

Improving patient tolerability in immunoglobulin treatment: focus on stabilizer effects

Expert Rev. Clin. Immunol. 9(6), 577–587 (2013)

Adam Sun¹,
Wolfgang Teschner²
and Leman Yel^{3*}

¹Baxter Biosciences, 4501 Colorado Blvd, Los Angeles, CA 90039, USA

²Baxter Innovations GmbH, Industriestr. 131, Vienna, Austria

³Baxter Healthcare Corporation/ BioScience, One Baxter Way, Westlake Village, CA 91362, USA

*Author for correspondence:

Tel.: +1 805 372 3572

Fax: +1 805 372 3492

leman_yel@baxter.com

Various types of excipients are added to immunoglobulin preparations to stabilize the product and prevent aggregation and dimer formation. These excipients, which are also called stabilizers or additives, are not inert chemicals and may have clinical implications. This is one reason why immunoglobulin products are not interchangeable. Herein, immunoglobulin preparation, excipient types and the differences among sugar stabilizers and the amino acids, glycine and proline as excipients, are presented. Preclinical studies that unravel the complexities of dimer reduction are summarized. Details of patient considerations with respect to excipient content are outlined focusing on patients with renal insufficiency, diabetes, corn allergy, hereditary fructose intolerance, inborn errors of proline metabolism, DiGeorge Syndrome and neuropsychiatric disorders associated with hyperprolinemia. Excipients are essential components of immunoglobulin preparations and their presence should be a consideration when matching patient needs to product characteristics.

KEYWORDS: adverse event • contraindications • excipient • IgG • immunoglobulin • stabilizer

Immunoglobulin preparations are used in a variety of diseases, that is, as a replacement therapy in primary and secondary immunodeficiency diseases and as an immunomodulator in auto-immune and inflammatory disorders. This wide-range use of immunoglobulin preparations highlights the need for a complete understanding of the clinical effects that the stabilizing components of these products may have [1–3]. Stabilizers, excipients and additives (terms often used interchangeably) refer to compounds such as sugars, polyols or amino acids that are added to immunoglobulins to enable the product to maintain a specific molecular form. Impurities are not stabilizers. The focus of this review is to discuss the clinical aspects of the various stabilizer components. Many reviews have already addressed the various efficacy and safety issues of the immunoglobulins as a class and by product [1–9]. It is not the authors' intent to review product efficacy, adverse events, warnings and precautions, drug interactions or administration protocols again as this scope is very large; rather, the authors hope to bring awareness to an area which is not well recognized – how product excipients might affect immunoglobulin choice when tailoring treatment to the individual patient.

There are currently 11 US FDA-approved commercial immunoglobulin preparations; ten products are approved for intravenous (iv.) immunoglobulin (IVIG) administration in various concentrations, one product is approved for subcutaneous (sc.) immunoglobulin administration and three products are approved for either iv. or sc. administration (TABLE 1).

Numerous clinical investigations have suggested that these products are similar in their efficacy due to the human IgG content [3–6]. However, they may not be similar in their manufacturing processes and final container formulation. Clinical effects may be different based upon the type of stabilizer used to maintain IgG integrity or levels of non-IgG proteins. The American Academy of Allergy Asthma & Immunology outlines eight guiding principles for the safe, effective and appropriate use of immunoglobulin for primary immunodeficiency [1]. The 8th principle cautions clinicians that IVIG is not a generic drug and IVIG products are not interchangeable. A specific IVIG product needs to be matched to patient characteristics to ensure patient safety. A change of immunoglobulin product should ideally occur only with the active participation of the prescribing physician. Stabilizers may

Table 1. Commercially available IgG preparations.

Product	Manufacturer	Formulation	Stabilizer	Osmolality [†] (mOsm/kg)	Storage/shelf life [‡]
<i>IVIG (human)</i>					
Bivigam® 10% IgG	Biotest Pharmaceuticals Corporation (FL, USA)	Liquid solution	Glycine (0.2–0.29 M)	Not listed	Refrigerated: 36–46°F (2–8°C)/till expiration date
Carimune NF® Nanofiltered	CSL Behring AG (Bern, Switzerland)	Lyophilized powder	Sucrose (1.67 g)	In 0.9% NaCl: 498 (3%), 690 (6%), 882 (9%), 1074 (12%)	RT not to exceed 86°F (30°C)/shelf life not stated
Flebogamma® 5% DIF; 10% DIF	Instituto Grifols, SA (Barcelona, Spain)	5% Liquid 10% Liquid	Sorbitol (5 g)	240–370	77°F (2–25°C)/24 months at RT
Gammagard Liquid	Baxter Healthcare Corporation (Westlake Village, CA, USA)	10% Liquid	Glycine (0.25 M)	240–300	Refrigerated: 36–46°F (2–8°C)/36 months RT: 77°F (25°C)/12 months within the first 24 months of manufacture
Gammagard S/D (solvent detergent treated)	Baxter Healthcare Corporation	Freeze/dried	Glycine (22.5 mg/ml) and Glucose (20 mg/ml)	636	Not to exceed 77°F (25°C)/24 months
IgA <2.2 µg/ml IgA <1 µg/ml					
Gammaplex 5%	Bioproducts Laboratory, LTD (UK)	5% Liquid	Sorbitol (5 g) Glycine (0.6 g)	Typically 420–500	Refrigerated 36°F (2°C) to RT 77°F (25°C)/24 months
Gamunex®-C Caprylate/ chromatography purified	Talecris Biotherapeutics, Inc. (Research Triangle Park, NC, USA)	10% Liquid	Glycine (0.16–0.24 M)	258	Refrigerated: 36–46°F (2–8°C)/36 months RT: 77°F (25°C)/6 months
Gammaked™ Caprylate/ chromatography purified	Talecris Biotherapeutics, Inc. (Research Triangle Park, NC, USA)	10% Liquid	Glycine (0.16–0.24 M)	258	Refrigerated: 36–46°F (2–8°C)/36 months RT: 77°F (25°C)/6 months
Octagam® 5%	Octapharma Pharmazeutika Produktionsges, m.b.H. (Vienna, Austria)	5% Liquid 10% Liquid	Maltose (100 mg/ml)	310–380	36–77°F (2–25°C)/24 months
Privigen 10%	CSL Behring AG (Bern, Switzerland)	10% Liquid	L-Proline (~250 mmol/l; range: 210–290)	~320 (range: 240–440)	RT up to 77°F (25°C)/36 months
<i>sc. immunoglobulin (human)</i>					
Gammagard Liquid			Same as reported in IVIG (human)		
Gamunex®-C			Same as reported in IVIG (human)		
Gammaked™			Same as reported in IVIG (human)		
Hizentra®	CSL Behring AG	20% Liquid	L-Proline (~250 mmol/l; range: 210–290)	380	RT up to 77°F (25°C)/30 months
[†] Physiologic osmolality: 285–295 mOsm/kg. [‡] Do not freeze any IgG product. DIF: Dual inactivation plus nanofiltration; IVIG: Intravenous immunoglobulin; RT: Room temperature; sc.: Subcutaneous. Data taken from [32,44–51]					

