Stabilizers Used in Intravenous Immunoglobulin Products: A Comparative Review

Introduction
There are several product characteristics that contribute to the tolerability of intravenous immunoglobulin (IVIG); many relate to the processes employed to extract and purify immunoglobulin G (IgG) from human plasma. Such characteristics include product purity; the presence of IgG aggregates, dimers, and fragments; level of immunoglobulin A (IgA); osmolality; and use of excipients, such as stabilizers and sodium.1,6

Stabilizers are added to IVIG products to prevent aggregation of IgG molecules during manufacturing and storage.1,6 In the absence of stabilizers, IVIG preparations must be formulated at a pH range of 4.0 to 4.5 in order to limit the formation of aggregates.1,7 Various types of compounds are used for this purpose, including sugars, such as sucrose, glucose, and maltose; polyols, such as sorbitol; and amino acids, including glycine and L-proline. The selection of a particular stabilizer influences the formulation, osmolality, and stability of the IVIG preparation, as well as the product’s tolerability and risk profile with regard to adverse events.
Inhibition of IgG Aggregation by Stabilizers

All plants, animals, and microorganisms have developed ways to adapt to environmental stresses, including extremes of temperature, desiccation, and high extracellular salt concentrations, all of which can denature proteins and disrupt normal cellular function. Adaptation to these environmental stresses involves cellular accumulation of small organic molecules, called osmolytes—regulators of cell volume. Such use of organic osmolytes appears to be widespread, from microorganisms to the mammalian kidney and brain. Unlike salt ions—inorganic osmolytes—organic osmolytes maintain cell volume without perturbing protein structure and function, even at high concentrations.

Naturally occurring organic osmolytes that protect against protein denaturation include polyols, sugars, amino acids and their derivatives, methylamines, and ureas. All IVIG stabilizers in use today function as organic osmolytes in stabilizing IgG. Some of these have very specific physiologic protective roles. For example, sorbitol is 1 of 5 organic osmolytes utilized by the mammalian kidney to maintain osmotic equilibrium with the hypertonic extracellular environment and glycine appears to be osmoprotective in early embryos.

Various mechanisms by which organic osmolytes stabilize proteins have been described. Protein stabilization, however, appears to involve the preferential exclusion of osmolytes from protein surfaces—along with the preferential hydration of the protein—which favors the native, folded state of the protein molecule. Multiple forces (eg, solvophobic, surface tension, electrostatic repulsion) have been proposed to account for the preferential exclusion phenomenon and its effect on protein stability. However, a solvophobic thermodynamic force, also called the “osmophobic effect,” appears to dominate; the solvophobic force preferentially raises the free energy of the unfolded (denatured) state, thereby shifting the equilibrium in favor of the native state. Thus, the osmophobic effect of organic osmolytes stabilizes proteins by forcing them to fold.

Impact of Osmolality

A product’s osmolality—defined as the concentration of osmotically active particles in solution and equal to the sum of the osmoles of all the solutes in solution—may influence the tolerability of IVIG. Hyperosmotic IVIG products—those that exceed the physiologic range of 280 to 296 mOsm/kg—have a possible association with thrombotic events (eg, stroke, myocardial infarction). The concentration of sugar, salt, and non-IgG proteins, such as albumin, contribute to the osmolality of IVIG, which varies widely among available products. Additionally, the osmolality of lyophilized IVIG products is variable, and depends on both the concentration of the reconstituted solution and the diluents used. Thus, the use of products that are close to physiologic osmolality has been recommended.

Below are the specific characteristics and important clinical effects of 6 IVIG stabilizers. Sucrose and glucose, used in lyophilized IVIG products, are discussed first, followed by maltose, sorbitol, glycine, and L-proline.

