Some characteristics of aggregates of IgG and plasma proteins in heat-treated factor VIII concentrates

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Summary. Eight batches of commercial heat-treated and one untreated factor VIII concentrate from 6 producers were analyzed for their content of IgG, IgG subclasses, IgG aggregates and the presence of other plasma proteins combined with the IgG as well as for anticomplement activity. Methods used were thin-layer gel filtration, immuno-gel filtration, spot immuno-precipitate assay in a double antibody version and an agarose plate haemolysis inhibition assay of complement fixation. The IgG content varied from 0.1-6.90 g/l. In all preparations IgG existed as monomers and aggregates. Associated with the IgG were also found, at significantly increased amounts compared to normal serum and intravenous immunoglobulin, one to four of the following plasma proteins; fibronectin, fibrinogen, von Willebrand factor antigen, Clq, albumin and IgA. Three batches from two producers had high anticomplementary activity, presumably caused by the IgG aggregates. Two of these deviated strikingly from normal human serum pools in percent distribution of IgG subclasses. It is hypothesized that these aggregates can induce side effects or cause immunological aberrations.

Key words: Factor VIII – IgG aggregates – IgG plasma protein coprecipitates – IgG subclasses – Complement activation

Introduction

In a recent preliminary study we demonstrated that factor VIII concentrates, not virus-inactivated with heat, contain from 20-700 mg IgG per 1,000 IU of factor VIII and that much of this IgG occurs in aggregated form [38]. There were also indications that other plasma proteins were “complexed” with the IgG, since they coprecipitated.

IgG polymers appearing in commercial Ig preparations have been related to the risk of side effects on intramuscular as well as intravenous injection of Ig [4-6]. It seems possible that the IgG-containing aggregates in factor VIII concentrates may also cause side effects on injection and perhaps be related to the immunological abnormalities noted in some haemophiliac patients without HIV exposure [18, 23, 34].

In the present investigation we have analyzed heat-treated factor VIII concentrates for content of IgG, for IgG subclass and aggregate composition, for the presence of other plasma proteins complexed with IgG and for anticomplementary activity.

Materials and methods

Seven commercial factor VIII concentrates from six firms in eight batches, heat-treated for virus-inactivation, were studied. A pair of samples with the highest concentration as well as heat-treated and unheated batches of a concentrate with the lowest concentration of factor VIII, all from one firm were included to test batch consistency and the influence of heating. The concentrates were designated according to IgG content.

Nos. 1 and 2 Octonativ (KabiVitrum); 50 IU factor VIII/ml, heat treated in dry form at 68°C for 24 h; lot Nos. 55082 and 56320. No. 3 Profilate 250 HS (Alpha); 25 IU factor VIII/ml; N-heptane-suspended and heat-treated at 60°C for 20 h; lot No. 585900/A6 0250. No. 4 Hemofi-T (Travenol); 38 IU factor VIII/ml; heat-treated in dry state at 60°C for 24 h; lot No. 150825 A 346 A. No. 5 Kryobulin TIM 3 (Immunon); 25 IU factor VIII/ml; steam-heated at 60°C for 10 h; lot No. 09A 298409-T. Nos. 6 and 7 AHF konc. (KabiVitrum); 3 IU factor VIII/ml; heat-treated in dry state at 60°C for 24 h; lot No. 54423 and No. 7 an unheated preparation (lot No. unknown). No. 8 Koate-HT (Cutter); 26 IU factor VIII/ml; heat-treated in dry state at 68°C for 72 h; lot No. 50A 005. No. 9 Haemate HS (Behringwerke, Marburg, FRG); 25 IU factor VIII/ml; heat-treated in solution at 60°C for 10 h; lot No. Ch-B. 654010. No. 10 in Fig. 1 was a semipurified laboratory scale preparation (provided by B. Blombäck, Stockholm and described by Thorell and Blombäck [35]).

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The preparations were reconstituted according to the producer's instructions, divided into aliquots of one ml volume and stored at -70°C. For testing, the aliquots were rapidly thawed in a 30°C waterbath and kept at room temperature. Usually tests were performed within two days, but some repeat assays were done up to a week after thawing. Preliminary analyses showed that freezing and thawing did not alter the proportion or molecular size of the aggregates in the gel filtration spectra, nor were radial immunodiffusion (RID) measurements affected (<10%) by two-months' storage at room temperature. No sedimentation was noted in the samples.

