Delivery of Recombinant Peptide and Protein Drugs

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Introduction

Advances in cell and molecular biology have led to a greater cognizance of the therapeutic value of peptides and proteins. Recombinant DNA technology has enabled high-level expression of these biological response modifiers (Sharma, 1989) and biotechnology has permitted large-scale production for clinical use (Baum, 1987). Examples of recombinant peptide and protein drugs already commercially available are human insulin, tissue plasminogen activator (tPA) (Collen, 1985), human growth hormone (hGH), interleukin-2 (IL-2) (Rosenberg et al., 1987; Negrier et al., 1989; Stoter et al., 1989) and human erythropoietin (Erslev, 1987). Others such as human interferon (Merigan, 1988), human tumour necrosis factor (TNF) (Chapman et al., 1987), interleukin-1 (IL-1) (Dinarello et al., 1986) and haemopoietic growth factors (Morstyn and Burgess, 1988) are undergoing clinical trials. It is abundantly clear that novel peptide and protein drugs having unique pharmacological properties will be used clinically in the coming decades (Check, 1984). These will include a vast range of molecules with markedly different physical and chemical properties and physiological actions (see Table 1). Size alone will vary enormously from some metabolism-modulating peptides such as luteinizing hormone-releasing hormone (LHRH), a decapeptide of molecular weight 1182, to cardiovascular-active peptides such as tPA, 567 amino acids of molecular weight 59 050 (Banga and Chien, 1988b). A number of proteins, such as interferon-β1, interferon-β2 and erythropoietin, are naturally glycosylated whereas many, for example insulin, albumin and

Abbreviations: AAT, α1-antitrypsin; ACTH, adrenocorticotropin hormone; BSA, bovine serum albumin; Cbl, cobalamin; CHO, Chinese hamster ovary; GH, growth hormone; GHRFs, growth hormone-releasing factors; GI, gastrointestinal; hGH, human growth hormone; HRP, horseradish peroxidase; IL-1, interleukin-1; IL-2, interleukin-2; LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone; LPC, lysophosphatidylcholine; PEG, polyethylene glycol; SOD, superoxide dismutase; STDHF, sodium taurodihydrofusidate; TNF, tumour necrosis factor; tPA, tissue-type plasminogen activator.
interferon-α, are not. In addition, the ranges of biological effects and modes of action are immense.

Table 1. Examples and application of recombinant peptide and protein drugs in clinical use or proposed for clinical use

<table>
<thead>
<tr>
<th>Peptide or protein drug</th>
<th>Application</th>
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<tbody>
<tr>
<td>IL-2</td>
<td>Renal carcinoma</td>
</tr>
<tr>
<td>LHRH</td>
<td>Prostate carcinoma</td>
</tr>
<tr>
<td>IL-1</td>
<td>Carcinoma</td>
</tr>
<tr>
<td>TNF</td>
<td>Carcinoma</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>Wound healing</td>
</tr>
<tr>
<td>Transforming growth factor</td>
<td>Wound healing</td>
</tr>
<tr>
<td>Fibroblast growth factor</td>
<td>Wound healing</td>
</tr>
<tr>
<td>Insulin-like growth factors</td>
<td>Wound healing</td>
</tr>
<tr>
<td>Hirudin</td>
<td>Fibrinolytic</td>
</tr>
<tr>
<td>tPA</td>
<td>Fibrinolytic</td>
</tr>
<tr>
<td>Urokinase</td>
<td>Fibrinolytic</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Erythropoiesis stimulation</td>
</tr>
<tr>
<td>Insulin</td>
<td>Glucose regulation</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Haemophilia</td>
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<tr>
<td>Factor IX</td>
<td>Christmas disease</td>
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<tr>
<td>Macrophage-inhibiting factor</td>
<td>Immunoregulation</td>
</tr>
<tr>
<td>Macrophage-activating factor</td>
<td>Immunoregulation</td>
</tr>
<tr>
<td>Muramyl dipeptide</td>
<td>Immunoregulation</td>
</tr>
<tr>
<td>Colony-stimulating factor</td>
<td>Immunoregulation</td>
</tr>
<tr>
<td>Interferons</td>
<td>Gaucher's disease</td>
</tr>
<tr>
<td>Glucocerebrosidase</td>
<td>Bone disease</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Labour induction</td>
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<tr>
<td>Oxytocin</td>
<td>Dwarfism</td>
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<tr>
<td>GH</td>
<td>AAT deficiency</td>
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</tbody>
</table>

A number of issues need to be addressed when considering the clinical use of these macromolecules (see Tomlinson, 1987). These include aspects of recombinant DNA technology and biotechnological production, pharmacological safety and regulatory affairs (Sharma, 1989) and will not be considered in this review. Other aspects also need to be addressed, viz. The route of administration and the mode of delivery. It is only in the recent past that these issues have gained the necessary prominence that will lead to more appropriate delivery systems for therapeutic peptides and proteins and offer clinicians better drugs to combat disease. Although some excellent reviews have been published regarding one or other of these aspects, there is a paucity of literature dealing with the intimate nature of both. Consideration will be given to advances made in the administration of recombinant peptide and protein drugs and also the rational design of delivery systems.

