, stabilize the endogenous content of this blood coagulation protein and, on the other hand, increase the efficiency of an exogenous blood coagulation protein.

According to the present invention said object is realized by a pharmaceutical preparation for the treatment of blood coagulation disorders, which preparation contains at least one protein selected from the group of a pro-protein of blood coagulation. In addition, said pharmaceutical preparation is characterized in that it
contains a receptor binding competitor, which, by itself, is
coaulation physiologically inert, such as e.g. lipoproteins. Thus,
the action of the protein on blood coagulation is not directly
influenced, and the receptor binding competitor only contributes to
stabilizing said protein in vivo.

In the preparation according to the present invention the
protein preferably is a blood coagulation protein selected from the
group of factor II, V, VII, VIII, IX, X, XI, XII, vWF and protein
C. These proteins are the pro-forms of the coagulation
physiologically effective active forms. For example, factor II
(prothrombin) is the pro-form of coagulation active thrombin, which
enzymatically cleaves fibrinogen to fibrin and thus causes a blot
clot. Among these proteins are, on the one hand, the vitamin K
dependent proteins, which are also present in a prothrombin complex
preparation based on factors II, IX and X and optionally factor VII
and protein C. In addition, vWF or its analogue proteins are
possible proteins in the preparation according to the present
invention, too. Particularly in platelet rich plasma vWF proves
that it is effective as a blood coagulation factor by itself,
independent of its role as a carrier and stabilizer of factor VIII
(Beguin et al., Thrombosis and Haemostasis 78 (1), 590-594 (1997)).
vWF is e.g. derived from plasma or a plasma fraction, but it may
also be prepared by recombinant DNA technology by way of expression
of a transformed cell. For example, a recombinant vWF is prepared
according to the teachings of Pannekoek (EP 0 197 592) or Fischer
et al. (WO 96/10584), respectively.

The group of blood coagulation zymogens or precursors of the
coagulation physiologically active blood coagulation proteins may,
on the one hand, be derived from plasma or a plasma fraction and
correspond to the native proteins. On the other hand, the native
proteins or their derivatives may also be derived from cell culture
supernatants. In this process, the cells are preferably transformed
by means of recombinant DNA technology in order to obtain the
recombinant forms of the blood coagulation proteins.
Mutants or analogues are contained in the preparation according to the present invention as long as they may be used in the treatment of blood coagulation disorders and may bind to a receptor functionally, i.e. competitively.

Particularly the lipoprotein receptor, or LRP, may be mentioned as a receptor for the proteins of the preparation according to the present invention. Another receptor to which the proteins bind is e.g. the mannose receptor. Surprisingly it has been found that according to the present invention the ligands of LRP demonstrate a positive effect in vivo particularly for pro-proteins of blood coagulation as these ligands competitively bind to the receptor. This came as a surprise, because it could not be anticipated that pro-proteins of blood coagulation and vWF are able to bind to LRP. LRP had mainly been known for binding enzymes or their complexes with inhibitors. Thanks to the competitive nature of their binding to LRP, premature binding of the protein in the pharmaceutical preparation or of the endogenous blood coagulation zymogen and vWF are inhibited, which considerably improves biological availability.

A number of proteins may be used as receptor binding competitors, with those being preferred whose physiological tolerance has been tested.

According to the present invention, the receptor binding competitor is preferably selected from the group of enzymes, particularly tissue type plasminogen activator (tPA), urokinase (u-PA), pro-urokinase (pro-u-PA), lipoprotein lipase (LPL) and kallikrein; inhibitors, particularly plasminogen activator inhibitor-1 (PAI-1), tissue factor pathway inhibitor (TFPI) and aprotinin; enzyme inhibitor complexes, particularly t-PA-PAI-1, u-PA-PAI-1 and thrombin-PAI-1, α2-macroglobulin proteinase (fast form) α2M, α2M, pregnancy zone protein proteinase, elastase-α1-antitrypsin, thrombin antithrombin III, thrombin heparin-cofactor II and u-PA-protease nexin I; lipoproteins, particularly low density lipoprotein (LDL), apolipoprotein E enriched β-very low density lipoprotein, (apo E-β-VLDL), LPL enriched VLDL (LPL-VLDL)
and LPL enriched β-VLDL (LPL-β-VLDL); matrix proteins, particularly thrombospondin 1 and 2; toxins and viruses, particularly pseudomonas endotoxin A, and minor group human rhinovirus; or other ligands, particularly apolipoprotein E (apo E), lactoferrin, receptor associated protein (RAP), FVIII and vWF.

