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(71) Applicant(s)
Rotkreuzstiftung Zentrallaboratorium Blutspendedienst SRK

(72) Inventor(s)
Markus Rentsch

(74) Agent/Attorney
GRIFFITH HACK,GPO Box 3125,BRISBANE QLD 4001

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(71) Anmelder *(für alle Bestimmungsstaaten ausser US)*: ROTKREUZSTIFTUNG ZENTRALLABORATORIUM BLUTSPENDEDIENST SRK [CH/CH]; Wankdorfstrasse 10, CH–3000 Bern 22 (CH).

(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: RENTSCH, Markus [CH/CH]; Eystrasse 4, CH–3400 Burgdorf (CH).

(74) Anwalt: BOVARD AG; Optingenstrasse 16, CH–3000 Bern 25 (CH).

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(54) Title: METHOD FOR PRODUCING AN IgM PREPARATION FOR INTRAVENOUS ADMINISTRATION

(54) Bezeichnung: VERFAHREN ZUR HERSTELLUNG EINES IgM–PRÄPARATES FÜR DIE INTRAVENÖSE APPLIKATION

(57) Abstract

An IgM–containing immunoglobulin solution is treated with a protease in a method for producing an immunoglobulin solution that is adequate for intravenous administration and having an Igm proportion higher than 5 weight % with respect to the immunoglobulin proportion. The iv–tolerant preparation obtained is characterized by not being chemically modified and by having low anticomplementary ACA activity.

(57) Zusammenfassung

Im Verfahren zur Herstellung einer für die intravenöse Applikation geeigneten Immunglobulinlösung mit einem IgM–Anteil von mehr als 5 Gew.–%, bezogen auf den Immunglobulinanteil, wird eine IgM–haltige Immunglobulinlösung mit einer Protease behandelt. Das erhaltene i.v. verträgliche Präparat zeichnet sich dadurch aus, dass es nicht chemisch modifiziert ist und eine niedrige antikomplementäre Aktivität ACA aufweist.

Abstract

In the method for producing an immunoglobulin solution suitable for intravenous application with an IgM proportion of more than 5% by weight with respect to the immunoglobulin proportion, an IgM-containing immunoglobulin solution is treated with a protease . The intravenously well tolerated preparation obtained is characterized by not being chemically modified and by having low anticomplementary activity ACA.

Method of Producing an IgM Preparation for Intravenous Application

This invention relates to a method of producing an immunoglobulin solution suitable for intravenous application. Used as the starting material is a protein fraction obtained from human or animal blood which contains the immunoglobulins s in concentrated form.

As is well known, immunoglobulins play an important role in the immune system of man and mammal in fighting off infections. The immunoglobulins are divided up into different classes (e.g. IgG, IgA,]gM, IgD and lgE) with differing biochemical and physiological properties. Until 1980 only IgG was isolated and used as an IV-well-tolerated product for prophylaxis and therapy. In EP-A-0 013 901, EP-A-0 413 187 and EP-A-0 352 500 IgM preparations are described, which have been made intravenously well tolerated mainly through treatment with β-propiolactone. EP-A-0 413 188 describes a method in which the IV-tolerance is achieved through anion exchange chromatography with selective elution of the IV-tolerant fraction.

It would therefore be desirable to provide means for production of a highly purified, IgM concentrate suitable for intravenous administration for therapy and prophylaxis. The product should have a low anticomplementary activity (ACA), and demonstrate a low blood pressure drop in a rat model, but the]gM molecules should not be chemically modified, however. In at least preferred forms of the present invention this has surprisingly been achieved through treatment of a IgM-containing immunoglobulin solution with a protease.

According to the present invention there is provided a method of producing an intravenously administrable polyclonal, chemically unmodified immunoglobulin preparation with an IgM proportion of more than 5% by weight with respect to the total immunoglobulin

proportion, and a low anticomplementary activity, characterized in that an aqueous solution or suspension of a corresponding IgM-containing plasma fraction is treated with a protease.

It will also be appreciated that the present invention provides an intravenously administrable polyclonal, chemically unmodified immunoglobulin preparation when prepared by this method.

The protease treatment is preferably an incubation at raised temperature in the presence of pepsin, papain, plasmin or thermolysine. The proteases can also be chemically modified, immobilized on a substrate and/or produced through genetic engineering. The reparation according to the inventive method can be transferred into an IV-administrable solution. Such a solution displays a reduction

of the ACA, of the blood pressure drop in a rat model and of the C1q binding activity.

Suitable as starting materials for the method according to the present invention are immunoglobulin-containing solutions, such as, e.g. plasma, precipitate A or B from Kistler-Nitschmann-fractionation; Cohn fraction I/II/III; II/III; III; or other IgM-containing plasma fractions from human or animal plasma. For example, a immunoglobulin-containing fraction, such as precipitate B according to Kistler-Nitschmann, can be dissolved in a buffer, most of the impurities being removed through a precipitation with 0.5 to 5% octanoic acid at pH 4 to 6, preferably pH 5. Afterwards the solution is incubated at low ionic strength for 1 to 48 hours, preferably 9 hours, at a temperature of 20 to 50 °C, preferably 37 °C with addition of at least 50 U/g of pepsin, preferably 600 U/G.