Sucrose

Sucrose (table sugar) is a disaccharide comprised of the monosaccharides glucose and fructose. Sucrose is one of several organic osmolytes that are used by bacteria and plants subjected to salt stress. Sucrose has been used as a stabilizer in several IVIG products. Compared with other organic osmolytes, sucrose is an intermediate-strength protein stabilizer. In one study, 10% sucrose effectively prevented aggregation of IgG molecules and reduced dimer formation in 10% solutions of IgG at a pH range of 4.1 to 6.8.

Effect on Blood Glucose

Following ingestion, sucrose is enzymatically hydrolyzed in the stomach or within the duodenum (via sucrase) into its component sugars, which are then readily absorbed into the bloodstream. Thus, sucrose provides a rapid source of energy by provoking a rapid rise in blood glucose after eating. However, after intravenous infusion, sucrose cannot be hydrolyzed and is eliminated exclusively by the kidneys. Therefore, intravenous administration of sucrose has no impact on blood glucose levels.

Effect on Renal Function

IVIG products have been associated with renal dysfunction, acute renal failure (ARF), osmotic nephrosis, and death; the vast majority of these adverse events occurred in patients who received IVIG products that were stabilized with sucrose.

In 1999, the Centers for Disease Control and Prevention (CDC) reported a series of 88 cases of renal adverse events (ie, ARF or renal insufficiency) following IVIG administration. Of these cases, 90% occurred in patients who...
received sucrose-stabilized IVIG. Most patients had pre-existing conditions, including diabetes (56%) and prior renal insufficiency (26%). Of the 88 patients, 13 (15%) died; all had severe underlying conditions. Histologic data available in 7 patients showed extensive vacuolization of the proximal tubules consistent with osmotic nephrosis—a form of renal injury associated with osmotically active molecules. Since neither the liver nor kidneys express sucrase, after intravenous administration, sucrose cannot be metabolized and is excreted unchanged in the urine. Thus, particularly at high doses of IVIG, considerable levels of sucrose can accumulate in the proximal tubules, causing osmotically driven cellular vacuolization and swelling, with subsequent narrowing and occlusion of the tubular lumen. Therefore, although sucrose is a naturally occurring organic osmolyte that protects various organisms against high salt concentrations, it does not have a physiologic protective function within the kidney.

Despite the strong association of sucrose-stabilized IVIG with ARF, it also has been reported with other IVIG products in lower frequency. Thus, labeling for all IVIG products contains a black box warning regarding the potential for nephrotoxicity. Patients at risk for ARF include those with any degree of preexisting renal insufficiency, diabetes mellitus, volume depletion/dehydration, sepsis, paraproteinemia, age greater than 65 years, and those receiving known nephrotoxic drugs.

Carimune® NF, Nanofiltered (CSL Behring) is a sucrose-stabilized IVIG marketed in the United States. This lyophilized product contains 1.67 g of sucrose per gram of protein (IgG), and has a shelf life of 24 months at room temperature (≤30°C). Ninety-two percent of the IgG in this product exists as monomers. Carimune® NF can be reconstituted with protein concentration ranging from 3% to 12%. The reconstituted solution has a pH of 6.4 to 6.8.

The osmolality of Carimune® NF reconstituted as a 6% solution in sterile water is 384 mOsm/kg, and of a 12% solution, 768 mOsm/kg. When reconstituted in 0.9% NaCl, a 6% solution is 690 mOsm/kg, and a 12% solution, 1,074 mOsm/kg.

### Glucose

Glucose is a simple sugar used by cells as a source of energy and metabolic intermediate. Most dietary carbohydrates contain glucose; however, the liver and kidneys can synthesize glucose from noncarbohydrate intermediates. Following ingestion and digestion of glucose-containing compounds, glucose is absorbed in the small intestine and may be used directly as an energy source by intestinal, brain, and red blood cells. Under the influence of insulin, the remaining glucose is stored as glycogen, primarily in the liver and muscles. When needed again for energy, liver glycogen is broken down into glucose and released for use throughout the body.