The ligand acting competitively is preferably selected from those physiological proteins which are preferably present as (human physiological) extracellular proteins in the circulation. They may be used in their native forms or as analogues characterized by their binding to the receptor.

Preferably the ligands acting competitively are RAP or a derivative thereof. Particularly preferred is a RAP fragment comprising the domain which keeps proteins from binding to LRP and thus inhibits internalization and clearance of the proteins (I. Warshawsky et al., J. Biol. Chem., 1993, vol. 15, pp. 22046-22054; Bu G., EMBO J., 1995, vol. 14, pp. 2269-2280). They may be RAP or a derivative thereof of human origin, or animal RAP or a derivative thereof, e.g. from mice, rats or other animals.

In order not to impair the effect of the pharmaceutical preparation or the endogenous proteins, respectively, said receptor binding competitor is selected from the coagulation physiologically inert proteins, among which are, for example, low density lipoprotein (LDL) and apolipoprotein.

Mixtures of receptor binding competitors may also be used as said coagulation physiologically inert receptor binding competitors which, as mixtures, though probably not as single proteins, do not exert any undesired influences on the coagulation system. Examples for such mixtures are thrombin with ATIII or with heparin cofactor II, or with plasminogen activator inhibitor-1 (PAI-1), and elastase and α1-antitrypsin, tPA with PAI-1. For example, a preferred mixture is tPA with aprotinin, which is highly suitable for stabilizing e.g. factor VIII in vivo. This means that, in this case, the selected receptor binding competitor is a ligand whose coagulation physiological activity is inhibited, optionally by antagonizing with the respective inhibitor.
Instead of a mixture of a, in principle, effective receptor binding competitor and a suitable antagonist, a mutated form of said receptor binding competitor may be used, too, which competitor is inactivated by suitable mutations (e.g. in the active center) (whose affinity to the receptor, however, is not essentially changed (reduced)). Examples are mutated forms of tPA, urokinase, kallikrein, renin, thrombin (WO 96/41868) etc., among others.

It came as a surprise that recombinant tPA (rtPA) stabilized the endogenous factor VIII content in a vWF deficient dog over a longer period of time, which allowed for hemophilia treatment. Although the natural stabilizer of factor VIII, i.e. vWF, was lacking, the LRP ligand, or the mixture of LRP ligands, respectively, was able to take over the role of vWF. The factor VIII content was increased by about 50% and remained at that level for several days. The enzyme tPA is a known ligand of LRP. The administration of rtPA to a mammal blocks LRP and thus inhibits metabolic degradation of the coagulation factors, such as e.g. factor VIII. This blocking effect is yet increased by aprotinin. The effect of aprotinin alone was also demonstrated in a vWF deficient dog which received a combination of rvWF and aprotinin. This lead to a rise in the factor VIII level over a longer period of time.

Another preferred receptor binding competitor is aprotinin, which is excellently suitable for stabilizing vWF. For example, aprotinin may not only contribute to improved biological availability in a vWF preparation, but, administered as a single active substance, it may also contribute to improving the condition of a vWF deficient patient.

In many cases a patient suffering from a certain protein deficiency will be treated with the respective receptor binding competitor in order to provide the possibility of formation of the endogenous protein or to stabilize the protein, respectively. This is possible in patients suffering from phenotypic coagulation protein deficiency.
The preparation according to the present invention, or the active substances mentioned above, respectively, are preferably used as treated preparations or proteins, respectively, in order to rule out any transfer of potentially present pathogens, such as viruses or prions (TBE). If they are derived from biological materials, such as blood, plasma, plasma fractions or cell cultures, they carry the risk of contamination with human pathogens, which risk, however, may be eliminated by a suitable inactivation or depletion treatment. Among the effective measures for virus inactivation are, for example, treatment with organic solvents and/or detergents (EP-0 131 740, EP-0 050 061, virus inactivating eluent), treatment with chaotropic means (WO 90 15 613) heat treatment processes, preferably in lyophilized, dry or wet state (cf. EP-0 159 311), combination processes, such as that in EP-0 519901, and physical methods. The latter lead to virus inactivation e.g. by exposure to light, e.g. in the presence of photo sensitizers (EP-0 471 794 and WO/AT 97/00068).

Among the human pathogen depletion processes are, in particular, filtrations using ultrafilters, deep-bed filters or nanofilters (A 341/98). In addition, precipitation steps and other protein purification measures, such as adsorption, also contribute to the depletion of probably present pathogens.

