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Preface

Introduction and overview of peptide and protein pegylation

Introduction

It has long been a dream in medicine and pharmacy to use peptides and proteins as drugs. The driving force for this interest is the ability of these compounds to eliminate toxic or overproduced compounds in the body and to mimic endogenous hormones, cytokines and antibodies. Two major hurdles were apparent. First, there was the difficulty in obtaining sufficient quantities of the materials. Small peptides could be made by chemical synthesis, but larger molecules could only be obtained by laborious extraction and purification from natural sources. This problem has been overcome by the development of genetic engineering, so that now it is routine to prepare kilogram quantities of pure proteins. The second major hurdle, and one that remains a serious challenge, is to deliver the molecules to the desired bodily target. Oral delivery of proteins remains unavailable because proteins are routinely destroyed by the digestive system. Even injected proteins generally have poor pharmacokinetics because of rapid renal excretion and proteolytic digestion. There can also be significant immunological reactions. Finally, proteins are difficult to formulate because of their intrinsic instability.

Many approaches to enhancing protein delivery have been examined, including protein entrapment in insoluble matrices [1] and liposomes [2] and immobilization onto polymer resins for use with extracorporeal shunts through which blood could flow [3]. By far the most successful approach has been to mask the protein surface by covalent coupling of soluble poly(ethylene glycol) (PEG) or, as it has become known, “pegylation”.

A large amount of literature is available on pegylation, including several books and reviews [1–10]. The prime purpose of the present volume is to present, for the first time, a review of the extensive human and animal data that have recently become available on pegylated proteins and peptides. Previous reviews have focused on chemistry for pegylation and on in vitro examination of pegylated molecules. Until recently only two proteins, PEG-asparaginase and PEG-adenosine deaminase, had been approved for human use, and little clinical information was available on other pegylated molecules. However, the past few years have seen a dramatic expansion of clinical trials, and applications for approval as drugs have been filed for two PEG-proteins (PEG-granulocyte colony stimulating factor from Amgen and PEG-human growth hormone antagonist from Pharmacia). Also two pegylated molecules with major commercial potential have been approved (PEG-alpha interferon 2b, Schering Plough's PEG-Intron[®], and PEG-alpha-interferon 2a, Hoffman-La Roche's Pegasys[®]). Results from these studies have revealed tremendously valuable information on strengths and weaknesses of pegylation, and it is clear that pegylation is well on its way to becoming a standard component of the pharmaceutical tool box.

Historical background

The pioneering first steps in pegylation were taken in late 1970s in the laboratory of Professor Frank Davis of Rutgers University, and the Commentary in this volume is a reminiscence of these early days

written by Davis. It is important to remember that the technique of coupling polymers to proteins was originated in the 1950s and 1960s with investigations of protein structure and function by site-directed chemical modification. These studies led to so-called “gentle chemistry” for protein manipulation. Important also were discoveries made in the 1970s that enzymes could be covalently linked to insoluble matrices for biocatalytic applications. From such studies we came to understand that the delicate and complex protein molecules, under appropriate conditions, could be treated as a common chemical entity.

Other soluble polymers, including polysaccharides [11] and albumin [12], have been used for protein conjugation, primarily to stabilize proteins toward proteolysis and to increase residence time in the body. One product, dextran-streptokinase, has been marketed in Russia for thrombolytic therapy [13]. However, it was the development of pegylation that provided the real breakthrough in enhancing the pharmaceutical properties of proteins and peptides. PEG possesses a unique set of properties, including absence of toxicity, immunogenicity and antigenicity; low, mass-dependent elimination via the kidney; high flexibility and high solubility in water and certain organic media. In turn, PEG-proteins possess reduced toxicity, immunogenicity and antigenicity, reduced rates of kidney clearance and proteolysis, and enhanced solubility and stability. Valuable improvements in various pharmacokinetic kinetics parameters (e.g., absorption rate and volume of distribution) are also seen. Details of these various properties are provided in the reviews we have referenced and in the following sections.