Parenteral delivery
To date, almost all therapeutic peptides and proteins are administered parenterally and many will have to be delivered in this manner, at least for the foreseeable future. A major challenge for pharmaceutical development is to implement this in an effective manner. Regarding parenteral administration per se, several novel delivery systems have already been developed. This is
because most peptides and proteins have a short circulating half-life and have to be injected repeatedly, often several times a day, to achieve the necessary biological response. Considerable resources within the pharmaceutical industry are directed towards developing delivery systems that will offer prolonged and controlled drug-release patterns (see Ritschel, 1989). Long-acting injectable formulations of insulin have been used for several years and are still popular. They are based on the finding that insulin can form a water-soluble zinc–insulin complex with zinc ion in a suitable buffer medium. The crystallinity of the complex is controllable and the degree of crystallinity determines the rate of dispersion and hence the duration of action following administration of the preparation (Halebian and McCrone, 1969).

SYSTEMS

Non-degradable and degradable polymeric systems have been used to sustain the release of several drugs (Gimbrone et al., 1974; Hutchinson and Furr, 1987) and these date back to the early seventies when Davis (1972, 1974) reported the sustained release of insulin, luteinizing hormone (LH), bovine serum albumin (BSA) and a prostaglandin, using a non-degradable cross-linked polyacrylamide and polyvinylpyrrolidone gel as a drug carrier. Biocompatible non-degradable carriers have been used, such as hydroxyethylmethacrylate polymer and ethylene–vinyl acetate copolymer, for the sustained release of macromolecules (Langer and Folkman, 1976, 1978). Although it was demonstrated that sustained release from these polymeric implants could occur over several months, reproducibility of release was erratic. Methods to improve the reproducibility of release kinetics proved difficult, although progress has been made using alternative techniques for the preparation of the polymer matrix (Cohen, Sieger and Langer, 1984; Siegel et al., 1984). Magnetism has also been used to release macromolecules from polymeric devices (Langer et al., 1985; Kost and Langer, 1986). Magnetically modulated drug delivery systems have been developed that consist of the drug dispersed within a polymer matrix together with magnetic beads. The rate of release is controlled by an oscillating magnetic field. When the matrix is exposed to the magnetic field, around 30 times more drug is released and the rate returns to normal when the field is discontinued. Implants composed of the polymer/magnetic bead matrix and insulin were tested in diabetic rats by placing subcutaneously. After implantation the blood glucose levels decreased due to diffusion of the insulin from the matrix. However, when exposed to an oscillating magnetic field, the blood glucose levels were further depleted. Clinical usage of this system will demand extensive in vitro and in vivo studies.

Degradable polymeric systems offer a significant advantage in that they need not be withdrawn from the patient. A highly potent synthetic analogue of LHRH, which is normally administered parenterally once or twice daily, has been successfully developed into an injectable, biodegradable depot formulation that can be administered to animals over a period of one month or longer (Hutchinson and Furr, 1987). The formulation is based on
poly[lactide-co-glycolide] and the mechanism of release was shown to be biphasic. The first phase of release results from leaching of the peptide from surface domains and the second phase is due to degradation of the polymer leading to a generation of microporosity and subsequent release of the peptide. This formulation has been tested in clinical trials with prostate carcinoma patients (Walker et al., 1984) and considerable therapeutic potential was demonstrated. Holland, Tighe and Gould (1986) cite numerous examples of polymeric matrices suitable for controlled release of macromolecules and include poly[lactic acid], poly[glycolic acid], polydioxanes, polylactones, polyester hydrogels and polyoxalates. The authors suggest that this range of material offers a considerable modification of release rates. However, many more studies are necessary to show the value of this approach with peptides and proteins. Microcapsules have also been developed that offer a constant and extended release of peptides and proteins. de Nijs and co-workers (1988) encapsulated several peptides and model proteins, including a modified adrenocorticotropic hormone (ACTH), an LHRH antagonist, porcine insulin, bovine GH and BSA into low molecular weight poly[lactic acid-co-glycolic acid] microspheres. Long-acting dosage forms resulted but it was concluded that due to the variations in the physical properties of peptides, optimal encapsulation process conditions would have to be determined for each drug.

