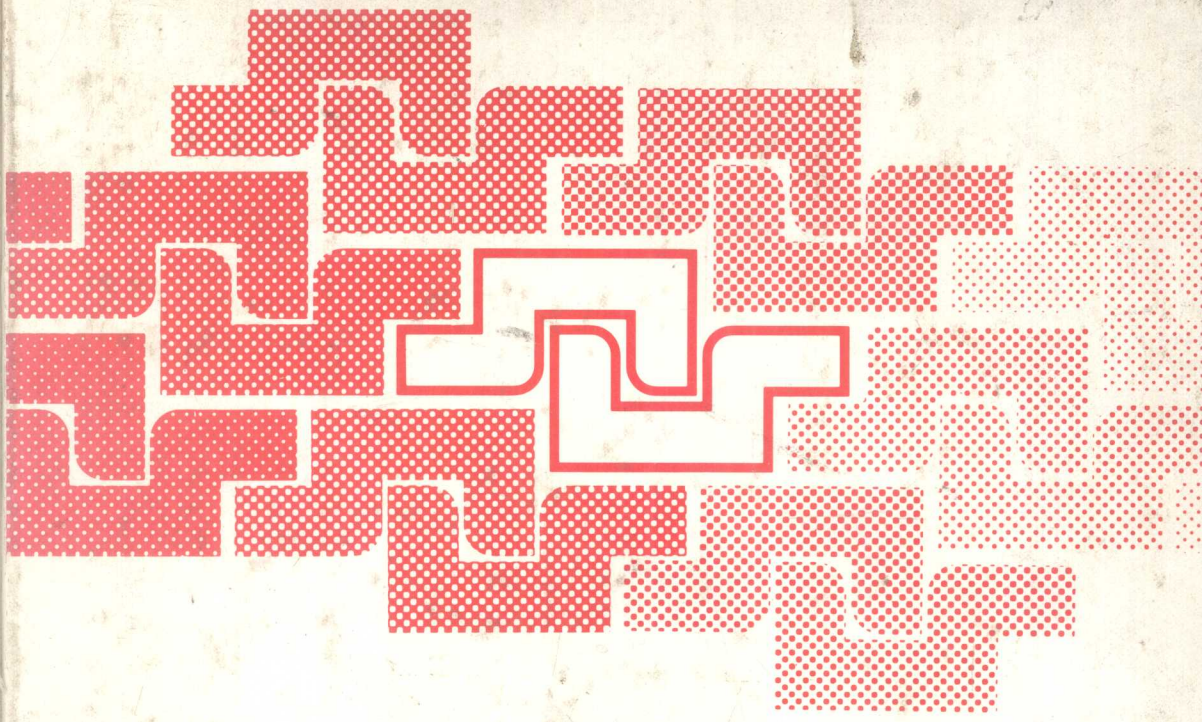

Delivery Systems for Peptide Drugs

Edited by
S. S. Davis
Lisbeth Illum and
E. Tomlinson

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Edited by

S. S. Davis

University of Nottingham
Nottingham, England

Lisbeth Illum

The Royal Danish School of Pharmacy
Copenhagen, Denmark

and

E. Tomlinson

Ciba-Geigy Pharmaceuticals
Horsham, England

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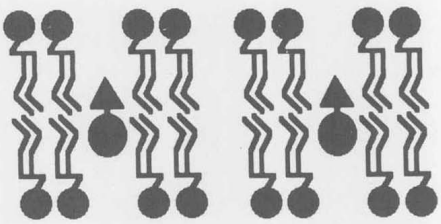

