Safety of Biotechnology Products

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Introduction

Medicines based on the application of recombinant technology are relatively new to the therapeutics industry. This chapter will focus specifically on purified and recombinant proteins, collectively termed protein therapeutic agents (Gallupi et al., 2001), used in the treatment of disease that are able to affect the growth or metabolism of cells in vivo. This rather broad definition includes proteins derived from a variety of different sources, including the purification of proteins from various sources (e.g. serum and urine), and proteins created using recombinant DNA technology in either bacterial or mammalian cells, including hormones, enzymes and antibodies. This is in contrast to the traditional method of developing medicines by the application of organic chemistry; medicines developed in this manner are usually referred to as ‘small molecules’. It should be noted that other molecules, such as steroids and vaccines, as well as gene therapy techniques and various diagnostic tests, might also be considered biologics. However, for the purposes of this discussion, these molecules will be excluded from this chapter; vaccines will be discussed in the Chapter 14.

Protein therapeutic agents have had an enormous impact on the treatment of many diseases that previously did not have adequate therapies. For example:

- Prior to the widespread availability of therapeutic human growth hormone, children with growth hormone deficiency had no viable treatment options, and uniformly developed short stature as adults (Mehta and Hindmarsh, 2002).

- Prior to the development of recombinant granulocyte-colony-stimulating factor (G-CSF), patients with severe chronic neutropenia had no protection against bacterial infections and usually succumbed to the disease at a young age. With the advent of G-CSF, these patients are now able to lead much more normal lives (Cottle et al., 2002).

These conditions, along with many others, are now successfully treated with protein therapeutic agents. Now that the human genome has been successfully mapped (International Human Genome Sequencing Consortium, 2001; Venter et al., 2001), newer, perhaps more complex recombinant proteins will be developed to treat diseases that are currently untreatable. However, with the development of new therapeutics comes a need to increase
our understanding of the differences in safety profiles seen with these molecules from those seen with traditional small-molecule therapeutics. Clinicians in particular need to be aware of a few classes of adverse reactions seen most commonly with biological products.

Properties of proteins

To be able to understand adequately and anticipate the types of adverse reactions that are likely to be observed for new protein therapeutic agents, it is necessary to have a fundamental understanding of the general properties of proteins. There are several important differences between small molecules and proteins created from the application of biotechnology. As many companies are now using biotechnology to develop new medicines, it is imperative that these properties, as contrasted with those of small molecules, are understood by research and development personnel, as well as healthcare providers, so that patient safety is optimal.

Proteins, which are composed of specific sequences of amino acids, are the building blocks of all life. In general, protein therapeutic agents are large molecules composed of many amino acids. However, the size of currently available biotechnology-based medicines ranges from a few amino acids for small oligopeptides, such as sermorelin, which is a 29 amino acid amino terminal segment of naturally occurring growth-hormone-releasing hormone (GHRH), to large proteins with hundreds of amino acids. For example, the \( \alpha \) and \( \beta \) chains of recombinant follicle-stimulating hormone (FSH) have 92 and 111 amino acids respectively. The amino acid backbone of these molecules means that they are highly degradable by the various proteases released by the stomach and small intestine during digestion, effectively decreasing the bioavailability of orally administered proteins to zero. Therefore, proteins must be given by injection, generally by the intravenous, subcutaneous, or intramuscular routes. Because of the routes of administration necessary to administer these molecules, patients must be closely monitored for the onset of hypersensitivity reactions (discussed in more detail below).

Proteins produced by the human body, as well as those created in mammalian cells using recombinant DNA technology, may contain carbohydrate modifications to the amino acid backbone (Lehninger et al., 1993). The process of adding carbohydrate moieties to the protein structure, called post-translational modification, begins in the endoplasmic reticulum and is completed in the Golgi complex. These are cellular organelles found only in cells of eukaryotic organisms (i.e. those that contain a nucleus). Therefore, recombinant versions of naturally occurring proteins produced in prokaryotic cells, such as bacteria, lack these carbohydrate additions. Some molecules, such as recombinant human growth hormone, have been successfully and safely developed using both bacterial and mammalian cells.

