

TECHNOLOGICAL ASPECTS OF ENSURING THE SPECIFIC SAFETY OF HUMAN IMMUNOGLOBULIN AND ALBUMIN PREPARATIONS

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Modern technological approaches to ensuring the specific safety of human immunoglobulin and albumin preparations are analyzed. Introduction into human immunoglobulin preparation technology of donor screening for group- and rhesus-affiliations and chromatographic purification steps to reduce the contents of anti-A and anti-B hemagglutinins and anti-D antibodies are shown to be promising. Technological capabilities for reducing the anticomplementary activity and thrombogenic potential of immunoglobulin preparations and decreasing the contents of prekallikrein activator in human i.v. immunoglobulin and albumin preparations are discussed.

Keywords: human immunoglobulin preparations, human albumin preparations, specific safety, anticomplementary activity, anti-A and anti-B hemagglutinins, anti-D antibodies, prekallikrein activator, manufacturing technology, plasma fractionation.

Effects of immunoglobulin therapy on complement systems, kallikrein-kinin, and the hemostasis system are manifested more strongly with more massive infusions to treat hematological and neurological diseases in transplantology and immune-deficit conditions [1 – 6]. Use of albumin can be associated with disturbance of kallikrein-kinin functioning. Use of human immunoglobulin and albumin preparations can be made safer by eliminating the corresponding risk factors. An aggregate content of $\leq 3\%$ and anticomplementary activity (ACA) of $\leq 1 \text{ CH}_{50}$ unit per mg of immunoglobulin in preparations for infusion can eliminate adverse reactions such as hot flashes, headache, fever, chills, nausea, vomiting, myalgia, dyspnea, and tachycardia [7 – 9]. An anti-D antibody content of 0.05 IU/mL in these preparations can be associated with intravascular hemolysis with a sharp drop of hemoglobin and hematocrit, reticulocytosis, hyperbilirubinemia, hemoglobinuria, and other symptoms up to kidney and multiple organ failure in Rh+ patients with disturbances of the immune system [6, 10 – 12]. Hemagglutinins (anti-A and

anti-B antibodies or class G immunoglobulins) are responsible for extravascular adverse effects associated with macrophage activation in the first 10 d after i.v. injection of human immunoglobulin preparation to patients not of blood type O [1, 5, 10, 13 – 17]. Hypotensive effects that affect the kallikrein-kinin system are minimal during infusion of human immunoglobulin and albumin preparations with $<35 \text{ IU/mL}$ of prekallikrein activator (PKA) [18, 19]. PKA activates prekallikrein by converting it to kallikrein, which in turn activates the kinin pathway via positive feedback and leads to vasodilation and hypotension. Thromboembolic complications including stroke, myocardial infarct, pleural artery thromboembolism, and deep vein/artery thrombosis can arise after i.v. injection of immunoglobulins and are related to not only increased blood viscosity after injection of the preparation but also residual blood clotting factors in the preparations and their influence on thrombin formation kinetics [17, 20 – 24]. The goals of the work were to analyze modern technological approaches to ensuring the specific safety of human immunoglobulin and albumin preparations and to assess the specific safety assurance profile during manufacturing of human immunoglobulin and albumin preparations registered in the Russian Federation.

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Technological features of blood preparation manufacturing are related to the special properties of the starting material (substance), i.e., plasma for fractionation. Plasma with the longest qualitative and quantitative shelf-life can produce highly efficacious non-allergenic preparations for replacement therapy. However, the risk is high for contaminating the isolated target proteins (immunoglobulins of various specificity, albumin, blood clotting factors, etc.) with those having adverse effects. Furthermore, various conformational alterations that also cause adverse effects during use can occur during manufacturing and/or storage of human immunoglobulin and albumin preparations. Human immunoglobulin and albumin preparations are traditionally manufactured via Cohn cold alcohol fractionation and its Cohn–Oncley, Hink, and Kistler–Nitschmann modifications [25–28]. However, quality requirements applicable to the preparations have necessitated a search for new technologies that can ensure their safety.

Reduction of the anticomplementary activity (ACA) of human i.v. immunoglobulin preparations is facilitated using several methods aimed at preventing immunoglobulins (IgGs) from aggregating. IgGs partially lose their native structure, aggregate, and become capable of activating spontaneously the complement system during fractionation of blood plasma. Furthermore, the ACA results from intramolecular restructuring and intermolecular interactions of IgGs that are induced by heating, treatment with organic solvents and chemical reagents, and spontaneous dimerization (up to 40% of IgG molecules). The following critical steps in the manufacturing process for human immunoglobulin preparations for infusion were presumed to affect the IgG characteristics [29]:

pH regulation can alter the properties of natural immunoglobulin (up to denaturation) if the NaOH or HCl concentration is too high or the stirring is too slow;

ultra-/diafiltration addresses the membrane area, protein concentration, liquid flow rate, transmembrane pressure, and membrane regeneration procedure;

chromatography includes the flow rate, pressure, regeneration procedure, etc.;

manufacturing of finished dosage form relates to pH regulation, osmolality, and protein concentration.

