Reducing or Eliminating Polysorbate Induced Anaphylaxis and Unwanted Immunogenicity in Biotherapeutics.

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ABSTRACT

An increasing use of biotherapeutics across a growing spectrum of neoplastic, autoimmune, and inflammatory diseases has resulted in a corresponding increase in hypersensitivity reactions. The origins of anaphylaxis are often attributed to undefined intrinsic properties of the biotherapeutic protein itself, ignoring the broader potential negative contributions of functional excipients, in particular polyoxyethylene containing surfactants such as polysorbate 80 and polysorbate 20 (Tween 80 and Tween 20). These surfactants allow biotherapeutics to meet the stringent challenges of extended shelf-life, increased solubility, protein aggregation prevention, reduced administration volume, and satisfactory reconstitution properties in the case of lyophilized biotherapeutics. The potential negative impact of certain functional excipients on product performance characteristics such as anaphylaxis and immunogenicity is often overlooked. While regulatory authorities understandably focus heavily on comparable efficacy in evaluating biosimilars, similar efficacy does not necessarily imply a similar safety profile between the originator and biosimilar products. Both unwanted immunogenicity and anaphylaxis do comprise major components of safety assessment, however, few if any attempts are made to differentiate drug-related from excipient-related anaphylaxis. The replacement of anaphylactogenic and immunogenic functional excipients with equally effective but safer alternatives will allow biotherapeutic developers to differentiate their biotherapeutic, biosimilar, or biobetter from the large number of nearly identical competitor products, simultaneously providing a substantial commercial benefit as well as critical clinical benefits for all concerned, that is, patients, physicians, and third party payers.

KEY WORDS: Polysorbate, Tween-80, functional excipient, allergic reaction, anaphylaxis, hypersensitivity, immunogenicity, alkylsaccharide, polyoxyethylene, surfactant

INTRODUCTION

Biosimilars are important contributors to improved healthcare and as more and more are receiving regulatory approval, they are gaining greater acceptance and broader utility. The rapidly increasing use of monoclonal antibodies in the treatment of neoplastic, autoimmune, and inflammatory diseases has resulted in a dramatic increase in hypersensitivity reactions worldwide, complicating the use of first-line therapies and impacting patients’ survival and quality of life (1).
The origins of anaphylaxis are not well understood and are often attributed to undefined intrinsic properties of the biotherapeutic, in spite of the fact that biotherapeutic formulations are necessarily complex, employing a host of functional excipients beyond the biotherapeutic protein itself. These functional excipients are necessary in order to meet the stringent challenges of extended shelf-life, stabilization, solubility, aggregation prevention, especially at the high concentrations typically used to reduce administration volume and time, and in the case of lyophilized products, satisfactory reconstitution properties. The potential negative impact of certain functional excipients on product performance characteristics such as anaphylaxis and immunogenicity is often overlooked. For example, a title search on PubMed, The U.S. National Library of Medicine, using the truncated stem biotherapeutic* in combination with the truncated stems anaphyla* or hypersensit* or allerg* yielded 332 journal articles with biotherapeutic/biotherapeutics in the title, but only a single combination with any of the other stems. Similarly, a title search using the truncated stem biosimilar* in combination with the truncated stems anaphyla* or hypersensit* or allerg* yielded 1218 journal articles having biosimilar/biosimilars in the title, and only a single return was received, in this case relating to anaphylaxis of Glargine, a modified human insulin. Interestingly, Glargine contains a polysorbate surfactant. Two other commonly prescribed human insulins, Humalog and Humulin, do not contain polysorbate surfactants and do not show any reports of anaphylaxis. The NLM MedLine searches were conducted between May 11 and May 15, 2017 and reflect NLM database content within this time frame.

To examine the level of interest in the role of polysorbates, the most common class of surfactant excipients used in formulating biotherapeutics, in connection with anaphylaxis, a title search using the truncated stem polysorbate* in combination with the truncated stems anaphyla* or hypersensit* or allerg* or immunogenic* yielded 362 journal articles with polysorbate/polysorbates in the title, with 4, 4, 1, and 1 in combination with anaphyla* or hypersensit* or allerg*, respectively (i.e., 10 articles returned in total with 9 being unique). Of this small number of articles dealing with anaphylaxis, 6 reported observations of human anaphylactic/allergic responses and three related to in vivo animal studies.