For further purification, the solution can be subjected to an adsorption, for example with a gel containing a DEAE-group in batch or column method. If the IgM concentration in the end product is supposed to be increased further, the IgM solution is put on an ion exchanger (e.g. TMAE-Fraktogel®). Through a selective elution, e.g. by means of a salt gradient or pH gradient, the IgM fraction can be isolated. Through ultrafiltration and diafiltration, for example a gel filtration, the solution can be concentrated and the electrolyte content can be adjusted to a final, intravenously well tolerated formulation. The protein concentration can amount to 1 to 20%, preferably 3 to 6%. The product can contain in addition proteins, preferably albumin, as well as sugar, preferably glucose or sucrose, or amino acids.

To assess the intravenous compatibility of immunoglobulin preparations, the anticomplementary activity (ACA) is usually used. To determine the ACA, a defined quantity of the product to be tested is incubated with a defined quantity of guinea pig complement and the remaining quantity of complement titrated. The ACA is indicated as consumption of CH50 per g of immunoglobulin. The indicted results of the ACA were determined to a large extent according to the method published by M. Mayer (Mayer, M.M. (1961), "Complement and Complement Fixation" in *Experimental Immunochemistry*, 2nd edition, pp. 133-240, C Thomas,

Springfield, IL). Valid as a guide value for intravenously usable IgG products is an ACA of < 1000 CH50 per g of protein.

To assess intravenous compatibility, the binding of the C1q complement components to the immunoglobulin can be further used. For determination, a defined quantity of test product is incubated with a defined quantity of purified, radioactively labelled C1q complement in buffer and in serum. The C1q binding activity of the test product is determined through precipitation in the presence of polyethylene glycol. The higher the radioactivity in the precipitate, the greater the C1q binding activity of the product. Finer predictions about the type of C1q binding and thereby the quality of the product can be achieved if the C1q is radioactively labelled with two different methods. On the one hand, under as mild as possible oxidative conditions with lactoperoxidase (LPO), and, on the other hand, under drastic oxidative conditions with chloramine T (CT). The tests were carried out to a large extent according to the method published by P. Späth (P.J. Späth, A. Corvetta, U.E. Nydegger, R. Büttler: "An Extended C1q-Binding Assay Using Lactoperoxidase- and Chloramin-T-iodinated C1q," *Scand. J. Immunol.* 18, 319-328, 1983). Expected of an intact, intravenously well tolerated preparation is that the C1q binding activity is as minimal as possible. A model for testing the IV compatibility of immunoglobulins is the rat model according to Bleeker et al. [W.K. Bleeker, J. Agterberg, G. Rigter, A. de Vries-van Rossen, J.C. Bakker: "An Animal Model for the Detection of Hypotensive Side Effects of Immunoglobulin Preparations, ", *Vox. Sang.* 52: 281-290 (1987)]. Tolerance parameter in this model is blood pressure. Intravenously poorly tolerated products lead to a significant drop in blood pressure.

Examples

Reference Example 1

1 kg of precipitate B according to Kistler-Nitschmann was suspended in 4 kg of 0.1 M acetate buffer, pH 5.1, and 2% octanoic acid was added at room temperature. 0.15 g of tricalcium phosphate was added per g of octanoic acid, and the precipitate filtered off. The filtrate was diafiltered against 20 mmol/l piperazine, 60 mmol/l NaCl, pH 5.8. The diafiltered solution was treated with 75

mg DEAE-Sephadex® per g of protein. Then the protein concentration was adjusted to 20 mg/ml, and the solution was treated with 1% Tween® 80 and 0.3% TNBP (tri-n-butyl-phosphate) for 8 hours at 25 °C. The solution was then put on a TMAE-Fraktogel® column, and the IgM fraction was eluted with 20 mmol piperazine, 160 mmol NaCl, pH 5.8. The end product was concentrated to 5% protein, and the pH value adjusted to 4.5.

Reference Example 2

1 kg of precipitate B according to Kistler-Nitschmann was suspended in 4 kg of 0.1 M acetate buffer, pH 5.1, and 2% octanoic acid was added at room temperature. 0.15 g of tricalcium phosphate was added per g of octanoic acid, and the precipitate filtered off. The filtrate was diafiltered against 20 mmol/l NaCl diafiltered, and the solution brought to 20 mg/ml protein. The pH value was adjusted with 0.2 M HCl to 4.0, and the solution incubated for 9 hours at 37 °C. After cooling down to 20 °C, the pH was adjusted to 5.8, and piperazine ad 20 mmol/l and NaCl ad 60 mmol/l added. The solution was subsequently treated with 1% Tween® 80 and 0.3% TNBP (tri-n-butyl-phosphate) for 8 hours at 25 °C. The solution was then put on a TMAE-Fraktogel® column, and the IgM fraction was eluted with 20 mmol piperazine, 160 mmol NaCl, pH 5.8. The end product was concentrated to 5% protein, and the pH value adjusted to 4.5.