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PREFACE

Recent years have seen enormous advances in the field of protein and peptide engineering and a greater understanding in the way in which biological response modifiers function in the body. It is now possible through the use of recombinant DNA techniques, or by solid phase protein synthesis, to produce significant quantities of a wide variety of regulatory agents that are therapeutically applicable. The list of these response modifiers expands almost daily to include interferons, macrophage activation factors, neuropeptides and agents that may have potential in cardiovascular disease, inflammation, contraception etc. Prospects to use some of these materials in medicine have reached the stage where products have either been approved by regulatory authorities or are the subject of applications as investigatory drugs or as new therapeutic agents. In some uses the pertinent agent will be administered on an acute basis in the form of a simple injection, as, for example, the use of a tissue plasminogen activator for the treatment of coronary infarct. In other cases regulatory proteins and peptides are indicated for chronic therapy and here they will need to be administered by an appropriate delivery system. Unfortunately, the research on delivery systems for peptides and proteins has not kept pace with the rapid progress in biotechnology and, consequently, there are presently few systems that are entirely appropriate for the administration of macromolecular drugs according to complex dosage regimens, (eg intermittent and pulsed therapy). Furthermore essential pharmacokinetic and pharmacodynamic data may be missing. For example, questions like where and how does the peptide function?, how much is needed and how often should it be dosed? Thus, so far, the construction of delivery systems for peptides and proteins has been largely based upon conjecture rather than sound rationale.

In order to correct this inbalance, groups or individuals, charged with the role of developing delivery systems for peptides and proteins have become established within biotechnology companies and large pharmaceutical organisations. Pharmaceutical researchers in academia are also turning their attention to this complex problem. With this background in mind we considered that a useful role would be served by bringing together the leaders in the field in a workshop where advanced delivery systems for peptides and proteins could be discussed. A three-day meeting (Advanced Science Workshop), sponsored by the NATO Science Foundation and various pharmaceutical and biotechnology companies, was held in Copenhagen during May 28-June 1 1986 and this book comprises the various contributions presented.

The book considers first the overall pharmaceutical considerations for the rational design of delivery systems for peptides and proteins, to include relevant aspects of the production of peptides and proteins by biotechnology, pharmacokinetic analysis and strategies for chemical

modification. The major routes of access into the body are examined in turn, and include parenteral, oral, rectal, nasal, buccal and transdermal. In each case attention is given to relevant physiology and the critical role of biological barriers to drug uptake to include membranes and enzymes. Attention is focused on the role of absorption enhancers for the oral and nasal routes. A number of important and representative regulatory peptides are then selected for more detailed examination as case histories. These include calcitonin, insulin, enkephalins, somatostatin, TPA, macrophage activation factors etc.

The penultimate Chapter reviews the regulatory implications relevant to the delivery of peptides and proteins and the requirements that might be demanded by regulatory authorities.

The participants at the meeting were allocated to small syndicate groups, and each syndicate was given two topics to discuss and report upon at the conclusion of the workshop. The deliberations of the syndicate sessions have been summarised in the final Chapter of the book.

We wish to express our appreciation to the NATO Science Foundation for financial support of the workshop as part of the Double Jump Programme and to the various pharmaceutical and biotechnology companies who also provided assistance.

September 1986

S.S. Davis
Lisbeth Illum
E. Tomlinson

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ADVANCED DELIVERY SYSTEMS FOR PEPTIDES AND PROTEINS - PHARMACEUTICAL CONSIDERATIONS

S. S. Davis

Department of Pharmacy
University of Nottingham
University Park
Nottingham NG7 2RD, UK

Presently, a wide variety of routes of administration and delivery systems exists for drug substances, but by far the most popular approach is oral delivery where the drug is intended to be absorbed from the gastrointestinal tract. Injectable systems (that include implants), suppositories and transdermal devices, have a more limited place in current therapy. Some of these administration systems can be employed directly for the delivery of peptides and proteins, however, others cannot be used in their present form and will require extensive modification. In particular, the delivery of peptides and proteins via the gastrointestinal tract will be especially difficult because of the inherent instability of such materials and the poor permeability of the intestinal mucosa to high molecular weight substances. Indeed it can be claimed that the process of evolution, over many thousands of years, has resulted in the gastrointestinal tract being impermeable to large molecular weight molecules in the adult mammal and that serious immunological consequences could arise if such materials happened to be taken up intact. This point will be discussed further below. In future the less well known routes of administration, namely nasal, vaginal and buccal, could play a more important role in the delivery of peptides and proteins because of their superior permeability characteristics or the fact that the dosage form can be retained at the site of administration for a prolonged period of time to maximise absorption.

