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Microparticles and Nanoparticles Delivered in Intravenous Saline and in an Intravenous Solution of a Therapeutic Antibody Product

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ABSTRACT

Intravenous (IV) infusion is used for administration of a large proportion of biologic therapeutics, including most monoclonal antibody products. In this study, we determined the subvisible particle levels in IV solutions and after the solutions were processed with an IV administration setup that mimicked the typical clinical method of administration. IV saline in bags manufactured by both Hospira and Baxter contained 1600-8000 microparticles/mL and 4-73 $\times$ 10^6 nanoparticles/mL in solution. When IV immunoglobulin was diluted into the IV saline, 3700-23,000 microparticles/mL and 18-240 $\times$ 10^6 nanoparticles/mL were detected. During processing of the solution through the IV system, in-line filters removed most microparticles. However, there were still 1-21 $\times$ 10^6 nanoparticles/mL in IV saline and 7-83 $\times$ 10^6 nanoparticles/mL in IV immunoglobulin diluted in saline. Finally, in samples processed through in-line filters, we found relatively large microparticles (20-60 μm) that were composed of protein or polycarbonate. These particles resulted from shedding of polycarbonate and sloughing off of protein films downstream from the filter membrane. Overall, the results document that even with in-line filters in place, high levels of subvisible particles are delivered to patients and there is a need for improved, more effective filters and IV solutions with lower particle levels.

Introduction

Therapeutic protein products are commonly administered parenterally, which include intramuscular, subcutaneous, or intravenous (IV) routes of administration. As of 2015, 30 of the 47 monoclonal antibodies (mAbs) on the market were formulated for delivery by IV infusion. Administering therapeutic proteins intravenously can lead to hypersensitivity and infusion reactions in patients, as well as immunogenicity.[1,2] Hypersensitivity and infusion reactions usually occur within 72 h of administration with symptoms ranging in severity from mild headaches, flushing, and itching, to tightness in throat, dizziness, and even anaphylactic shock.[3] For intravenous immunoglobulin (IVIG), which contains IgG molecules from pooled plasma of human donors and is the model protein for this study, incidences of infusion reactions vary from 1% to 81% of patients or infusion cycles.[4] In most cases, these reactions are frequent among patients during initial treatment and decrease over subsequent infusion cycles.[5,6] Immunogenic reactions caused by therapeutic proteins may compromise drug product efficacy because of the production of neutralizing antibodies against the therapeutic protein. More than 20% of the patients experience loss of efficacy during treatment with different mAb products including infliximab, daclizumab, adalimumab, abciximab, and efalizumab.[7]

Protein aggregates and particles resulting from physicochemical instability of drug products represent important factors in eliciting adverse infusion reactions and immune responses.[1,8-11] In an IV infusion system, numerous factors can lead to protein aggregation. Foremost, a diluent incompatible with a therapeutic protein product could cause protein aggregation. Packaging inserts for some mAbs including abciximab, alemtuzumab, and rituximab recommend using either 0.9% saline or 5% dextrose as a diluent. But for mAbs such as panitumumab, pertuzumab, and trastuzumab, saline is the only recommended diluent, and for trastuzumab it is specifically noted that dextrose should not be used as a diluent. Demeule et al.[12] characterized trastuzumab diluted into saline or dextrose, and found much higher aggregate formation in the 5% dextrose solution.

Furthermore, because of the high level of dilution occurring when IV solutions are prepared, protein stability provided by the product formulation is greatly reduced, increasing the likelihood of

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protein aggregation. For example, Kumru et al.[13] found that upon dilution into IV saline, an IgG4 mAb formed soluble aggregates and subvisible particles, which were measured by microflow imaging (MFI) and nanoparticle tracking analysis (NTA). Other studies have reported no changes in protein stability over at least 24 h of incubation after dilution in saline for cetuximab, panitumumab, trastuzumab, pertuzumab, and infliximab.[14-16] However, in these cases either light obscuration or an enzyme-linked immunosorbent assay was used to determine the presence of aggregates or changes in protein concentration, respectively. Such techniques are typically not sensitive enough to detect levels of damage that may account for <0.1% of the total protein concentration, but can still be associated with formation of significant numbers of protein particles.[17,18]

During processing through an IV infusion system, protein drug products are exposed to a number of surfaces—IV bags, IV tubes, in-line filter units, and so on. IV bags are usually made of polyvinyl chloride (PVC) or polyolefin. The IV infusion sets are composed of PVC tubing and silicone tubing in the section on which the pump operates. The connectors are made of polycarbonate. The housing chamber for most filters and the framework on which the filter membrane rests are also made of polycarbonate; and there is typically a tube downstream from the filter membrane made of PVC that connects to the needle. Protein molecules readily adsorb onto such solid surfaces at the liquid-solid interface. For example, factor VIII diluted in saline and stored for 48 h in an IV bag made of PVC resulted in reduction in protein activity, which was attributed to adsorption of the protein molecules onto the bag surface.[19] With all of the solid-liquid interfaces mentioned above, along with the air-water interface in the drip chamber and the IV bag, adsorption of protein molecules can result in the formation of surface films. The sloughing off and mechanical rupture of such films leads to subvisible particles in solution.[20,21] Protein particle formation in IV bags due to protein adsorption to interfaces was also suggested by Kumru et al.,[13] and they observed a reduction in protein particles in the presence of polysorbate 20, an effect attributed to inhibition of protein adsorption to the bag walls and to the air-water interface.