Polysorbates are used in formulations of non-biotherapeutic drugs as well. Norris et al. conducted extensive analysis of the rates of anaphylaxis for the taxanes docetaxel and paclitaxel (2). Docetaxel is formulated with PS-80 and paclitaxel is formulated with Cremophor EL, another polyoxyethylene based surfactant that is not typically used in biotherapeutic formulations but often used in small molecule drug formulations. The taxanes are small molecule drugs, not biotherapeutics, however, like many biotherapeutics, hypersensitivity reactions are common side effects. Because the hypersensitivity reactions do not appear to be IgE mediated, it is hypothesized that the polyoxyethylene-based surfactants are the causative agents.

The reported rate of hypersensitivity reactions with docetaxel is estimated at 30% for patients who do not receive pre-medications such as dexamethasone. Of 67 cases of docetaxel-induced anaphylaxis, 34% represented fatalities. Paclitaxel induced hypersensitivity reactions occur in up to 41% of patients. A more recently introduced taxane, cabazitaxel, also formulated with PS-80, lists severe hypersensitivity in its associated the Black Box warning, and it is suggested that an antihistamine, a corticosteroid, and an H2 blocker be administered prophylactically.

The development cost of a biosimilar is not insignificant and is expected to represent a much larger fraction of the corresponding development cost and ultimate pricing of small
molecule generics. While this has been cited as a negative aspect of biosimilars, it opens the door to another entirely different perspective. Since the development cost in some cases may already be relatively high, the additional cost of modifying the formulation to improve performance, for example by circumventing the use of anaphylactogenic or immunogenic functional excipients, represents a proportionately smaller fraction of the total development cost. And in cases where high development costs will make price competition a less desirable commercial strategy, product acceptance, which ultimately translates to market share, will largely be driven by improved product performance characteristics rather than lower pricing.

The large, and growing number, of nearly identical biosimilars and ‘biobetters’, illustrated in Table 1 (3), provides a significant, if not essential, commercial incentive to differentiate one product from another. It should be noted that the term “biobetter” is not an approved term from a regulatory perspective. No separate regulatory pathway exists for ‘biobetters’ and they are treated essentially as New Chemical Entities. Nevertheless, the term is being used with increasing frequency. A simple intuitive definition is that ‘biobetters’ are improvements to originator biological molecules, offering some degree of superiority over the originator drug and its biosimilar competitors (4). Anour provides an illustrative example of a biobetter in the form of Roche’s anti-CD20 monoclonal antibody obinutuzumab, which has shown superior efficacy in the treatment of chronic lymphocytic leukemia (CLL) compared to its ‘originator’ rituximab (5). Barbosa et al. reviewed the process for the development of biosimilars and ‘biobetters’, focusing on how the various steps impact immunogenicity and the development of anti-drug antibodies (6). While the total market for many of these originator products is substantial, the likelihood that more than a small handful of each competitive biosimilar product will achieve a significant percentage of the total market is slim. ‘Biobetters’ are likely to fare better than biosimilars if a clear benefit can be demonstrated in clinical trials. Minimal, or somewhat esoteric changes, for example related to longer shelf life or the elimination of the need to reconstitute in the pharmacy, are not likely to be persuasive in having a meaningful impact on the purchasing decisions of treating-physicians or institutional purchasers. In contrast, significant improvements, such as reduced or eliminated anaphylaxis and immunogenicity, or reduced likelihood of developing neutralizing antibodies in comparison to the originator biotherapeutic or

<table>
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<tr>
<th>PRODUCT OR PRODUCT CLASS</th>
<th>SALES (US$B)</th>
<th>BIOSIMILARS</th>
<th>BIOBETTERS</th>
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<td>Herceptin</td>
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Table 1 Some major recombinant originator products and classes with biosimilars and biobetters currently in development
alternative biosimilars would likely have a very substantial and beneficial effect on prescribing and purchasing decisions.

**Polysorbates are among the most common functional excipients in biotherapeutics**

Surfactants, such as the polysorbates Tween-80 and Tween-20 (PS-80 and PS-20 respectively) are routinely used to prevent or limit the highly significant and very common problem of protein aggregation (7-19). Polysorbates are highly effective in preventing aggregation but polysorbates suffer from significant limitations. Specifically, polysorbate surfactants are not single or discrete chemical entities. They are complex and highly variable mixtures of fatty acid esters of polyoxyethylene sorbitan with residual amounts of polyoxyethylene sorbitan, polyoxyethylene, and isosorbide polyoxyethylene fatty acid esters, as well as, spontaneously forming protein-damaging hydro- and alkyl-peroxides, epoxy acids, and reactive aldehydes (20-25). Lot-to-lot variability in the concentration of chemically reactive species such as peroxides has been found to exceed an order of magnitude (26).