Liposomes have been advocated as potential vehicles for controlled delivery, and many peptides and proteins have been tested (see Weiner, 1989; J. Senior, Ch. 9 this volume). The sustained delivery of interferons has been extensively examined using various liposomal formulations (Eppstein et al., 1985, 1986; Felgner and Eppstein, 1986). After intramuscular injection into mice, liposome-associated (stearylamine with or without cholesterol) interferon-α in reverse-phase evaporation vesicles was retained at the injection site for 3 days, significantly longer than free interferon (Eppstein and Stewart, 1982). The formulation of the liposomes proved critical. For example, phosphatidylserine-containing liposomes without a cholesterol component resulted in no increased local interferon retention compared to administration of the free drug. The later work showed that some vesicle formulations were capable of prolonging interferon delivery for 9 days after intramuscular or subcutaneous injection. Sustained release of liposomal calcitonin ranged from a few hours to several days (Fukunaga et al., 1984). However, after several years of study, clinical success has been limited and it remains to be seen whether the success of liposomes in the field of vanity creams will be reproduced for sustained peptide and protein delivery.

Mechanical devices have been tested on a range of peptides, most notably insulin, and continuous infusion devices have been used successfully (Pickup et al., 1978; Tamburlane et al., 1979; Nathan, 1982). However, concerns regarding the result of mechanical failure have been raised. Computer-driven infusion pumps (Sefton et al., 1979, 1984; Brown, Nelson and Bottoms, 1987) and piezoelectric-controlled micropumps (Watler and Sefton, 1987) have also been developed. It is difficult to envisage widespread use of such mechanical
devices, but they clearly offer a considerable advantage when used in controlled release feasibility studies.

More sophisticated systems have been proposed that could offer drug on demand or are self-regulating (see Goosen, 1987). Once again, insulin has been the drug of choice in these studies and several groups have developed physiologically relevant feedback systems. For example, insulin-encapsulated glucose-sensitive hydrogel polymers containing pendant amine groups have been formulated. Membrane permeability is a function of glucose concentration on the outside of the polymer and as the glucose concentration increases more insulin is released (Albin, Horbett and Ratner, 1985). An enzymatically controlled feedback system has also been developed (Fischel-Ghodsian et al., 1988). This approach is based on the observation that changes in pH can cause dramatic shifts in the solubility of peptide drugs, and solubility is one of the prime determinants of diffusion rate. The concept was tested on an ethylene–vinyl acetate copolymer containing insulin and immobilized glucose oxidase (EC 1.1.3.4). The reaction of glucose to gluconic acid (catalysed by the glucose oxidase) reduces the pH, and insulin solubility increases with decreasing pH thereby releasing more insulin in response to glucose. The system has been tested successfully in vitro and in a diabetic rat model.

Goosen (1987) articulates the rationale for the encapsulation of insulin-producing cells as ‘the search for an artificial pancreas’. The immobilization of transplanted cells from the islets of Langerhans within semi-permeable microcapsules offers the potential for a bioartificial pancreas (Sun et al., 1980; Sun, O’Shea and Goosen, 1983). However, although these systems have been tested in animals, to date, little is known about efficacy in humans. Testing these systems in humans will prove difficult and therefore extensive in vivo data will need to be provided to satisfy regulatory authorities of the potential benefits. Although the more sophisticated approaches remain speculative, the potential for regulated release or release of the drug on demand is immense for conditions such as diabetes.

PHARMACOLOGY

Given that the parenteral route will be used extensively for peptide and protein drugs, it will be essential that delivery is compatible with the physiological action of the therapeutic peptide or protein. For endocrine-like peptides and proteins some form of sustained release may be appropriate and is compatible with the biological response desired. This type of mediator normally enters the vasculature at the site of biosynthesis, is sufficiently stable to reach its target cell, which is often remote, and can extravasate if the target cell is in the extravascular parenchymae. Therefore, conventional routes of administration allied to correct dosage regimes are consistent for this type of mediator. Nevertheless, pulsatile release would often better mimic the normal physiological state and could offer advantages in terms of disease management. This issue will be considered in the next section. For many growth factors that can be paracrine or autocrine mediators and which, in normal circumstances, are produced and act locally over very short distances,
sustained release within the vasculature will almost certainly be inappropriate. The target cells for many of these molecules are extravascular and the molecules are of a size which precludes extravasation. Intravenous administration is therefore completely inappropriate.

