

Theme Issue Article

Plasma-derived biological medicines used to promote haemostasis

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Summary

Several biological medicines derived from human and animal plasmas can effectively improve haemostasis in individuals with inherited or acquired defects in haemostasis. Factor VIII and factor VIII/vWF and factor IX concentrates are used to treat haemophilia A, von Willebrand disease and hemophilia B respectively. Cryoprecipitates are used to treat hypofibrinogenemia and von Willebrand disease where desmopressin (DDAVP) is ineffective or when plasma-derived factor VIII/vWF concentrates are unavailable. Thrombin-containing topical haemostatic agents and fibrin sealants are used to control perioperative bleeding. Intravenous immunoglobulin has several uses, including management of patients with autoimmune thrombocytopenias and patients with acquired factor VIII deficiency. Similar to most protein-based biological medicines, all the above products can elicit some level of antibody response, with clinical consequences that vary from mild anaphylaxis to loss of product efficacy. An on-

going potential safety concern with any biological medicine derived from blood/plasma is transmission of blood-borne pathogens. This safety concern has lessened significantly in the past decade as a result of the institution of more effective pre- and post-donation screening that tests for potential pathogens, and institution of pathogen reduction strategies to which many plasma-derived biological medicines are now routinely subjected. This article considers the manufacture, standardization, clinical efficacy and adverse event profiles of the plasma-derived biological medicines currently used to promote haemostasis in patients with inherited or acquired functional defects in haemostasis. It also considers approaches employed to minimize infectivity of biological medicines derived from human and animal plasmas and to manage patients who develop antibodies (inhibitors) to clotting factor concentrate infusions.

Keywords

Adverse events, biological medicines, cryoprecipitate, factor VIII, factor IX, haemostasis, IVIG, thrombin

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General considerations

A number of biologicals (protein-based medicines) important for haemostasis and manufactured from both human and non-human plasmas have been approved for clinical use (Table 1), although plasma-derived biologicals important for haemostasis only represent a small fraction of the more than 250 approved biological products (1). Many therapeutic proteins, including coagulation factors, topical haemostatic agents, hormones, enzymes, cytokines and fully humanized monoclonal antibodies, have elicited antibody formation in some treated patients (2–4). While the clinical consequences of immune responses to biopharmaceutical proteins are generally benign, immune responses to some biological proteins can have serious adverse clinical consequences (2–5).

Immune responses arising after the parenteral administration of a biological medicine normally results from the body recog-

nizing non-self proteins (i.e. therapeutic proteins not normally synthesized by the recipients) in the product. For example, up to 30% of severely factor VIII-deficient patients develop factor VIII-neutralizing antibodies after repeated prophylactic or therapeutic treatment with factor VIII concentrates (3). Another mechanism proposed for the immunogenicity of biopharmaceuticals is the breaking of self-tolerance (6). In these instances, medically warranted administration of therapeutic proteins that the patients normally synthesize may elicit the generation of antibodies that neutralize both the therapeutic proteins and autologous proteins (6). Several recipient-related and product-related factors contribute to immunogenicity of biological medicines. Recipient-related factors include genetic predisposition, immune status and susceptibility of the recipient. Product-related factors include differences in the amino acid sequences between biological medicines and the patients' homologous proteins, differences in glycosylation patterns between the thera-

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Table 1: Biological medicines important for haemostasis that are derived from human or animal plasmas.**Clotting factors (human and porcine in origin)**

- Human factor VIII concentrates
- Human factor VIII/von Willebrand factor concentrates
- Human cryoprecipitate
- Human plasma fraction with factor VIII inhibitor bypassing activity (FEIBA)
- Activated and non-activated human prothrombin complex concentrates
- Porcine factor VIII (no longer used clinically)
- Human factor IX concentrates
- Human fibrinogen
- Human factor XI concentrates
- Human factor XIII concentrates

Topical haemostatic agents derived from animal plasmas and/or tissues or from human plasmas**Intravenous immunoglobulins (IVIg)**

peutic and patients' proteins, drug formulation, doses, frequency and duration of treatment, routes of administration, presence of impurities and/or aggregates or degraded forms of the therapeutic proteins (6–11).

Source materials for biological medicines

While human blood is a valuable source of many therapeutic proteins, inadequately screened human blood or blood components can transmit a variety of pathogens, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and West Nile virus (12). Additional blood-borne pathogens include *Treponema pallidum*; human parvovirus B19 and, more rarely, hepatitis A; malaria, the tick-borne disease babesiosis, human granulocytic ehrlichiosis and Lyme disease (12–14). The transmissibility of prion diseases, such as scrapies in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease (CJD) in humans, became a concern in the 1990s after a BSE epidemic and a new form of CJD (variant CJD) were reported in the United Kingdom (13). Even adequately screened blood is now deemed to always carry a very low risk for the transmission of pathogens, since screening tests currently available cannot exclude all of the potential human pathogens nor completely anticipate future blood transfusion-transmitted agents (12).

