INTRODUCTION

PROTEIN PHARMACEUTICALS – RECENT ACHIEVEMENTS AND TRENDS FOR FORMULATION AND DELIVERY

In this introductory article to the Injectable Drug Delivery (Formulations Focus) issue of ONdrugDelivery, we will focus on the delivery of protein therapeutics. The application of delivery technologies to formulate protein therapeutics in order to optimise or enable their development as viable pharmaceutical products is one of most important and fastest-growing areas of injectable drug delivery nowadays. However, this has not always been the case.

Personally, we remember from our own time as undergraduate students that certain professors declared protein pharmaceuticals to be “a temporarily existing hype that will go away”.

The so-called hype has not only gone on to become one of the most active and intense periods of pharmaceutical R&D to date, but also one of the fastest growing segments, today almost matching the more conventional small-molecule market in terms of revenues.

Protein pharmaceuticals have become a mainstay in the pharmaceutical product pipeline and today even the most traditional Schools of Pharmacy cannot ignore them. There have also been some very dramatic events with rises and falls of small, promising biotech start-ups in the late-1990s/early-2000s period that perhaps even Edgar Allan Poe could not plot in better words. Many lessons have been learnt both from the scientific perspective and in the financial world too. And probably, the learning curve still has to yet reach its apex.

In this transitional process we can look back and state: Yes, it is feasible to develop, and safely and economically to manufacture, most biopharmaceuticals for therapeutic use.

Challenges are still represented by the process to include the patient’s perspective in terms of convenience of administration, and with regard to the tax payer, who wishes to have all therapeutic options available without paying too much for health insurance cover.

From the patient’s perspective, much is being accomplished. As the first biopharmaceuticals put on the market are almost exclusively bound to needle-based routes of administration to be able to enter the systemic circulation, the second generation is aiming to reduce injection frequency (by using PEGylation strategies and depot technologies, for example), targeting the drug more efficiently to the site of action to reduce unwanted side-effects or to switch the administration from intravenous (requiring ambulant administration) to auto-injectors for subcutaneous or intramuscular administration to be handled by the patient him/herself.

After the recent withdrawal of Exubera™ insulin, the only systemically acting protein drug that had been made available as a pulmonary formulation, it may be another decade until serious attempts outside the parenteral route will reach the submission stage for market authorisation.

For the purpose of this editorial we will review a few examples of recent progresses in the field of parenteral protein delivery.

These are also discussed in much more detail in the recently published book: “Protein Pharmaceuticals – Formulation, Analytics and Delivery” 1, made possible through the strong support of the European non-profit organisation, APV (www.apv-mainz.de), which has also organised a series of seminars related to this same topic over the past ten years.

PEGYLATION

PEGylation, the covalent attachment of one or more polyethylene-glycol (PEG) molecules to a protein drug, was invented in the 1970s. Major players in the field of PEGylation today are, among others: Enzon Pharmaceuticals, Inc (Bridgewater, NJ, US); Nektar Therapeutics, Inc (San Carlos, CA, US); Mountain View Pharmaceuticals, Inc (Menlo Park, CA, US); Celltech (now UCB SA, Brussels, Belgium); Amgen, Inc (Thousand Oaks, CA, US); F. Hoffmann-La Roche Ltd (Basel, Switzerland); Schering-Plough (now Merk & Co, Inc, Whitehouse Station, NJ, US); and Eyetech, Inc (Palm Beach Gardens, FL, US).

So far, nine PEGylated products are on the market (see Figure 1). With the exception of Pfizer/Eyetech’s Macugen, which is a PEGylated aptamer based on an oligonucleotide backbone, all marketed products are proteins.

The application of therapeutic proteins is facing several challenges, among which are:

- the mandatory application by injection
- low enzymatic resistance
- rapid renal elimination and resulting low t½ and AUC
- potential immunogenicity

PEGylation is posed to improve on these properties through shielding the protein against immunological recognition and enzymatic attack by steric hindrance, and reducing renal filtration by increasing the overall molecular weight, with 40kDa signifying the threshold molecular weight for PEGylated compounds. Reduction of local irritation or immune reactions at the injection site have also been reported after PEGylation.