Unlike most low molecular weight drugs, many proposed therapeutic peptides and proteins will need to arrive at their site of action in a discontinuous manner. The administration pattern of a peptide or protein drug can be a powerful determinant of its resultant pharmacological effect. This is particularly so for autocrine and paracrine mediators, which are often produced and released in order to act in concert with other mediators in a cascade-like process. A striking example of the potential advantages of pulsatile delivery relates to work with GH (Clark et al., 1985). Pulsatile intravenous infusions of human and bovine GH led to a greater effect on the growth of hypophysectomized rats than did continuous infusion. The number of pulses also affected growth, with nine pulses per day more effective than three pulses per day. Pulse duration did not affect growth rates. In addition, the authors demonstrated that the intravenous route was three times more effective than the subcutaneous route. It was concluded that long-term pulsatile intravenous infusions of GH mimicked the endogenous secretory pattern and were most effective when administered at the physiologically appropriate pulse frequency. In another study, Clark and Robinson (1985) administered growth hormone releasing factors (GHRFs) in both continuous and pulsatile fashion to normal and GHRF-deficient rats. They showed a greater effect on GH release, and therefore growth, from the pulsatile infused peptide in both rat types. The same group has repeated their findings with somatostatin delivered by pulsatile infusion (Clark and Robinson, 1988). In this case, intermittent delivery of somatostatin led to increased body weight and pituitary GH content in rats, whereas constant infusion of equivalent amounts of drug had no effect on body weight gain.

Dunn et al. (1989) demonstrated the pathological effects in mice of slow-release ethylene–vinyl acetate formulations of three cytokines, viz. IL-1, IL-2 and interferon-γ. Their observations suggested that continuous endogenous local release of IL-1 and IL-2 in vivo was sufficient for the development of specific pathological features characterizing chronic immuno-inflammatory diseases. However, interferon-γ failed to elicit a significant tissue response. Although this study was performed to adjudge the role of cytokines in the pathogenesis of immuno-inflammatory diseases, it issues a warning regarding constant release for therapeutic purposes.

On the topic of concerted therapy, Aggarawal and colleagues (1985) showed that interferon-γ influences the cytotoxic effects of TNF-α and β on the human cervical carcinoma cell line, ME-180. Preincubation of the cells with interferon-γ increased the total number of TNF receptors two- to threefold without any change in the affinity constant. It is therefore apparent that many autocrine and paracrine mediators do not exert a biological response in isolation and are single steps in cascade processes. Pulsatile delivery and the need to understand the exquisite interaction of some mediators are only two of many issues that will have to be addressed for the
effective therapeutic use of a future generation of therapeutic peptides and proteins.

SITE-SPECIFIC DELIVERY

It is now evident that conventional types of therapy, while being appropriate for some peptides, will not satisfy the criteria required for the delivery of numerous paracrine and autocrine mediators. Unless drug delivery strategies keep pace with the production of these mediators then their therapeutic potential will be compromised and largely unfulfilled. The concept of site-specific delivery as a means of circumventing many potential problems gained much attention in the eighties, and several excellent reviews were published (Tomlinson, 1987, 1989). It is a major challenge of the coming decades to translate our theories into practical strategies for routine clinical use. The concept of site-specific drug delivery envisages a drug which reaches its site of action, often previously inaccessible, in a manner appropriate to the disease and the drug. This will lead to a less unfavourable disposition of the drug, a reduction in side-effects and will improve on the benefit : risk ratio. Several strategies to achieve this have been proposed and examples of recent approaches will be considered.

Controlling the dispersion of a peptide or protein would satisfy the constraints described above. This could be achieved either by incorporation into a macromolecular carrier which would protect drug and body alike until release at the target cell, or by modifying the peptide or protein structure. The use of a carrier offers the potential for adjusting drug access to its site of action and its pharmacological response. Carriers have been broadly categorized into soluble macromolecular drug conjugates and drug-bearing particulate systems, and both categories have been discussed previously (O’Mullane, Artursson and Tomlinson, 1987). The potential for these carriers to target to lung, liver, bone marrow and cancer cells has been pin-pointed, but to date there are few specific examples of peptide delivery in humans.

More examples exist where attempts have been made to control the biological dispersion of peptides and proteins by modifying their structure. For example, a series of deletion mutants of tPA were constructed by deletion mutagenesis, expressed in Chinese hamster ovary (CHO) cells and purified to homogeneity (Collen, Stassen and Larsen, 1988). Natural tPA and the deletion mutants were infused intravenously into rabbits and pharmacokinetics and thrombolytic properties determined. The mutants were shown to have altered pharmacokinetic profiles and thrombolytic properties. In this way sequences responsible for liver clearance, fibrin affinity and fibrin specificity were shown to be in different parts of the structure. It has been suggested that the circulating half-life of the native protein can be extended without decreasing efficacy, and the altered protein may prove to be a useful alternative to the natural molecule for thrombolytic therapy in patients with thromboembolic disease.