On the one hand, the preparation according to the present invention may be provided as a ready-to-use formulation, i.e. as a mixture of the protein with the receptor binding competitor. On the other hand, it is also possible to provide a set containing A) the protein, and B) the receptor binding competitor. Said set has the advantage that the doses of the various components are variable and that the way of administration may be adapted to the prevailing circumstances. For example, apart from i.v. administration, intramuscular, subcutaneous or oral administration of the active substances is possible, too. The active substances may be administered to the patient together, i.e. simultaneously, or in parallel or consecutively. If a set is used, the preparation
according to the present invention is preferably provided in ready-to-use syringes containing the individual components.

An indication according to the present invention for the preparation is, for example, the treatment of a patient suffering from phenotypic coagulation factor deficiency, e.g. a vWF deficient patient. According to the present invention, a preparation is prepared based on the coagulation factor the patient is lacking, using the combination according to the invention of the receptor binding competitor. Moreover, the receptor binding competitor alone also corresponds to the indication according to the present invention. For example, in case of functional factor VIII deficiency, the receptor binding competitor for factor VIII is administered, which may be a mixture of tPA and aprotinin.

Another object of the present invention is the use of an LRP ligand preparation for the preparation of a means to treat phenotypic coagulation factor deficiency (e.g. vWF, factor VIII or factor IX deficiency) or to increase the biological half-life of a protein.

The invention will be explained in greater detail using the following examples and drawings, to which, however, it is not restricted.

Fig. 1 shows factor VIII determination in a vWF deficient dog
Fig. 2 shows vWF and factor VIII determination in a vWF deficient dog

**EXAMPLE 1:**

A male Kooiker dog suffering from severe type 3 von Willebrand factor deficiency (Rieger et al., Thromb. Haemost. (1998), 80: 332-337), 8.5 kg of body weight, 2 years old, was anesthetized using 10 mg/kg body weight of ketamine (Ketasol, Dr. E. Gräub AG, Bern, Switzerland) and 1 mg/kg body weight of xylaxine (Xylasol, Dr. E. Gräub AG, Bern, Switzerland). Subsequently, a permanent venous access via a lower arm was established, and aprotinin (Pantinol 100.000 K.I.E. vials, Gerot Pharmazeutika, Vienna, Austria) at a dose of 10 000 K.I.E. per kg of body weight was administered as a
bolus via a hollow needle. Then recombinant tissue plasminogen activator (Actilyse, Boehringer Mannheim) (rtPA) was reconstituted using distilled water according to the producer's instructions, and a dose of 0.25 mg/kg body weight was administered the von Willebrand deficient dog as a bolus. At the same time, a blood sample on citrate was taken. Within the first hour of treatment a co-infusion of aprotinin (3000 K.I.E. per kg of body weight) and rtPA 0.75 mg/kg body weight was administered, and after one hour this was exchanged for an infusion containing aprotinin at a dose of 3000 K.I.E. per kg of body weight and 0.5 mg/kg body weight of rtPA, which infusion was administered for one hour. After that, i.e. after two hours, aprotinin was infused for another hour at a dose of 3000 K.I.E./kg body weight. At the time points of 30 min, 1, 3, 24, 48, 92, and 96 hours after the beginning of the experiment blood samples were taken on citrate, platelet depleted plasma was prepared from them by means of centrifugation, and they were deep-frozen at -20°C and stored until further analysis.

After completion of the infusion experiment, blood coagulation factor VIII was determined quantitatively in the frozen blood samples using two different determination methods. Firstly, the two step coagulation method according to the method of Austen, D.E.G. and Rhymes, I.L., A Laboratory Manual of Blood Coagulation, Oxford, U.K., Blackwell Scientific (1975) was employed using the reagents of the two step factor VIII test kit of IMMUNO AG, Vienna, Austria. Secondly, factor VIII was determined using the chromogenic factor VIII test kit, Immunochrom FVIII:C of Immuno AG, Vienna, Austria (Lang H., Oberreiter M., Moritz, B., Thromb. Haemost. 65: 943 (1991)). Factor VIII was measured with respect to a human plasma factor VIII standard, which had been calibrated against the Third International Standard 91/666. Factor VIII activity is expressed in human factor VIII units per ml. The result of this determination is summarized in fig. 1. Both factor VIII determination methods, which were carried out independently of each other, showed a rise in factor VIII plasma activity which lasted over a period of 24 to 48 hours and then remained stable at a level of about 150% of the
initial level for a period of up to 96 hours after the beginning of the experiment, irrespective of the test method used. As von Willebrand factor is known to be sensitive against proteolytic degradation by plasmin, it had to be assumed that the administration of rtPA would result in an increase in von Willebrand factor inactivation in combination with a secondary reduction of the plasma factor VIII level due to a lack in factor VIII stabilizing protein. The simultaneous administration of aprotinin as an inhibitor of fibrinolysis and of plasmin only gave rise to the expectation that the rtPA effect would be inhibited. The seemingly paradoxical rise in the plasma factor VIII level can therefore only be explained by the interference of aprotinin and/or rtPA with the factor VIII metabolizing mechanisms.