be problematic in patients with certain conditions, especially since immunoglobulin is used in a wide range of indications and varying patient populations. Some of the per-product contraindications differ due to the stabilizer content. This review article focuses on the similarities and differences found in the various immunoglobulin products based on the excipient used to stabilize the IgG component. Medical evidence for the proper use of each product based on the potential stabilizer effects in a variety of patient populations is presented. This evidence was obtained by a comprehensive internet search and search of the National Library of Medicine databases for relevant articles. The search criteria included English language articles published in 2013 and earlier. Search terms included: immunoglobulin, immunomodulatory therapy, IgG replacement therapy, primary immunodeficiency, immune deficiency, gammaglobulin, excipients and stabilizers. Qualifiers such as adverse events, contraindications, warnings and therapy were considered. Using Boolean strategy, a multilayered search of articles related to topical articles assisted in making the search as comprehensive as possible. All IgG product manufacturer's websites and full prescribing information were reviewed. The full text of the articles was retrieved and reviewed for inclusion.

Preparation of immunoglobulin products

Commercial immunoglobulin products are derived from pooled plasma and consist of a distribution of IgG subclasses as close as possible to its source – normal human plasma [8,9]. During the manufacturing process, IgG is isolated from the plasma of hundreds to thousands of healthy donors followed by sequential fractionation, chromatography, purification and viral inactivation processes. Different manufacturing processes used to purify the product and to render it free of viral contaminants can result in product differences with respect to the content of IgG monomers, dimers and insoluble IgG aggregates.

Preventing aggregation of IgG molecules

Dimers form when the antigen-binding region of one IgG molecule binds to the variable region of another [10]. The amount of dimers in immunoglobulin preparations increases with the number of donors contributing to the plasma pool [8]. Early intramuscular preparations of immunoglobulin contained high levels of aggregates and other impurities that activated the complement system causing anaphylaxis or other adverse events and hence, could not be given by iv. [9]. Immunoglobulin preparations containing high levels of dimers may cause headache, fever and flushing [11]. While dimers in some instances have been proposed to confer therapeutic efficacy, they may also contribute to side effects [12]. Despite the removal of IgG aggregates associated with complement activation, immunoglobulin preparations may still have the ability to induce a long-lasting hypotension, which is usually attributed to the presence of IgG-polymers and dimers [13]. The mechanism of action of the immunoglobulin-associated hypotension is unrelated to complement activation and is thought to be mediated by the release of platelet-activating factor from macrophages, monocytes and neutrophils [14,15].

Manufacturers follow the WHO Expert Committee on Biologic Standardization recommendations for the preparation of immunoglobulin, which state that IgG must be as unmodified as possible; maintain its biological function; contain certain levels of specific antibody and meet accepted safety standards [16]. These criteria are met by all of the IVIG or sc. immunoglobulin products. One of the major differences in commercial products is the type of excipient used to maintain IgG stability [17]. The six excipients used in immunoglobulin preparations are shown in TABLE 2.

Soluble excipients, when added, stabilize the IgG molecule by preferential interaction. Sugars, polyols and amino acids employ a preferential hydration approach to stabilization that is very effective [18]. IgG prefers to interact with the excipient rather than other IgG molecules [19]. This molecular stabilization helps the product maintain its functional status and prolongs its shelf life [19].

Excipients, which are not impurities, are often considered inert, but chemical additives in any product can have untoward effects especially in certain patient populations. Excipients are an important factor for the product's stability, but also the product's osmolality, tolerability and risk profile.

Stabilizing ability of sugars

Sucrose, glucose, maltose and sorbitol are effective sugar excipients used in immunoglobulin preparations to stabilize proteins. Sugars increase the hydrophilicity of the protein surface causing the IgG molecules to preferentially bond with water [20]. This interaction minimizes aggregation and also protects the proteins from chemical degradation [19].

Sucrose

Sucrose is a crystalline disaccharide composed of fructose and glucose extracted mainly from sugar cane and sugar beets. The side chains of the IgG molecule prefer to bind to sucrose versus other IgG molecules, keeping the protein folded and stabilized [19]. Sucrose is widely used as an excipient in pharmaceutical products but in general is not an inert ingredient. Approximately 100 cases of acute renal failure (ARF) associated with the administration of immunoglobulin have been published in the medical literature [21]. The majority (>90%) of cases implicate the excipient sucrose in renal failure associated with immunoglobulin administration [22,23]. All of the IVIG products carry a black-box warning noting that these products have been associated with renal dysfunction, ARF, osmotic nephrosis and death [24]. Sucrose is not metabolized in the kidney and when administered by iv., it is excreted unchanged in the urine. In renal compromise, sucrose can accumulate in the kidney tubular epithelium and cause osmotic nephrosis. Renal insufficiency typically develops 1–10 days after administration of IVIG. Serum creatinine levels peak around day 5. In most patients, renal failure is reversible after discontinuation of IVIG; 30% of patients require hemodialysis [25]. Although ARF after IVIG therapy occurs rarely [26], it would be prudent for clinicians to identify patients at risk for renal failure including patients with any degree of pre-existing renal insufficiency, cryoglobulinemia, diabetes mellitus and/or hypertension, age greater than 65, volume depletion,

Table 2. Excipients used in immunoglobulin formulations as stabilizing agents.

Type	Example	Formulation/administration
Sugar	Sucrose	Lyophilized, iv.
	Glucose	Lyophilized, iv.
	Maltose	Liquid, iv.
Polyol	Sorbitol	Liquid, iv.
Amino acid	Glycine	Liquid, iv.; liquid, sc.
	Proline	Liquid, iv.; liquid, sc.

iv.: Intravenous; sc.: Subcutaneous.

sepsis, paraproteinemia and patients receiving known nephrotoxic drugs. In patients at risk, IVIG should be administered at the minimum concentration and at the minimal rate of infusion possible.

Glucose

Glucose is a monosaccharide sugar that possesses a free ketone or aldehyde group, allowing it to donate electrons to other molecules and, therefore, act as a reducing agent. Glucose is produced commercially by digestion of starch. Excipient grade D-glucose (dextrose) is frequently used for its various chemical properties (osmotic, diluent, sweetening) and its ability to improve the stability of oxidation-sensitive active materials. Some of the medical literature draws attention to the use of glucose-stabilized IVIG in patients with diabetes [27]. However, intravascular glucose is rapidly distributed to extracellular fluid and is expediently catabolized or converted to glycogen [28]. In general, the monthly increment afforded by immunoglobulin administration would be less than that found in a typical candy bar; it would have nominal clinical impact. Although glucose, when used as an excipient in immunoglobulin preparations, does not appreciably increase glucose levels in blood, it is metabolized to fructose; hence, should be avoided in patients with hereditary fructose intolerance, which is a deficiency of liver enzymes that metabolize fructose [3].