PS-80 is more prone to the generation of oxidative species compared with PS-20 as a result of the greater content of unsaturated alkyl side chains in PS-80 (27). In aqueous formulations polysorbates can also hydrolyze with an apparent half-life of five months at 40°C (28-30). While aggregation alone is known to cause unwanted immunogenicity, progressive oxidative damage to the protein’s aminoacyl side chains caused by the contaminating peroxides, other reactive species cause neoantigen formation over time further increasing or altering immunogenicity. The peroxides principally damage methionine and tryptophan side chains (31-37). The aldehydes react with primary amino groups on proteins and are known to induce immunogenicity of proteins in the absence of aggregation or adjuvants. The epoxy acids can react with any exposed nucleophile. Oxidative damage also leads to peptide bond hydrolysis, altering protein tertiary structure, yet another source of protein aggregation and releasing free fatty acids which can cause increased turbidity, a separate but not insignificant issue altogether (28,30).

**Polysorbate induced anaphylaxis**

Polysorbates, as well as certain polysorbate degradation products, are intrinsically anaphylactogenic. Examples of hypersensitivity reactions to polysorbates in humans and animal models, have been demonstrated by such indicators as histamine release, hemodynamic effects, skin prick testing, enzyme-linked immunosorbent assay, IgE immunoblotting, flow cytometric detection of basophil activation, complement activation, determination certain humoral factors, and the absence of polysorbate specific IgE (to confirm the non-immunologic nature of the anaphylactoid reactions). These are summarized below.

Anaphylaxis is a serious allergic reaction associated with the administration of some biotherapeutics. There has been minimal research into the specific causes in the case of either the originator or biosimilar biotherapeutics, as indicated above by the small number of articles dealing with the subject. One may reasonably speculate that since proteins are well known initiators of anaphylaxis, the role of other formulation components, such as surfactants in initiating anaphylaxis has generally been overlooked. While polysorbate-induced unwanted immunogenicity is well documented in the literature (30, 38-40) little attention has been paid to polysorbate induced anaphylaxis.

Anaphylaxis symptoms occur over minutes to hours with an average onset of 5 to 30 minutes if exposure is intravenous, as it is for many biotherapeutics, and can affect the skin, respiratory system, gastrointestinal tract, the heart and vasculature, and the central nervous.
system. Symptoms include hives, itchiness, flushing, or swelling (angioedema), swelling of the tongue or throat, runny nose and swelling of the conjunctiva. Respiratory symptoms include shortness of breath, bronchial spasm, and upper airway obstruction (41-47). Coronary artery spasm and associated drop in blood pressure or shock, and subsequent myocardial infarction, dysrhythmia, or cardiac arrest may also occur. In some cases it may cause death (44,45).

Intramuscularly injected epinephrine is the primary treatment for anaphylaxis along with antihistamines, steroids, intravenous fluids, and positioning the person flat (41,46). While regulatory authorities understandably focus heavily on comparable efficacy in evaluating biosimilars, similar efficacy does not necessarily imply a similar safety profile between the originator and biosimilar products. Both unwanted immunogenicity and anaphylaxis do comprise major components of the safety assessment requirements of biotherapeutics, however, few or no attempts have been made to distinguish functional excipient-induced anaphylaxis from anaphylaxis arising from the biotherapeutic protein itself. In the case of monoclonal antibodies, drug hypersensitivity and anaphylaxis have been reported for rituximab, ofatumumab, obinutuzumab, trastuzumab, cetuximab, tocilizumab, infliximab, etanercept, adalimumab, abciximab, golimumab, certolizumab, brentuximab, bevacizumab, and omalizumab, all of which contain a polysorbate surfactant (1).

PS-80 and PS-20, found in more than 70% of monoclonal antibody and other protein biotherapeutic drugs, have now been shown to cause anaphylaxis in patients receiving biotherapeutics. The anaphylactogenic properties of PS-80 are now increasingly well documented in the clinical literature. The precise mechanistic cause, or causes, of polysorbate induced anaphylaxis is complicated by their complex chemical nature. A number of specific molecular species that induce anaphylaxis have been identified in preclinical animal studies. For example, as early as 1985, Masini et al. demonstrated that polysorbate induced histamine release in peripheral tissues and isolated mast cells, as well as hemodynamic responses (47). In 1997, Bergh et al. reported that air exposure of aqueous solutions of PS-80 produced formaldehyde and acetaldehyde (48), the latter shown to react with proteins and produce anaphylactogenic moieties (49) and be anaphylactogenic in amounts that may be eliciting allergic reactions in some individuals. The authors prophetically warned that allergenic compounds are formed during manufacture, storage, and handling of products containing polysorbates and chemically similar surfactants and that this should be taken into consideration by drug developers.