Another property of proteins is that they are often somewhat unstable and are prone to denature, which means that they lose their structural conformation and, therefore, efficacy, under specific known physical conditions. For example, this can occur if the storage temperature is not maintained within a tight range and the molecule becomes too warm. Many of these products require refrigeration to ensure that they do not denature.

Categories of protein therapeutic agent

Protein therapeutic agents can be classified in several ways. The most obvious way is to organize the molecules by function. Currently approved protein therapeutic agents include:
• hormones and cytokines (or smaller sequences of amino acids that retain the properties of the naturally occurring hormone);
• enzymes; and
• antibodies, both monoclonal and polyclonal.

However, for the purposes of safety monitoring, proteins can also be organized by manufacturing method. Currently, proteins are either purified from various sources, such as serum and urine, or are created in bioreactors using recombinant DNA technology.

Grouping by function

The most familiar proteins used in a therapeutic setting are recombinant versions of naturally occurring hormones. Hormones are molecules released from various glands in the body that act on target organs or cells. Examples include insulin and human growth hormone. Recombinant versions of both of these hormones were developed in the 1970s, early in the biotechnology era. Since that time, recombinant versions of other naturally occurring hormones have been developed, including recombinant erythropoietin and recombinant FSH. These recombinant molecules have very specific actions \textit{in vivo} and normally have well-characterized safety profiles.

More recently, recombinant versions of naturally occurring cytokines have been developed using recombinant DNA technology, such as various interferons and growth factors. Examples include recombinant G-CSF and various types of recombinant interferon. Similarly, molecules have been developed to block the actions of naturally occurring cytokines, such as recombinant IL-1 receptor antagonist (Bresnihan, 2001) and etanercept, which is a hybrid molecule (used to treat active rheumatoid arthritis) that contains the extracellular ligand binding portion of the tumour necrosis factor receptor bound to the Fc portion of human IgG1 (Alldred, 2001).

Enzymes are proteins that catalyse chemical reactions in the body. Several recombinant versions of enzymes have been developed or are being developed to treat rare genetically inherited disorders, such as alpha galactosidase A for the treatment of Fabry disease and iduronate-2-sulfatase in Hunter syndrome.

Antibodies are glycoproteins that are created by B cells against foreign antigens. Therapeutic antibodies are of two types: monoclonal and polyclonal. Monoclonal antibodies arise from a single clone of cells and are specific for a single antigen. Rituximab is a monoclonal antibody directed specifically against the CD20 antigen found on normal and malignant B cells and is used to treat non-Hodgkin’s lymphoma. Polyclonal antibodies arise from multiple clones and interact with many antigens. For instance, intravenous immune globulin can be used to provide passive immunity to infectious agents, such as hepatitis A (Stapleton, 1992).

Organization by manufacturing method

The earliest proteins to be used in a therapeutic setting came from the purification of proteins from various sources, including serum, urine, and even organs. Immune globulin (pooled immunoglobulin from donor serum) has been available since the 1950s. Similarly, for many years, urine-derived FSH was used to treat female infertility. Pituitary-derived human
growth hormone, which was derived from pooling growth hormone from the pituitary glands of cadavers, was for years the only source of human growth hormone. Depending on the level of purity achieved, the possibility exists for contamination, including by proteins and infectious organisms. Most products that were originally derived from purification are now manufactured using recombinant technology.

The biotechnology era truly began in the late 1970s, when recombinant insulin was first produced in 1978, followed by recombinant human growth hormone in 1979. Whereas prior to this time, any protein that was used for therapeutic purposes was derived from purifying it from a biological source (see previous section), now the technology became available to insert the gene for a protein into prokaryotic (i.e. bacteria) host cells. Next, technology was developed that enabled eukaryotic cells, such as Chinese hamster ovary cells, to be used as host cells. Since that time, many different proteins have had their genes sequenced and expressed. The use of recombinant DNA technology during the past two decades has revolutionized the practice of medicine.

The earliest host cells were bacteria, as the generally much smaller genomes of prokaryotic organisms were much easier to manipulate. However, because they are simple one-celled organisms that lack a nucleus, as well as cellular organelles such as the endoplasmic reticulum and Golgi complex, they also lack the capacity to post-translationally modify proteins. This means that protein therapeutic agents produced by bacteria will not contain the carbohydrate modifications found on recombinant proteins produced by eukaryotic organisms. Also, as the protein must then be purified from the bacteria, the finished product could theoretically contain bacterial breakdown products, such as bacterial cell walls, that can result in toxic side effects (Lauta, 2000).