Maltose

Maltose is a water-soluble disaccharide formed from two units of glucose produced by enzymatic digestion of corn. Because maltose is derived from corn, avoidance in patients with a corn allergy is recommended [29]. Unlike sucrose, maltose is metabolized by kidney cells, and is not excreted in the urine [21]. However, osmotic nephropathy and renal failure with maltose-stabilized immunoglobulin preparations have been reported in patients with poor glycemic control [30,31]. Maltose is converted to glucose by the enzyme maltase. Since the conversion to glucose occurs intracellularly in kidney cells, maltose does not increase glucose levels in the blood. However, immunoglobulin preparations stabilized with maltose have been associated with falsely high blood glucose meter readings using systems based on glucose dehydrogenase pyrroloquinoline quinone or glucose-dye-oxidoreductase methods [32]. These systems falsely interpret

maltose as glucose when blood maltose levels exceed 0.9 mmol/l and could potentially lead to a life-threatening hypoglycemic state if insulin is administered based on a falsely elevated blood glucose reading [33]. Therefore, it is important to inquire as to which blood glucose monitoring systems are being used by diabetic patients receiving a maltose-containing immunoglobulin and make sure patients use a meter that employs a glucose oxidase system that does not react with maltose. Alternatively, clinicians may consider switching the patient to an immunoglobulin product with a different excipient [21].

Sorbitol

Sorbitol, also known as glucitol, is a white, crystalline sugar alcohol that is found naturally in fruits. Sorbitol can be synthetically manufactured by the hydrogenation of sucrose. Sorbitol may be further metabolized to fructose, which can cause an increase in osmotic pressure, intracellular edema, Schwann cell swelling, anoxia and nerve demyelination. Sorbitol has been implicated in diabetic neuropathy [34]. Medically, sorbitol is used as a laxative. When sorbitol is given intravascularly in high amounts, it produces osmotic diuresis. As a 3.3% solution, sorbitol is recommended for irrigation of the urinary bladder and prostatic bed during transurethral prostatectomy. Unlike dextrose solutions, sorbitol solutions are not sticky and if administered in the above dilution, are not hemolytic. Some research suggests that diabetics should avoid foods containing sorbitol [35]; how this relates to the effects of iv. sorbitol from immunoglobulin products is unknown. Symptoms of sorbitol intolerance include gastrointestinal distress, flatulence, bloating, diarrhea, fatigue, low iron or other nutrient deficiency. Because sorbitol is metabolized to fructose, patients with rare hereditary problems of fructose intolerance should not be administered products with sorbitol excipients. Fructose intolerance may not yet be diagnosed in infants and young children and therefore caution is advised in this patient population [4].

Sugars are not equal: case in point

Our review of the literature finds most discussions regarding immunoglobulin excipient effects to mistakenly group the sugars together as a class implying their properties and clinical effects would be similar. The chemical structures of sugars are different, hence their properties are different. It is important to consider the specific attributes and detriments of the different sugars as detailed above as the clinical effects of sugar excipients are not a 'class effect'. The different clinical effects of sugar-stabilized preparations are supported in the case study reported by Chapman *et al.* who evaluated the renal effects on a patient administered with sucrose-stabilized immunoglobulin, which was subsequently switched to a D-sorbitol-stabilized immunoglobulin [36]. While being treated with sucrose-stabilized immunoglobulin, a 44-year-old male developed ARF, thus requiring hemodialysis, while awaiting a heart transplant. After his renal function normalized, the patient was switched to a D-sorbitol-stabilized immunoglobulin during which no renal complication was observed. Vo *et al.* compared the adverse events seen with a sucrose-stabilized

product to two products not containing sucrose in a retrospective analysis of 279 patients treated with immunoglobulin at a single center between 1997 and 2004. Of the immunoglobulin products administered, one contained sucrose, one contained glycine and the other contained glucose+glycine as excipients [37]. Of the patients treated with sucrose-stabilized immunoglobulin, eight out of 98 (8.2%) ($p < 0.001$) experienced ARF compared with no patients receiving glycine (0 out of 76) or glucose+glycine (0 out of 105) stabilized products. All eight patients had identifiable risk factors for ARF [37].

Stabilizing ability of amino acids

Amino acids are the principal building blocks of proteins and enzymes. The 3D structure and stability of human proteins are determined by amino acid sequences. Glycine and proline are two of the 20 naturally occurring amino acids that are used as immunoglobulin excipients. As such, these amino acids stabilize IgG by preferential exclusion, that is, the protein takes on water and excludes the excipient, thus preventing destabilization and unfolding [18,19].

Glycine

Glycine is an organic compound with only two hydrogen atoms as its side chain. It is the smallest of the 20 amino acids and is unique among the amino acids in that it is not chiral – not able to be superimposed on its mirror image. It can fit into hydrophilic or hydrophobic environments due to its two hydrogen atom side chains. Unlike proline, glycine has the ability to potentiate *N*-methyl-D-aspartate receptor-mediated neurotransmission and is being considered as a new potential therapy for schizophrenia [38]. No specific excipient-related patient cautions are noted for glycine-containing immunoglobulin preparations.

L-Proline

L-proline, a nonessential amino acid, has a molecular structure with a polar, water-soluble group attached to a nonpolar, water-insoluble hydrocarbon chain making it amphiphilic. Its distinctive cyclic side chain gives L-proline an exceptional conformational rigidity compared with other amino acids. When acting as a stabilizer, interactions between proline's hydrophobic side chains and the antibody's variable (antigen-binding) regions are thought to inhibit the binding responsible for dimer formation [10]. According to prescribing information, proline, as an excipient, is contraindicated in patients with hyperprolinemia, a rare autosomal recessive inherited disorder caused by a deficiency of the enzymes proline dehydrogenase and catechol-*O*-methyl-transferase (COMT), which metabolize proline. Hyperprolinemia type I is characterized by high (three- to ten-times normal) proline levels in the blood that may contribute to seizures, intellectual disability or other neurological or psychiatric problems although it is often asymptomatic, making prevalence estimates difficult. Hyperprolinemia type II is characterized by very high (ten- to 15-times normal) proline levels in the blood and high levels of pyrroline-5-carboxylate. Neurological symptoms vary in severity from benign to refractory convulsions [39].

Preclinical & clinical studies with immunoglobulin stabilizers

Dimer reduction by immunoglobulin stabilization has been investigated in two recent comparative studies. Bolli *et al.* studied the percentage of IgG dimer reduction obtained by the use of proline, glucose and glycine. Approximately, 16.2% dimer content is found in unstabilized immunoglobulin solutions without excipient [10]. Stabilization significantly reduced the percentage of dimer content (reduction by proline: 1.4%; by glucose: 1.2% and by glycine: 0.5%), demonstrating the efficacy of these excipients in dimer reduction.