#### Effect on Blood Glucose

Intravenous delivery of glucose produces an appreciable rise in blood glucose and plasma insulin levels. At infusion rates comparable to those used for administration of IVIG, intravenous infusion of glucose in normal subjects produced a rapid and significant elevation of blood glucose and insulin levels. This observation suggests that the use of glucose-stabilized IVIG products may influence diabetic control and insulin requirements in diabetic patients, and that diabetic patients treated with such products may be at risk for hyperglycemia.

#### Effect on Renal Function

Per the CDC report cited previously, glucose-containing IVIG products also were associated with several cases of ARF; it is noteworthy that 56% of reported cases overall involved diabetic patients. Like sucrose, glucose functions as an osmolyte in bacteria and plants. In addition, sugar osmolytes such as glucose, are accumulated by diverse organisms for survival under freezing conditions. In one striking example, glucose plays a central role in the freeze tolerance of wood frogs during winter hibernation. However, these protective functions appear to be species-specific; excess accumulation of glucose has a deleterious effect on proximal kidney tubules in humans.

Glucose has been used as a stabilizer in several IVIG products. Of those available in the United States, only Gammagard S/D (Baxter) is stabilized with glucose. Gammagard S/D is supplied as a lyophilized product, with 96.4% of the IgG existing as monomers. When reconstituted to 5% IgG, the glucose concentration is 2%; at 10% IgG, the glucose concentration is 4%. Gammagard S/D also contains glycine (discussed later) as a stabilizer; at 5% IgG, the glycine concentration is 2.25%, and at 10% IgG, the glycine concentration is 4.5%. The pH range of the reconstituted solution is 6.4 to 7.2. Gammagard S/D has a shelf life of 24 months at room temperature (≤25°C). The osmolality of Gammagard S/D as a 5% solution is 636 mOsm/L, and as a 10% solution, 1,250 mOsm/L.

### Maltose

Maltose (malt sugar) is a disaccharide composed of 2 units of glucose. Maltose itself is not found commonly in food, but is formed through the digestion of starch, catalyzed by amylase found both in human saliva and the intestine (pancreatic amylase). Once formed, maltose is further broken down to glucose by the action of maltase. Maltase is widely distributed in mammalian tissue, including heart, liver, skeletal muscle, and the brush border of
the proximal renal tubules. Therefore, after intravenous administration, maltose is primarily metabolized in the kidney and generally very little is excreted in the urine.

Studies in plants suggest that maltose accumulation protects proteins, membranes, and the photosynthetic electron transport chain inside the chloroplast against heat stress. Maltose has been used as a stabilizer in several IVIG products. At a concentration of 10%, maltose prevented aggregate formation in 10% solutions of IgG within an acidic pH range of 4.1 to 5.3.

**Effect on Blood Glucose**

In contrast to intravenous administration of glucose, infusion of maltose does not result in an appreciable elevation of blood glucose levels in normal or diabetic individuals, due to its renal metabolism and competitive reabsorption of glucose.

**Effect on Renal Function**

Osmotic nephropathy and ARF have been reported in patients following treatment with maltose-stabilized IVIG. A possible mechanism for maltose-induced nephrotoxicity is inhibition of maltase present in the brush border of the proximal renal tubules under conditions of co-existing hyperglycemia. Thus, patients with diabetes with poor glycemic control may be predisposed to ARF after administration of IVIG containing maltose. Nephrotoxicity also may occur in patients with diminished maltase activity secondary to renal tubular damage associated with their underlying disease.

**Effect on Glucose Monitoring**

Maltose-containing IVIG interferes with certain glucose-monitoring systems that are not glucose-specific, resulting in falsely elevated blood glucose readings. These include glucose dehydrogenase pyrroloquinoline quinone (GDH-PQQ) or glucose-dye-oxidoreductase–based monitoring systems. Several cases of inappropriate administration of insulin based on false-positive results using such systems, including 2 fatalities in the United States, have been reported among patients who received maltose-containing IVIG products. Therefore, only glucose-specific monitoring systems should be used in patients with diabetes who are receiving maltose-stabilized IVIG therapy.

