Protein Instability and Immunogenicity: Roadblocks to Clinical Application of Injectable Protein Delivery Systems for Sustained Release

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ABSTRACT: Protein instability and immunogenicity are two main roadblocks to the clinical success of novel protein drug delivery systems. In this commentary, we discuss the need for more extensive analytical characterization in relation to concerns about protein instability in injectable drug delivery systems for sustained release. We then will briefly address immunogenicity concerns and outline current best practices for using state-of-the-art analytical assays to monitor protein stability for both conventional and novel therapeutic protein dosage forms. Next, we provide a summary of the stresses on proteins arising during preparation of drug delivery systems and subsequent *in vivo* release. We note the challenges and difficulties in achieving the absolute requirement of quantitatively assessing the degradation of protein molecules in a drug delivery system. We describe the potential roles for academic research in further improving protein stability and developing new analytical technologies to detect protein degradation byproducts in novel drug delivery systems. Finally, we provide recommendations for the appropriate approaches to formulation design and assay development to ensure that stable, minimally immunogenic formulations of therapeutic proteins are created. These approaches should help to increase the probability that novel drug delivery systems for sustained protein release will become more readily available as effective therapeutic agents to treat and benefit patients. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:946–954, 2012

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INTRODUCTION

Therapeutic protein drugs have revolutionized human healthcare, providing unique treatments of numerous diseases and disorders. A major drawback, however, is that they typically must be administered repeatedly by intravenous (i.v.) infusion or subcutaneous injection. Pharmaceutical dosage forms that achieve the goal of long-term sustained delivery of therapeutic proteins could provide significant benefits for patients such as a decrease in the number and in the amount of required doses, better control

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of drug levels *in vivo,* and thereby, an increase in patient compliance rates. In cases when the pharmacokinetic profile of the native drug is inadequate for a normal dosage regimen (e.g., in the case of very short half lives in the range of minutes), rational therapies with new drugs may only be possible with the use of controlled drug delivery systems. These beneficial effects are attributed to the ability of delivery systems to increase the time over which a protein's concentration is maintained at the appropriate therapeutic levels and/or by tailoring delivery to a specific site. To date, however, these benefits have not been realized to any great extent with commercially available therapeutic protein–drugs, despite a large pharmaceutical research focus on numerous "advanced" protein delivery systems. Shorter peptides such as luteinizing hormone-releasing hormone (LHRH) antagonists are delivered over a period of 1–6 months in the form of poly(lactic-co-glycolic acid) (PLGA) microspheres and provide effective, temporary therapy for prostate cancer.[1] Delivery of proteins, however, has proved more problematic. Although novel delivery products for recombinant human growth hormone and recombinant human insulin have been commercialized (i.e., injected PLGA microspheres and inhaled aerosol powder technology, respectively), both products have been withdrawn from the marketplace[*,†].

A Web of Knowledge[SM] search for the terms "PLGA," "protein," and "delivery" resulted in more than 1000 scientific publications, which have been cited on average 27 times each. Similar results were obtained for the same search with "chitosan" or "liposome" in place of "PLGA." This large number of publications stands in stark contrast to the current absence of marketed products that use these delivery systems for therapeutic protein drugs. Our thesis is that protein instability associated with the preparation and storage of these more complex dosage forms as well as their inherent propensity for immunogenicity limit the medical applicability and commercial success of such controlled-release products.

In this commentary, we will address two significant technical obstacles to clinical success of advanced protein drug delivery systems, with a special emphasis on injectable systems for sustained systemic delivery of therapeutic proteins. First, we discuss the need for more extensive characterization in relation to concerns about protein instability in drug delivery systems, and second, we will briefly address immunogenicity concerns. Although we will not discuss other macromolecular-based pharmaceutical drugs such as plasmid DNA and viral vectors in this commentary, the comments made about proteins are generally applicable to the delivery of these other large biomolecules as well. Next, following a section summarizing current stability and assay expectations for therapeutic protein products, based on the collective knowledge acquired through decades of formulation work devoted to developing commercial protein-based products, we will provide a summary of the stresses arising in such drug delivery systems during preparation, storage, and administration to the patient. We also describe the difficulties that need to be overcome to quantitatively assess the degradation profile of protein molecules in a drug delivery system. Furthermore, we describe the potential roles for academic research in further improving protein stability in novel delivery systems and developing improved analytical technologies to better detect degradants in novel delivery systems. Finally, we provide some recommendations for the appropriate approaches and assays for enhancing the probability of assuring that stable, minimally immunogenic formulations of therapeutic proteins are developed, with the overall goal of enhancing the likelihood that novel delivery systems for protein-based drugs will become effective therapeutic agents with which to treat patients.

PROTEIN INSTABILITY IN DRUG DELIVERY SYSTEMS: HOW MUCH OF A PROBLEM?

Efforts to develop commercially successful "advanced" drug delivery systems for protein-based drugs must be considered in the larger, critically important context of maximizing the safety and efficacy of the given product candidate for the targeted patient population. As such, it is generally not sufficient to demonstrate only some level of sustained delivery by monitoring recovery of activity (or other desired biological properties) of the protein molecules that are released. Rather, the fate of the vast majority of the protein molecules in the drug delivery system must be rigorously and quantitatively determined. For example, if even a small percentage of the protein molecule aggregates within the delivery system, the resulting micron- or nanometer-sized aggregates (particulates) may cause serious safety issues such as adverse immunological responses in a percentage of the treated patient population.

Importantly, the design and assessment of drug delivery systems must be in accordance with 21st century analytical technology based on proper, rigorous application of fully qualified, high-resolution

[*]A PLGA polymer-based delivery formulation for recombinant human growth hormone was approved for commercial use in 1999 and marketed until 2004. In addition, an aerosol-delivered powder formulation of insulin was approved for commercial use in 2006 and marketed until 2007.

[†] Also in 2004, following the withdrawal of the controlled-release human growth from the market, commercial development efforts for a PLGA-based delivery system for recombinant human follicle stimulating hormone were discontinued.