It is interesting that it has taken over 20 years for protein pegylation to approach becoming a standard technique. In part this was due to the time required to improve protein manufacturing, but also it has been necessary for the organic and polymer chemistry of PEG activation to mature. Of course there has been tremendous advancement as well in understanding of protein structure and properties and of the effects of pegylation. Quite probably, various market forces have also played a role in slow adoption of the technology, as with any revolutionary new technology.

Articles in this issue (Parts One and Two)

The seventeen chapters of these two issues of *ADDR* will review many major achievements of pegylation. As mentioned above, the Commentary in this first volume is a review by Davis of the discovery of pegylation and its benefits. It is indeed fascinating to read this first-hand accounting of the creative and the mundane forces that led to such an important invention. We see the professor's eternal pursuit for funding, the delight and value of old-fashioned library detective work, the light-bulb flash of a good idea, the juxtaposition of the idea with protein availability and just the right polymer catalogue, and finally appreciation of unexpected laboratory results. We suspect it is just this type of experience that keeps most of us in science. Admittedly, however, Davis' work has had far greater impact than most.

The first chapter by Roberts and co-workers reviews the contributions from many different research groups on improving conjugation chemistry, analytical methods for conjugate characterization, and the influence of mass and shape of the polymer on conjugate properties.

The second chapter by Kinstler and co-workers teaches how PEG may be specifically linked to the amine terminus of a protein. As noted here, site-specific conjugation chemistries are a critical need in protein pegylation because of the many isomers that can result from non-specific chemistries. Two important applications of this chemistry to therapeutically useful proteins are reported also. The problem of specificity of conjugation is also faced by Sato in Chapter 3 where conjugation to glutamine residues is described.

Hinds and Kim report specific conjugation of PEG to two of the three amino groups of insulin in Chapter 4. PEGs of low mass were shown in this work not to alter the conformation or activity of the hormone, but they did eliminate immunogenicity, antigenicity and allergenicity and lengthen circulation lifetime.

In Chapter 5 Chapman and co-workers discuss results obtained in the pegylation of antibody fragments. Antibodies have remarkable potential as drugs, but their high production cost is a serious

barrier to commercial application. Antibody fragments are much less costly to manufacture, but their circulation life times in the body are quite short. The half-life problem can be solved by pegylation. Chapman and co-workers describe this approach and show that PEG–antibody fragment conjugates have great therapeutic potential.

The next two chapters present pivotal papers from groups at Schering-Plough and Hoffman-La Roche and Shearwater on pegylation of alpha-interferon. The scientific community is fortunate to receive these important papers on two very important commercial products (Pegasys® and PEG-Intron®). This kind of information is frequently not published. These papers reveal chemical and clinical results for pegylated proteins in unprecedented detail. We believe the information will be used for years to design new PEG-protein pharmaceuticals. The pegylated alpha-interferons have been approved for treatment of hepatitis-C, a disease of great societal importance, and they are under investigation for other important indications.

The last chapter by Veronese and colleagues reviews the hundreds of laboratory studies of superoxide dismutase pegylation. This enzyme is involved in many pathological states, and it would seem reasonable to expect commercial development, but this has not occurred, and one is left to wonder why. Nonetheless these works have served as a proving ground for new chemical methods of PEG coupling and new analytical procedures for conjugate characterization, and they illustrate the influence of PEG molecular weight on clearance and activity.

The ADDR issue following this one will report nine additional reviews regarding other products, including pegylated asparaginase, growth hormone releasing factor, growth hormone antagonist, tumor necrosis factor, a TPO-mimetic and staphylokinase, and a chapter on pharmacokinetic and immunological properties of conjugates.

Conclusions

In summary, protein pegylation has come a long way toward becoming a standard tool for the pharmaceutical sciences. We believe these volumes will

assist the scientific community in applying the knowledge that is now available.

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