PEGylated Protein Drugs: Basic Science and Clinical Applications
Edited by F.M. Veronese

Preface

PEGylated protein conjugates: A new class of therapeutics for the 21st century

Ruth Duncan[1] and Francesco M. Veronese[2]

[1] *Centre for Polymer Therapeutics, Welsh School of Pharmacy, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3NB, UK*
[2] *Department of Pharmaceutical Sciences, University of Padova, 35131 Padova, Italy*

Introduction

The collected Chapters in this volume describe the current status of poly(ethylene glycol) (PEG) modification of proteins, peptides, oligonucleotides and small molecule drugs, the recent advances in conjugation chemistry, and new clinical products. The book provides an excellent update in this rapidly evolving field, and the comprehensive collection of Chapters complements well past reviews/volumes that have documented the evolution of PEGylation. For example, a reader new to this field is encouraged to gain the historical perspective by reading the following reviews [1–8]. Only then is it possible to see just how far this field has come and understand that it has already established a new class of therapeutics as we start the 21st Century!

In 1990, the Regulatory Authority's approval of the first PEGylated enzymes (PEG-adenosine deaminase; ADAGEN® and PEG-L-asparaginase; ONCASPAR®) was an important landmark. This achievement was the culmination of the pioneering research of Davis, Abuchowski and colleagues in the 1970s that led to the development of these first PEG-enzyme products by Enzon Inc., a company still today contributing important new advances in PEGylation technology. These beginnings, together with the parallel research efforts of a relatively small number of academic groups in the 1980s, gave the credibility to this novel class of drugs, viewed with much scepticism by the pharmaceutical industry at the outset. As with many new ideas, PEGylation was rated as interesting science but impractical to commercialise. How wrong could they be! Today there are thousands of researchers worldwide working in the field and many companies have been founded on the back of this technology. The smaller ones offer speciality PEGs, new conjugation chemistries, and/or they are developing PEGylated liposomes/nanoparticles and PEG-based conjugates of proteins, peptides, oligonucleotides and small molecules as new medicines. Today almost all Pharma sell highly profitable, PEGylated

products; for example the two PEG-interferon alpha products and PEG-human-GCSF all have an ~1 billion $US market.

We all know that it is relatively easy now to review the literature-and speculate, although sometimes dangerously as to the likely future directions of a scientific field. Due to the vast wealth of emerging literature, most authors are encouraged to limit their review to those studies published over the last 3–5 years. While this is important, and defines the state of the art, it is also wise to remember the historical evolution of any field, acknowledge its roots, the advances made and the challenges/disappointments encountered. This ensures a realistic starting point for any new developments, avoids repeating mistakes of an earlier generation and allows new technologies to be built on firm foundations, and most rightly gives credit to those who came before [1–8]. It is sometimes too easy to reinvent the wheel on the back of hype! Scientific progress is always evolution and rarely revolution, to quote Einstein "*… my life is based to such a large extent on the work of my fellow human beings, and I am aware of my great indebtedness to them…*" (From 'My Credo', a speech by Albert Einstein to the German League of human Rights, Berlin 1932). This short introduction makes some brief comments relating to the 'recent' historical evolution of the fields of drug targeting and drug delivery, polymers as therapeutics, and the strategic importance of PEG-protein conjugates. These topics are meant to provide a link with the other chapters in the textbook which describe almost all recent progress in chemistry and purification of conjugates, potential issues relating to toxicity and immunogenicity, and also the recent extension of PEGylation strategy to oligonuceotide delivery.

Historical perspective

This year we are celebrating the centenary of Paul Ehrlich's Nobel Prize in Physiology and Medicine (awarded 1908). Ehrlich's vision not only gave important new insights into immunological mechanisms, but he also discovered the first synthetic low molecular weight chemical drug. This was arguably the beginning of drug development as we know it today and medicinal chemistry is still the mainstay of the modern pharmaceutical industry. Moreover, Ehrlich coined the term 'magic bullet', still popular today as an embodiment of the dream of effective disease-specific, targeted therapy. The phrase 'magic bullet' has proved easier to 'say' than achieve in practice. However, it is clear as we enter the 21st Century there is a paradigm shift, both in terms of the changing societal healthcare needs (e.g., increased incidence of diseases relating to the aging population, and emergence of drug resistant infectious diseases), and in parallel, the emergence of exciting new tools that have real potential to help tackle more effectively life-threatening and chronic, debilitating diseases in clinical practice.