Coors et al. conducted a thorough examination of PS-80 as an inducer of severe anaphylaxis in patients receiving intravenous drug formulations. They employed an extensive complement of well accepted and sensitive detection methodologies including skin prick testing, enzyme-linked immunosorbent assay, IgE immunoblotting, and flow cytometric detection of basophil activation, in control patients and patients with a medical history of anaphylactic shock due to intravenous administration of a multivitamin product as a surrogate for intravenously administered drugs. Polysorbate specific IgE antibodies were not identified in enzyme-linked immunosorbent assay and immunoblot examinations, confirming the nonimmunologic nature of the anaphylactoid reaction. Their study unambiguously demonstrated that PS-80 can cause severe nonimmunologic anaphylactoid reactions (50).

Sun et al. evaluated the sensitization effect of PS-80 from different manufacturing lots in beagle dogs. Varying degrees of anaphylactoid reaction were observed (51). Similarly, Yang et al. assessed 10 batches of PS-80 solutions from various suppliers and found that spontaneously formed PS-80 impurities such as peroxides and
oxidized fatty acid residues, present in varying levels in each of the tested batches, induced anaphylactoid reactions in an *in vivo* zebrafish model (52).

Qiu et al. demonstrated that polysorbate 80 induces typical non-immune anaphylactic reactions (pseudoallergy) in dogs characterized by the release of histamine and unvaried IgE antibodies. PS-80 induced the release of histamine, a 2-fold increase in SC5b-9, a 2.5-fold increase in C4d, and a 1.3-fold increase in Bb, while IgE remained unchanged. PS-80 caused cardiopulmonary distress in dogs and activated the complement system through classical and alternative pathways as indicated in both *in vivo* and *in vitro* assays (53).

With the increasing importance and routine use of a growing number of biotherapeutics, clinical reports describing polysorbate induced anaphylaxis are increasing as well. For example, in two patients receiving omalizumab, reactions after administration have been reported. Intradermal testing produced significant wheal/flare reaction to PS-20 but not in the negative control subject. The *in vitro* and *in vivo* immunologic data support the conclusion that the adverse reactions experienced by the two patients after more than a year of successful omalizumab therapy were likely anaphylactoid in nature (54). An earlier report of unexplained omalizumab anaphylaxis appeared but the possible association with PS-20 was not considered at that time (55).

Patients receiving the red cell growth hormones darbepoietin and erythropoietin developed hypersensitivity reactions. Based on subsequent skin testing and the observed clinical effects it was concluded that the cause of these reactions was due to the excipient PS-80, and that this might have contributed to the incidence of pure red cell aplasia (56).

In a study comparing etoposide formulations with and without PS-80 using the same premedication protocol, the patient exhibited hypersensitivity reaction with the PS-80 containing formulation but none with the etoposide formulation not containing PS-80. The authors concluded that the hypersensitivity reaction was likely due to PS-80 rather than the etoposide itself (57).

Badiu et al. reported multiple cases of PS-80 induced anaphylaxis arising from administration of different vaccines. A female patient experienced generalized urticaria, eyelid angioedema, rhino-conjunctivitis, dyspnoea and wheezing 1 hour after her third intramuscular dose of quadrivalent human papilloma virus vaccine, Gardasil, which contains PS-80 (58). Gardasil also yielded positive intradermal tests, while skin tests with the bivalent vaccine not containing PS-80 were negative. Prick tests performed with PS-80 were positive in the patient and negative in ten healthy controls. The CD203 basophil activation test result was negative for PS-80 at all the tested dilutions and specific IgE was not found. The authors also skin tested patients receiving two flu vaccines, one containing PS-80 (Fluarix, GSK), which resulted in a positive reaction and another flu vaccine with no adjuvant or preservative (Vaxigrip, Sanofi Pasteur MSD), which yielded negative results.

Limaye et al. reported an allergic reaction to erythropoietin which included generalized pruritis, erythema, and orofacial angioedema (59). The Eprex erythropoietin formulation contained recombinant human erythropoietin and PS-80 as excipient (0.15 mg/ml). Skin prick and sequential intradermal testing with increasing concentrations of Eprex and Neupogen (Amgen, Thousand Oaks, CA) also containing polysorbate at 0.04 mg/ml, gave positive reactions, whereas a polysorbate-free erythropoietin preparation yielded negative test results. Intradermal testing with pharmaceutical-grade polysorbate resulted in a positive local reaction followed by mild orofacial angioedema 1 hour later. No reaction was observed in a control subject. Purcell et al. identified polysorbate 80 as the likely cause of
immune response to erythropoietin when human albumin was replaced by polysorbate 80 and glycine (60). In addition to the vaccine examples cited above, polysorbate induced anaphylactic responses have also been reported in non-biologic drug classes containing polysorbate, such as vitamin A, certain steroids, and acyclovir (61, 62).