Those reading popular fiction may have been led to believe that the problem in delivering peptides had already been solved. To quote from Arthur Hailey's book entitled 'Strong Medicine' (Hailey, 1984) one learns that

> *"The way in which the drug would be ingested .... was important. We've researched this exhaustively and recommend delivery by nasal spray. This is the modern coming system .... Peptide 7 will be in an inert saline solution mixed with a detergent (that) assures the best absorption rate .... The best non-toxic (detergent) creating no irritation of nasal membranes had been found".*

If only it was so easy! While it is true that certain peptides are well taken up via the nasal route (Su, 1986), totally innocuous absorption enhancers have yet to be discovered and the implications of long term therapy on possible damage to the mucosa need to be resolved.

A universal delivery system for peptides and proteins is neither possible nor perhaps desirable, largely because the types of materials being considered for therapeutic application comprise a diverse range of biological response modifiers (Table 1). They have different physical and chemical characteristics (molecular size, stability, conformation etc) as well as different sites and modes of action within the body. For example a delivery system for tumour necrosis factor or an interleukin by necessity, will be rather different from that for insulin or calcitonin. Furthermore, unlike conventional low molecular weight drugs, biological response modifiers are often involved in complex processes where a whole group of regulatory materials can be involved. The "classical" situation of a single pharmacodynamic agent acting independently on one particular receptor type may not necessarily pertain. For example, it is known that materials such as interferon can act to prime or "up-regulate" the action of another material (Aggarwal et al, 1985). Similarly, the action of one agent may lead to a whole process of actions, often in the form of a cascade process. Consequently the resultant biological response may be determined by an alteration in a delicate balance between a number of inter-related regulatory materials. Clearly, in the development of any potential therapeutic agent and its delivery system it will be essential that such aspects of molecular and biochemical pharmacology are elucidated. The complexity of the properties and actions of regulatory peptides and proteins will mean that the successful development of pharmaceutically elegant delivery systems for peptides and proteins will be a multidisciplinary venture, to include input from disciplines such as biochemistry (the action of enzymes), physiology (membrane permeability), immunology (immunogenicity), physical chemistry (molecular characteristics; solubility, stability).

The main emphasis of the present contribution will be to consider the possibilities and problems for peptide and protein delivery and the different options available for rational drug delivery systems. By necessity comment will also be made on the pharmacokinetics of peptide systems, their assay and preformulation studies and the possibilities of immunogenic reactions.

Kinetic profiles

In many cases the simplest form of a delivery system for peptides and proteins will be the hypodermic syringe containing the drug in a buffered aqueous solution. The appropriate dose of agent is then injected intravenously or subcutaneously according to the desired time pattern. This mode of administration, although ideal from the standpoint of clinical pharmacology, is obviously limited in its widespread applicability to the patient population at large. Even for a simple system (and especially for complex systems providing sustained therapy), essential information is necessary. This includes the dose, dose frequency and the site of action of the drug. Generally speaking, natural peptides and proteins acting as agonists are short-lived in their action and are rapidly metabolised. Furthermore, the body provides these agents as pulses rather than continuously to a particular receptor site (Knobil, 1980; Urquhart et al, 1984). From the outset, the ability to mimic such a pattern of delivery using a novel delivery system will present substantial challenges to the pharmaceutical scientist. Moreover, it is often found that the desired pharmacokinetic profile for a regulatory material in man has yet to be determined since animal models, although useful, may not be entirely suitable. An obvious starting point is studies with infusion pump systems in animal models and more recently the implantable osmotic pump (Alzet) has been employed to provide a response without the necessity of tethering the animal (Obie et al, 1979; Knobil, 1980; Lynch et al, 1980; Ewing et al, 1983). This

Table 1. Selected polypeptides and proteins (and analogues) with potential for therapeutic use