Octagam® (Octapharma) is the only maltose-stabilized IVIG product available in the United States. Supplied as a liquid (5% protein), Octagam® contains greater than 99% monomers or dimers, and has a pH range of 5.1 to 6.0. Octagam® has a 10% maltose concentration, with an osmolality of 310 to 380 mOsm/kg. Octagam® has a shelf life of 24 months at room temperature (≤25°C). The labeling for Octagam® contains the appropriate warning regarding the use of glucose-monitoring systems.

**Sorbitol**

Sorbitol (also called D-sorbitol)—a naturally occurring polyhydric alcohol, or polyol, found in stone fruits (eg, apricots, cherries, peaches) and berries—often is used as an artificial sweetener in foods, such as diet drinks and ice cream. Polyols are chemically defined as saccharide derivatives in which a ketone or aldehyde group is replaced by a hydroxyl group; sorbitol is a monosaccharide derived from glucose through the reduction (hydrogenation) of an aldehyde group to a hydroxyl group.

Sorbitol is metabolized within the mammalian liver and kidneys. In the liver, the metabolism of sorbitol may proceed along several pathways: Sorbitol may be oxidized completely to carbon dioxide; converted to fructose or glucose and released for use elsewhere in the body; or converted to glucose and temporarily stored in the liver as glycogen. Only a fraction of ingested sorbitol is excreted in the urine.

**Effect on Blood Glucose**

Multiple in vivo studies in animals and in humans have demonstrated that in contrast to intravenous administration of glucose, infusion of sorbitol does not affect glucose homeostasis (ie, does not produce an appreciable elevation of blood glucose or insulin levels). Additionally, in one study, compared with glucose, sorbitol was utilized (oxidized) much more slowly (100% vs 27%), suggesting that a large fraction of the infused sorbitol was not directly metabolized. Considering the metabolic options for sorbitol, and that only a small fraction is lost in the urine, it is likely that a large portion of the infused sorbitol served for the synthesis of liver glycogen. These results suggest a plausible explanation for the minimal impact of a sorbitol infusion on blood glucose levels.

Moreover, in a clinical study of carbohydrate utilization during pharmacologic suppression of endogenous insulin secretion, sorbitol was oxidized at a higher rate than glucose following intravenous administration, without any significant rise in blood glucose levels. In contrast, blood glucose levels rose during suppression of insulin secretion and even further during glucose infusion. These data indicate that sorbitol does not require insulin to enter its metabolic pathways, and that insulin levels above baseline are not required for its utilization. Therefore, because sorbitol is metabolized in the liver in an insulin-independent fashion, it should not influence diabetic control and insulin requirements in diabetic patients, providing a safe option in such patients. Additionally, there have been no reports of sorbitol interference with blood glucose-monitoring technology nor of any clinical impact on patients.
**Effect on Renal Function**

Compared with IVIG products stabilized with sugars, sorbitol-stabilized IVIG appears to be associated with a lower risk for acute renal failure. As already discussed, the majority of reports of ARF involved sucrose-containing products, and less frequently glucose- or maltose-containing IVIG.34,27,29,30 In one case of ARF involving sucrose-stabilized IVIG, after a return to baseline renal function, subsequent administration of sorbitol-stabilized IVIG was well tolerated.25 One report of reversible ARF associated with sorbitol-containing IVIG was identified in the literature; however, this occurred in a patient with focal segmental glomerulosclerosis and nephrotic syndrome.53

Sorbitol plays a physiologically protective role as an organic osmolyte in the kidneys. In renal medullary cells, osmotic equilibrium with elevated extracellular NaCl concentrations is achieved primarily by intracellular accumulation of organic osmolytes; the principal organic osmolytes in these cells are sorbitol, betaine, inositol, glycerophosphocholine, and taurine. In response to extracellular hypertonicity, sorbitol is synthesized within the kidney from glucose, catalyzed by aldose reductase (AR); hypertonicity increases transcription of the AR gene. When the total intracellular concentration of organic osmolytes is sufficient for osmotic regulation, sorbitol can be metabolized to fructose, catalyzed by sorbitol dehydrogenase.11-13 Thus intracellular accumulation of sorbitol, along with other organic osmolytes, is finely regulated within the kidney, thereby preventing excess accumulation. These dynamics may provide, at least in part, a plausible explanation for the relatively low incidence of ARF when using sorbitol-stabilized IVIG products.