Sun *et al.* conducted a comparative study of IVIG 10% solution formulated in either 0.25 M glycine or 0.25 M L-proline at a target pH of 4.8 [40]. This study demonstrated that both amino acids provided similar stabilization of the IgG molecules in liquid formulations at low pH. There were no statistically significant differences in total 'monomers + dimers' (96.5 vs 96.1%, $p = 0.62$), in aggregates and fragment content (0.29 vs 0.26%, $p = 0.75$; 3.20 vs 3.63%, $p = 0.54$) and in the level of protective antibodies to hepatitis B surface antigen (3.73 vs 3.81 IU/ml, $p = 0.53$) between the two formulations after 6 months storage (FIGURE 1) [40]. Dimer level in both formulations was below 10% (7.5% in glycine, 5.8% in L-proline), which is considered to be the limit for good tolerability [10].

In clinical testing, proline was not found to accumulate after treatment with L-proline-containing immunoglobulin preparations and was not associated with adverse events [41]. Hagan *et al.* retrospectively evaluated the safety of proline as a stabilizer in IVIG, which is contraindicated in patients with hyperprolinemia per prescribing information [41]. Transient elevations in serum proline levels resulting from administration of proline-stabilized IgG were not associated with adverse events in patients with primary immunodeficiency and no evidence could be found to demonstrate a higher risk of administering proline-containing products in patients with 22q11.2 deletion, half of whom would be expected to have hyperprolinemia. However, no exclusive study on the use of proline-stabilized IgG in patients with 22q11.2 deletion syndrome has been reported so far.

Product selection considerations

The list of medical considerations, warnings and precautions for immunoglobulin preparations are extensive, including a black-box warning regarding the potential for ARF. This article is not intended to review the general warnings and precautions associated with immunoglobulin use. Briefly, the most common adverse effects of immunoglobulin occur soon after infusions and can manifest as headache, flushing, chills, myalgia, wheezing, tachycardia, lower back pain, nausea and hypotension. Other potential AEs include aseptic meningitis, hemolysis and thrombosis/thromboembolism. Thrombosis/thromboembolism may be related to high osmolality, contamination with procoagulants and existing patient risk factors. Our literature search did not reveal any information regarding the relationship between any excipient and a thromboembolic event related to immunoglobulin treatment. Adverse effects are extensively covered in each product's full prescribing information and in other review articles

[5–7,15,22–27,30–33,37,42–52]. It is essential to take into account all information related to the immunoglobulin product to be given and the medical characteristics of each patient. Immunoglobulin is contraindicated in any patient who has had an anaphylactic or severe systemic reaction to the administration of human immunoglobulin or is allergic to any component of the preparation. If an allergic reaction occurs, it should be treated with epinephrine and/or antihistamines based on the severity of the reaction. A summary of other general precautions with immunoglobulin use is listed in Box 1.

All products contain small/trace amounts of IgA and IgM impurities in different concentrations. Iv. administration of products with high IgA levels should be avoided in patients with IgA deficiency, who may have IgE and/or IgG isotype antibodies against IgA, as severe anaphylactic reactions may occur [53]. In

these circumstances, preparations with low IgA content should be a choice for iv. administration. Another alternative would be to give immunoglobulin via the sc. route. Immunoglobulin should be administered with caution in patients with volume depletion, sepsis, paraproteinemia or in those taking concomitant nephrotoxic drugs. The use of higher than recommended doses, concentration and rapid infusion rates of immunoglobulin products have resulted in higher infusion-related adverse events [37,54]. The active immunoglobulins in immunoglobulin preparations are considered as similar; hence, specific medical conditions require consideration of the excipient used to prevent untoward effects (TABLE 3). Product manufacturing considers the amount of stabilizer required to prevent aggregation, and manufacturers follow guidelines for immunoglobulin preparation from blood products. Each immunoglobulin preparation may have a different

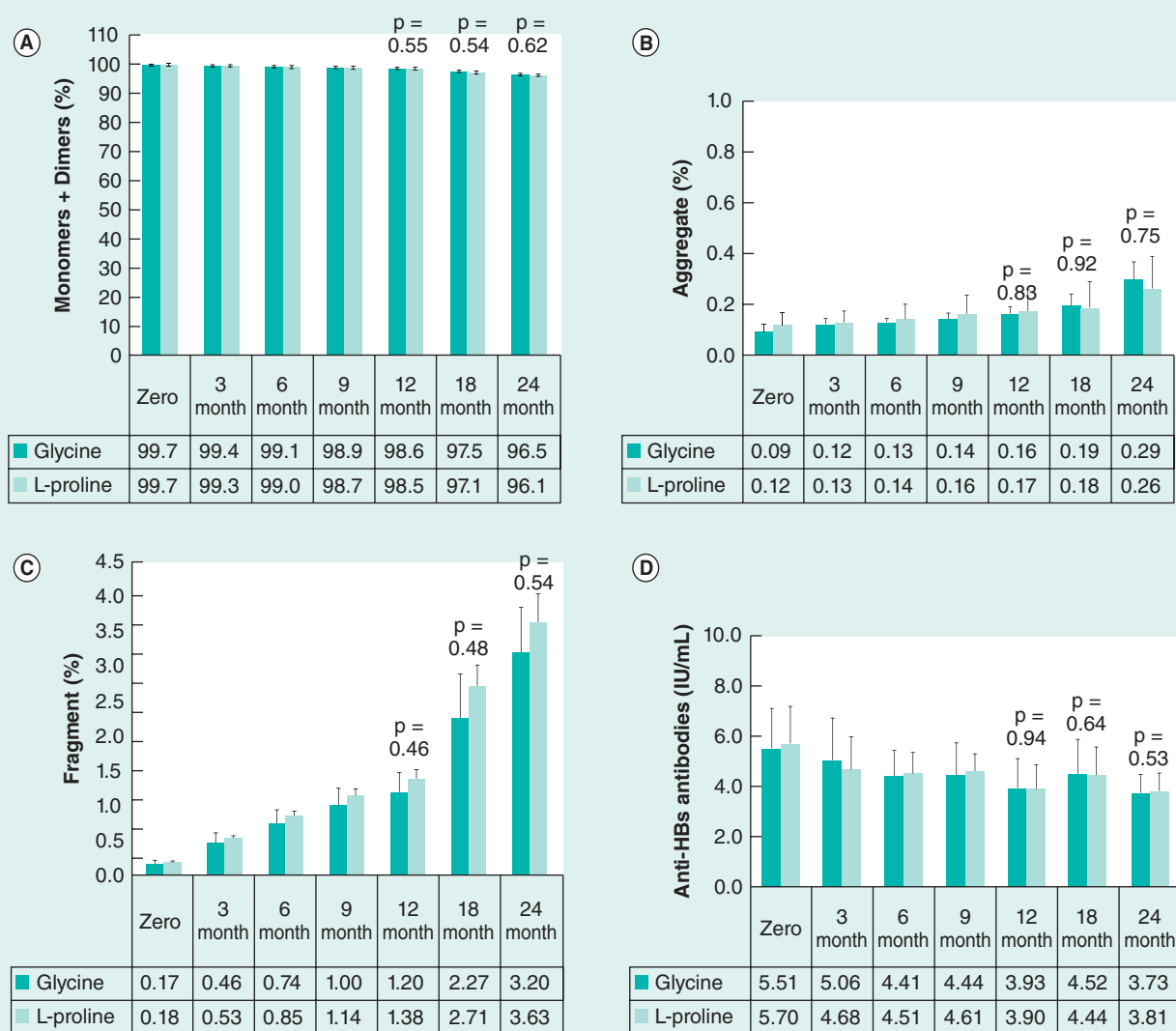


Figure 1. Comparison of intravenous immunoglobulin 10% containing 0.25 M glycine with intravenous immunoglobulin 10% containing 0.25 M L-proline. (A) Percent of 'Monomers + Dimers'; (B) Percent of aggregates; (C) Percent of fragments and (D) Anti-HBs antibody activity.