Today, many of the more recent protein therapeutic agents have been developed using eukaryotic cells, such as Chinese hamster ovary cells. As previously discussed, eukaryotic cells contain nuclei and cellular organelles that are able to post-translationally modify proteins. Post-translational modification allows the glycosylated recombinant protein to resemble the naturally occurring protein more closely.

Safety monitoring for protein therapeutic agents

Owing to the above properties of protein therapeutic agents, and the differences cited between them and small molecules, some general observations can be made regarding the safety profiles of proteins as a class. By grouping together various types of protein therapeutic agent, including hormones, cytokines, enzymes and antibodies, and looking at their common characteristics, it is possible to recognize certain categories of adverse reactions that are common to all groups. Whereas individual molecules will obviously have unique overall safety profiles, these categories of adverse reactions must be carefully monitored to ensure the safety of patients. These categories, which are discussed in detail below, include the following:

- allergic and hypersensitivity reactions;
- infectious disease transmission;
- decreased efficacy secondary to neutralizing antibody development; and
- adverse reactions related to the efficacy of the molecule itself.
It should be noted that, practically speaking, there is no difference in the regulations that govern safety monitoring of protein therapeutic agents or small molecules. Some biologics, such as vaccines, and methods of treatment, such as gene therapy, require specific pharmacovigilance efforts as defined by regulatory agencies, and certain events, such as the development of neutralizing antibodies, require specific assays to confirm a diagnosis. However, the therapeutics described below are managed in the same way, from a regulatory perspective, as small molecules, and the monitoring procedures are essentially the same between the two.

Allergic and hypersensitivity reactions

As previously discussed, proteins are generally much larger than traditional small molecules and are easily digestible by the gut. Therefore, they must be administered by injection. While this decreases the potential of direct irritation of the gastrointestinal tract when compared with orally administered products, adverse reactions associated with the injections themselves are relatively common. These reactions can range from local oedema, erythema and pain at the injection site to injection site necrosis, severe rashes, including erythema multiforme and Stevens–Johnson syndrome, and anaphylactic shock, possibly leading to death. The mechanisms of these reactions vary from local tissue irritation and release of inflammatory mediators at the injection site to systemic reactions that involve antibody production.

The types of hypersensitivity seen with protein therapeutic agents that involve antibodies are:

- type I (immediate) hypersensitivity, and
- type III, or immune-complex hypersensitivity.

Type I, or IgE-mediated, hypersensitivity usually occurs after re-exposure to the protein and occurs within minutes of injection, when the antigen binds to IgE on the surface of mast cells, causing release of inflammatory mediators such as histamine. The signs and symptoms of type I hypersensitivity can range from urticaria and asthma to systemic anaphylaxis, which is characterized by laryngeal oedema, bronchoconstriction and hypotension, and can be fatal. Whereas reports of type I hypersensitivity are rare for most products, owing to the serious nature of these reactions, patients must be closely monitored for any evidence of anaphylaxis and appropriate treatments should be readily available.

Immune-complex hypersensitivity occurs when antibody binds to antigen and subsequently precipitates out of solution and is deposited in tissues, causing an inflammatory response. Antithymocyte globulin (ATGAM) is a collection of equine-derived polyclonal antibodies against human thymocytes. These antibodies are produced by repeatedly inoculating horses with human thymocytes, which causes the horse to produce antibodies against the thymocytes. These antibodies are then purified from the horse serum. Antithymocyte globulin is used primarily to treat acute renal allograft rejection. As these antibodies are not of human origin, hypersensitivity reactions are of particular concern, including serum sickness. When given to humans, the patient may develop ‘serum sickness’, which is a form of type III hypersensitivity characterized by fever, urticaria, joint pain, lymphadenopathy and splenomegaly. The symptoms usually develop between 1 and 2 weeks after administra-
tion of ATGAM. As the immune complexes are cleared from the tissues, the symptoms begin to resolve (Lawley et al., 1984, 1985).