Many site-specific hybrid proteins have been produced by covalently linking peptide fragments or using recombinant DNA methods to express
ligated proteins (see Tomlinson, 1987). These hybrids can be regarded as
distinct proteins which comprise the combined properties of the parent
molecules. This offers the advantages of adding recognition moieties or
re-ordering effector sequences such that an improved pharmacological profile
results.

Peptides and proteins have also been glycosylated, deglycosylated,
pegoylated and a number of other molecules conjugated to modify their
surface characteristics and therefore change their pharmacodisposition. Rela-
tively simple methods have been developed which enable the synthesis of
polyethylene glycol protein adducts, and several PEG-conjugated proteins
have been investigated for preservation of activity and increased circulating
half-life, including superoxide dismutase (SOD) (EC 1.15.1.1), lactoferrin
and α2-macroglobulin (Beauchamp et al., 1983). SOD coupled to PEG
retained 95% of its original activity and its plasma half-life increased from
around 3 min to 9 or more hours, depending on the PEG derivative studied.
Other proteins, tPA (Berger and Pizzo, 1988), IL-2 (Katre, Knauf and Laird,
1987) and human TNF (Katre, Thomson and Knauf, 1987), show similar
retention of activity and increased circulating half-life.

Several means are therefore available to modify peptide and protein
structure that can result in improvemens in stability, site specificity, cell
binding and tissue distribution. However, the use of carrier molecules or the
modification of endogenous proteins to improve efficacy raises a number of
concerns relating to toxicology (for example, immunotoxicity) and other
regulatory issues. Adequate attention must be paid to these questions in
coming years.

Parenteral administration can lead to problems in terms of disease manage-
ment, particularly in instances where the condition demands home treatment
but is not sufficiently threatening to ensure adequate patient compliance. A
great deal of attention has focused on the search for non-parenteral alternat-
ives, and insulin has been the peptide most actively studied in this respect.
The increasing importance of therapeutic peptides and proteins in the clinic
has led to considerable resources being provided within academia and the
pharmaceutical industry to meet this challenge. It is fair to say that every
human orifice has been explored with a plethora of systems and, not
surprisingly, success has been highly variable. The alternatives to parenteral
delivery will be dealt with in the following section.

Non-parenteral delivery

Unfortunately, the physical and chemical properties of proteins do not make
them model candidates for alternative routes of administration. Their size,
often complex secondary, tertiary and even quaternary structure, and their
surface characteristics suggest that there are likely to be problems. For
example, for a drug to be administered orally it must withstand the following
hostilities: chemical and enzymic degradation in transit, the mucosal barrier,
metabolism during transepithelial transport and hepatic elimination. Table 2
shows the physical and physiological factors involved in the absorption of
peptides and proteins after oral administration. The challenges associated with oral delivery and unique to peptides and proteins have been listed (Humphrey, 1986).

Table 2. Physical and physiological factors affecting the extent of absorption of peptides and proteins

<table>
<thead>
<tr>
<th>Physical</th>
<th>Physiological</th>
</tr>
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<tbody>
<tr>
<td>Solubility</td>
<td>pH, regional and microclimate</td>
</tr>
<tr>
<td>Dissolution rate</td>
<td>Drug binding and complexation</td>
</tr>
<tr>
<td>Stability profile</td>
<td>Proteolytic enzymes</td>
</tr>
<tr>
<td>Molecular size and conformation</td>
<td>Mucosal barrier</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>Intestinal permeability</td>
</tr>
<tr>
<td>Charge</td>
<td>Transcellular transport</td>
</tr>
<tr>
<td>Delivery system</td>
<td>Intestinal and hepatic metabolism</td>
</tr>
</tbody>
</table>

However, a better comprehension of the nature of the limiting steps for non-parenteral absorption of peptides has revealed opportunities for administration via more acceptable and convenient routes. Progress is being made in developing non-parenteral systems, particularly for small (3–12 amino-acid residues) or medium-sized (13–60 amino-acid residues) peptides.