**Hereditary Fructose Intolerance**

Hereditary fructose intolerance (HFI) is a rare genetic disorder of fructose metabolism due to a deficiency of the enzyme aldolase B in the liver, kidneys and small intestine.54 Because sorbitol is metabolized to fructose, patients with HFI should not receive sorbitol-stabilized IVIG.

Two sorbitol-stabilized liquid products are marketed in the United States: Flebogamma® 5% DIF (Grifols) and Gammaplex® (Bio Products Laboratory). Flebogamma® 5% DIF is formulated with 5% sorbitol, a pH ranging from 5.0 to 6.0, and an osmolality of 240 to 370 mOsm/L, which is within the normal physiologic range. The product contains greater than 99.8% IgG monomers and dimers.31 Flebogamma® 5% DIF can be stored at room temperature (≤25°C) for 24 months.

In addition to 5% sorbitol, Gammaplex® is stabilized with 0.6% glycine, and approximately 5 mg polysorbate 80/100 mL. The product’s pH ranges from 4.8 to 5.0, and its osmolality is typically 420 to 500 mOsm/kg. Gammaplex® has a shelf life of 24 months when stored between 2°C and 25°C.

**Glycine**

Glycine is a nonessential amino acid that has both inhibitory and excitatory functions within the central nervous system.55 It also functions as a protective osmolyte in mollusks and crustaceans living at shallow depths and in early mammalian embryos.14,20

Amino acid stabilizers have been employed in IVIG products as an alternative to sugars, and are formulated at a generally lower pH to optimize IgG stability. In one study, 250 mmol/L glycine was less effective than sucrose and maltose in preventing aggregate formation in 10% solutions of IgG within an acidic pH range of 4.1 to 5.3.21

**Effect on Blood Glucose**

Gluconeogenesis refers to the synthesis of glucose within the liver and kidneys from noncarbohydrate precursors, including amino acids. Gluconeogenesis is important in maintaining glucose homeostasis in the postabsorptive state and in hypoglycemia after liver glycogen is depleted.52 Although alanine and glutamine are preferred substrates for the liver and kidneys, respectively, both glycine and L-proline also are glucogenic.52,56,57 Nevertheless, supraphysiologic concentrations of glycine infused intravenously in postabsorptive obese individuals produced no change in plasma glucose, insulin, or glucagon levels.58 No data are available in human subjects with diabetes, although no difference in the rate of gluconeogenesis from glycine was found between fasted normal and diabetic rats.56 Therefore, it appears unlikely that infusion of glycine-stabilized IVIG would have any impact on glucose homeostasis in normal individuals or those with diabetes.

**Effect on Renal Function**

ARF due to osmotic nephrosis has not been reported with glycine-stabilized IVIG. In one report of ARF with documented osmotic nephrosis following treatment with sucrose-stabilized IVIG in a patient with IgA nephropathy, subsequent treatment with glycine-containing IVIG produced no renal impairment.59

Two glycine-stabilized products are marketed in the United States, Gammagard® Liquid 10% (0.25M glycine; Baxter) and Gamunex® (0.16-0.24M glycine; Talecris). Gammagard® Liquid is formulated at a pH range of 4.6 to 5.1, at an osmolality of 240 to 300 mOsm/kg. Gamunex® is formulated at a pH range of 4.0 to 4.5, at an osmolality of 258 mOsm/kg. Both products may be stored for 36 months at 2°C to 8°C. At room temperature (≤25°C), the shelf life of Gammagard® Liquid and Gamunex® is 9 and 6 months, respectively.