Seven commercial Ig products for intravenous use (IV Ig) were included in the assays for IgG-protein complexes and IgG subclass content. They were Nos. I-IV and VI-VIII in an earlier study [6] produced by KabiVitrum, Stockholm, Sweden; Immuno, Wien, Austria; Travenol, Glendale, CA, USA; Sandox, Basel, Switzerland; Biotest, Frankfurt, FRG; Cutter, Berkeley, CA, USA and Behringwerke, Marburg, FRG respectively.

Cohn fraction II from Sigma, St Louis Mo USA, was also tested as a reference for IgG subclases.

The following sera were used as human serum standards: HSI from Behringwerke, Marburg, FRG, the World Health Organization reference serum 67/97 (HS2), a pool of >100 blood donor sera stored at -20°C (HS3) and a similar pool (HS4) stored for 5 years at -70°C or at +4°C.

For quantification of IgG and characterization of IgG-protein complexes in the factor VIII concentrates the following antisera were used: immunoglobulin fractions of rabbit anti-IgG (gamma-chain specific), anti-fibrinectin, anti-fibrinogen, anti-factor VIII-related antigen, i.e. anti-von Willebrand-factor-antigen (anti-vWF:Ag), anti-C1q, anti-albumin and anti-IgA (alpha-chain specific) obtained from Dakopatts, Copenhagen, Denmark. Monoclonal anti-IgG subclass 1, 2, 3 and 4 sera from Unipath, Birmingham, UK, (clones JLS12, GOM1, 2G4 and RJ4) were employed for the IgG subclass determinations.

Thin-layer Sephadex G-200 superfine gel filtration (GF) [36] was performed to characterize the protein content of the factor VIII concentrates according to molecular size. They were run immediately after thawing the samples.

References used were: the HS4 -70°C pool, an IgG aggregate reference consisting of an outdated IgG preparation for intramuscular use heated at 63°C for 10 min (ref IgG 63°C) which contained components from fragments to polymers in size. A preparation with albumin and monomeric IgG (ref alb IgG) was also included, see Fig. 1.

For characterization of protein with GF, samples were filtered through the Sephadex thin layer, the gel dried and overlayed with 0.8% agarose. After fixation with picric acid and drying, the stabilized proteins were stained with Coomassie Blue (Fig. 1). Peak positions in the filtered spectra were measured in mm from the start on spectrophotometric tracings and molecular weight (MW) was calculated from least squares regression curves for the simultaneously filtered HS4 -70°C standard, with the albumin peak as 68 kiloDaltons (kD), the IgG peak as 155 kD and the alpha2-macroglobulin as 820 kD.

Immune gel filtration (IGF) [15] was used for demonstrating the presence of IgG in the separated components; an agarose slab containing anti-IgG was overlaid after the gel-filtration step. At completion of the antigen-antibody binding, unprefixed proteins were eluted from the agarose and the gel dried and stained as before. The IgG in 5 representative factor VIII concentrates is illustrated in Fig. 2.

Quantification of albumin was done with the spot immunoprecipitate assay (SIA) [37] which utilized applications of 3 ul volumes of samples and an albumin (KabiVitrum) standard on a thin agarose layer followed by equal-sized antisera aliquots. After the antigen-antibody binding unprefixed components were eluted, the gel dried and stained. Thereafter the spot stain intensity was read spectrophotometrically as absorbance (O.D.) on a Titertek Multiskan (Flow Laboratories, Ingwood CA, USA) at 579 nm and albumin content calculated from standard curves with least squares regression analysis.

Mancini's radial immunodiffusion (RID) method was used for IgG quantification with HS4 -70°C as standard (14.6 g IgG/L) and for the IgG subclass determinations with HS2 as the standard and polyethylene glycol as precipitate enhancer.

Total protein was assessed with a protein spot-in-gel method, stained protein assay (SPA) [37] with the IgG standard (69 g protein/L according to Lowry assay).

A double antibody version of SIA [37] was employed to estimate the amounts of proteins other than IgG found in the immunoprecipitated IgG of the factor VIII samples. Series of 2 to 4 two-fold dilutions of the test samples in the agarose were first reacted with anti-IgG; after elution of the unprefixed protein from the gel, the second-step antisera were applied on the IgG-anti-IgG precipitates, e.g. as in Fig. 3, where the precip-