EXAMPLE 2:

A von Willebrand factor deficient dog as in example 1 was anesthetized and subsequently primed with aprotinin (Pantinol) at a dose of 100,000 K.I.E. as an intravenous bolus. Then the animal was given a recombinant von Willebrand factor preparation at a dose of 70 RCoF E/kg. The preparation and characterization of the recombinant von Willebrand factor preparation is described in Fischer et al., FEBS Lett. 375: 259 (1995). Immediately after the administration of the recombinant von Willebrand factor preparation, an infusion of 100,000 K.I.E. of aprotinin was administered intravenously over a period of three hours. Blood samples on citrate were taken prior to the beginning of the experiment (= time 0), 15 min, 30 min, 1 h, 3 h, 24 h, and 48 h after treatment with recombinant von Willebrand factor, and plasma was prepared therefrom as described in example 1 and deep-frozen at -20°C until further analysis. Then von Willebrand factor antigen was measured in these samples using a rabbit anti-human von Willebrand factor antibody with the test Asserachrom vWF, Boehringer Mannheim. In addition, ristocetin cofactor activity was determined by ristocetin induced aggregation of formaldehyde fixated human blood platelets and according to the method of Evans and Austen, Brit. J. Haematol. 37: 289 (1977). A human plasma
standard served as von Willebrand factor standard. In addition, factor VIII was tested as described in example 1. The results of the blood sample determinations of von Willebrand factor and factor VIII activity are shown in Fig. 2. In this figure a mean level of factor VIII activity, determined according to the two factor VIII determination methods, is given as a graph. As known from literature (Turecek et al., Blood 90: 3555 (1997)), von Willebrand factor was eliminated at a half-life of 10 to 20 hours, so that after 48 hours von Willebrand factor was hardly detectable in the circulation. Simultaneously with the administration of recombinant von Willebrand factor and aprotinin, the plasma factor VIII level rose to about 150% of the initial level within 3 h and remained stable at this level or even showed a slightly rising tendency up to 48 h post administration. This result may also be interpreted as in example 1, which means that by the administration of aprotinin the factor VIII plasma level remains increased due to the interference with factor VIII metabolism, although factor VIII is not stabilized by recombinant von Willebrand factor.

EXAMPLE 3:

EFFECT OF RAP ON FACTOR VIII OBTAINED IN KNOCK-OUT MICE SUFFERING FROM SEVERE FACTOR VIII DEFICIENCY

By means of genetic engineering a mouse strain was created suffering from severe factor VIII (FVIII) deficiency by purposefully disrupting the mouse factor VIII gene according to Bi et al., Nature Genetics 10: 119-121 (1995). Factor VIII knock-out mice were created by inserting a neo-gene at the 3'-end of exon 17 of the mouse factor VIII gene. The relevant male animals (X'Y) had non-detectable factor VIII levels of < 0.02 ± 0.01 E/ml when measured using either a chromogenic factor VIII test, Hyland Immuno, Vienna, Austria, as recently described (Turecek et al., Thromb. Haemostas. Suppl. 769 (1997)) or an antigen-ELISA as described below.

Two relevant hemizygous male mice (X'Y) were treated intravenously with a dose of 200 E/kg of body weight of a recombinant human factor VIII (rhFVIII) preparation derived from
Chinese hamster ovary cells, which had been prepared as described (PCT WO/85/01961) and formulated pharmaceutically without stabilizing protein.

One hour after said treatment, the tail ends of the anesthetized mice were cut in with the blade of a scalpel as described by Novak et al., Brit. J. Haematol. 69: 371-378 (1998). A volume of 50 μl of blood was collected by means of capillary tubes (Ringcaps, Hirschmann, Germany) from the tail wounds, said capillary tubes being coated with lithium heparin as anticoagulant. The capillary tubes were sealed and centrifuged in order to separate the blood cells from the plasma. Then the capillary tubes were opened, and the cell and plasma fractions were collected by further centrifugation. Finally the plasma samples were subjected to factor VIII determination using factor VIII antigen ELISA, test set IMMUNOZYM FVIII Ag, Hyland Immuno, Vienna, Austria, using monoclonal antifactor VIII antibodies for scavenging as well as for detection, as described (Stel et al., Nature 303: 530-532 (1983); Lenting et al., J. Biol. Chem. 269: 7150-7155 (1994); Leyte et al., Biochem. J. 263: 187-194 (1989)). The resulting factor VIII levels were expressed in international units of human factor VIII. The results of the factor VIII plasma levels are given in the table.