As is well known, most haemophilia A patients who received regular infusions of plasma-derived factor VIII concentrates in the 1980s in North America had already been infected with HIV before the first clinical case of acquired immune deficiency syndrome (AIDS) was described (12). Because plasma-derived proteins are manufactured from large pools of human plasmas, special precautions are now mandated during therapeutic protein production to minimize the possibility of the final products transmitting infectious diseases to recipients (14). Prior to the introduction of these pathogen-reduction strategies, 75% of North American patients with haemophilia A had serological evidence of exposure to hepatitis B (12). Exposure to HCV was almost universal (15, 16), and about 80% of severe haemophilia A North American patients developed chronic hepatitis (17). The pathogen reduction procedures to which plasma fractionation products are now subjected have significantly reduced the risk of

pathogen transmission. No transmission of HBV, HCV or HIV attributable to manufactured plasma derivatives licensed for use in the US has been reported since the introduction of effective virus inactivation procedures in 1985 (18). The strategies adopted to reliably minimize infectivity of human and animal plasmas and products derived from them include improved donor screening, which, in North America for example, includes obtaining a detailed medical history of blood donors to exclude individuals whose blood has a high risk for transmitting blood-borne pathogens. The institution of a variety of sensitive serologic and nucleic acid-based tests has restricted most new cases of transmission to the window between the time of donor infection and the point at which current tests can detect infection (12). Once blood or plasma donations have been collected and screened for major clinically relevant viruses, most potentially infectious units are eliminated (13). The pooled plasmas made from the acceptable units of plasma are processed to isolate the biological medicines of interest. Because plasma pools may contain window period donations, viral inactivation or reduction strategies are necessarily employed during the manufacture of plasma-derived therapeutic products (12).

The Cohn-Oncley fractionation process is an efficient method developed in the mid-1940s and modified subsequently to isolate many therapeutic proteins from human plasma pools. This process allows multiple stepwise extractions of therapeutic proteins using cold ethanol precipitation (Fig. 1) (13). The process involves slowly thawing frozen plasma at 2–4°C and centrifuging it in the cold to remove cryoprecipitate (the cold insoluble plasma fraction that contains most of the factor VIII and von Willebrand factor and some fibrinogen and the other cold insoluble globulins) (13). The solubilities of different protein fractions in the remaining supernatant are then manipulated by increasing the concentration of ethanol and altering temperature, pH and/or ionic strength of the remaining plasma pool (13). Centrifugation or filtration is then used to partition the fractions containing different plasma proteins. Nanofiltration has been implemented at the production scale since the early 1990s to complement viral reduction treatments such as solvent/detergent and heat treatments to enhance product safety against non-enveloped viruses and provide a possible safeguard against new infectious agents potentially entering the human plasma pool (19–21).

Since some manufacturing steps, such as sterile filtration and freeze-thaw cycling, remove large pathogens such as bacteria and parasites, only viruses continue to pose serious threats to the safety of plasma-derived medicinal proteins (13). Although some viruses may be removed coincidentally during Cohn-Oncley fractionation (13), an additional layer of safety is now achieved by including effective and specific viral clearance steps during the manufacturing process (14). Recent improvements in protein processing have increased the purity and yields of human plasma-derived proteins (13). Since 1991, the Committee for Human Medicinal Products (CHMP) (20) also requires all manufacturers of blood and plasma products used in the EU to implement viral inactivation/reduction steps in their processes to target pathogens and prevent transmission of potential emergent viruses (13). The most promising approaches used currently involve pathogen-reduction strategies that reliably and effectively reduce the infective doses of model viruses in laboratory spiking

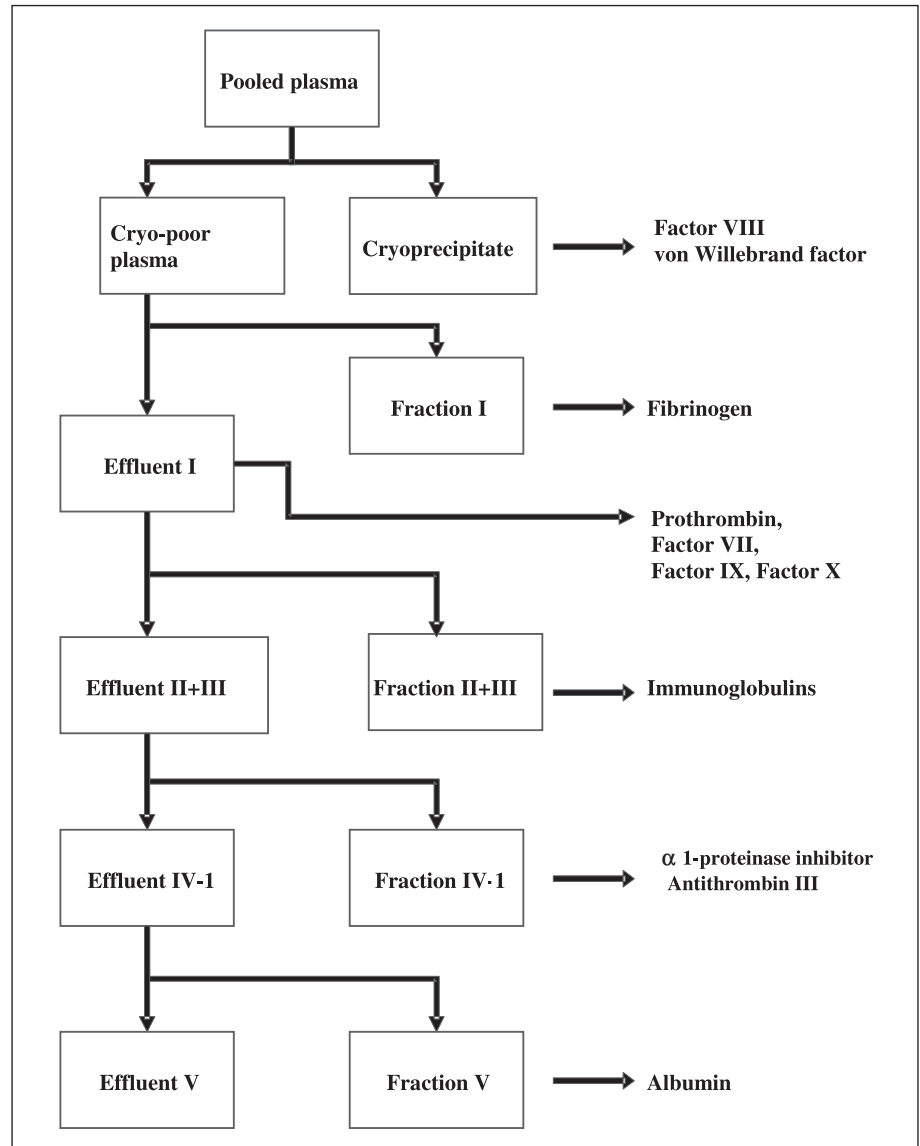


Figure 1: Cohn-Oncley plasma fractionation scheme. Adapted from Cai 2005 (13).