We hypothesized that dilution of formulation excipients and exposure to various interfaces in the infusion system will cause protein aggregation in the form of micro- and nanoparticles. In this study, we started by characterizing IV saline for its micro- and nanoparticle concentration. IV saline bags of 2 sample volumes (100 and 250 mL) from 2 manufacturers (Hospira and Baxter) were examined for particle concentration. In-line filters manufactured by Baxter (BX; pore size 0.2 and 1.2 μm) and CareFusion (CF; pore size 1.2 μm) were also tested for their efficiency in filtering out these particles. Thereafter, we characterized micro- and nanoparticles formed by a model therapeutic protein, IVIG, upon dilution into IV saline and during processing through a conventional infusion system. The IV system was composed of IV saline bag, IV tube, infusion pump, and filter units that are used routinely for clinical administration of therapeutic protein products. Finally, an automated Raman microscope was used to identify some of the microparticles observed in solutions of IVIG that had been processed through the infusion system.

Materials and Methods

Materials

The IV administration arrangement employed an Alaris 8100 pump module and an Alaris 8015 pump controller. IV tubes (CF, serial # 2426-0500), in-line filters (CF: 1.2-μm pore size, serial # 20128E; BX: 1.2-μm pore size, serial # 2C1103, and 0.2-μm pore size, serial # 2C8671), and IV saline bags (Hospira, Lake Forest, IL: 100 mL serial # NDC 0409-7984-23 and 250 mL serial # NDC 0409-7983-02; BX: 100 mL serial # NDC 0338-0049-48 and 250 mL serial # NDC 0338-0049-02) were purchased from various medical equipment suppliers and distributors. Gammagard® (100 mg/mL IVIG, Lot# LE12N107AB, Expiry: May 2016) manufactured by Baxter Healthcare Corporation (Westlake Village, CA) was used as a model protein. The IV tube was made of PVC and was 126″ long. Polycarbonate components of the tube system consisted of 2 male lure lock fittings and 3 y-connectors. The section of the IV tube on which the IV pump operated was made of silicone.

Methods

Processing of Samples Through IV System

In the laboratory, we mimicked the infusion system and the protocol used by the Outpatient Infusion Center at the University of Colorado Hospital (Fig. 1). First, 10 mL of saline was removed from the injection port of the IV bag using a siliconized plastic BD syringe. IVIG was then diluted in this saline and introduced into the IV bag for a final protein concentration of 0.4 mg/mL. Using a non-siliconized syringe, 10 mL of the IVIG saline solution was withdrawn from the bag for particle concentration analysis in the initial IVIG saline sample. The tube was primed using the roller clamp to control the flow rate, ensuring no air bubbles were generated during this step. The primed IV tube was then connected to the infusion pump and the flow was set to 140 mL/h. The solution was then pumped through the tube without an in-line filter in place. Thereafter, 3 different in-line filters (a 1.2-μm CF filter, a 1.2-μm BX filter, and a 0.2-μm BX filter) were sequentially attached to the IV tube to collect filtered IVIG saline samples. Five measurements were conducted on the initial sample collected from the injection

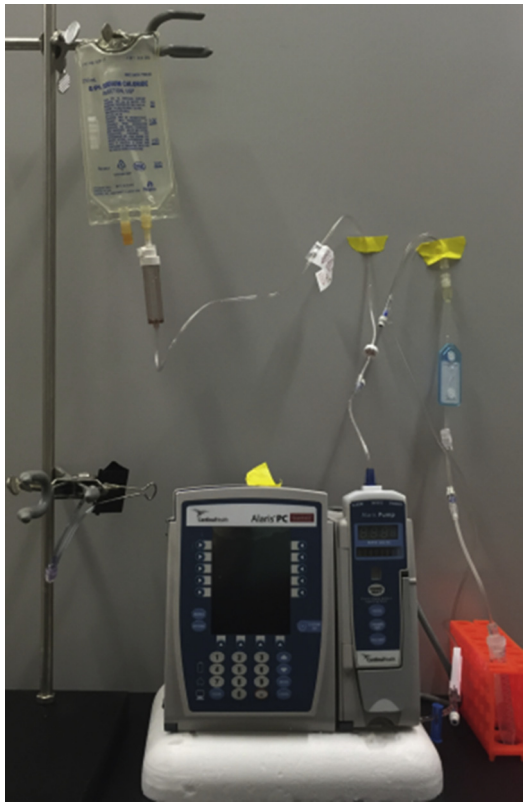

Figure 1. Setup in the laboratory replicating a typical IV infusion system used at the Outpatient Infusion Center of the University of Colorado Hospital.