Chapter 1

Formulation and Delivery of Proteins and Peptides

Design and Development Strategies

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The success of most peptide and protein drugs is dependent upon the delivery of the biologically active form to the site of action. In the design and development of formulations to achieve this goal, the formulation scientist must consider the clinical indication, pharmacokinetics, toxicity, and physicochemical stability of the drug. The development of a stable formulation is a necessary step for each new protein or peptide therapeutic. The degradation pathways and their impact on stability should be systematically analyzed and competing degradation rates must be balanced to arrive at the most stable formulation possible. Several routes of administration should also be considered and future development of new formulations may expand the number of potential options. Formulations for each route of administration may be unique and, therefore, have special requirements. In the case of depot formulations, there are many potential matrices, each of which has distinct characteristics that affect its interactions with the drug and its behavior *in vivo*. The formulation characteristics may have a dramatic impact on the *in vivo* stability of the drug as well as the pharmacokinetics and pharmacodynamics. The optimization of formulations, the routes of delivery, the design of depot systems, and the correlation between physicochemical stability and *in vivo* behavior are discussed in detail with recent examples. For new biotechnology-derived drugs including nucleic acids (DNA vectors and antisense RNA) to reach commercialization, all of the issues involved in the design and development of a drug formulation must be considered at an early stage of the overall development process.

Many aspects of biopharmaceutical process development have been well studied over the past twenty years. Difficulties in fermentation, cell culture, and, to some extent, purification and recovery have largely been overcome and these process steps have been well characterized for the production of many protein pharmaceuticals. However, one important field lags behind these others in its development. The design and production of protein and peptide drug formulations is not well developed and many of the mechanisms for stabilization and delivery of these drugs have not been

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determined. In many cases, companies may initially neglect formulation and stability issues, resolving to simply store proteins or peptides in phosphate buffered saline or other solutions that have not been optimized for stabilizing the drug. Several unknowns still exist when developing a stable dosage form for peptides and proteins. Each molecule has its own unique physical and chemical properties which determine its *in vitro* stability. The formulation scientist must also be concerned about the *in vivo* stability of the drug. Thus, the development of successful formulations is dependent upon the ability to study both the *in vitro* and *in vivo* characteristics of the drug as well as its intended application.

Effect of Formulation Design and Delivery on Drug Development

As shown in Figure 1, a formulation scientist is confronted with a complex decision in choosing a formulation for delivery of a therapeutic protein or peptide. In the literature, the most common discussions of protein and peptide formulations focus on the physicochemical stability of these molecules. Indeed, the properties of the drug molecule are critical in determining the appropriate formulation for successful delivery and stability. The vast majority of the literature on protein and peptide formulations describes the degradation pathways for the drug. Many degradation pathways have been well characterized and, in some cases, degradation may often be predicted from the primary sequence of the protein or peptide (see *1* for examples). Once the formulation scientist has found a set of conditions that provide extensive stability (>2 year shelf-life), the formulated drug is tested in animal models for toxicity and pharmacokinetics. In many cases, this testing phase does not occur until the drug has moved from research into development. At this stage, many problems can occur including poor bioavailability due to the instability of the drug *in vivo,* rapid clearance, or the distribution of the drug in the body. Furthermore, an attempt is often made to resolve these difficulties by administering excess drug to achieve the desired biological effect. However, excessive drug doses often lead to toxicity problems. By this stage, the development of the drug has reached a critical decision point. The tendency in most organizations is to reconsider the development of the drug, sometimes resulting in the 'death' of the development project. However, the formulation scientist has the unique opportunity to work with the scientists in pharmacokinetics and toxicology to 'save' the development of the drug. By altering the formulation or the route of delivery, a drug can often have another opportunity to reach the stage of an Investigational New Drug (IND) filing. Unfortunately, the formulation scientist may not become involved until the drug has already encountered difficulties in animal studies. Thus, it is essential for the formulation scientist to work closely with the discovery research team, the pharmacokinetics department, and the toxicology department prior to the decision to move the drug into full scale development.