Anti-HBs: Antibody to Hepatitis B surface antigen.

Adapted with permission from Baxter BioSciences (CA, USA).

IgG concentration and osmolality, which may have an effect on the efficacy and safety profile of the product regardless of the stabilizer content.

Pre-existing renal insufficiency

Patients with impaired renal function, the elderly and patients with diabetes are at increased risk for ARF with the infusion of immunoglobulin. Rarely, ARF has been associated with immunoglobulin products containing high concentrations of sucrose. Renal complications have also been reported with non-sucrose-containing immunoglobulin products and may be related to volume depletion, rapid infusion rates, immune complex deposition in glomeruli and ischemia secondary to immune complexes [21]. Immunoglobulin products should be administered at the lowest dose and slowest infusion rate possible to prevent renal complications. A thorough discussion of immunoglobulin-related renal failure is not within the scope of this manuscript; interested readers can review the references [21–24].

Diabetes/prediabetes

The majority of past and current literature cautions against the administration of sugar-containing immunoglobulin products in patients with diabetes mellitus or prediabetes. The authors feel this information has been promulgated from review article to review article without compelling evidence. Glucose, when used as a stabilizer, is not expected to appreciably raise blood glucose levels (see glucose discussion above). A search of the literature finds no reports of hyperglycemia induced by the administration of glucose-containing immunoglobulin products. Furthermore, none of the 11 reviewed immunoglobulin product inserts list diabetes mellitus as a contraindication, warning or precaution. A 2013 search of MedWatch found no reports of hyperglycemia associated with any of the approved IVIG products. Sucrose, once used as an osmotic diuretic agent, is directly excreted from kidneys when administered intravenously. Because kidney cells do not have disaccharidase, sucrose cannot be hydrolyzed in the kidney and therefore is eliminated via the urine unchanged. Thus, iv. administration of sucrose would not impact blood glucose levels [42]. Interestingly, IVIG has been investigated in patients with diabetic demyelinating polyneuropathy [55–58]. The excipient types in immunoglobulin products used in these investigations are unknown but, considering the novelty of amino

Box 1. Summary of precautions for immunoglobulin administration.

Intravenous administration

- Provide adequate hydration; assure patient is not volume-depleted – serum sodium level >135 mEq/l
- Exercise caution in patients at risk for acute renal failure:
 - Any degree of pre-existing renal insufficiency; fluid and electrolyte imbalance and
 - <400 ml urine per day in adults
 - <0.5 ml/kg/h in children
 - <1 ml/kg/h in infants
 - Diabetes mellitus
 - Age greater than 65
 - Volume depletion
 - Sepsis
 - Paraproteinemia
 - Use of known nephrotoxic drugs (ACE inhibitors, NSAIDs, cyclosporine, tacrolimus, contrast agents/medications, amphotericin B, aminoglycosides, cisplatin, methotrexate, intravenous acyclovir, sulfonamides, penicillins, cephalosporins, fluoroquinolones)
- Weigh the risk/benefit when administering a sucrose-containing IVIG preparation with respect to renal failure. Infuse sucrose-containing IVIG preparations at rates lower than 2 mg IgG/kg/min
- Do not exceed the recommended dose. Reduce dose, concentration, and/or infusion rate in patients at risk of renal failure to minimize risk
- Determine baseline urine output and BUN/serum creatinine before infusion; monitor levels at appropriate intervals. Avoid concomitant use of loop diuretics. Discontinue IVIG if renal function deteriorates

Subcutaneous administration

- Watch for signs of local, transient skin reactions: swelling, redness, itching, burning; possible pearl-sized nodules that persist for 1–2 days

Postinfusion and laboratory monitoring

- Continuously monitor vital signs during infusion. Observe patients for 20 min postinfusion. Watch carefully for signs of rise in temperature, chills, nausea or vomiting, potentially leading to shock
- Monitor for:
 - Hemolytic anemia
 - TRALI
 - Thrombotic events; assess blood viscosity
 - Aseptic meningitis
 - Hyperproteinemia, serum osmolyte changes and electrolyte imbalances

ACE: Angiotensin-converting enzyme; BUN: Blood urea nitrogen; IVIG: Intravenous immunoglobulin; TRALI: Transfusion-related acute lung injury.
Data taken from [32,44–52].

acid excipients (2003), sugar excipients were probably the norm in products used in these investigations without compromising the blood glucose levels in diabetic patients. Prospective clinical studies to investigate the effect of sugar-containing immunoglobulin preparations on the blood glucose levels of patients with diabetes mellitus are needed for a definite conclusion.

Corn allergy

Corn or maize allergy is an emerging IgE-mediated hypersensitivity reaction to corn or corn syrup, which is found in a majority of commercially prepared food products. Corn allergy may affect both adults and children [29]. As maltose is derived from corn,

Table 3. Medical considerations for specific excipients in immunoglobulin preparations.

Condition	Sucrose	Glucose	Maltose	Sorbitol	Glycine	Proline
Pre-existing renal insufficiency	X					
Diabetes			X [†]			
Allergy to corn			X			
Hereditary fructose intolerance		X		X		
Hyperprolinemia						X

[†]It may interfere with some glucose monitors.

X: Consider avoiding use in this condition.

maltose-containing immunoglobulin preparations may potentially cause adverse reactions in patients with corn allergy [43].

Hereditary fructose intolerance

Administration of sorbitol in patients with fructose intolerance could lead to severe and often fatal hepatic failure [4]. Immunoglobulin containing sorbitol needs to be administered with caution to infants and young children, who may not yet be diagnosed with hereditary fructose intolerance.

Inborn errors of proline metabolism

Proline is an indispensable amino acid for the neonate. Additionally, it is an inhibitory neuromodulator and a metabolic precursor for glutamate in the CNS [59]. Abnormalities in proline metabolism are found in hyperprolinemia I and II, Δ^1 -pyrroline-5-carboxylic acid synthetase deficiency, ornithine aminotransferase deficiency, hydroxyprolinemia and imino-glycinuria [60]. Because these conditions are often asymptomatic, prevalence is difficult to estimate. A deficiency of proline oxidase or pyrroline-5-carboxylate dehydrogenase, the enzymes that metabolize proline, results in a buildup of proline in the body, which can cause seizures, intellectual disability or other neurological or psychiatric problems. Thus, immunoglobulin products with proline are deemed contraindicated in patients with hyperprolinemia.