**L-Proline**

L-proline is a nonessential amino acid, derived from the amino acid L-glutamate. L-proline functions as a protective...
osmolyte in bacteria, plants, mollusks, and crustaceans, and also accumulates in some freeze-tolerant animals. L-proline has a modest protein-stabilizing effect compared with other osmolytes. At a concentration of 250 mmol/L, L-proline was comparable to maltose, but less effective than sucrose, in preventing aggregate formation in 10% solutions of IgG at a pH range of 4.1 to 6.8. L-proline, however, was more effective in preventing IgG dimer formation than sucrose or maltose. Additionally, L-proline was superior to glycerine in preventing aggregate formation and reducing dimer levels. It has been proposed that the ability of L-proline to both enhance the stability of IgG in solution and reduce dimer formation is due to its amphiphilic nature.

Effect on Blood Glucose

As noted above, L-proline is a glucogenic amino acid. However, a 3-hour intravenous infusion of an amino acid mixture containing L-proline in healthy male volunteers did not result in increased glucose release from the kidneys. Therefore, increased blood levels of glucose are not anticipated following L-proline-stabilized IVIG infusion.

Effect on Renal Function

ARF due to osmotic nephrosis has not been reported after infusion of L-proline-stabilized IVIG. However, pigment-mediated acute kidney injury secondary to hemoglobinuria following administration of L-proline-stabilized IVIG has recently been reported.

Currently, Privigen® (CSL Behring) is the only L-proline-stabilized IVIG product available in the United States. Privigen® is a 10% IgG preparation formulated with 250 mmol/L L-proline at a pH range of 4.6 to 5.0, and an osmolality of 240 to 440 mOsm/kg. The product contains at least 98% IgG monomers and dimers. Privigen® can be stored for 36 months at room temperature (<25°C).

Potential for Neurotoxicity

Privigen® is contraindicated in patients with hyperprolinemia. Hyperprolinemia is associated with neurotoxic effects, including seizures and mental retardation. The mechanism underlying these effects are not clearly defined. However, the pathogenesis of brain dysfunction may involve proline-induced oxidative stress, and inhibition of creatine kinase and Na⁺-K⁺-ATPase activity.

Conclusion

IVIG stabilizers in current use are all organic osmolytes that effectively prevent aggregation of IgG during storage at room temperature. However, their risk profiles with regard to serious adverse events differ. Sugar-stabilized IVIG, particularly with sucrose, poses the highest risk for ARF due to osmotic nephrosis. Sorbitol-stabilized IVIG has a lower risk, possibly due to the physiologically protective role of sorbitol within the kidneys. Amino acid-stabilized IVIG has not been associated with ARF due to osmotic nephrosis. Nevertheless, in patients predisposed to ARF, all IVIG products should be administered according to directions included in their FDA-approved labeling. Only glucose increases blood sugar levels following intravenous infusion; therefore, glucose-containing IVIG should be used with caution in patients with diabetes. Additionally, due to interference of maltose with certain glucose-monitoring technologies, only glucose-specific monitoring systems should be used in patients with diabetes who are receiving maltose-stabilized IVIG therapy. Because sorbitol is metabolized to fructose, patients with known HFI should not receive sorbitol-stabilized IVIG. In addition, because hyperprolinemia is associated with neurotoxicity, the use of L-proline-stabilized IVIG is contraindicated in patients with hyperprolinemia. Finally, the occurrence of pigment-mediated acute kidney injury following administration of L-proline-stabilized IVIG raises new concerns regarding risk factors for severe hemolysis and the potential contribution of the L-proline stabilizer. Hopefully, continued experience with such products will permit an accurate assessment.

Note: Unless otherwise specified, all product-specific information is cited from FDA-approved labeling.

References


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