Example 1

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piperazine, 160 mmol NaCl, pH 5.8. The end product was concentrated to 5% protein, and the pH value adjusted to 4.5.

Example 2

I kg of precipitate B was prepared analogously to reference example 2, instead of 600 U of pepsin per g of protein of the solution, 1200 U of pepsin per g of protein being added, however, before the pH 4 incubation.

I. Characterization of the Experimental Products

The immunoglobulins IgG, IgA and IgM were nephelometrically determined with antisera. The total protein content was determined with the Kjeldahl method.

	Protein mg/g	IgG mg/g	IgA mg/g	IgM mg/g	Isoagglutinins Anti-A	Anti-B
Reference example 1	44.4	4.1	16.8	45.0	1:64	1:64
Reference example 2	48.1	8.6	19.3	43.5	1:128	1:64
Example 1	51.3	7.0	22.0	50.5	1:128	1:64
Example 2	46.9	4.7	20.2	51.0	1:128	1:64

II. Table: Tolerance Parameters

	Treatment	ACA	Rat model	C1q-binding			
				Puffer		Serum	
		CH50/g	Blood pressure drop %	LPO %	CT %	LPO %	CT %
Reference example 1	Without pH 4	515	19	1.5	0.1	43	5.5

Reference example 2	pH 4	179	18	0	0.5	42	6.7
Example 1	pH 4 with 600 U pepsin	125	7	0	0.3	34	3.9
Example 2	pH 4 with 1200 U pepsin	89	2	0	0.5	23	3.7

The addition of pepsin causes a reduction of the ACA, a lessening of the blood pressure drop in the rat model as well as a reduction in C1q binding activity.

Claims

1. A method of producing an intravenously administrable polyclonal, chemically unmodified immunoglobulin preparation with an IgM proportion of more than 5% by weight, with respect to the total immunoglobulin proportion, and a low anticomplementary activity, characterized in that an aqueous solution or suspension of a corresponding IgM-containing plasma fraction is treated with a protease.

2. A method according to claim 1, characterized in that the preparation has an anticomplementary activity of less than < 500 CH50/g protein.

3. A method according to claim 2 characterized in that the preparation has an anticomplementary activity of less than < 200 CH50/g protein.

4.A method according to claim 2 characterized in that the preparation has an anticomplementary activity of less than < 150 CH50/g protein.

5. A method according to any one of claims 1 to 4, characterized in that the aqueous solution or suspension of the IgM-containing plasma fraction is incubated with the protease at an acidic pH value with and a temperature of at least 15 °C.

6. A method according to claim 3, characterized in that the incubation is temperature is 20 to 50 °C.

7. A method according to claim 6, characterized in that the incubation temperature is 35 °C to 40 °C.

8. A method according to any one of claims 5 to 7, characterized in that the incubation period is 1 to 48 hours.

9. A method according to claim 8, characterized in that the incubation period is 6 to 12 hours.

10. A method according to one of the claims 1 to 9, characterized in that the protease concentration in the aqueous solution or suspension of the IgM-containing plasma fraction is at least 50 U/g protein.

11. A method according to claim 10, characterized

in that the protease concentration in the aqueous solution or suspension of the IgM-containing plasma fraction is 300 to 1200 U/g protein.

12. A method according to one of the claims 1 to 11, characterized in that the pH value of the aqueous solution or suspension of the IgM-containing plasma fraction during the protease treatment is 3.5 to 5.5.

13. A method according to claim 12, characterized in that the pH value of the aqueous solution or suspension of the IgM-containing plasma fraction during the protease treatment is 3.7 to 4.3.

14. A method according to one of the claims 1 to 12, characterized in that the protease is an endopeptidase.

15. A method according to claim 14, characterized in that the protease is at least one endopeptidase selected from pepsin, papain, plasmin or thermolysine, which can be immobilized on a substrate if necessary.

16. A method according to one of the claims 1 to 15, characterized in that the ionic strength in the aqueous solution or suspension of the IgM-containing plasma fraction is less than 0. 1.

17. A method according to claim 16, characterized in that the ionic strength in the aqueous solution or suspension of the IgM-containing plasma fraction is less than 0.04.

18. A intravenously administrable polyclonal, chemically unmodified immunoglobulin preparation, when prepared by a method according to any one of claims 1 to 17.

19. A method of producing an intravenously administrable polyclonal, chemically unmodified immunoglobulin preparation substantially as herein described with reference to the examples, other than the reference examples.