**ORAL**

The oral route is the favoured one for administration for most drugs and, indeed, would be the preferred route for most therapeutic peptides and proteins. Gastrointestinal (GI) absorption of drugs has been reviewed recently (Morré, 1985; Aungst and Shen, 1986; Jackson, 1987; O’Hagan, Palin and Davis, 1987; Kararli, 1989). It has been known for some time that peptides and proteins do cross the GI tract, albeit in small amounts (Wilson and Walzer, 1935; May and Whaler, 1958; Danforth and Moore, 1959; Warshaw et al., 1971; Warshaw and Walker, 1974; Warshaw, Walker and Isselbacher, 1974; Galant, 1976; Warshaw, Bellini and Walker, 1977; Udall and Walker, 1982; Gardner and Wood, 1988; Pusztai, 1989). These peptides and proteins include horseradish peroxidase (HRP) (EC 1.11.1.7), chymotrypsin (EC 3.4.21.1), elastase (EC 3.4.21.37), *Clostridium boulardii* type A toxin, a variety of food antigens and even insulin. It is clear that more than one mechanism exists by which intact peptides enter the blood compartment via the GI tract and these include both passive and specific pathways. The question as to whether these normal physiological processes can be exploited for the delivery of therapeutic peptides and proteins remains unanswered. It is generally accepted that these processes only enable the transport of minute quantities of macromolecule and therefore would be insufficient to achieve therapeutic levels of a peptide or protein. However, at least one natural
receptor-mediated endocytic process has been exploited for the delivery of peptides. Russell-Jones and co-workers (1987) conjugated an LHRH analogue (d-lys-6 LHRH) and BSA to cobalamin (Cbl, vitamin $B_{12}$) via the monocarboxyl derivative and orally administered the conjugates to mice. They showed biological effects after oral administration, viz. ovulation in the case of the Cbl–LHRH conjugate and the presence of significant anti-BSA antibody titres with the Cbl–BSA conjugate. No account was given of the pathway used by the conjugates and it remains to be seen whether the receptor-mediated route was invoked. Although a biological response was detected, the nature of the response makes it difficult to adjudge availability. Pharmacokinetic studies would provide an answer to the question of whether this pathway can be exploited for the delivery of therapeutic amounts of peptides and proteins.

More work has focused on increasing the low amounts normally associated with peptide and protein availability rather than exploiting physiological transport processes. Attempts have been made to circumvent the difficulties associated with the capacity of the oral route with varying degrees of success. One reason for slower progress than was realistically expected has been our lack of understanding of the nature and complexity of the problem. The belief that by simply protecting peptides from proteolytic degradation one could solve low availability after oral administration was naive and did not, for example, take into account the barrier function of the epithelial membrane and associated layers (von Heijne, 1985; Vetvicka and Fornúsek, 1988; Kleinman and Walker, 1989). The co-administration of protease inhibitors with insulin demonstrated little increase in absorption (Danforth and Moore, 1959; Inouye and Vars, 1962). However, more recent reports have shown that by protecting insulin against luminal degradation, absorption is increased (Fuiji et al., 1985) and probably reflects the improvement in the inhibitors used.

Similarly, attempts to bridge the epithelial membrane using ‘enhancers’, ‘accelerators’ or ‘promoters’, thereby allowing marked peptide absorption, has often been rather primitive, resulting in pathological damage to the epithelial cell lining. However, progress has been made and should provide a platform for more rational approaches for at least some therapies. Several workers have attempted to increase the transport of peptides across the GI tract using so-called flux enhancers or absorption promoters. Comprehensive reviews are presently available with respect to this field (see van Hoogdalem, de Boer and Breimer, 1989) and consideration will be given to more successful approaches. Various adjuvants have been used over several decades to enhance the absorption of macromolecules across the GI tract, including salicylates (Peters et al., 1987), water–oil–water emulsions (Shichiri et al., 1978), surfactants (Galloway and Root, 1972), bile salts (Matsumura and Saito, 1989), surfactant–lipid mixed micelles (Muranishi, 1985) and combinative promotion effects of azone and fusogenic fatty acids (Fukui et al., 1986). Although these agents undoubtedly increase absorption, there have been few attempts to determine the mechanism of action or, for that matter, the chronic toxic effects resulting from such application. It is now
clear that many ‘enhancers’ merely perturb the epithelial membrane, albeit transiently, such that endogenous or food-derived molecules in close proximity to the drug and enhancer will also cross into the vascular compartment. In addition, bile salts have been shown to affect the mucosal barrier in different ways. For example, by alteration of the physico-chemical properties of the mucus, solubilization of mucus components and even by stimulation of mucus secretion. Another major concern is the lack of specificity of some enhancers and the inevitable uptake of endogenous molecules in the vicinity of the drug–enhancer dosage form. The consequence of this in terms of toxicity can only be assessed in chronic studies, of which there are precious few. However, one could predict that the absorption of endogenous intestinal enzymes would lead to clinical pathologies. A greater understanding of cell physiology and biophysical forces ought to lead to the development of ‘safe’ enhancement systems which do not cause pathological damage to the epithelial cells and are specific for the drug to be transported.