The observation that PEGylated compounds or delivery systems accumulate preferably in tumor tissues or in joints under inflammatory conditions has caused the group of Maeda et al 2 to postulate the enhanced permeation and retention (EPR) effect. Overall, PEGylation results in a significant increase in retention time of the API in the systemic circulation, passive targeting by the EPR effect, reduction of immunological side effects, reduction in application frequency and thus general enhancement of patient compliance and adherence to therapy.

However, PEGylation also faces several challenges. Early N-hydroxysuccinimide chemistries involving attachment of PEG to amino functions did result in random PEGylation. This resulted in considerable challenges to production uniformity and quality assurance. In addition, PEG sites may be closely located to the reactive or binding site of the protein, which may impair its activity or binding affinity to the API’s receptor. This, however, may not be true in every case.

While PEG-aldehyde chemistry allowed targeting of the N-terminus more specifically by variation of the reaction pH, more advanced thiol and maleimide chemistries allowed the specific PEGylation of free sulfhydryl moieties.

PEGylation can lead to a reduction of in vitro activity of the modified molecule, which does not necessarily correspond to a loss in biological activity in vivo. It is thought that biopharmaceuticals may have higher binding affinity to their targets than
needed for cellular activity, and that this affinity is only partially reduced by PEGylation. In general, a poor in vitro/vivo correlation is observed for PEGylated compounds, making candidate selection processes tedious and time-consuming.

New avenues for permanent PEGylation have been developed by companies such as Neose (now in liquidation) and Polytherics (London, UK). The former has developed technology to glycosylate proteins expressed in Escherichia coli, or optimise glycosylation patterns by specific enzymatic GalNAc glycosylation of serine and threonine. The technology has been successfully applied to E. coli-expressed G-CSF, interferon-alpha, and GM-CSF. The glycopegylation technology was sold to Ratiopharm subsidiary BioGenerix AG, in 2008.

Another PEGylation approach, developed by PolyTherics, includes the reduction of intramolecular bonds in disulfide bridges and successive annealing of these bonds by using a spacer molecule, which is itself attached to PEG. Studies in the PEGylation of interferon alpha-2b show that PEGylation occurs completely site specifically, at ‘accessible’ disulfides only, and that the native protein conformation is maintained, the company states. In March 2010, PolyTherics entered into a research collaboration with Zealand Pharma (Copenhagen, Denmark) on the PEGylation of peptide therapeutics.

Disadvantages of the PEGylation process, such as possible reduction in bioactivity, may be overcome by a new class of linker molecules, binding PEG reversibly to the protein, virtually creating a pro drug from which the active principle is released by hydrolysis or enzymatic activity over a prolonged period of time. An advantage of this technology, which is considered as the next PEG linker generation, is that the native compound is recovered, and may have better access to compartments within the body than the high-molecular-weight PEGylated compound. In addition, releasable linkers can be designed to show a certain release profile, or even site-specific cleavage.

Since its invention in the 1970s, PEGylation has matured into a technology that offers the opportunity to improve on the properties of the full spectrum of peptide-, protein- and oligonucleotide-based drugs. Although not a trivial feat, PEGylation using permanent linkers is generally regarded as a commodity. Permanent linker chemistries – useful or not – have been vastly patented, leaving little room for new IP, with the technologies developed by Neose Technologies and Polytherics being among the few exceptions.

The development of releasable linker chemistries appears as a step forward, possibly offering the opportunity to create true biogenerics, as the API is regenerated from the PEG linkage, regaining its specific pharmacokinetic and bioactivity profile. Though a few “releasables” have been developed, the proof of concept still needs to be shown in clinical trials.