studies (12). Pathogen reduction processes include filtration, affinity chromatography, ion-exchange chromatography, polyethylene glycol fractionation, heat and solvent/detergent treatments that inactivate viruses and processes such as ethanol fractionation that both remove and inactivate viruses that might be present in the fractionated products (14). Other pathogen-reduction techniques include irradiation and chemical and photochemical techniques (12, 14). Nanofiltration has been implemented recently in addition to solvent/detergent treatment during the manufacture of factor VIII/vWF concentrates (21). However, some loss of von Willebrand factor was noted when factor VIII/concentrates were subjected to nanofiltration (21). The benefits of each pathogen-reduction strategy must be weighed against a variety of risks: loss of or damage to specific plasma proteins, the toxicity of residual antiviral agent to product recipients, risk of infection of the personnel processing the plasmas and potential environmental contamination (12).

In general, products such as albumin, intravenous immunoglobulin and α 1-proteinase inhibitor, which are obtained after several fractionation steps, present a lower risk for pathogen transmission than products such as coagulation factors which are obtained at the earlier steps (19, 20). Prions may be removed to varying degrees during precipitation, filtration and chromatography steps used to fractionate plasma, depending on the purification conditions (22). As with any biological product, the manufacturing and purification of human- and animal-derived biological medicines involve a degree of risk, although measures taken by the manufacturers under careful supervision of the appropriate regulatory agencies ensure the relative safety and efficacy of biological medicines used clinically today. The remainder of this review will consider the steps used to manufacture the specific biological medicines important for haemostasis from animal and human plasmas, their standardization, clinical effectiveness and safety considerations.

Plasma-derived human factor VIII concentrates

Factor VIII is an essential coagulation factor that provides the cofactor that enhances coagulation factor X activation by the enzyme coagulation factor IXa. This essential cofactor of coagulation is absent or dysfunctional in patients with haemophilia A. Haemophilia A affects one in 5,000 males, who generally receive prophylaxis and/or treatment with infusions of factor VIII concentrates two or three times a week (23–26). Together with von Willebrand factor, human factor VIII concentrates are manufactured from the cryoprecipitate fraction isolated during Cohn-Onley fractionation (13). Pathogen-reduction validation studies with early factor VIII concentrates derived from cryoprecipitate showed no viral clearance across the initial precipitation step, but many of the newer methods for manufacturing factor VIII concentrates incorporate additional chromatographic steps to remove contaminants, including viruses (13). During an affinity chromatography purification stage, immobilized monoclonal anti-factor VIII antibodies can be used to specifically capture factor VIII while allowing contaminant proteins and small non-enveloped viruses such as parvovirus and poliovirus to flow through the affinity column (13, 19, 21, 27). Solvent/detergent treatment can also inactivate enveloped viruses such as HIV, bovine viral diarrhoea virus (BVDV) and pseudorabies virus (PRV) to below the levels of detection (13). The final containers for some freeze-dried factor VIII products undergo terminal dry heat treatment at temperatures of up to 80°C for up to 72 hours to inactivate HIV and other enveloped viruses such as HBC and HCV (13). Plasma-derived factor VIII products used most widely in the US are prepared from pooled human plasmas by immunoaffinity chromatography with a murine monoclonal antibody followed by ion exchange chromatography for further purification, plus a solvent/detergent virus inactivation step or by pasteurization of the concentrate at 60°C for 10 hours (28, 29). The resulting concentrates contain factor VIII formulated with either albumin, polyethylene glycol, histidine and/or glycine as stabilizing agents, small amounts of mouse protein, organic solvent and detergent or calcium chloride, albumin, mannitol and histidine and trace amounts of murine protein. The number of units of factor VIII administered is expressed in International Units (IU) that are assigned by reference to the current World Health Organization (WHO) International Standard for factor human VIII concentrates (20).

Several highly purified plasma-derived factor VIII concentrates also used widely are formulated to contain von Willebrand factor, and factor VIII/von Willebrand factor concentrates are approved for treating inherited von Willebrand disease or factor VIII deficiency. Ion exchange chromatography procedures, rather than monoclonal anti-factor VIII affinity columns, are used to produce plasma-derived factor VIII/vWF concentrates. The marketed products include Alphanate, Biostate, Factane, Fanhdi, Humate-P/Haemate-P, Immunate, Koate-DVI and Wilate. While some of these products are registered for use in several countries others are only registered for use in three or fewer countries. Use of some plasma-derived FVIII/vWF concentrates to induce immune tolerance to factor VIII is discussed below.

With viral transmission now less of an issue, and the risk of viral transmission more theoretical, the most serious clinical

complication of factor VIII replacement therapy is the risk of developing inhibitory antibodies (12, 23, 24, 26, 30). This risk may be higher in patients with severe than moderate or mild factor VIII deficiency (30). Anti-factor VIII antibodies that inactivate this protein (inhibitors) can develop after treatment with cryoprecipitate, plasma-derived or recombinant factor VIII concentrates (30). Up to 30% of severe haemophilia A patients treated with factor VIII concentrates develop anti-factor VIII antibodies (24–26, 30). These inhibitory antibodies tend to appear after the first 10 to 20 treatments for factor VIII replacement (26). The development of inhibitors in stable, previously treated patients (especially in moderate and mild haemophilia A patients) may reflect the presence of neo-antigens in the factor VIII concentrates that are lacking in these patients who normally synthesize low levels of factor VIII (30). Although factor VIII inhibitors may be transient (26), some persist and can inhibit factor VIII activity by interfering with its binding to components of the factor Xase complex, and thus reduce the efficacy of treatment with factor VIII concentrates (24). This major complication in the management of haemophilia A can significantly impact both the effectiveness and cost of treatment (31–36).