Two other relevant male hemizygous mice (X′Y) were primed with recombinant receptor associated protein (GST-RAP) at a dose of 40 mg/kg body weight 10 min prior to treatment with the recombinant human factor VIII. The receptor-related protein (RAP) used in this study, which interacts with the low density lipoprotein receptor, was obtained by bacterial fermentation as described by Hertz et al., J. Biol. Chem. 266: 21232-21238 (1991). A fusion protein of RAP with glutathione-S-transferase was expressed in E. coli and purified by means of affinity chromatography on glutathione agarose. The resulting protein was mainly comprised of the fusion protein and cleavage products of RAP and glutathione-S-transferase. The fusion protein was formulated in an injectable buffer ready for administration to the factor VIII knock-out mice. As in the control group (treatment with only factor VIII), blood samples were taken
one hour after administration of recombinant factor VIII and measured for factor VIII activity using factor VIII antigen ELISA. The results are given in the table.

<table>
<thead>
<tr>
<th>mouse No.</th>
<th>treatment</th>
<th>dosage</th>
<th>obtained 1 h after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GST-RAP</td>
<td>rhFVIII</td>
<td>FVIII:Ag (E/ml plasma)</td>
</tr>
<tr>
<td>1</td>
<td>40 mg/kg</td>
<td>200 E/kg</td>
<td>1.92</td>
</tr>
<tr>
<td>2</td>
<td>40 mg/kg</td>
<td>200 E/kg</td>
<td>1.88</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>200 E/kg</td>
<td>0.73</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>200 E/kg</td>
<td>0.83</td>
</tr>
</tbody>
</table>

In mice primed with GST-RAP, factor VIII was obtained at a level of more than 200% of the plasma levels after treatment with recombinant factor VIII alone.
1. A pharmaceutical preparation for the treatment of blood coagulation disorders containing at least one protein selected from the group of a pro-protein of blood coagulation and, in addition, a coagulation physiologically inert receptor binding competitor.

2. A preparation according to claim 1, characterized in that said pro-protein of blood coagulation is selected from the group of factor II, V, VII, VIII, IX, X, XI, XII, vWF and protein C.

3. A preparation according to claim 1, characterized in that said protein is vWF.

4. A preparation according to any one of claims 1 to 3, characterized in that said protein is derived from a biological material selected from the group of human plasma, a plasma fraction and a cell culture supernatant.

5. A preparation according to any one of claims 1 to 4, characterized in that said receptor binding competitor is a ligand of lipoprotein receptor related protein (LRP).

6. A preparation according to any one of claims 1 to 5, characterized in that said receptor binding competitor is RAP.

7. A preparation according to any one of claims 1 to 6, characterized in that said receptor binding competitor is a mixture of a coagulation physiologically active protein and its inhibitor, particularly tPA and aprotinin.

8. A preparation according to any one of claims 1 to 7, characterized in that said mixture contains tPA and aprotinin.

9. A preparation according to any one of claims 1 to 8, characterized in that it is provided as a set containing

   a) the protein and

   b) the receptor binding competitor.

10. A preparation according to any one of claims 1 to 9, characterized in that said protein is factor VIII and said receptor binding competitor is aprotinin, optionally together with tPA.

11. A preparation according to any one of claims 1 to 9, characterized in that said protein is vWF and said receptor binding competitor is aprotinin.
12. A preparation according to any one of claims 1 to 11, characterized in that said receptor binding competitor is a human physiological extracellular protein or an analogue thereof.
Wirkung von Aprotinin und rtPA bei einem vWF-defizienten Hund

Primen mit Aprotinin (3000 KIE/kg)

 rtPA (0,25 mg/kg)

Aprotinin (3000 KIE/kg)

FVIII:2 Schritt (E/ml) Zeit (h)

Aprotinin (3000 KIE/kg) + rtPA (0,50 mg/kg)

FVIII: Chrom (E/ml)

Aprotinin (3000 KIE/kg) + rtPA (0,75 mg/kg)

FIG. 1
Wirkung von Aprotinin und rWF bei einem vWF-defizienten Hund

VWF:Ag

VWF:RCoF

FVIII

Präven mit Aprotinin (100000 kIE)

rWF (70 RCoF / Kg Bolus) Aprotinin (100000 kIE)

zeit (h)

FIG. 2