DiGeorge syndrome

The 22q11.2 deletion syndrome, also known as velocardiofacial syndrome or DiGeorge Syndrome, is a primary immunodeficiency disease caused by abnormal migration and development of cells and tissues during fetal development. It is the most common microdeletion genetic disorder, occurring in an estimated one in 6000 newborns; however, it is often underdiagnosed and its true prevalence is probably higher than current estimates [61]. Recurrent infections, congenital heart disease, especially conotruncal deformities, palatal defects, characteristic facial features – high broad nasal bridge, narrow palpebral fissures, low-set ears and micrognathia – and hypocalcemia due to hypoparathyroidism are common [62]. DiGeorge syndrome is also associated with learning disabilities, mental retardation and behavioral/psychiatric problems in children and schizophrenia in

adults [60,63]. Immunoglobulin therapy protects against the recurrent infections that are mostly due to the immunodeficiency related to thymus gland dysfunction and impaired T-lymphocyte production. Half of all individuals with DiGeorge syndrome are hyperprolinemic [64], in whom immunoglobulin stabilized with proline may be concerning.

Neuropsychiatric disorders associated with hyperprolinemia

The association between the 22q11.2 deletion syndrome and psychiatric disorders,

particularly psychosis, suggests a causal relationship between *22q11DS* genes and abnormal brain function [65]. The COMT and proline dehydrogenase genes reside within the commonly deleted region of 22q11.2; missing these genes and the enzymes they code for, which breakdown proline, can result in hyperprolinemia. Some animal models have demonstrated that dysfunctional COMT and inadequate proline dehydrogenase cause an increase in proline, which leads to altered brain function [66–69]. Human investigations confirm these preclinical findings [65,70,71]. The neuropsychiatric disorders possibly associated with hyperprolinemia include: schizophrenia, attention deficit hyperactivity disorder, oppositional defiant disorder, anxiety and affective disorder, autism and obsessive-compulsive disorder. Schizophrenia has a global frequency of approximately 1% and approximately one-third of individuals with the 22q11.2 deletion syndrome develop schizophrenia-like psychotic disorders [59,63]. Therefore, proline-containing products would be inadvisable in patients with neuropsychiatric disorders associated with hyperprolinemia.

Conclusion

Patients with primary or secondary immunodeficiencies, or autoimmune and inflammatory disorders may need immunoglobulin treatment. Excipients are essential components of all immunoglobulin preparations for their molecular stabilizing properties; however, excipients should not be considered inert. Additionally, the sugar excipients should not be considered similar in clinical effects. There is evidence that the various IgG products, which contain different excipients, have different tolerability profiles based solely on the excipient component. Rather than randomly selecting one product for every patient, each patient's specific needs and risk factors with the product attributes or deficiencies should be assessed carefully in therapeutic decision making. Additional research into the clinical effects of product excipients will add to the scant body of literature found on this subject.

Expert commentary

Immunoglobulins are a fundamental component of the human immune system, a deficiency of which causes disease. The evolution of immunoglobulin replacement therapy has allowed immunodeficient individuals to live longer productive lives. There

are 11 FDA-approved immunoglobulin products on the market and the future will probably bring more. It is important to become familiar with all products available to treat patients and know the pros and cons, warnings and precautions of not only the active component but also the so-called inactive components of all medications, immunoglobulin products included. A periodic review of a patient's product usage and a yearly review of product insert information with the patient would facilitate tailoring an optimum treatment and potentially avert any adverse effects. It is often difficult to keep up with the literature, especially with respect to tried and true products used to manage chronic diseases. We hope this review keeps the reader abreast of an area regarding immunoglobulin therapy that is often overlooked – namely stabilizer/excipient effects.

Five-year view

Although immunoglobulin replacement therapy has been used for decades, our understanding of its nuances is still in its infancy. The mechanisms of the immune system, while currently being unraveled at the molecular level, still leaves mysteries to be

solved. Refinements in manufacturing processes and dosing regimens have improved the safety of immunoglobulin replacement therapy while also improving the prospects for optimal clinical outcomes. We expect these refinements to continue bringing more safety to all human-derived products. Improvements in product stability and delivery modes will expand self-administration. Therefore, sc. administered immunoglobulin will probably surpass the use of IVIG providing patients with a more convenient treatment.

Financial & competing interests disclosure

A Sun is an employee of Baxter Bioscience. W Teschner is an employee of Baxter Innovations GmbH, Vienna, Austria. L Yel is an employee of Baxter Healthcare Corporation/BioScience, Westlake Village, California. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Writing and editorial assistance was provided, which was contracted by Baxter Healthcare Corporation.

Key issues

- Patients with primary antibody deficiencies usually require lifelong treatment with immunoglobulin.
- Generally, these preparations have been rendered safe using tested plasma from qualified donors and various manufacturing processes to eliminate pathogen contamination.
- Immunoglobulin preparations contain various excipients, which are not inert chemicals.
- These excipients stabilize the monomeric IgG molecule.
- Stabilizers can have various clinical effects and may influence product safety and tolerability in certain populations.
- Major chemicals used as excipients in immunoglobulin formulations include sugars (sucrose, glucose, maltose and sorbitol) and amino acids (proline and glycine). These excipients do not have equal properties and tolerability for immunoglobulin preparations should not be considered the same.
- All stabilizers have a unique safety profile.
- Patient considerations including contraindications, warnings and precautions are presented for the various stabilizer components of immunoglobulin.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- 1 Orange JS, Hossny EM, Weiler CR *et al.*; Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. *J. Allergy Clin. Immunol.* 117(Suppl. 4), S525–S553 (2006).
- 2 Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. *N. Engl. J. Med.* 367(21), 2015–2025 (2012).
- 3 Kivity S, Katz U, Daniel N, Nussinovitch U, Papageorgiou N, Shoenfeld Y. Evidence for the use of intravenous immunoglobulins – a review of the literature. *Clin. Rev. Allergy Immunol.* 38(2–3), 201–269 (2010).
- 4 Chérin P, Cabane J. Relevant criteria for selecting an intravenous immunoglobulin preparation for clinical use. *BioDrugs* 24(4), 211–223 (2010).
- Recent review regarding the safety and tolerability of IgG preparations including comparative charts of pharmacologic agents.
- 5 Berger M, Pinciari PJ; Flebogamma 5% Investigators. Safety, efficacy, and pharmacokinetics of Flebogamma 5% (immune globulin intravenous [human]) for replacement therapy in primary immunodeficiency diseases. *J. Clin. Immunol.* 24(4), 389–396 (2004).
- 6 Church JA, Leibl H, Stein MR *et al.*; US-PID-IGIV 10% – Study Group 10. Efficacy, safety and tolerability of a new 10% liquid intravenous immune globulin (IGIV 10%) in patients with primary immunodeficiency. *J. Clin. Immunol.* 26(4), 388–395 (2006).
- 7 Ochs HD, Pinciari PJ; Octagam Study Group. Octagam 5%, an intravenous IgG product, is efficacious and well tolerated in subjects with primary immunodeficiency diseases. *J. Clin. Immunol.* 24(3), 309–314 (2004).
- 8 Nezlin R. Interactions between immunoglobulin G molecules. *Immunol. Lett.* 132(1–2), 1–5 (2010).