After all the difficulties are resolved in the early development stages, many protein and peptide drugs can still encounter problems in the clinic. The major clinical hurdles may be similar to those observed in the pre-IND animal studies. However, the company may have filed an IND for a therapeutic indication that will encounter complex formulation and delivery problems. The route and frequency of administration and the bioactivity or potency of the drug in humans are critical issues that are often not addressed in the pre-IND animal studies. If difficulties in delivery or potency of the drug arise during clinical trials, the formulation scientist along with others on the development team must reconsider the design of both the drug formulation and the clinical plan. These pitfalls may often be avoided by testing the drug in a suitable animal model, if available, and an extensive analysis of the patient population including a marketing survey of the end users (physicians, nurses, and/or patients). By establishing early in the development stage (e.g. between research and Phase I clinical trials) the best route and formulation for the drug, the potential for a

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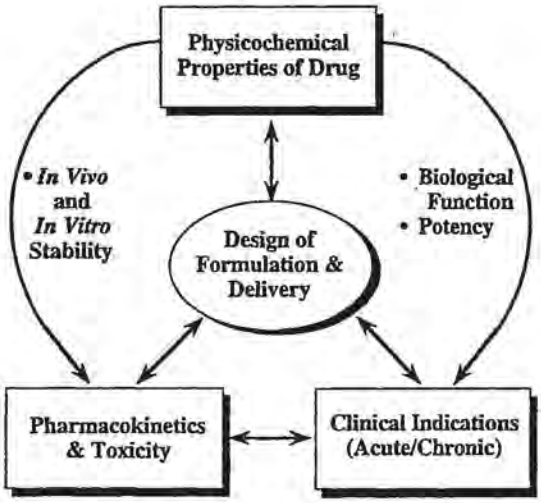

Figure 1: Key factors influencing the design of drug formulations and delivery. The physicochemical properties of the drug can affect the pharmacokinetics and toxicity as well as the clinical indication. The *in vitro* and *in vivo* stability of the drug determines its fate upon administration. The potential clinical utility of the drug is dependent upon the drug characteristics, biological function, and potency. To obtain the desired pharmocological response, a drug must be administered with a stable formulation. The design of a delivery system must also consider the clinical indication, pharmacokinetics, pharmacodynamics, toxicology, and drug properties.

clinically successful product and, ultimately, a marketed product increases dramatically.

The best route for delivery of a protein or peptide drug is often not investigated during the research stage or early in development. The protein or peptide is commonly administered systemically through an intravenous (i.v.) injection in initial animal testing. Thus, for indications that require a high local dose of the drug at the target site, high drug doses are required by i.v. injections. Due to toxicity problems, the efficacious dose may not be reached via i.v. administration. More recently, alternative routes of delivery have been studied. In particular, the therapeutic protein, recombinant human deoxyribonuclease I (rhDNAse), must be delivered directly to the lung of cystic fibrosis patients to degrade the DNA in the mucus. rhDNAse delivered systemically would clearly have little effect on the target site. While this example is an obvious candidate for an alternate delivery route (aerosol delivery of rhDNAse), many other proteins and peptides may also benefit from alternative routes of delivery for therapeutic or clinical reasons. It is therefore essential to investigate the site of action and assess any side effects before choosing a route of administration.

In addition, when companies are developing competitive products, the future sales of the product may rest upon the superior formulation and delivery of the drug, assuming that the efficacy of the competing products are similar. For example, many existing therapeutic proteins such as human growth hormone and insulin are administered chronically requiring daily injections. Competitors with superior drug formulations that release a sustained level of the protein and, thus, require less frequent injections would dominate the market. An example of competing products is the development of sustained release formulations for a luteinizing hormone-releasing hormone (LHRH) agonists. Takeda Pharmaceuticals developed an LHRH agonist (leuprolide acetate) - polylactide-coglycolide formulation that could be administered monthly and provided a continuous sustained therapeutic level of LHRH for one month (*2-5*). This product, Lupron Depot®, had a ¥57 billion (~$570 million) market in 1992 for prostate cancer, precocious puberty and endometriosis indications and competition from other types of LHRH agonist formulations, including daily injections and daily nasal delivery, have been insignificant (*6*). Similar competitive products also consist of controlled release systems using polylactide-coglycolide with different LHRH agonists (goserelin acetate, Zoladex®, *7*, triptorelin, Decapeptyl®, *8*). Overall, the clinical administration, patient compliance, pharmacokinetics, toxicity, and physicochemical properties of the drug must be considered to successfully develop a pharmaceutical protein or peptide drug.