Several currently known methods for reducing the ACA of human immunoglobulins are based on altering, removing, and destroying components with ACA [30–36]. The anticomplementary properties of immunoglobulins are eliminated using methods of practical value for preparing drugs for i.v. injection:

enzymatic cleavage (pepsin, plasmin);
treatment with acid or acid with pepsin;
treatment with β -propiolactone or dithiothreitol;
precipitation by Rivanol;
treatment with sorbents (charcoal, calcium phosphate);
purification using poly(ethylene glycol) and chromatography.

ACA is reduced during manufacturing of various generations of immunoglobulin preparations using appropriate methods aimed at preventing IgG aggregation:

first-generation preparations are prepared using enzymatic cleavage (pepsin, plasmin) and acid hydrolysis at 37°C for 1 d in weakly acidic solution (pH 4.0);

second-generation preparations are prepared using chemical modification by β -propiolactone, dithiothreitol, and sorbents (charcoal, calcium phosphate) [36–38];

third- and fourth-generation preparations are practically free of aggregates with ACA because of treatment with a small amount of pepsin at low pH values (4.0) and use of poly(ethylene glycol) and chromatographic purification [35, 38–40].

However, immunoglobulin preparations with increased tolerance for i.v. injection are also significantly less efficacious therapeutically. Thus, the ACA of IgGs is restored during storage if small amounts of enzyme are used whereas IgG is significantly cleaved and eliminated rapidly from the body if the enzyme hydrolysis is increased. Therefore, the specific safety due to reduction of ACA by removing or preventing IgG aggregates and the therapeutic efficacy of im-

TABLE 1. Stabilizers Used for Manufacturing Foreign Human Albumin Preparations Registered in the Russian Federation

Drug trade name	Stabilizer	Shelf life, storage conditions
Alburex®	Sodium caprylate. Sodium acetyltryptophan	3 yr, $\leq 30^{\circ}\text{C}$
Human albumin	Sodium caprylate. Sodium acetyltryptophan	3 yr, 2 – 25°C
Zenalb®-4.5	Caprylic acid	3 yr, 2 – 25°C
Uman albumin	Sodium caprylate. <i>N</i> -Acetyltryptophan	3 yr, 15 – 25°C
Human albumin	<i>N</i> -Acetyltryptophan. Caprylic acid	3 yr, 2 – 25°C
Biotest human albumin	Sodium caprylate. Sodium acetyltryptophan	3 yr, $\leq 25^{\circ}\text{C}$
Zenalb®-20	Caprylic acid	3 yr, 2 – 25°C
Human serum albumin	Sodium caprylate. Sodium acetyltryptophan	3 yr, $\leq 25^{\circ}\text{C}$
Plasbumin 20	Sodium caprylate. <i>N</i> -Acetyltryptophan	3 yr, $\leq 30^{\circ}\text{C}$

munoglobulin infusions must be balanced. Introduction into the manufacturing process of additional virus inactivation/removal steps should be accompanied by a study of their influence on the ACA level. For example, treatment with solvent and detergent could increase the anticomplementary properties of the IgG [36].

Reduction of anti-A and anti-B hemagglutinins and anti-D antibodies in human immunoglobulin preparations during manufacturing is accomplished by screening donors for blood type and Rh-factor and forming donor plasma pools of blood type O and/or Rh-negative donors [41]. Incorporation of immunoaffinity chromatography purification steps can also reduce the hemagglutinin and anti-D antibody contents in the finished preparations [10, 16, 42, 43]. Glycine should be excluded as an IgG stabilizer because its use in the preparation was associated with an increased risk of hemolysis [2].

Prekallikrein activator (PKA) is a biologically active fragment of blood clotting factor XII (Hageman factor) and is present in human immunoglobulin and albumin preparations because of incomplete removal during processing [44]. Cases are known where filters with certain properties that were used in manufacturing steps of human i.v. immunoglobulins activated enzymes and increased PKA activity in the finished preparation [45]. The PKA content could also change during storage of the preparations, e.g., human albumin, for a shelf life. This was related to the albumin properties (stability) and effects of the packaging material, i.e., glass. Technological aspects of ensuring the specific safety of human albumin preparations should consider the manufacturing technology and the stabilizers of monomeric albumin. Most global manufacturers of human albumin preparations use several chemical compounds to stabilize albumin (Table 1) [46].