Whereas the majority of pharmaceuticals are still natural products or synthetic low molecular weight drugs, the last two decades have seen growing

commercialisation of biotech macromolecular therapeutics, particularly antibodies, proteins, peptides and oligonucleotides. The small interfering ribonucleic acids (siRNAs) have most recently entered clinical trials with much anticipation of important new therapeutic benefits. Moreover, genomics and proteomics research is bringing remarkable advances in the understanding of molecular mechanisms of many diseases, which together with the identification of new molecular targets, is leading to an ever-increasing number of biotech drugs. Although these advances have brought many exciting new therapeutic opportunities, it is well acknowledged that effective targeting/delivery of such macromolecular drugs both to diseased cells, and, furthermore, to the particular intracellular compartment they must reach for activity, is very difficult to achieve in practice. The issue of effective drug delivery, and, hopefully, targeting is ever more evident and these challenges are stimulating parallel interest in the design of complementary drug delivery systems (DDS) needed to realise the potential of macromolecular therapeutics.

In the DDS field, the explosion of innovative thinking in the 1970s marked a renaissance period for enabling technologies. A number of distinct classes of DDS appeared that were recently extensively described and reviewed [9]. They included antibody-conjugates, reviewed in [10], liposomes reviewed in [11], nanoparticles reviewed in [12] and polymer–protein [1–8] and polymer-drug conjugates [13, 14]. In these early days, each technology was viewed as competing with the others, and it was naively suggested that one would emerge as the 'best' universal platform for all drug delivery applications. However, clearly each technology has individual advantages and disadvantages [9], and there was increasing realisation that 'the' ideal DDS must be designed on a case-by-case basis, being optimised in respect to the nature of the drug payload to be carried and the specific target for pharmacological action. During the 1980s, a sound biological rational for design of DDS emerged and many modern systems are hybrid, nano-sized technologies, (e.g., PEG-coated liposomes) incorporating multiple components that harness the benefits of several of the original technologies. Moreover, they can be viewed as the 'first generation' nanopharmaceuticals and many have become established clinical products as discussed in [9]. Indeed, the number of Regulatory Authority approved products of this type have grown year on year, and in 2002/2003 the FDA approved more macromolecular drugs and drug delivery systems than small molecules as new medicines [15].

In the context of DDS, it is also important to acknowledge the rapidly rising interest in the application of nanotechnology in medicine [16, 17]. The European Science Foundation's Forward Look in Nanomedicine defined 'nanomedicine' (i.e., nanopharmaceuticals) as "*nanometre size scale systems consisting of at least two components one of which being the active ingredient*". This definition embraces the PEG conjugates as described herein, and the convergence of the basic scientific disciplines relating to 'nano' research is bringing a wealth of new opportunities. For example, to apply existing and new technologies to important emerging clinical challenges, e.g., use of stem

cells, and promotion of tissue engineering and repair, design of systems that self-assemble in the patient, and to fabrication of hybrid systems combining DDS technologies and miniaturised devices. Real opportunities exist to design nano-sized, bioresponsive systems able to diagnose and then deliver even macromolecular drugs, so-called theranostics, and to design systems able to promote tissue regeneration and repair in disease, trauma, and during ageing so perhaps in the future it will be possible to circumvent the need for chemotherapy. Although many of the ideas circulating today are still science fiction, it is likely that some facets of 'nanotechnology applied to medicine' will become practical reality within the foreseeable future.

What is increasingly clear, however, is the growing role of natural and synthetic polymers as components of complex DDS, as nanopharmaceuticals and to make nanodevices. Those PEG conjugates described in this volume are nanopharmaceuticals according to the above definition, and they were certainly well ahead of time!

Polymer therapeutics

So, let us begin a brief introduction to 'polymer therapeutics'. In the beginning, the idea of using water-soluble polymers as components of innovative polymer-based therapeutics, particularly for parenteral administration, was viewed by the industry with much scepticism as another totally impractical, scientific curiosity that was much too risky. This was a peculiar stance since natural polymers have been active components of herbal remedies for several millennia, and polymers were widely used as biomedical materials, to fabricate medical devices, as pharmaceutical excipients, and for controlled drug delivery in the form of hydrogels, rate-controlling membranes and biodegradable implants for local delivery. However, it is worthy to remember that the many synthetic polymers we use in society everyday in many different forms (from plastics to computers and mobile phones, to consumer products, etc.) do have a relatively short history. From the outset critics were right to point out that most synthetic and natural polymers are not suitable, and moreover never designed for human administration.