Major improvements have been made in treating haemophilia A patients who develop factor VIII inhibitors as most of these patients can now be managed successfully (26). Patients with low titre inhibitors (<5 Bethesda units/ml) can generally be managed by infusions with more factor VIII than usual to overpower the inhibitors. Patients with inhibitors of high Bethesda titer (>5 Bethesda units/ml) require different approaches. Human prothrombin complex concentrates, activated prothrombin complex concentrate [aPCC], a plasma product known as FEIBA (factor VIII inhibitor bypassing activity), porcine factor VIII and recombinant human factor VIIa have been used to successfully manage bleeding in patients with high titer factor VIII inhibitors (26, 37–44). Plasma-derived porcine factor VIII was used in patients whose anti-human factor VIII antibodies did not cross-react with porcine factor VIII (36). While porcine factor VIII supplied the missing factor to deficient patients, it was proposed to also enhance coagulation and haemostasis by activating human platelets (45). Concerns about potential porcine parvovirus infectivity have led to the withdrawal of porcine factor VIII from the market. While therapeutic plasma exchange generally has not been successful in the management of patients with high levels of inhibitor to factor VIII, success has been reported using extracorporeal immunoadsorption of the factor VIII-neutralizing antibodies (46, 47).

Activated prothrombin complex concentrates contain activated clotting factors other than factor VIII that can stimulate clotting and may thus bypass the need for factor VIII (37, 39, 40). In a study of 14 patients with acquired haemophilia, Holme et al. concluded that aPCC is an effective treatment for acute bleeds in patients with acquired haemophilia with high levels of inhibitors and that it also provided effective haemostasis during minor surgery. Furthermore, a combination of oral corticosteroids and cyclophosphamide seemed to be effective in eradicating factor VIII inhibitors (37). Tjønnfjord concluded from a series of 10 patients that minor and major surgeries are feasible, with a low risk of severe bleeding complications in patients with haemophilia and factor VIII inhibitors and patients with acquired haemophilia using aPCC (48). Unfortunately, use of aPCC has been associ-

ated with deep vein thrombosis, pulmonary embolism and disseminated intravascular coagulation (44). Clinical data show that one marketed plasma product (FEIBA[®]) is safe and well tolerated in a wide variety of clinical settings when used according to dosing guidelines (49). Recombinant factor VIIa is also an effective treatment for both minor and life-threatening bleeds and for preventing surgical bleeding in patients with factor VIII inhibitors (44). In the only randomized study to compare FEIBA, aPCC and recombinant factor VIIa, all three had similar effects on joint bleeds, although the efficacy of the two plasma-derived products was rated differently in a substantial proportion of patients, particularly in the first 12 hours after symptoms started (50). Because of the very short half-life of recombinant factor VIIa, patients with factor VIII inhibitors receive recombinant factor VIIa infusions every 2 to 4 hours (44). Use of recombinant factor VIIa is the preferred approach in patients with a known anamnestic response to aPCC and in patients waiting to begin immune tolerance induction (51).

Immune tolerance induction aims to modify the patient's immune system to tolerate treatment with factor VIII. This is accomplished by the administration of large doses of factor VIII concentrates twice daily, daily or every other day for weeks, months or even years and sometimes in combination with immunosuppressive therapy (34, 37, 44). Immune tolerance induction is time consuming, expensive and not always successful in patients with high titres (> 5 Bethesda units/ml) of factor VIII antibodies (37) but it is the recommended approach for children with inhibitors (44). A number of small studies have shown that immune tolerance induction is successful in 70% to 85% of cases, with dosage of factor VIII used apparently not relevant to success rate (55). Registries, with a total of 505 patients with haemophilia A undergoing immune tolerance induction, have identified factors predictive of successful treatment: including age, time between inhibitor diagnosis and initiation of immune tolerance induction and dose (52–55). Hodges et al. suggested that inhibition of T-cell CD132 by plasma-derived factor VIII may make it more appropriate for inducing tolerance to factor VIII than recombinant factor VIII (56), and this is supported by data from the North American Immune Tolerance Registry, which suggested better success rates with monoclonal antibody immunoaffinity chromatography purified factor VIII (55). Interestingly, data from Germany suggest a reduced success rate when patients were switched to higher purity factors and an improved success rate (80%) when patients were switched back to lower purity factors (57, 58). Tamura et al. found continuous infusion of recombinant factor VIII effective at inducing immune tolerance, with inhibitors becoming undetectable after three weeks of continuous infusion and remaining undetectable six months after initiation of prophylactic treatment with recombinant factor VIII three times weekly (34). Low-dose, ongoing prophylaxis thus might be protective against the formation of inhibitors in some patients, but an optimum regimen has not yet been established, and very early prophylaxis is likely to have its own set of problems (52). Whether the presence of high levels of vWF in plasma-derived factor VIII concentrates moderate the risk of inhibitor development in previously untreated patients or improves the chances for immune tolerance induction remains controversial (52, 57–63).