PEPTIDE	AA	ORAL*	NASAL*	BRAIN
Adrenocorticotrophic hormone (ACTH)	39	X	X	X
Atrial natriuretic factor				
Calcitonin	32	X	X	
Cholecystekinin (CCK)	33			X
Colony stimulating factor				
DDAVP	9	X		X
Delta sleep inducing peptide				X
β Endorphin	31			X
Enkephalin	5		X	X
Fibroblast growth factor				
Glucagon	29		X	
Growth hormone				
Growth hormone releasing factor	40-44			
Insulin	51	X	X	
Inhibin				
Interferon			X	
Interleukin 2				
Leuprolide		X	X	
Leutinising hormone releasing hormone (LHRH)	10		X	X
Lipmodulin				
Melanocyte inhibiting factor I	3	X		X
Melanocyte stimulating hormone		14		
Muramyl dipeptide	2	X		X
Migration inhibiting factor				

(continued)

Table 1 (continued)

PEPTIDE	AA	ORAL*	NASAL*	BRAIN
Oxytocin	9			X
Parathyroid hormone				
Relaxin				
Somatostatin	14	X		X
Superoxide dismustase			X	
Thyrotropic releasing hormone	3	X	X	X
Tissue plasminogen activator				
Tumour necrosis factor				
Vasopressin	9		X	

* Activity following oral or nasal administration of peptide or analogue (Samanen, 1985; Lee, 1986; Su, 1986).

Table 2. Analytical methods for peptides. Studies carried out on a peptide (Somatostatin-14) prior to use in man (after Wunsch, 1983 and Wada, 1983)

A. Chromatographic methods

1. Amino acid analysis of acid and enzymatic hydrolysates
2. Gas chromatographic analysis of derivitised acid hydrolysate for postsynthetic chiral analysis
3. TLC on normal and high performance plates in different solvent systems
4. HPLC using different reversed phase supports and eluant systems
5. Electrophoresis on supports or free flow systems to characterise homogeneity
6. Micropreparative gel filtration to detect polymeric species

B. Spectroscopic Methods

1. UV measurements

2. NMR (^{1}H and ^{13}C) to detect possible synthetic side products or residual protecting groups

3. IR to detect possible contamination by column material from repeated chromatographic steps

4. MS (chemical ionisation, field desorption and fast-atom bombardment) to characterise enzymatic fragments or entire molecule for sequence

5. Fluorescence methods to detect contaminants from solvents, resins etc

C. Enzymatic Methods

1. Amino-peptidase-M digest

2. Trypsin digest followed by chromatographic separation

3. Dipeptidyl-peptidase digest followed by chromatographic separation

D. Biological Methods

1. In vitro assay

2. In vivo assay

3. Toxicological evaluation

E. Immunological Methods

1. Cross-reactivities with various antisera

F. Other methods

1. Circular dichroism to study conformation changes

2. Light scattering to follow aggregation

device, originally developed to provide a zero order input of drug, can also be used to provide the necessary driving force for pulsed delivery (Ewing et al, 1983). This is achieved by attaching the pump to a reservoir where boluses of the agent are separated by placebo. For example, thin plastic tubing filled with alternate doses of drug dissolved in water and separated by vegetable or mineral oil has been employed for the delivery of melatonin.

The pulsed delivery of peptides and proteins may well be the desired mode of delivery for therapeutic effect rather than the more conventional steady state levels that have been popular with conventional drug molecules. In many cases the continuous administration of a regulatory material can result in the phenomenon of tolerance or "down-regulation" (Catt et al, 1979; Obie et al, 1979; Koch and Lutz-Bucher, 1985). That is, the continual presence of the agent at a receptor site can lead to a reduction of activity. Here again osmotic pumps have been a useful aid. Continuous infusion and resultant steady state levels of an agent, while not necessarily desirable for agonist action, may be entirely appropriate for antagonist action.

In therapy a pulsed delivery pattern could be achieved by a programmed series of injections and possibly by nasal administration. Programmable, transportable pump systems, although bulky and inconvenient to use, have been developed and are used in clinical practice. For example, a device developed in Sweden (the Ferring product Zyklomat) has been used to deliver LHRH in order to induce ovulation. The pump contains a 10 day supply and is active every 90th minute for one minute duration delivering on each occasion 50 ug of drug. More complex patterns of delivery on a daily, weekly or even monthly basis are presently beyond the capabilities of conventional pharmaceutical dosage forms.