Steiner and Rosen (1988) have recently patented a proteinoid microsphere system for the delivery of insulin, heparin and physostigmine, and claim that the encapsulating microspheres pass readily through the stomach wall. This would seem contrary to known intestinal physiology as the size of the microspheres ranged from 0.1 μm to 10 μm. Additional work is necessary to determine the mechanism. Damge and co-workers (1988) have demonstrated a significant pharmacological response after the oral administration to rats of insulin encapsulated into very small isobutyloxyacrylate capsules. When administered orally by force-feeding to diabetic rats, insulin capsules decreased fasted glycaemia by 50–60% and this effect was maintained for either 6 to 20 days depending on the dose administered. In normal rats, hyperglycaemia induced by oral glucose load was reduced by 50%. Once again, the route of transepithelial transport was not determined but the authors speculated that the nanocapsules protected insulin from intestinal degradation and that a paracellular mechanism was invoked. The effectiveness of this delivery system was also shown after subcutaneous administration.

Recently, Cho and Flynn (1989) have published on the enhanced absorption of insulin in studies with volunteers. The system comprises a water-in-oil microemulsion in which the aqueous phase contains insulin and the oil phase contains lecithin, non-esterified fatty acids and cholesterol. The formulation is designed such that a proportion of the dose will be delivered to the liver associated with chylomicrons and chylomicron remnants while the remainder enters the systemic circulation. The dose of oral insulin was only 1–3 times higher than would be administered in normal practice and this resulted in a significant reduction in blood glucose levels. It remains to be seen whether this can be developed into a clinically usable system with a suitable toxicological profile, but does represent an exciting development in the field. The authors concluded by describing the potential of this oral delivery system for other recombinant peptide and protein drugs, such as human GH, erythropoietin and calcitonin.

Dosage forms that deliver peptide drugs to specific regions of the GI tract
have been proposed, based on either a ‘trigger’ mechanism (Saffran et al., 1986) or a timed-release element. This in itself may overcome the stability problems arising from chemical and enzymic degradation during GI transit, particularly if the dose can be released in regions such as the proximal colon, thought to be less hostile to proteins. Irrespective of the mechanism, novel dosage forms will have to be developed suitable for carrying and protecting therapeutic peptides and proteins until release at specific regions of the GI tract. To date, few studies have been performed in man.

It is difficult to believe that the tremendous activity in the search for oral delivery systems will not be successful in coming years. A better understanding of the nature of the limiting steps and, in particular, the barrier function of the epithelial cells can only facilitate this. Effective cell-model systems have been developed recently (Wilson et al., 1990) which enable peptide and protein transport across epithelial cells to be studied and may prove essential for the development of clinically effective drug delivery systems.

NASAL

Models of disposition of drugs administered to the human nasal cavity have recently been developed and factors involved in absorption across the nasal cavity mucosa discussed (Gonda and Gipps, 1990). Of course, the nasal route has been extensively used for the delivery of drugs, particularly locally acting vasoconstrictors such as decongestants, antibiotics and anti-inflammatory steroids (Bond, 1987). It has also been used for the delivery of small peptides and many, such as LHRH and LHRH agonists (Anik et al., 1984), vaso-pressin and vasopressin analogues such as desmopressin (Harris et al., 1988) and oxytocin (Hoover, 1971) are now commercially available. Nevertheless, bioavailability of these small peptides after nasal administration is still low, often less than 1%. The nasal route has also been investigated for delivery of larger therapeutic peptides and proteins (Eppstein and Longenecker, 1988; Pontiroli et al., 1989) such as interferon-β (Maitani et al., 1986). For example, insulin has been administered intranasally using a plethora of enhancers, including sodium glycocholate (Pontiroli et al., 1987; Aungst, Rogers and Shefter, 1988), polyacrylic acid gel (Morimoto, Morisaka and Kamada, 1985) and sodium taurodihydrofusidate (STDHF) (Longenecker, 1986). STDHF has an advantage in that there are no local histopathological changes to the nasal mucosa after chronic administration and therefore may prove a good candidate for long-term use (Moses, 1988). However, STDHF has been shown to decrease the ciliary movement of human nasal tissue in vitro (Hermens and Merkus, 1987; Hermens et al., 1990). Given the importance of ciliary movement for mucociliary clearance in the upper airways, long-term application of ciliostatic agents is undesirable. The effects of STDHF on ciliary movement in vivo will have to be examined to determine if this represents a serious concern.