Domestic manufacturers of albumin preparations use sodium caprylate as a stabilizer. For this, the shelf life is 5 yr if stored at 2–10°C [46]. However, it is noteworthy that a study of the PKA content in domestically manufactured human albumin preparations upon release and during storage was introduced as an obligatory quality assessment parameter in the pharmacopoeial monograph Human Albumin only in 2014 [47]. Adsorption on silica-containing substances is used to reduce the PKA content in human albumin preparations. However, this technological approach is not widely used because of potential contamination of the finished preparation by traces of heavy metals [48]. Addition of C_1 -esterase inhibitor and use of pasteurization for 10 h at 60°C also reduced effectively the probability of PKA enzymatic activity in the preparations [49]. A high safety level with respect to PKA content can be achieved by adding antithrombin III at >0.03 mg per gram of protein in the albumin preparation [50]. Chymotrypsin was also used to inactivate PKA. Chymotrypsin in solution or immobilized chymotrypsin could be added at any step of plasma fractionation. Chymotrypsin was removed from the intermediates in later process steps [51]. Pasteurization, i.e., heating at

50–70°C for at least 5 h of precipitate paste (fraction V), could reduce the PKA content if the traditional Cohn fractionation technology was used [52]. PKA was reported to be inactivated more at the maximum of the pasteurization temperature range (60.5°C) than at a lower value (59.5°C) [53]. This pasteurization temperature difference could produce a 2- or 3-fold difference in the PKA content of the finished product. A heat-treatment step not only inactivates viruses but also has a denaturing action that can reduce selectively the amount of individual undesired proteins that are usually associated with Cohn fractions II+III, i.e., prekallikrein, plasmin, plasminogen, and prothrombin complex proteins. Then, fractionation by poly(ethylene glycol) can remove denatured proteinaceous products formed during heat treatment [54]. A procedure combining anion-exchange treatment before inactivation of viruses by solvent and detergent followed by cation-exchange treatment had special advantages.

Treatment with octanoic acid during fractionation steps can not only reduce the virus load but also remove blood clotting factors (II, V, VII, XI) and their activated forms [55, 56]. Pasteurization can also decrease considerably the thrombogenic potential of human immunoglobulin preparations [27]. Multi-stage chromatographic purification (combination of cation- and anion-exchange and immunoselective chromatography) can be used after the fractionation steps to produce human immunoglobulin preparations with high specific safety profiles [57–61]. For example, contamination of immunoglobulin solutions by PKA and procoagulant blood clotting factors can be avoided by using cation-exchange chromatography at pH 3.8–5.3 followed by incubation at low pH values of the finished preparation. Kallikrein and blood clotting factor XI contamination can be avoided by using anion-exchange chromatography at pH 7.0–8.2 [62].

Chromatography has recently been applied to the manufacture of human i.v. immunoglobulin preparations from plasma for fractionation [63]. Most therapeutically significant proteins can be removed from the plasma if the chromatography steps are carefully chosen. The intermediates containing the target proteins are not contaminated by undesired blood plasma components. The thawed pooled plasma fraction obtained after filtration through a 45- μ m filter is placed onto a size-exclusion chromatography column packed with a resin such as Sephacryl, Cellufine, or other similar resins. The column is eluted by a buffer containing phosphate, citrate, or analogous buffer salts at pH 6.0–7.5. The buffer molarity ranges from 20 to 200 mM, preferably <150 mM. Also, the buffer contains an additive such as NaCl in the range 0.1–0.2 M.

The fraction containing IgG is fractionated in a second anion-exchange chromatography column to separate other therapeutic proteins that may be present together with the immunoglobulins in this plasma fraction. Finally, immuno-adsorption can remove undesired hemagglutinins and anti-D antibodies from the immunoglobulin fraction [64]. Currently, human i.v. immunoglobulin and albumin preparations on the

Russian market have safety profiles meeting both global and Russian requirements. Thus, the technology for Privigen® preparation (CLS Behring) included alcohol fractionation, treatment with octanoic acid, incubation at pH 4.0, fine filtration, anion-exchange chromatography, immunoaffinity chromatography (to remove hemagglutinins), and nanofiltration (pore size 20 nm). Manufacturing of Flebogamma preparation (Grifols) is based on alcohol fractionation, precipitation by poly(ethylene glycol) followed by ion-exchange chromatography, solvent and detergent treatment, incubation at pH 4.0, pasteurization, and double sequential nanofiltration (filters with pore sizes 53 and 20 nm). The technology for Octagam (Octapharma) and Gamunex® C preparations (Grifols) is based on Cohn–Oncley alcohol fractionation followed by solvent and detergent treatment, chromatographic purification, and ultrafiltration. Incubation at low pH values (4.0) is also used to manufacture Gamunex® C preparation [65, 66].

Existing technologies are constantly being improved to maintain a balance between efficacy and safety of human blood preparations for infusion. Global trends in pharmaceutical development show a gradual but definitely justified transition to continuous separation of all therapeutically significant human blood plasma proteins by chromatographic methods. This could produce highly purified safe and efficacious preparations. Drugs currently registered in the Russian Federation include human i.v. immunoglobulin and albumin preparations with controlled specific safety profiles. This provides a reasonable selection for patient prescriptions.

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