Plasma-derived human factor IX concentrates

Haemophilia B is caused by an inherited deficiency of factor IX and affects one in 30,000 males (26). For many years, prothrombin complex concentrates, which contain prothrombin and factors VII, IX and X, were the only treatment for patients with haemophilia B (64). Use of these combined clotting factor products was associated with thrombotic events, particularly in cases of repeated infusions for surgery (65). Patients are now treated prophylactically with purified human plasma-derived factor IX concentrates (26), isolated during Cohn-Onclay fractionation, in this case from Effluent 1 (see Fig. 1) (13) or with recombinant factor IX (66). Hampton et al. showed that high-purity preparations of plasma-derived factor IX concentrates produced significantly less coagulation activation than prothrombin complex concentrates (67). The global use of purified factor IX concentrates has reduced the incidence of thrombotic and bleeding events in patients with haemophilia B (64). The number of units of factor IX administered is expressed in IU, which is related to the current WHO standard for factor IX concentrate products (68). Calculation of the desired dosage is based on how much 1 IU/kg body weight of a specific factor IX product increases the plasma factor IX activity (68). Unlike plasma-derived factor VIII, the recovery of plasma-derived factor IX is around 50%, and this has to be taken into account when calculating the dose.

As was described for factor VIII concentrates, plasma-derived factor IX concentrates have also benefited from various viral inactivation methods, such as heating, thiocyanate treatment and solvent/detergent treatment, which offer patients protection from enveloped viruses such as HCV, HBV and HIV (64). In 1996, Lawlor showed that factor IX concentrates can transmit hepatitis A virus (HAV) and that appropriate steps to inactivate non-enveloped viruses and enveloped viruses were needed to ensure the safety of plasma-derived factor IX concentrates (69). In 1998, a double-blind, randomized study by Goudemand et al. showed that the addition of a 15-nm filtration step to the manufacture of a solvent/detergent-treated factor IX concentrate did not alter the biological properties of the molecule, yet was highly effective in removing the loads of non-enveloped viruses (64). Schulman et al. found that perioperative and postoperative replacement therapy by continuous infusion with a highly purified factor IX concentrate (treated with solvent/detergent and nanofiltration to improve viral safety) was safe and efficacious in haemophilia B patients (70). Evans et al. reported similar efficacy and safety profiles for a high-purity factor IX concentrate given by continuous infusion to treat bleeding and as a cover for surgical procedures (71). Zakarija reported a preference for plasma-derived products, claiming they provide more reliable control of factor levels in patients than recombinant factor IX and thus better control of bleeding (72). This study also confirmed the two-fold greater in-vivo recovery of plasma-derived than recombinant factor IX reported Ewenstein et al. (73).

The prevalence of inhibitors in patients with haemophilia B is much lower than in patients with haemophilia A, with factor IX inhibitors prevalence ranging from 0% to 33% in patients with severe haemophilia B (68). Parquet et al. found only one patient with inhibitors in a cohort of 15 previously untreated children with severe haemophilia B treated with a high purity, solvent/de-

tergent-treated plasma-derived factor IX concentrate developed inhibitors (74). Mutation analysis showed a total gene deletion in this patient, partial gene deletion in one patient and missense mutations in the other patients (74). The authors concluded that the risk of factor IX inhibitors is higher in patients with factor IX gene deletions and recommended that factor IX gene analysis be undertaken at diagnosis of haemophilia B to identify and provide safer management for patients at high risk (74), 50% of whom may be at risk for severe allergic reactions and anaphylaxis (36). In 2005, Ruiz-Sáez et al. showed that, in 12 previously treated patients with haemophilia B, a factor IX concentrate and a plasma-derived prothrombin concentrate (both plasma-derived and double inactivated: solvent/detergent treatment; heating at 100°C for 30 minutes) effectively controlled bleeding episodes and both well tolerated six months of follow-up, and none of the patients developed factor IX inhibitors (75). However, the authors highlighted the need for caution with respect to potential thrombogenicity with repeated doses or the presence of other thrombogenic risk factors (75). As with haemophilia A, haemophilia B patients who develop low Bethesda titre inhibitors may be treated with more frequent doses of factor IX concentrates. However, recombinant factor VIIa or aPCC may be more appropriate in patients with high levels of factor IX inhibitors (44).

Cryoprecipitates, fibrinogen, factor XI and factor XIII concentrates

Cryoprecipitate is a human plasma component that was the treatment of choice for factor VIII replacement in patients with haemophilia A or von Willebrand disease until factor VIII and factor VIII/vWF concentrates subjected to pathogen reduction became available (21, 76–78). The current primary indication for cryoprecipitates is the treatment of hypofibrinogenemia (fibrinogen levels <100 mg/dl) (79). Plasma-derived fibrinogen concentrates, factor XI concentrates and factor XIII concentrates also available for the treatment of congenital fibrinogen, factor XI and factor XIII deficiencies, respectively. Cryoprecipitates may also be used to treat uraemic bleeding diathesis that is unresponsive to 1-deamino-8-D-arginine vasopressin (DDAVP, or decompression) and von Willebrand disease when DDAVP is ineffective, and plasma-derived factor concentrates containing von Willebrand factor is unavailable (79).

Cryoprecipitate is obtained by cold-thawing (at 4°C) fresh frozen plasma prepared from a unit of whole blood to produce a white precipitate that is then separated from the supernatant plasma (79). The 10–20 ml of cryoprecipitate that results is rich in fibrinogen (150–250 mg), factor VIII von Willebrand factor (80–100 IU), factor XIII (50–100 units) and fibronectin (50–60 mg) (76, 79). It is stored frozen and must be transfused within six hours of thawing or four hours of pooling of 6–8 units of cryoprecipitates for each patient (76, 79). As noted elsewhere, manufactured clotting factor concentrates undergo pathogen-reduction measures and are thus safer with respect to the risk of transmitting blood-borne pathogens than cryoprecipitates (12–14, 76). Therefore, cryoprecipitates are usually reserved for use in emergency situations for the management of von Willebrand dis-

ease when plasma-derived factor VIII/vWF concentrates are unavailable in a timely manner (76, 79). As cryoprecipitates also contain haemagglutinins, intravenous haemolysis can occur if large volumes of incompatible cryoprecipitate are administered (76).