Even though recombinant versions of naturally occurring proteins are chemically very similar to the native proteins, allergic reactions can occur when these recombinant proteins are administered. Although these products are made using sophisticated recombinant technology, there still exists the possibility that the process of extracting the protein from the cellular reactors leaves some impurities in place. It is also possible that the excipients present in the formulation are able to elicit an allergic reaction.

**Infectious disease transmission**

Because several protein therapeutic agents, especially older products, are derived from purifying the target protein from a biological source, infectious disease transmission must be monitored very carefully. Monitoring of these types of reaction is complicated, because many of the infectious diseases contracted from the administration of tainted product, such as human immunodeficiency virus (HIV) and hepatitis C, may not manifest themselves for months, even years, after exposure. Also, these diseases are often difficult or impossible to treat effectively once they are diagnosed. Therefore, safety monitoring is crucial.

Perhaps the most well-documented example is the transmission of HIV to haemophiliacs through contaminated blood products. Patients with haemophilia A, the most common type, have extremely low circulating levels of factor VIII, causing them to be unable to develop clots effectively. Prior to the development of recombinant DNA technology, factor VIII was obtained by pooling sera from multiple donors, as it is normally present in small quantities in the blood. The availability of factor VIII concentrate had a profound impact on these patients’ lives. Unfortunately, in the early 1980s, a majority of patients with severe haemophilia A were infected with HIV, and ultimately died of AIDS, as there were no effective treatments of the disease at that time (Koerper, 1989). Since then, techniques have been developed to reduce or, in the case of recombinant factor VIII, eliminate the transmission of all infectious diseases, including HIV (Horowitz and Ben-Hur, 2000).

Another prominent example is pituitary-derived growth hormone and the transmission of Creutzfeldt–Jakob disease, which is classified as a transmissible spongiform encephalopathy (TSE). Prior to the 1980s and the advent of recombinant DNA technology, human growth hormone for therapeutic use was only available via pooling and purifying the pituitary glands of cadavers. Unfortunately, in 1985, the Food and Drugs Administration and the National Institutes of Health received the first three reports of patients that had developed Creutzfeldt–Jakob disease, which is a uniformly fatal neurological disease that has been associated with a prion infection of the brain (Fradkin et al., 1991; Preece, 1993). In these cases, it was discovered that the disease was transmitted through contaminated human growth hormone extract from the cadaver pituitary glands. These patients, who were adults at the time of diagnosis, had been treated with pituitary-derived growth hormone as young children, and had not developed symptoms of the disease for years after exposure. Human growth hormone is no longer available as a pituitary gland extract, and is now manufactured using recombinant DNA technology.

TSEs may also be transmitted from animal sources. Products containing bovine gelatin, tallow derivatives and serum are of particular concern. There has been increasing interest in this area due to recent reports of the development of bovine spongiform encephalopathy (BSE), or mad cow disease, in parts of Europe after ingesting infected beef. To date, there
have been no reports of patients developing a TSE after exposure to a biologic that contains animal by-product. However, because it is currently thought that all reports of BSE received to date have been transmitted orally, and it has been estimated that intravenous and subcutaneous inoculation of a particular pathogen responsible for a TSE is up to 100–1000-fold more potent than an oral inoculation, there is cause for concern (Rohwer, 1996). As the TSE agents are extremely difficult to detect and remove from animal tissue, it is of the utmost importance that all steps are taken to ensure that no infected animals are used in the manufacturing process (Asher, 1999). In spite of the fact that no reports have been seen, it is prudent to monitor both proteins and small molecules that contain animal-derived excipients for events suggesting possible prion transmission.

For immune globulin there is also an increased risk of transmitting blood-borne infectious diseases, such as hepatitis C. For instance, one preparation of IVIG resulted in 137 reported cases of hepatitis C infection (Bresee et al., 1996), prompting the manufacturer to recall the product. The manufacturer replaced the original preparation with one containing a solvent and detergent, which is effective in ridding the immune globulin of infectious agents. Immune globulin preparations are now considered to be safe with respect to known viruses, but companies and healthcare professionals must remain vigilant in monitoring for reports of infectious disease transmission.

