Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 300 (2005) 125–130
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Improving the therapeutic efficacy of peptides and proteins: A role for polysialic acids

Gregory Gregoriadis [a,b,*], Sanjay Jain [a,b], Ioannis Papaioannou [b], Peter Laing [b]

[a] *School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK*
[b] *Lipoxen Technologies Ltd, 29-39 Brunswick Square, London WC1N 1AX, UK*

Accepted 1 June 2005

Abstract

Peptide and protein drugs are a growing class of therapeutics. However, their effective application in the clinic is compromised by problems, for instance proteolysis in the circulating blood, premature clearance through the kidneys, and immunogenicity. A number of approaches have been used to circumvent such shortcomings including changes in the primary peptide structure, entrapment into nanoparticles (e.g. liposomes) and conjugation to polymers. Polysialylation, namely, conjugation of peptides and proteins to the naturally occurring, biodegradable α-(2→8) linked polysialic acid is a recent development, which promises to be at least as effective as PEGylation but without its potential toxicity. Polysialylation of a range of peptide and protein therapeutics has led to markedly reduced proteolysis, retention of their activity in vivo, prolongation of their half-life in the circulation and reduction in immunogenicity and antigenicity. It is anticipated that polysialylation will lead to a new generation of peptide and protein constructs with significantly improved pharmacological profiles.

Keywords: Polysialic acid; Peptide drugs; Protein drugs; Insulin interferon; Erythropoietin; Granulocyte colony stimulating factor

1. Introduction

The therapeutic use of peptide and protein drugs, for instance insulin, growth hormone and the interferons has a history of several decades. However, the potentially huge impact of this class of drugs in therapy has become apparent only recently, as a result of advances in genomics and proteomics. These have led to the discovery of numerous protein and peptide drugs of therapeutic potential, a number of which are already applied clinically (Walsh, 2003).

Effective use of peptide and protein drugs in the patient can, however, be compromised by their instability in the body, rapid rates of clearance, premature uptake by tissues (for instance the reticuloendothelial system), loss through the kidneys, and immunogenicity or antigenicity (Harris and Chess, 2003). Concerted efforts made over the years to circumvent such prob-

* Corresponding author. Tel.: +44 20 7753 5822; fax: +44 20 7753 5820.
E-mail address: gregoriadis@ulsop.ac.uk (G. Gregoriadis).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.06.007

lems include changes in the primary peptide structure to render it less prone to degradation, introduction of glycons into the structure or conjugation to polymers in order to improve residence in the circulating blood and, also, reduce immunogenicity, and entrapment into nanoparticles such as liposomes.

By far the most successful approach to date has been conjugation to monomethoxy poly(ethyleneglycol) (mPEG) (Mehwar, 2000). PEGylation (as conjugation to mPEG is commonly referred to) endows protein and peptide drugs with certain advantages which include longer circulatory half-lives and reduction of immunogenicity. An increasing number of PEGylated drugs are now used clinically (e.g. asparaginase, interferon α, tumour necrosis factor and granulocyte-colony stimulating factor) (Harris and Chess, 2003). However, PEG is not biodegradable, and although there is some evidence (Caliceti and Veronese, 2003) of enzyme driven low rate oxidation generating aldehydes and ketones, this is not a normal detoxification mechanism. When conjugated to therapeutic proteins that are large enough to escape kidney clearance, PEG will end up in the tissues participating in the uptake of the PEGylated constructs where it will accumulate intralysomally. Moreover, PEGylated proteins have been found to generate anti-PEG antibodies that could influence the residence time of the conjugate in the circulating blood. So far, however, no adverse effects of PEG immunogenicity have been observed, possibly because of the very small amounts of injected PEGylated drugs currently in use (Caliceti and Veronese, 2003).

2. Polysialic acids

As already mentioned, peptide and protein drugs interact with the biological milieu in ways that can curtail their therapeutic efficiency. An approach to circumvent this difficulty would be to modify drugs in a way that renders them “unnoticeable” in the body and yet allows them to retain their activity. One such approach is to be found in certain bacteria that have evolved to foil the body’s defences by coating their walls with polysialic acid. Arguably nature’s ultimate stealth technology, polysialic acids (PSA) (Fig. 1) are linear polymers of *N*-acetylneuraminic acid (sialic acid) abundantly present on the surface of cells and many proteins. Interestingly, the role of PSA in protecting invading bacteria by interfering with host complement activation and phagocytic activity is extended to such functions in the body as modulating cell to cell inhibition thus facilitating neural tissue development, or helping cancer cells to metastasise by reducing their adherence and thus promoting migration.

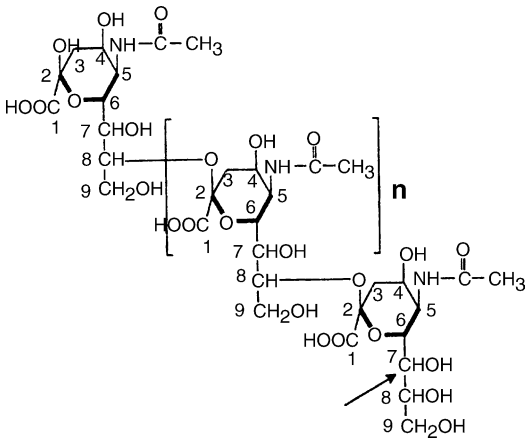

Fig. 1. Structure of polysialic acid (colominic acid). *N*-acetylneuraminic acid units are linked via α-(2→8) glycosidic linkages. The arrow indicates the carbon atom (C_7) at the non-reducing end of the sugar where periodate oxidation introduces an aldehyde group.

It was proposed in 1993 (Gregoriadis et al., 1993) that the unique ability of polysialic acid to insulate microbes and cells alike from external insults could be used to protect therapeutic molecules from the biological milieu and improve their pharmacokinetics. The rationale was that by forming a “watery” cloud around the therapeutic molecule by virtue of the extreme hydrophilicity of PSA, interaction with other molecules (e.g. proteolytic enzymes, opsonins, neutralizing antibodies or receptors on phagocytic cells), would be interfered with, thus allowing the therapeutic to preserve both its structure integrity and activity and prolong its presence in the body. In the case of small size therapeutics (e.g. short peptides and conventional drugs), a considerable increase in size as a result of conjugation with PSA, together with the highly ionic state of PSA could contribute to reduced loss of therapeutic through the kidneys. A schematic representation of polysialylated constructs in Fig. 2 shows two different types. In the case of relatively large peptides and proteins, a number of polymer chains of appropriate length attached randomly or strategically, would