In conclusion, the lessons learned in PEGylation technology are:

- An ideal PEG reagent is derived from simple, proven, straightforward chemistry that produces linkages at predictable sites, contains non-immunogenic and non-toxic spacers or linkers and produces reaction byproducts that are innocuous.
- In vitro activity of PEG products is not predictive of their biological activity.
- PEGylation at or near binding domains may not necessarily result in loss of biological activity.
- Site-specific PEGylation of antibodies or antibody fragments alleviates loss of antigen binding usually seen for random PEGylation and maintains binding affinity.
- Releasable PEGylation offers the opportunity to develop conjugates releasing the original API in a sustained-release pattern.

**LIPID TECHNOLOGIES**

Although delivery of proteins in connection with non-covalently associated lipids, such as liposomes, is still in its infancy, several remarkable attempts have been made in recent years.

Not only scientifically remarkable, but also from a financial point of view, is the ApoA1Milano story. The background for the discovery of this variant of the HDL-bound ApoA1 is from an observation of a family living close to Milan, Italy, who have an unusually high life expectancy without any noticeable cardiovascular diseases. The company Pharmacia (now Pfizer), before merging with Upjohn, found the Milano variant and started initial development. Later it was spun-out to a new biotech company in the late 1990s, called Esperion Therapeutics, Inc (Plymouth, MI, US).

What makes the molecule so special? Lipoproteins circulate in the bloodstream in the form of natural, lipid-containing nanoparticles such as high- and low-density lipoprotein (HDL and LDL, respectively). Natural HDL particles can have either a spherical or discoidal shape and contain about 50% protein (predominantly ApoA1) and about 15% non-esterified cholesterol and 35% triglycerides. Small discoidal HDL contains primarily ApoA1, and a lipid monolayer consisting of phospholipids and free cholesterol. ApoA1Milano is a rare variant of ApoA1, which is associated with high HDL-cholesterol blood levels without increased risk for atherosclerosis. Administration of ApoA1Milano (apoA-I) halved plaque formation in animal models and resulted in more efficient efflux of cholesterol from existing atherosclerotic plaques.

Interestingly, Esperion was bought in 2003 by Pfizer in a breathtaking deal for US$1.3 billion. In 2008, Esperion Therapeutics regained independence through a financing round of US$22.75 million, buying back the product rights from Pfizer (which retains an undisclosed stake in the company).

**MICROPARTICULATE DEPOT TECHNOLOGIES**

Also remarkable are the up and downs of microparticulate protein depot systems. After considerable research efforts and probably a still more embracing regulatory environment the first depot formulation, containing human growth hormone (hGH), was brought to the market by Genentech (South San Francisco, CA, US) as Nutropin® Depot in the late 1990s. However, after about five unsuccessful years on the market it was withdrawn for several reasons. Up to now, Nutropin® Depot was the only protein depot product to have reached the pharmaceutical market place.

What lessons have been learned and what are the new trends?

To provide an answer, two aspects must be considered closely.
The first is the polymer itself and the second is the process by which the protein is formulated into PLA/PLGA microparticles. The polymer is a highly lipophilic macromolecule, which requires particular organic solvents to dissolve adequately. Proteins, on the other hand, are mostly amphiphilic with a defined three-dimensional structure which is maintained by intramolecular Van der Waals forces and interaction between polar groups. If such a molecule is forced to interact with a highly lipophilic polymer structure it may irreversibly alter its conformation, for example, to a conformation which may not refold adequately after being released back into an aqueous environment.

Secondly, the process to make microspheres involves, for example, a double emulsion method in which, from the viewpoint of an amphiphilic protein, it is literally squeezed in-between two very different solvent phases and it will most likely choose to interact with its polar part with very different solvent phases and it will most likely choose to interact with its polar part with the aqueous phase and its lipophilic moieties with the polymer/organic solvent phase. When the solvents are in motion, forces may act on the protein molecule or the protein molecules may interact, both of which can impact protein stability. The stress situation is enhanced in the drying steps involving solvent evaporation and subsequently freeze-drying.