Development of neutralizing antibodies

Another major safety concern for all injectable proteins, especially for recombinant versions of naturally occurring human proteins, is the development of neutralizing antibodies against the protein. This can occur because the body recognizes the injected protein as foreign and develops antibodies against it. In the case of naturally occurring substances, such as hormones and cytokines, the antibodies may cross-react with the native protein. These antibodies have the potential to neutralize the actions of the native protein by binding to the active site of the protein. This can lead to profound medical conditions, such as aplasias of blood cell lines.

Patients with chronic renal failure are unable to produce adequate endogenous erythropoietin to maintain normal haemoglobin levels (Eckhardt, 2000). As a result, these patients develop anaemia, causing them to feel fatigued and short of breath. The introduction of recombinant erythropoietin to treat anaemia has helped patients maintain adequate haemoglobin levels and reduce their need for transfusions. Beginning in 1999, however, there has been a significant increase in the number of cases of pure red cell aplasia (PRCA) in patients receiving epoetin alfa in Europe, Australia, and Canada (Casadevall et al., 2002; Gershon et al., 2002). PRCA is a condition in which there is an absence of erythroid precursors in the bone marrow, causing patients to develop profound anaemia requiring red cell transfusions to maintain haemoglobin levels. An increased incidence of PRCA has not been observed with erythropoietic agents or in other geographic areas. The majority of these patients have developed neutralizing antibodies to this treatment, which cross-react with the patient’s endogenous erythropoietin, as well as with other erythropoietic agents. At this time, the reason for this outbreak of cases is not known. Although data on the treatment and outcome of these patients is limited at this time, some of these patients remain transfusion dependent for years after diagnosis.

The availability of factor VIII has made living with haemophilia far more tolerable. However, 25 to 50 per cent of patients with severe haemophilia A that require treatment with
factor VIII concentrate develop antibodies (called ‘inhibitor’) against factor VIII (Lusher, 2000). These antibodies may neutralize the action of the factor VIII therapy and can greatly complicate treatment. Patients may require enormous doses of factor VIII during the treatment of haemorrhagic episodes, and some of these patients might not respond to at all. Once other potential aetiologies for non-response have been excluded, neutralizing antibodies should be considered.

**Adverse reactions related to efficacy of the protein**

Patients can experience adverse reactions as a direct result of the mechanism(s) of action of the recombinant version of a naturally occurring protein. In effect, these patients develop adverse reactions that result from the efficacy of the molecule itself, sometimes resulting in syndromes similar to genetic or malignant processes. For example, recombinant proteins have been developed to treat rheumatoid arthritis by blocking the effects of inflammatory mediators, such as tumour necrosis factor. Although these products are efficacious in relieving the pain and inflammation associated with rheumatoid arthritis, they also can increase the incidence of serious infections in these patients by blocking these inflammatory mediators and thereby decreasing their ability to develop an appropriate immunologic response against infection (Keane et al., 2001). Patients are at increased risk of developing serious infections, possibly requiring hospitalization. Some patients have also developed opportunistic infections normally seen only in severely immunocompromised patients (i.e. patients with HIV).

Infertility is routinely treated with injections of FSH and luteinizing hormone to stimulate the ovaries to develop multiple follicles, in preparation for *in vitro* fertilization. During the process of *in vitro* fertilization, high doses of FSH are given to the patient daily during the early part of her menstrual cycle. Because of the high doses, a common, treatable, but extremely serious associated condition is the ovarian hyperstimulation syndrome (Whelan and Vlahos, 2000). The mechanism for this syndrome is unknown, but the patient develops increased vascular permeability, which manifests itself in pleural and pericardial effusions, ascites and extreme hypovolaemia, predisposing the patient to intravascular thrombosis. Patients are hospitalized and treated with fluids; infertility injections are stopped. Once the patient’s FSH levels begin to decrease, the patient usually fully recovers. However, it is imperative that the condition be diagnosed and treated early by a physician experienced in this field.

**Off-label use**

The use of therapeutic agents for indications other than those that have been approved by the appropriate regulatory agencies is a significant safety issue for all pharmaceutical products, not just proteins. Once an agent has been approved for one use, a treating physician is free to prescribe the drug for any indication that he or she chooses, with few restrictions. However, these drugs are also often obtained and distributed illegally. In the case of some biologics, often the aim is to give supraphysiologic doses to people to increase one’s physical strength or endurance. In other cases, the use is based on a perceived benefit to the patient that has little or no scientific data to support its use. In either case, this is a
major area of concern in safety monitoring for biologics, and is likely to continue to increase in frequency.