It is sometimes forgotten that the efforts of Hermann Staudinger and his contemporaries led to the birth of polymer science only in the 1920s (post Paul Ehrlich!), and moreover, it was not until 1953 that Staudinger was honoured with the first Nobel Prize for 'polymer chemistry' as reviewed in [18]. Nevertheless, even in these early days, biomedical applications of polymers were envisaged. In the Second World War, synthetic water-soluble polymers were widely adopted as plasma expanders, e.g., poly (vinyl pyrolidone), and large amounts of synthetic polymer were safely administered. This encouraged further exploration of polymers as drugs (e.g., radioprotectants and immunomodulators) and began to underline the potential usefulness of water-soluble, biomedical polymers.

Pioneering work began to emerge in the 1960s and 1970s that lay the foundations for a clearly defined chemical and biological rational for the design of polymeric drugs [13, 19, 20], polymer–protein conjugates [1–8], polymer-drug conjugates [21] and block copolymer micelles [19]. Today, we use the umbrella term 'polymer therapeutics' to include all these classes of polymer-based drugs [13, 14]. From the industrial standpoint, these multicomponent nanosized medicines (typically 5–30 nm) are new chemical entities and macromolecular prodrugs rather than conventional 'drug delivery systems or formulations' which simply entrap, solubilise or control drug release without resorting to chemical conjugation.

There has been a growing realisation that the versatility of synthetic polymer chemistry provides a unique opportunity to tailor synthetic, biomimetic, macromolecular carriers of a specific molecular weight (typically 5,000–100,000 g/mole). Polymer structure can be customised to provide the multi-valency so often needed to promote effective receptor-mediated targeting. Moreover, using the flexibility of dendrimer chemistry, we have a tool kit able to build sophisticated three-dimensional architecture into the structure of synthetic macromolecules, as reviewed in [22], and this is increasingly being built into PEG chemistry via use of branched or dendronised PEGs. Importantly, the linking chemistries used for polymer conjugation have been refined over the years such to enable creation of macromolecular prodrugs (e.g., containing drugs, proteins, oligonucleotides) that are able to display sophisticated rate control and site-specific release of the bioactive moiety. The polymer therapeutics are, still today, often misreported as a rather minor contribution to the therapeutic armoury. This is largely because over the years large companies have made a very small investment in this area compared to biotech and medicinal chemistry/high throughput screening. However, review of the current polymer therapeutics market size (>5 billion US$) compared to antibodies (>17 billion US$) show just how wrong this conclusion is, especially taking in account the disparity in the relative historical economic investment in the two fields!

PEG conjugates

So within this complex landscape of drug delivery and polymers, how best can one summarise the current and future contribution of PEGylation? At the outset [4], PEGylation was developed as a tool to improve delivery of protein drugs and rectify their shortcomings. For example, proteins and peptides can have a short plasma half-life, poor stability, poor formulation properties and they can be immunogenic. Although other polymers, such as dextran, had been explored to address these shortcomings, PEG was initially chosen as the polymer for protein modification as it was already used as 'safe' in body-care products and approved for use as excipient in many pharmaceutical formulations. As a further advantage, it could be synthesised to have a molecular weight of

narrow polydispersity and also to have one terminal functional group making it ideal for protein modification without risk of crosslinking. Moreover, this highly hydrated polymer chain makes it theoretically ideal to 'mask' sites responsible for the immunogenicity of proteins to which it was bound. 30 years later, PEGylation is now a well-established tool able to address the limitations of proteins, peptides and oligonucleotides and, in addition a number of PEG-drug conjugates have been tested clinically for both parenteral and oral administration.