- 9 Lemm G. Composition and properties of IVIg preparations that affect tolerability and therapeutic efficacy. *Neurology* 59(12 Suppl. 6), S28–S32 (2002).
- 10 Bolli R, Woodtli K, Bärtschi M, Höfferer L, Lerch P. L-Proline reduces IgG dimer content and enhances the stability of intravenous immunoglobulin (IVIg) solutions. *Biologicals* 38(1), 150–157 (2010).
- 11 Cramer M, Frei R, Sebald A, Mazzeletti P, Maeder W. Stability over 36 months of a new liquid 10% polyclonal immunoglobulin product (IgPro10, Privigen) stabilized with L-proline. *Vox Sang.* 96(3), 219–225 (2009).
- 12 Teeling JL, Jansen-Hendriks T, Kuijpers TW *et al.* Therapeutic efficacy of intravenous immunoglobulin preparations depends on the immunoglobulin G dimers: studies in experimental immune thrombocytopenia. *Blood* 98(4), 1095–1099 (2001).
- 13 Bleeker WK, Teeling JL, Verhoeven AJ *et al.* Vasoactive side effects of intravenous immunoglobulin preparations in a rat model and their treatment with recombinant platelet-activating factor acetylhydrolase. *Blood* 95(5), 1856–1861 (2000).
- 14 Jenei B, Lázár G, Bartha K, Medgyesi GA. Hypotensive action of IgG preparations containing aggregates is suppressed by PAF-receptor antagonist BN 52021 and by gadolinium chloride (an agent blocking Kupffer cell function). *Agents Actions* 32(3–4), 333–338 (1991).
- 15 Knezevic-Maramica I, Kruskal MS. Intravenous immune globulins: an update for clinicians. *Transfusion* 43(10), 1460–1480 (2003).
- 16 WHO. Appropriate use of human immunoglobulin in clinical practice: memorandum from an IUIS/WHO meeting. *Bull. WHO* 60, 43–47 (1982).
- 17 Apte SP, Ugwu SO. A review and classification of emerging excipients in parenteral medications. *Pharmaceut. Technol.* 46–60 (2003).
- 18 Jorgensen L, Hostrup S, Moeller EH, Grohgan H. Recent trends in stabilising peptides and proteins in pharmaceutical formulation - considerations in the choice of excipients. *Expert Opin. Drug Deliv.* 6(11), 1219–1230 (2009).
- **Review of the role of excipients in medications including their safety, toxicity and immunogenicity.**
- 19 Wang W. Instability, stabilization, and formulation of liquid protein pharmaceuticals. *Int. J. Pharm.* 185(2), 129–188 (1999).
- **Biochemical background information on why pharmaceuticals require stabilization.**
- 20 Szczeni A, Kardos J, Medgyesi GA, Závodszky P. The effect of solvent environment on the conformation and stability of human polyclonal IgG in solution. *Biologicals* 34(1), 5–14 (2006).
- 21 Graumann A, Zawada ET Jr. Case report: acute renal failure after administering intravenous immunoglobulin. *Postgrad. Med.* 122(2), 142–147 (2010).
- 22 Carbone J. Adverse reactions and pathogen safety of intravenous immunoglobulin. *Curr. Drug Saf.* 2(1), 9–18 (2007).
- 23 Lin RY, Rodriguez-Baez G, Bhargava GA, Lin H. Intravenous γ -globulin-associated renal impairment reported to the FDA: 2004–2009. *Clin. Nephrol.* 76(5), 365–372 (2011).
- 24 Itkin YM, Trujillo TC. Intravenous immunoglobulin-associated acute renal failure: case series and literature review. *Pharmacotherapy* 25(6), 886–892 (2005).
- 25 Gürçan HM, Keskin DB, Ahmed AR. Information for healthcare providers on general features of IGIV with emphasis on differences between commercially available products. *Autoimmun. Rev.* 9(8), 553–559 (2010).
- **Recent review of the differences between commercially available IgG products. Stresses the need for monitoring patients for adverse events.**
- 26 Cayco AV, Perazella MA, Hayslett JP. Renal insufficiency after intravenous immune globulin therapy: a report of two cases and an analysis of the literature. *J. Am. Soc. Nephrol.* 8(11), 1788–1794 (1997).
- 27 Ochs HD, Siegel J. Intravenous immunoglobulin products. A comparative review. *Pharm. Pract. News* (2010).
- 28 Bauman LA. Fluid volume monitoring with glucose dilution. *Anesthesia Analg.* 105(4), 1117 (2007).
- 29 Scibilia J, Pastorello EA, Zisa G *et al.* Maize food allergy: a double-blind placebo-controlled study. *Clin. Exp. Allergy* 38(12), 1943–1949 (2008).
- 30 Centers for Disease Control and Prevention. Renal insufficiency and failure associated with immune globulin intravenous therapy – United States, 1985–1998. *MMWR Morb. Mortal. Wkly Rep.* 48(24), 518–521 (1999).
- **Key warning from the CDC regarding the potential for renal insufficiency when using intravenous immunoglobulin therapy with products stabilized with sucrose.**
- 31 Chacko B, John GT, Balakrishnan N, Kirubakaran MG, Jacob CK. Osmotic nephropathy resulting from maltose-based intravenous immunoglobulin therapy. *Ren. Fail.* 28(2), 193–195 (2006).
- 32 Octagam[®], prescribing information. Immune globulin intravenous (human) 5%, solvent/detergent treated. Octapharma USA, Inc., Hoboken, NJ, USA (2009).
- 33 Gaines A, Pierce LR, Bernhardt PA. Fatal iatrogenic hypoglycemia: falsely elevated blood glucose readings with a point-of-care meter due to a maltose-containing intravenous immune globulin product. FDA, Safety and Availability Bulletin (2009).
- **Warning from the US FDA about the potential for falsely elevated blood glucose readings with some glucose meters if a maltose-containing IgG preparation was administered.**
- 34 Burg MB, Kador PF. Sorbitol, osmoregulation, and the complications of diabetes. *J. Clin. Invest.* 81(3), 635–640 (1988).
- 35 Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414(6865), 813–820 (2001).
- 36 Chapman SA, Gilkerson KL, Davin TD, Pritzker MR. Acute renal failure and intravenous immune globulin: occurs with sucrose-stabilized, but not with D-sorbitol-stabilized, formulation. *Ann. Pharmacother.* 38(12), 2059–2067 (2004).
- 37 Vo AA, Cam V, Toyoda M *et al.* Safety and adverse events profiles of intravenous γ -globulin products used for immunomodulation: a single-center experience. *Clin. J. Am. Soc. Nephrol.* 1(4), 844–852 (2006).
- 38 Hashimoto K. Glycine transporter-1: a new potential therapeutic target for schizophrenia. *Curr. Pharm. Des.* 17(2), 112–120 (2011).
- 39 Onenli-Mungan N, Yüksel B, Elkay M, Topaloglu AK, Baykal T, Ozer G. Type II hyperprolinemia: a case report. *Türk. J. Pediatr.* 46(2), 167–169 (2004).
- 40 Sun A, Wu Y, Pot G *et al.* IgG stabilization in glycine or L-proline formulated