Preformulation studies

An essential part in the development of a delivery system for a peptide and protein, as for any other drug molecule, will be extensive preformulation studies to establish the physicochemical characteristics of the agent and possible limitations in terms of stability etc. Methods for examining the physicochemical properties of peptides are given in Table 2. High molecular weight proteins and peptides present some unique difficulties because of their molecular properties. For example, a peptide or protein may demonstrate aggregation and changes in conformation (unfolding and denaturation) (Dodson et al, 1983; Toniolo et al, 1986). Flexibility within a molecule can have a direct bearing upon biological response and such flexibility can arise in amino acid side chains, within certain parts of the molecule itself or in separate domains that are separated by more rigid covalent bonds (Glover et al, 1983). Immunoglobulin G is a good example of a molecule that has two flexible domains that are believed to be important in antigen binding. Such subtle changes may not only lead to a loss of biological activity (Goldenberg et al, 1983) but could also lead to immunogenic reactions. Self-association can involve intermolecular H-bonds and hydrophobic interactions and will lead to a decrease in the solubility of the peptide. The degree of aggregation will be controlled by the mean chain length, the solvent and the concentration of the peptide. Other external factors such as pH, metal ions, ionic strength, temperature and agitation have been implicated. It can be minimised by incorporation of residues promoting folding (Toniolo et al, 1986).

Insulin is a good example of a molecule where extensive studies have been undertaken to ascertain its properties in solution, the phenomenon

of aggregation, polymerisation of the molecule (gelling in solution) and how this can be controlled by pharmaceutical means through the addition of stabilising agents, eg nonionic surfactants (Dodson et al, 1983). The sites in the molecule responsible for aggregation have also been determined.

Peptide molecules can also change their structure and aggregation behaviour depending upon the environment in which they are placed. For example gramicidin A can undergo different conformational arrangements, depending on whether it is for example within a phospholipid bilayer (helical dimer structure) or a nonpolar solvent (double helix structure) (Wallace, 1983). Small changes in peptide sequences in a molecule can alter dramatically the physicochemical properties of such a species. These modifications can occur through instability or can be induced deliberately into a peptide molecule to improve its characteristics both for delivery and biological response (Samanen, 1985).

Proteins and peptides may cause special difficulties in their handling characteristics; particularly adsorption onto surfaces to include glass and plastic. Such adsorptive behaviour can lead to a significant loss in the amount of material available for delivery. Interestingly these properties can be exploited and the adsorption of peptides to microporous polyethylene has been used as a way of developing a controlled release system for the delivery of Vasopressin. The adsorbed material provides a reservoir effect and consequently pseudo zero order kinetics for the drug material (Kruisbrink and Boer, 1984).

Conventional preformulation studies such as those on stability-pH profile, solubility-pH profiles etc need to be supplemented with investigations involving the action of enzymes (proteases and peptidases) (Wunsch, 1983; Tobey et al, 1985). Peptides and proteins are particularly susceptible to hydrolysis of amide bonds (catalysed by peptidases) and oxidation of disulphide bonds (Carone et al, 1982). There are various ways in which the molecular structure might be altered to provide enhanced stability (Wunsch, 1983; Samanen, 1985). Table 3 (after Samanen, 1985) lists a variety of possible options. Proteins modified by the covalent attachment of carbohydrate and polyoxyethylene derivatives also possess enhanced stability characteristics and a higher resistance to proteolysis (Abuchowski and Davis, 1981).

Table 3. Modification to peptide backbone to reduce peptide degradation

- olefin substitution
- carbonyl reduction
- D-amino acid substitution
- N α-methyl substitution
- C α-methyl substitution
- C αC'-methylene insertion
- dehydro amino acid substitution
- retro-inverso modification
- N-terminal to C-terminal cyclisation
- thiomethylene modification