Undoubtedly, the Regulatory Authority approval of the first PEG-enzyme conjugates, ADAGEN® and ONCASPAR®, in the 1990s was a significant breakthrough. Indeed, this proof of concept immediately gave credibility to all the emerging classes of polymer therapeutics as a whole. However, although ADAGEN® and ONCASPAR® were important first products, they achieved limited clinical use and only a niche market; particularly ADAGEN®, which is used to treat severe combined immunodeficiency syndrome, a rare disease with few patients worldwide, and a disease that has more recently been treated with mixed success by gene therapy. Nevertheless, these beginnings paved the way for the subsequent application of PEGylation to cytokines such as the interferons (PEG-Intron® and PEGASYS®, (see the chapter by Pasut in this book), which have been successfully used to treat hepatitis C, and a granulocyte colony-stimulating factor (Neulasta®, see chapters by Molinex and by Sergi et al.) used as an adjuvant to repair the effects of neutropenia-inducing chemotherapy. These innovative medicines achieved significant therapeutic benefit, improved patient convenience as they need less frequent dosing compared to the free-protein drug, and achieved considerable economic success and they are now featured in the top marketed drugs lists. Recent Regulatory Approval of the PEG-aptamer Macugen® as a treatment for age-related macular degeneration, (reviewed in [23]), the PEG-anti-TNF antibody Fab' fragment (Cimzia®) for treatment of Crohn's disease, (reviewed in [24] and by Nesbitt et al.) also in clinical development for arthritis, as well as the suggestion to use the enzyme urate oxidase for the refractory gout treatment uricase (chapter by Hershfield et al.), are all showing a move towards application of PEG conjugates in the treatment of chronic diseases. It is important to note that such conjugates have not only therapeutic and formulation advantages, but also the potential to be cost-effective and even cost saving [25, 26].

Evolution of PEGylation chemistry over the last 30 years has been well documented [5–8]. Instrumental to the continuing success of the now emerging products has been the increasing degree of sophistication of the conjugation chemistry and methodology developed for product isolation and characterisation, as described and reviewed in detail by Fee. The first PEGylated enzymes contained multiple PEG chains per protein, whereas now a number of conjugation approaches (chemical and enzymatic also described herein by Bonora/Drioli, Sergi et al. and by Fontana et al.), combined with recombinant protein technology, can ensure 1:1 (polymer: protein) site-specific conjugation. The PEGs used vary in molecular weight from low (~3–5,000 g/mole) to

high (20–40,000 g/mole) molecular weight chains and both linear and branched PEGs are now being used (see the chapter by Veronese et al. for properties and limitations description). As PEG is not biodegradable, the use of high molecular weight PEGs and chronic administration of all molecular weights of PEG raise questions about fate and long term safety (see the chapter by Webster et al. on toxicity and the chapter by Armstrong on PEG immunogenicity) that may have regulatory implications in the future depending on proposed conjugate use, dose, frequency of dosing and whether the treatment is for an acute or a chronic disease [27, 28]. Additional chapters deal with the use of PEGylation for the improvement of anticancer drug therapy (see chapter by Mero et al.) and of acromegaly (chapter by Finn). As for all polymer therapeutics, a sound biological rational for design has always been applied to PEG-proteins and it has evolved with time as more has become known of the structure activity relationships in respect to the effect of PEG molecular weight and branching on the pharmacokinetic-pharmacodynamic profile.

As more and more polymer therapeutics are being developed, there is a need to continuously review and consider new Regulatory Guidelines for their approval (see the chapter by Viegas and Veronese).

The future?

It should not be forgotten that it was only the turn of the last century when Paul Ehrlich proposed the first synthetic small molecules as chemotherapy and Hermann Staudinger was suggesting that small molecules, monomer units, might be covalently linked to give us polymer chains! Who could have predicted the plastics revolution that followed?

Introduction of the first biotechnology and polymer-based products over the last two decades of the 20th Century was greeted with the same suspicion that Ehrlich encountered when introducing modern chemotherapy in his day. Things are now rapidly moving on. PEGylated proteins are now well established as therapeutics and PEGylated peptides are gaining momentum. Will they be the mainstay of therapy for all diseases within this Century? Probably not, but it seems certain that as we start the 21st Century we are entering a therapeutic era where low molecular weight chemotherapy, macromolecular drugs, including, antibodies, peptides and proteins, polymer therapeutics, and oligonucleotides and cell therapy will all play an important and complementary role in the prevention, control and cure of diseases. It is rapidly becoming apparent that the future is combination therapy. Many of the PEG conjugates already marketed and those in clinical development will increasingly be used in combination with small molecular chemotherapy and/or any of these new classes of therapeutic/nanopharmaceuticals to ensure successful treatment of complex pathologies. This itself will bring new healthcare challenges including treatment cost, the need to foresee and minimise potential new contraindi-

cations and/or drug–drug interactions. There is still much interesting/vital research remaining to be done.

To conclude, thanks to the efforts of a relatively small community (academic and industrial), PEGylation and PEG-proteins as polymer therapeutics are already well established. The recent progress documented in this volume shows that there is more, much more, yet to come and that this is just the beginning!

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