As can be concluded from the above, either alternatives to the PLA/PLGA polymer have to be considered, or the process has to be modified to a more protein-friendly method, preferably avoiding double emulsion techniques.

Concerning the choice of polymers, more amphiphilic structures are preferred apparently by the protein. Probably the most advanced, most promising product coming through the pipeline is BioloXide Therapeutics’ (Pittsboro, NC, US) Locteron™ for the treatment of hepatitis C infection. It contains Interferon-α encapsulated using the PolyActive™ technology from OctoPlus (Leiden, The Netherlands).

Recent interim Phase II data demonstrated that, in comparison with PEG-Intron™, Locteron™ was able to reduce the PEG-Intron™ related side effects by 65% while maintaining an equivalent reduction of virus titers.

Interestingly, and commercially probably just as relevant, is the dosing every other week for Locteron™ instead of once-weekly with PEG-Intron™ and other PEGylated Interferon formulations currently on the market.

Considering the market for PEGylated interferons for HCV therapy is about US$1.4 billion, successful approval and launch of Locteron™ would encourage the many other companies engaged in development of protein depot formulations.

Another trend to be recognised is the move towards processing approaches that offer an alternative to double emulsion.

Several established in situ solidifying hydrogel approaches are in development. For example, the Atrigel™ delivery system, developed by QLT Inc (Vancouver, BC, Canada) and now under development also by QLT spin-out Tolmar, Inc (Fort Collins, CO, US), was featured in the 2006 Safer Injections issue of ONdrugDelivery (http://www.ondrugdelivery.com/publications/safer_injections.html).

The ReGel™ system, initially developed by Protherics and Macromed and now owned by BTG PLC (London, UK), is being applied in oncology and ophthalmic indications, amongst others. It is a thermosetting biodegradable gel that solidifies when injected into the body and is designed to provide high local concentrations of a drug for a sustained period.

A third example is Durect’s (Cupertino, CA, US) SABER™ delivery system. It uses a highly viscous base component, such as sucrose acetate isobutyrate, to provide controlled drug release. When the base component is combined with drug, biocompatible excipients and other additives, the formulation is liquid enough to inject easily with a standard needle and syringe. After injection, the excipients diffuse away, leaving a viscous depot.

We would also like to point to what is in our personal opinion a still hidden jewel. This concerns the microsieve™ emulsification technology of Nanomi (Oldenzaal, The Netherlands), which utilises silicon membranes with defined pore sizes and shapes that are made by photolithographic techniques widely used in the semiconductor industry. In the microsieve™ emulsification process, monodisperse droplets are generated by dispensing one fluid into a second immiscible fluid through millions of tiny pores, where every pore has the same size and shape (see Figure 2).

Since every pore is essentially the same, every droplet generated by the membrane appears to be similar, resulting in highly uniform, reproducible and size-controlled droplets or, after an appropriate solidification step, highly uniform, reproducible and size-controlled particles. A unique feature of the microsieve™ emulsification technology is that the droplet size is mostly independent of the precise formulation and the transmembrane pressure, and solely determined by the membrane design.
A continuous process in which the size of the membrane and the number of membranes can be altered easily, the process appears to be readily scaled-up, compared with the established batch-controlled, double-emulsion process.

The derived particles are highly uniform and monodisperse (see Figure 2) which also opens the doors to vascular drug targeting or – in a more immediately practical sense – to improve syringeability compared with systems generating wider particle size distributions, since larger and smaller particles can be omitted.

CONCLUDING REMARKS

In this article, we could only take out short extracts on recent trends and developments in the field of protein drug delivery from our recently published book: Protein Pharmaceuticals. However, even this short piece shows clearly that the R&D landscape in this field to be highly dynamic and colourful.

Many different disciplines of scientific research come together creatively to solve the challenges inherently connected when aiming to make the needle-based route of protein drug delivery more convenient, safer and more efficient for the therapy of the patient. With this in mind we are quite confident that the coming decade will bring exciting new developments and significant commercial success. It is clear that our jobs are in no danger of becoming boring at all!

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