- immunoglobulin intravenous 10% (IGIV) solutions: 24-month data to support equally effective stabilization. Presented at: *Clinical Immunology Society Annual Meeting: Primary Immune Deficiency North America Conference*. Chicago, IL, USA, 18 May 2012.
- 41 Hagan JB, Wasserman RL, Baggish JS *et al.* Safety of L-proline as a stabilizer for immunoglobulin products. *Expert Rev. Clin. Immunol.* 8(2), 169–178 (2012).
 - 42 Zhang R, Szerlip HM. Reemergence of sucrose nephropathy: acute renal failure caused by high-dose intravenous immune globulin therapy. *South. Med. J.* 93(9), 901–904 (2000).
 - 43 Siegel J. IVIG medication safety: a stepwise guide to product selection and use. *Pharm. Pract. News* 77–84 (2011).
 - 44 Carimune® NF, prescribing information. Nanofiltered Immune Globulin Intravenous (Human), Lyophilized Preparation. CSL Behring AG, Bern, Switzerland (2010).
 - 45 Flebogamma® 5% DIF, prescribing information. Immune globulin intravenous (human), 5% liquid preparation. Instituto Grifols, Barcelona, Spain (2012).
 - 46 Gammagard, prescribing information. Immune globulin intravenous (human), liquid 10%. Baxter Healthcare Corporation, CA, USA (2011).
 - 47 Gammagard S/D, prescribing information. Immune globulin intravenous (human), solvent detergent treated IgA less than 2.2 µg/mL in a 5% solution. Baxter Healthcare Corporation, CA, USA (2011).
 - 48 Gammaplex®, prescribing information. Immune globulin injection (human) 5%. Bio Products Laboratory, Hertfordshire, UK (2009).
 - 49 Gamunex®-C, prescribing information. Immune globulin injection (human) 10%, caprylate/chromatography purified. Talecris Biotherapeutics, Inc., NC, USA (2010).
 - 50 Hizentra™, prescribing information. Immune globulin subcutaneous (human), 20% liquid. CSL Behring AG, Bern, Switzerland (2011).
 - 51 Privigen®, prescribing information. Immune globulin intravenous (human), 10% liquid. CSL Behring AG, Bern, Switzerland (2011).
 - 52 Pierce LR, Jain N. Risks associated with the use of intravenous immunoglobulin. *Transfus. Med. Rev.* 17(4), 241–251 (2003).
 - 53 Yel L. Selective IgA deficiency. *J. Clin. Immunol.* 30(1), 10–16 (2010).
 - 54 Feldmeyer L, Benden C, Haile SR *et al.* Not all intravenous immunoglobulin preparations are equally well tolerated. *Acta Derm. Venereol.* 90(5), 494–497 (2010).
 - 55 Jann S, Bramerio MA, Facchetti D, Sterzi R. Intravenous immunoglobulin is effective in patients with diabetes and with chronic inflammatory demyelinating polyneuropathy: long term follow-up. *J. Neurol. Neurosurg. Psychiatr.* 80(1), 70–73 (2009).
 - 56 Sharma KR, Cross J, Ayyar DR, Martinez-Arizala A, Bradley WG. Diabetic demyelinating polyneuropathy responsive to intravenous immunoglobulin therapy. *Arch. Neurol.* 59(5), 751–757 (2002).
 - 57 Cocito D, Ciaramitaro P, Isoardo G *et al.* Intravenous immunoglobulin as first treatment in diabetics with concomitant distal symmetric axonal polyneuropathy and CIDP. *J. Neurol.* 249(6), 719–722 (2002).
 - 58 Hahn AF, Bolton CF, Zochodne D, Feasby TE. Intravenous immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy. A double-blind, placebo-controlled, cross-over study. *Brain* 119(Pt 4), 1067–1077 (1996).
 - 59 Hu CA, Phang JM, Valle D. Proline metabolism in health and disease. Preface. *Amino Acids* 35(4), 651–652 (2008).
 - 60 Mitsubuchi H, Nakamura K, Matsumoto S, Endo F. Inborn errors of proline metabolism. *J. Nutr.* 138(10), 2016S–2020S (2008).
 - 61 Botto LD, May K, Fernhoff PM *et al.* A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics* 112(1 Pt 1), 101–107 (2003).
 - 62 Sullivan KE. Chromosome 22q11.2 deletion syndrome: DiGeorge syndrome/velocardiofacial syndrome. *Immunol. Allergy Clin. North Am.* 28(2), 353–366 (2008).
 - 63 Gothelf D, Feinstein C, Thompson T *et al.* Risk factors for the emergence of psychotic disorders in adolescents with 22q11.2 deletion syndrome. *Am. J. Psychiatry* 164(4), 663–669 (2007).
 - 64 Goodman BK, Rutberg J, Lin WW, Pulver AE, Thomas GH. Hyperprolinaemia in patients with deletion (22)(q11.2) syndrome. *J. Inherit. Metab. Dis.* 23(8), 847–848 (2000).
 - 65 Vorstman JA, Turetsky BI, Sijmens-Morcus ME *et al.* Proline affects brain function in 22q11DS children with the low activity COMT 158 allele. *Neuropsychopharmacology* 34(3), 739–746 (2009).
 - 66 Ferreira AG, Stefanello FM, Cunha AA *et al.* Role of antioxidants on Na⁺, K⁺-ATPase activity and gene expression in cerebral cortex of hyperprolinemic rats. *Metab. Brain Dis.* 26(2), 141–147 (2011).
 - 67 Bavaresco CS, Streck EL, Netto CA, Wyse AT. Chronic hyperprolinemia provokes a memory deficit in the Morris water maze task. *Metab. Brain Dis.* 20(1), 73–80 (2005).
 - 68 Delwing D, Bavaresco CS, Wannmacher CM, Wajner M, Dutra-Filho CS, Wyse AT. Proline induces oxidative stress in cerebral cortex of rats. *Int. J. Dev. Neurosci.* 21(2), 105–110 (2003).
 - 69 Moreira JC, Wannmacher CM, Costa SM, Wajner M. Effect of proline administration on rat behavior in aversive and nonaversive tasks. *Pharmacol. Biochem. Behav.* 32(4), 885–890 (1989).
 - 70 Raux G, Bumsel E, Hecksweiler B *et al.* Involvement of hyperprolinemia in cognitive and psychiatric features of the 22q11 deletion syndrome. *Hum. Mol. Genet.* 16(1), 83–91 (2007).
 - 71 Jacquet H, Raux G, Thibaut F *et al.* PRODH mutations and hyperprolinemia in a subset of schizophrenic patients. *Hum. Mol. Genet.* 11(19), 2243–2249 (2002).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.