**Enhanced athletic performance**

One of the most commonly misused biologic classes is anabolic steroids, which are commonly used illegally to increase strength in athletes (VanHelder et al., 1991; Silver, 2001). Though technically not proteins, these molecules help illustrate the types of safety monitoring that must be done when there is the potential for off-label abuse. Anabolic steroid use has been banned from most major sports worldwide since the mid-1980s, when use was considered to be rampant among professional and Olympic athletes. However, every year, several players receive mandatory suspensions for testing positive for steroid use. It is also becoming increasingly popular among adolescent athletes (Bahrke et al., 1998). The side effects of anabolic steroid abuse can be devastating, especially to younger athletes who are still growing. The side effects range from skin disorders, such as acne or increased rate of hair loss, to testicular atrophy, hypertension, amenorrhea, cardiac disease, and even mental disturbances, such as ‘roid rage’, characterized by aggressive behaviour and uncontrollable temper. Although some of these side effects can be reversible, many, such as cardiac disease, are not, and may result in an increased risk of early death (Lamb, 1984; Strauss and Yesalis, 1991).

Athletes looking for increased muscle mass and decreased body fat have also misused recombinant human growth hormone (Bidlingmaier et al., 2001). However, the long-term adverse event profile of supraphysiologic doses of human growth hormone is not well defined. In patients with AIDS wasting, a syndrome characterized by loss of muscle mass and total body weight in patients with advance HIV infection, high-dose daily injections of recombinant human growth hormone can help reverse some of these effects (Mulligan et al., 1999). In these patients, however, new-onset hyperglycaemia, to the point of diabetes mellitus, has been reported. Also, these patients tend to develop increased tissue turgor from fluid retention, sometimes precipitating carpal tunnel syndrome (Van Loon, 1998).

In contrast to athletes looking to increase strength, endurance athletes have been known to use recombinant erythropoietins to increase their haemoglobin concentrations to supra-physiologic levels, increasing oxygen carrying capacity and giving the athlete an edge in distance competition. Use of these drugs in distance cyclists and other endurance athletes, such as cross-country skiers, is well documented (Simon, 1994; Gareau et al., 1996). The side effects associated with increased haemoglobin levels are similar to those seen in patients with polycythaemia, including an increased risk of hypertension, myocardial infarction, stroke and sudden death (Sawka et al., 1996).

**Other off-label use**

In contrast to athletes using biologics for a performance advantage, use of some biologics is becoming more common for conditions that have very little scientific data to back them up. For instance, in the USA, some physicians are prescribing human growth hormone injections for middle-aged patients, citing the ‘anti-aging’ benefits of growth hormone, including its ability to increase muscle mass and decrease body fat (Cuttica et al., 1997). Although human growth hormone does in fact have these physiologic actions, there is little evidence to suggest that these properties in any way retard the aging process. There have also been
reports of patients being given growth hormone injections before and after surgery to help ‘increase healing’ (Carli et al., 1997). Also, it is unclear what benefit, if any, these patients derive at the doses given, considering that the patients usually have normal growth hormone levels for their age. At the same time, these patients are exposing themselves to all of the potential risks of taking protein therapeutic agents, including those cited throughout this chapter.

Conclusion

Protein therapeutic agents are still relatively new compared with their small-molecule counterparts. Whereas small molecules extracted naturally from plants have been used to treat all sorts of maladies for centuries, proteins have only begun being used therapeutically beginning in the 20th century, with recombinant technology only becoming available within the last 25 years. However, with the human genome recently being sequenced, it is anticipated that many new therapeutics, including both proteins and small molecules, will be developed with specific targets over the coming decade. Knowledge of the specific properties and safety profiles of proteins as a class will become increasingly more important for all therapeutics companies, as many traditional pharmaceutical companies develop their own in-house biotechnology divisions — or merge with companies that already have the expertise. Although each individual molecule will obviously have a unique side effect profile, having a working knowledge of the types of adverse reactions to be expected from proteins as a therapeutic class will ultimately benefit patients and companies alike.

References