**Separating mab induced anaphylaxis from PS induced anaphylaxis**

While it is clear that polysorbates can and do induce anaphylactic responses, current clinical trials do not appear to attempt to differentiate polysorbate-induced anaphylaxis from biotherapeutic-induced anaphylaxis. In order to do this, separate vehicle studies would have to be conducted. Considering the fact that anaphylaxis only occurs in a fraction of patients, and patients as a whole will likely have had varying exposure histories to polysorbates for earlier, and perhaps unrelated, disease treatments, selecting multiple control cohorts, i.e., no previous polysorbate exposure versus previous polysorbate exposure over varying timeframes, is difficult and prohibitively expensive and would add further costs to the already expensive clinical trial and regulatory approval process. Substituting alternative stable, non-chemically reactive, non-anaphylactogenic surfactants such as alkylsaccharides (38, 40, 63, 64) in biotherapeutics, and comparing anaphylaxis rates in clinical trials would begin to answer this question.

**CONCLUSION**

Replacing polysorbates with surfactants that minimize anaphylaxis episodes, and at the same time, do not result in progressive protein degradation and increased immunogenicity, would meet a critical need while providing a substantial differentiating clinical benefit for all concerned, that is, patients, physicians, and third party payers. The most advanced alternative appears to be a class of non-ionic surfactants termed alkylsaccharides. Alkylsaccharides are comprised of a sugar coupled to an alkyl chain (35, 37, 59, 60). While certain alkylsaccharides are GRAS for use in food and cosmetic products, no biotherapeutics employing alkylsaccharides have yet been approved. Impediments to approval of any functional excipient, including potential alternative surfactants, include the significant costs of demonstrating functional effectiveness, safety and tolerability, as well as the cost of establishing and qualifying multiple GMP manufacturing sources in order to guarantee an uninterrupted supply during manufacturing.

‘Biobetters’ may offer the benefit of earlier market entry compared to the corresponding biosimilar because the product launchability date for a biosimilar is determined by the latest relevant patent expiration date as well as any data and market exclusivity extensions. Because ‘biobetters’ involve full approvals, they are not affected by data exclusivity, so that their launchability dates would be determined solely by the latest date of patent expiration or market exclusivity grants (3).

The clinical and commercial incentives to replace polysorbates are clear. For originator biotherapeutics, minimization of anaphylaxis would offer significant clinical and safety benefits to patients, possibly reducing the time and cost of pretreatment with antihistamines and steroids. In the case of biosimilars, risks associated with high development cost, continued pricing uncertainty, with pricing estimates of ranging from 25% to 75% of the originator product price (65), and the large number of competitor products, with over 900 biosimilars and 600 ‘biobetters’ currently in development, the ability to differentiate a product based upon improved clinical or safety characteristics, may be critical to commercial success. Commercial success is key to achieving the objectives of reduced biotherapeutic cost and increased patient accessibility. The ability to offer patients some clear and substantial clinical benefit would likely facilitate the physician’s decision or recommendation to switch a patient.
away from a well characterized originator product to a newly introduced ‘biobetter’.

CONFLICT OF INTEREST STATEMENT

Dr. Maggio is the CEO of Aegis Therapeutics LLC (Aegis), as well as, a shareholder, and serves on its Board of Managers. Aegis commercializes drug formulation technologies based upon the use of alkylglycosides which were developed at the University of Alabama Medical Center, Birmingham, Alabama, by Professors Dennis Pillion and Eli Meezan. Issued Aegis patents cover the use of alkylsaccharides as absorption enhancers for peptide and protein drugs via intranasal and oral routes, and the prevention of aggregation of protein and peptide-based drugs, including monoclonal antibodies. Aegis is a party to a number of research and commercialization licensees with multinational pharmaceutical companies active in the biotherapeutics field.

REFERENCES


18 Purohit VS, Middaugh CR, Balasubramanian SV. Influence of Aggregation on Immunogenicity of


65 How cheap will biosimilars need to be Posted. 0 5 / 0 8 / 0 2 0 0 1 1 , http://gabionline.net/Biosimilars/Research/How-cheap-will-biosimilars-need-to-be.