Formulation Development Considerations

While development of novel delivery routes or systems is often necessary, the first step in development of any protein or peptide drug formulation involves the complete characterization of the drug properties and its stability in different formulations. Typically, a formulation scientist will begin by considering the physicochemical properties of the protein such as the isoelectric point, molecular weight, glycosylation or other post-translational modification, and overall amino acid composition. These properties along with any known behavior of the drug in different solutions (e.g. different buffers, cofactors, etc.) as well as its *in vivo* behavior should guide the choice of formulation components for testing in the initial screen of candidate formulations. The potential candidate formulations are composed of U. S. Food and Drug Administration (FDA) approved buffer components, excipients, and any required cofactors (e.g. metal ions). Often, the first choice of candidate formulations is based upon the previous experience of the formulation scientist with other proteins

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or peptides and, in many cases, a simple phosphate buffered saline solution may be one of the initial candidates.

A simplified approach to formulation development may proceed through the steps depicted in Figure 2. After obtaining all the available background information, one often evaluates several parameters in the initial screen of candidate formulations. One parameter that impacts all the major degradation pathways is the solution pH. Thus, the initial formulations also assess the pH dependence of the degradation reactions and the mechanism for degradation can often be determined from the pH dependence (*9*). The formulation scientist must quickly analyze the stability of the protein in each solution. Rapid screening methods usually involve the use of accelerated stability at elevated temperatures (e.g. 40° C; see references *10-13* for discussions of elevated temperature studies). Unfortunately, the FDA will only accept real time stability data for shelf life and accelerated stability studies may only serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature. The degradation of the protein for both accelerated and real time studies is then followed by assays developed for analysis of degradation products (see reference *14* for detailed review). The most common degradation pathways for proteins and peptides are listed in Table I. Several recent reviews have analyzed these pathways as well as potential methods to prevent degradation (*11*, *15-18*). In each case, the amount of degradation must be minimized to achieve greater than or equal to 90% of the original drug composition after 2 years (e.g. $t_{90} \geq 2$ years). The FDA usually requires that a pharmaceutical product is not more than 10% degraded and the company must demonstrate that the degradation products do not have any adverse effects on the safety or efficacy of the drug. Many proteins and peptides can degrade extensively without effecting either their safety or efficacy. For example, 70% deamidated recombinant human growth hormone (rhGH) is fully bioactive and non-immunogenic, but this extent of degradation is not acceptable by regulatory agency standards for a therapeutic protein (*19*). The effect of degradation on the safety and efficacy of a protein or peptide is difficult to ascertain without extensive testing. Thus, the more conservative standards of the FDA and other regulatory agencies may often provide a less expensive alternative if a stable formulation (> 2 year shelf-life) can be developed.

To fulfill the regulatory requirements for a stable formulation, the scientist must consider all of the major degradation routes and the potential conditions for optimization. In the case of aggregation, the addition of surfactants or sugars can prevent denaturation events that lead to irreversible aggregation. If the deamidation rate is the dominant degradation route, the use of amine buffers such as Tris, ammonium, or imidazole may slow the deamidation. Alternatively, a reduction in pH will also decrease the deamidation rate, but the reduced pH may also lead to cleavage or cyclization at Asp-X residues where X is usually a residue with a small side chain (e.g. Gly or Ser) and this degradation has been observed in several proteins (*1*). Proteins with Asp-X degradation must then be placed in a higher pH buffer to avoid cleavage or cyclization. High pH conditions (> pH 8) will however catalyze oxidation, thiol disulfide exchange, and β-elimination reactions. These degradation pathways may be inhibited by the addition of free radical and thiol scavengers such as methionine. In addition, the method used to prevent one type of degradation may influence another degradation pathway. For example, by adding surfactants or other polymers to prevent aggregation, the residual peroxide in the surfactant may cause a more rapid oxidation (*20*). In some cases, the formulation pH must be reduced to decrease the rate of deamidation. Reducing the pH may also alter the solubility of the protein since many proteins have isoelectric points at or near the optimal pH (pH 5-6) for minimizing the deamidation rate. For each protein formulation, all the degradation pathways must be evaluated and often a balance must be achieved between the different degradation pathways.

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