Plasma-derived factor XI concentrates have been available to treat the relatively rare congenital factor XI deficiency, especially in affected patients undergoing major surgery (80–83). Thrombotic complications as well as the development of factor XI-neutralizing antibodies have been reported in 10% of patients treated with factor XI concentrates (80–84). Recombinant factor VIIa was used to provide effective haemostasis in congenital factor XI-deficient patients who developed factor XI inhibitors (84). Plasma-derived factor XIII concentrates have also been available to treat the extremely rare but severe bleeding associated with homozygous factor XIII deficiency (85–87). No adverse events associated with the clinical use of congenital factor XIII deficiency have been reported.

Topical haemostatic agents

Excessive blood loss occurring during some major surgeries can be controlled by a variety of methods, including mechanical and thermal techniques as well as pharmacotherapies and topical agents (5, 88–92). For controlling brisker bleeding, topical haemostatic agents and fibrin sealants derived from plasma containing fibrinogen, thrombin and other clotting factors are useful adjuncts when mechanical and thermal haemostatic methods fail. In cases of oozing or minor bleeding in surgical procedures and trauma, use of haemostats comprising components of the normal clotting cascade has become a part of normal surgical routine (93). Numerous topical haemostatic agents are used in minor surgical bleeding, and their components and mechanisms of action differ greatly (94). Some topical haemostatic agents are considered passive, largely providing a barrier scaffold for the aggregation of platelets and subsequent soft clot formation. Other topical haemostatic agents, such as topical bovine thrombin are considered active as they directly participate in blood coagulation and platelet and cell activation. Topical bovine thrombin was first used as a topical haemostatic agent to treat surface bleeding in the 1930s, and its use became more widespread in the US after receiving Food and Drug Administration (FDA) approval in the late 1970s (95). In August 2007 and January 2008 the FDA approved human-plasma derived topical and recombinant human thrombin, respectively. Both were demonstrated in non-inferiority phase III trials to have equivalent safety and efficacy profiles as topical bovine thrombin (96, 97). Absorbable passive haemostatic agents have been available since the 1940s and are useful therapy during surgical procedures when conventional methods, such as ligation or the application of pressure, do not effectively control bleeding (98).

The first step in the manufacture of topical thrombin is the preparation of a crude concentrate containing factors VII, IX and X and prothrombin by adsorption of plasma or the plasma fractions from Cohn-Oncley fractionation onto insoluble barium salts or by ‘batch adsorption’ of plasma with an anion exchanger. Both approaches are followed by elution the four vitamin-K dependent proteins (76, 99–101). Prothrombin activation is then

initiated with tissue thromboplastin or the factor X activator from Russell's viper venom (102, 103). The thrombin generated is then purified using cation-exchange chromatography (104–106).

Patients exposed to topical bovine thrombin preparations or other haemostatic agents containing bovine thrombin may develop antibodies to bovine thrombin, factor V and other proteins in these topical preparations (107). Some human anti-bovine antibodies generated after patient perioperative exposures to topical bovine thrombin can cross-react with autologous human coagulation proteins to cause coagulopathies (108–110). Generation of other human anti-bovine antibodies has also been reported, including antibodies to galactose- α 1-3 galactose, a disaccharide foreign to humans (111). However, anti-galactose- α 1-3 galactose antibodies are also found in individuals who have never been exposed to topical bovine thrombin. In randomized control trials that compared the efficacy and safety of topical bovine and human thrombin, generation of anti-product antibodies were unrelated to postoperative development of adverse events in the patients (96, 97).

Intravenous immunoglobulin

Intravenous immunoglobulin (IVIG) is used widely including treatment of primary and secondary immunoglobulin deficiency diseases, infectious complications in various clinical states and

Table 2: Disorders of haemostasis in which intravenous immunoglobulin is considered first-line treatment (110).

Idiopathic thrombocytopenic purpura (ITP)
Fetal thrombocytopenia and neonatal alloimmune thrombocytopenia
Acquired haemophilias
Acquired von Willebrand disease
HIV-associated thrombocytopenia
Post-transfusion purpura
ITP in pregnancy

following its initial use by Imbach in 1981 to treat idiopathic thrombocytopenic purpura (ITP), it has been used in a number of autoimmune diseases (112, 113). Consensus groups have identified the haemostatic disorders for which IVIG can be considered a first-line treatment (Table 2) and as a consequence of the increasing use of IVIG for many indications, new evidence-based guidelines for the use of IVIG in haematologic and neurologic disorders have recently been published (114–117).

Immunoglobulin for clinical use was first isolated more than 50 years ago by Cohn as a plasma fractionation product. Prophylactic intramuscular administration of immunoglobulin became the first-line treatment for patients with an inherited deficiency of circulating immunoglobulin G (IgG) in the 1950s and 1960s (112, 114, 118, 119). These preparations could not, however, be

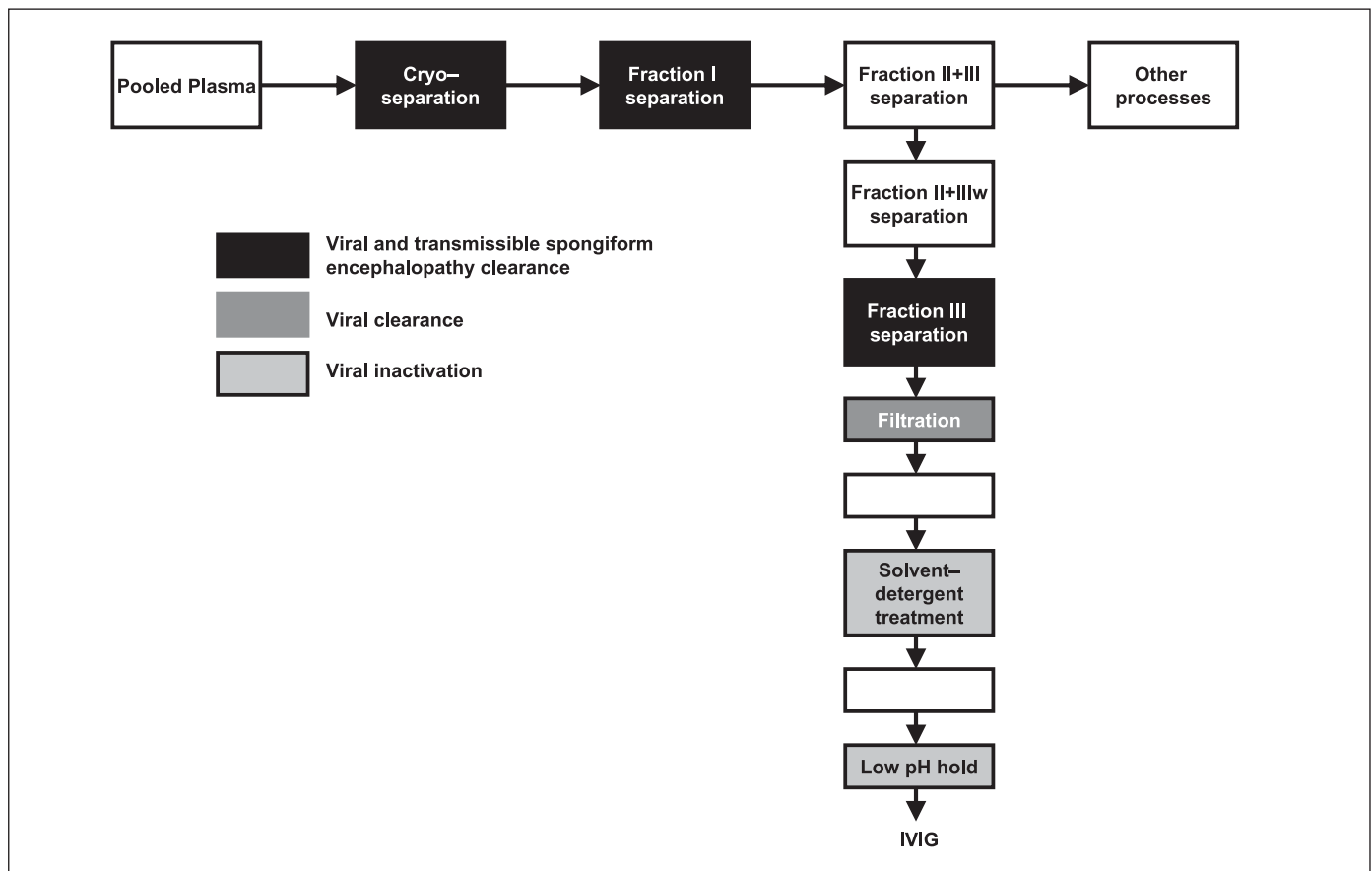


Figure 2: General processes for the isolation of IVIG from human plasma pools, indicating specific steps in which pathogens can be inactivated or removed. Adapted from Miller et al. 2001 (122).

Table 3: Guidelines of the WHO Expert Committee on Biologic Standardization for IVIG products (128).

Immunoglobulin must – Be as unmodified as possible – Maintain its biological function (opsonic activity, complement fixation, Fc receptor binding) – Contain certain levels of specific antibody – Meet accepted safety standards

given intravenously (IV) because they contained immunoglobulin aggregates and other impurities capable of activating complement, causing severe adverse reactions, including anaphylaxis (120). Modifications to the manufacturing process in 1979 led to the first safe IV form of intact immunoglobulin (IVIG) that was therapeutically effective with a low incidence of adverse reactions (104, 109). Currently, IVIG is produced from large pools of human plasma processed so that the final IVIG preparation contains primarily IgG with trace amounts of IgA and IgM (112, 116, 119–121). The IVIG is then concentrated (typically by ultrafiltration) and formulated for clinical use. Excipients are added to ensure product stability during storage (122).

In addition to the previously described donor screening processes to reduce transmission of pathogens, IVIG undergoes pathogen inactivation/reduction processes as summarized in Figure 2 (122–129). The effects of pathogen reduction measures on the stability of plasma-derived therapeutic producing proteins must always be evaluated on a product-by-product basis (pasteurization, for example, may render IVIG inactive), and consideration must also be given to reductions in yield that can result from such processes (122).

Although all IVIG preparations contain IgG (predominantly monomeric IgG and in subclass proportions reflecting normal human IgG [130]), differences in the methods of their production and their composition may affect their efficacy, tolerability and adverse events profiles (131). Differences in basic fractionation methods and the processes for product stabilization, virus inactivation and removal can yield in products with significant differences in antibody content, subclass distribution and electrophoretic profiles. It thus became necessary for the WHO Expert Committee on Biologic Standardization to provide guidelines for IVIG products (Table 3) (124, 132). IVIG preparations are not routinely tested for antibody titre, affinity or function (133, 134). The concentration of the product (usually available as a 5% or a 10% solution) determines the volume load and time required for administration, which can be a consideration for some patient populations, and the storage temperatures vary from product to product (134). Sugars are added to some preparations to prevent aggregate formation, but significant adverse events, including renal failure and renal insufficiency, can result, particularly in older patients with diabetes mellitus, hypertension, prior renal dysfunction, sepsis, volume depletion or paraproteinemia or in patients on low salt diets (124, 134). The presence of sodium, sugars, amino acids means that osmolarities of these preparations can be higher than physiologic osmolarity. Therefore, care must be taken to avoid rapid infusion of hyperosmolar solutions to minimize fluid shifts after IVIG administration. Other charac-

teristics that may influence properties of IVIG include pH, IgA content, isoagglutinin antibodies (124).

Indicated uses for IVIG in haemostatic disorders are shown in Table 2. The literature on which these recommendations are based has been recently outlined (115, 116). It should be noted that IVIG is not recommended for the treatment of heparin-induced thrombocytopenia (HIT) or for thrombotic thrombocytopenic purpura (TTP)/haemolytic uraemic syndrome (HUS). IVIG has also been used in the treatment of ITP and in the management of patients with severe acquired haemophilia A with inhibitors (136, 142, 143). The successful use of IVIG to treat patients with acquired haemophilia A has been attributed to the presence in IVIG of anti-idiotypic antibodies against idiotypes expressed by anti-factor VIII autoantibodies (135–143). Natural anti-factor VIII antibodies have been reported in the plasmas of up to 20% of normal blood donors and in plasma pools made from normal blood donors (136, 142, 143).

Plasma-derived anti-D is also used in the treatment of ITP. Whereas IVIG preparations are made from pools of human plasma collected from 3,000 to 10,000 donor units of plasma per lot, anti-D consists of IgG isolated from the plasma of donors immunized with the D-antigen intentionally or during prior pregnancy (130). Anti-D was first used in the early 1980s by Salama et al. to treat patients with ITP (144), and it can be used at lower doses than IVIG. While anti-D and IVIG appear to have similar efficacy in appropriate patients with ITP, anti-D is effective only in unsplenectomized Rh-positive patients. Anti-D has been suggested to be more effective than IVIG for HIV-associated ITP (145).

The mechanism of action of these two plasma-derived immunomodulatory agents in ITP remains unclear, but both IVIG and anti-D can block the function of the reticuloendothelial system to modulate immune responses in man and in animal models (130, 146–159). The clinical efficacy of IVIG likely results from a combination of modulation of the expression and function of Fc receptors, interference with the activation of complement and cytokine network, provision of anti-idiotypic antibodies, regulation of cell growth, and effects on the activation, differentiation, and effector functions of T and B cells. Optimal immunomodulatory effects require the infused IgG to be intact and functional. Further work is required to understand how these products work, but, given the potential for product shortages and cost, recent studies have examined the use of monoclonal antibodies as alternatives to IVIG and anti-D in murine models (159).

Unpredictable and unavoidable adverse reactions are a concern with IVIG products, although it is unclear whether such reactions result from the IVIG, the underlying disease or a combination (146). Minor and infusion-related adverse events, which seem to be systemic in nature, include headache, chills, flushing, low backache/hip pain, abdominal pain/cramps, myalgia/arthritis, tachycardia, nausea, vomiting, dyspnoea, hypotension, diaphoresis, anxiety, and infusion site pain (145, 160). Serious adverse reactions are rare and occur more frequently in adults with autoimmune disease, so they may be related to high-dose therapy or comorbid conditions (160–163). Rare immunoglobulin-related complications include acute Coombs' haemolysis, neutropenia and increases in blood viscosity resulting in thromboembolic events (153, 161). Non-haematologic complications include transient increases in levels of serum creatinine,

eczema, alopecia and erythema multiforme (161). Serious adverse reactions may include aseptic meningitis, anaphylaxis and thromboembolic events (153). IVIG-mediated anaphylaxis is rare, and anaphylactoid reactions secondary to aggregates are not seen (161). Systemic symptoms may be prevented and managed by starting with a slow infusion rate and reducing the infusion rate if a reaction occurs, treating with an antipyretic agent and an antihistamine, premedicating with an antipyretic agent and an antihistamine in patients who have had multiple reactions, treating or premedicating with steroids or switching to an alternative brand of IVIG (161). Recently, there have been several reports of severe intravascular haemolysis with anti-D and IVIG treatment for ITP (161–164); the mechanism for these reactions remains unclear.

Conclusions

A wide variety of biological medicines derived from human or animal plasmas and important for haemostasis are available today and efficacious in many indications. These therapeutic proteins are non-self, and thus could and do elicit immune responses, although the clinical consequences of immunogenicity vary considerably, ranging from no effect to serious adverse effects. For instance, antibodies directed against human plasma-derived factor VIII and factor IX develop in some patients treated with either product, with continued use (for prophylaxis or treatment), possibly increasing the titre of these problematic antibodies. Although patients with low levels of inhibitors may respond effectively to more frequent or higher doses of factor VIII

or IX concentrates, alternative approaches, such as aPCC, recombinant factor VIIa and immune tolerance induction, may be needed in subset of patients with high levels of factor inhibitors. Similarly, patients exposed to topical thrombin preparations may develop antibodies to bovine thrombin, factor V and other proteins found in some topical bovine thrombin preparations. Some of these antibodies have been reported to cause haemorrhagic complications in a small number of patients, relative to the large number of patients that are exposed to topical bovine thrombin. Intravenous immunoglobulin has many clinical uses, being effective in antibody-deficiency diseases and autoimmune and inflammatory diseases, possibly because of normalization of immunoregulatory pathways. Unpredictable and unavoidable adverse reactions are a concern with IVIG products, with a variety of minor and infusion-related adverse events and some rare serious adverse reactions, particularly in patients with autoimmune diseases.

Any therapeutic product derived from blood or plasma has the potential to transmit human pathogens. A variety of strategies are implemented during biological sourcing and manufacturing to minimize the infectivity of products derived from human and animal plasmas. These strategies have dramatically reduced the infectivity of plasmas and plasma-derived products, enabling many patients to benefit from these important and effective biological medicines with minimal risk of adverse outcomes. Regardless of whether a biopharmaceutical protein is derived from human or animal plasmas or is manufactured using recombinant technology, some immunogenic reactions with variable clinical consequences can be expected.

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