Lyosphosphatidylcholine (LPC) has been used to enhance the absorption of insulin in rats (Illum et al., 1989). Insulin co-administered with 0·5% LPC produced a 65% decrease in blood glucose levels, which is similar to that
obtained for intranasal insulin co-administered with other enhancers. However, LPC is known to have ulceration effects on the gastric mucosa of guinea-pigs (Maksem, Jacobson and Neiderhiser, 1984) and cause morphological changes to intestinal epithelial cells of rats (Bolin et al., 1986) and therefore should be treated with some caution. The effects have been shown to be concentration dependent and it may transpire that safe levels that still offer enhancement potential can be achieved. Toxicity of other enhancers used for intranasal delivery has been demonstrated (Hirai, Yashiki and Mima, 1981; Duchateau, Zuidema and Merkus, 1986; Hersey and Jackson, 1987).

The calcium-lowering polypeptide hormone calcitonin has been marketed for several years as a parenterally (subcutaneous or intramuscular) administered drug and until recently was prepared by chemical synthesis. However, recombinant calcitonin is now available and will soon supersede the chemically prepared molecule. In addition, alternative routes of administration have been investigated, including intranasal delivery (Zeigler et al., 1979; Nagant de Deuxchaisnes et al., 1985; Reginster, Albert and Franchimont, 1985; Sjoberg, Torring and Buch, 1985; Buclin et al., 1987; Hanson et al., 1987; Franceschini et al., 1989; Pontiroli et al., 1989), and clinical trials have been undertaken. For example, Overgaard et al. (1989) studied the effects of intranasal calcitonin on early postmenopausal bone loss over a 2 year period with 52 healthy women. A significant biological effect, as measured by bone mineral content of the spine, was achieved, showing the value of using this route of administration. In another study, 11 women with primary osteoporosis received nasal calcitonin and a biological effect was demonstrated over the short period of the study (Vega et al., 1989). A similar range of enhancers to those tested with insulin have also been applied with calcitonin and therefore suffers the same potential problems for long-term treatment.

Interestingly, Tarquini et al. (1988) have shown a significant circadian absorption of intranasal calcitonin in a human volunteer study. The serum concentration of calcitonin monitored 10 min after dosing was statistically more significant when inhaled at midnight than at other more conventional administration times such as morning or evening. Circadian frequency may be a critical determinant of intranasal calcitonin absorption, and the importance of time of administration should not be lost.

The nasal route offers advantages over the oral route in terms of avoidance of the intestinal milieu and hepatic ‘first-pass’ clearance. In addition, acceptable dosage forms have been developed over a number of years suitable for drug delivery. However, similar problems exist to those encountered with oral absorption, notably enzymatic degradation (Hussain, Bawarski-Nassar and Huang, 1985) and poor epithelial transport. Other problems include a limited surface area for absorption, dissolution problems and removal of drug during conditions such as rhinitis. Therefore, it is not surprising that poor availability has led to the widespread use of enhancers and promoters, many being the same as exploited for oral delivery and therefore raising the same concerns regarding toxicity. However, notable successes have been achieved, and it is
clear that this route of administration will be used for a number of recombinant peptide and protein drugs in the future.

RECTAL

The rectal route has been used to deliver many drugs and offers similar advantages over oral delivery to those enjoyed by the nasal route (de Boer et al., 1982; de Boer, de Leede and Breimer, 1984; Muranishi, 1984). It can be an extremely useful route for delivery of drugs to babies and young children where difficulties can arise using the oral route. Similar disadvantages are also evident, including limited surface area and dissolution problems. Interruption of drug absorption during defecation is another concern and patient acceptability is extremely variable. However, the rectal route does offer a more convenient way to control drug release using osmotic pumps and hydrogel cylinders, although to date they have only been tested with low molecular weight drugs such as propranolol (de Leede et al., 1984) and nifedipine (Kleinbloesem et al., 1984a, b).

An extensive array of enhancers has been used to overcome the difficulties associated with poor and erratic availability after rectal administration, with some limited success. Non-ionic surfactants (Ichikawa et al., 1980) and glycerol esters (Nishihata et al., 1983) have been used to deliver therapeutic amounts of insulin, and non-ionic surfactants in a polyacrylic acid gel to deliver insulin and calcitonin (Morimoto et al., 1980, 1985; Morimoto, Iwamoto and Morisake, 1987). Bile salts have been used to deliver interferon-α (Bocci et al., 1985) and insulin (Aungst, Rogers and Shexter, 1988). However, given the damaging effects of bile salts on the mucosa (Saunders et al., 1975) and the possible carcinogenic effects of rectally administered bile salts (Rainey, Maeda and Williamson, 1986), safer enhancers have been developed. Following on from the success of STDHF with intranasal insulin, van Hoogdalem and co-workers (1990) studied the absorption enhancement of rectally infused insulin with this enhancer. They found that at concentrations of 1% and 4% (w/w) STDHF insulin bioavailability was enhanced from 0-2% ± 0-2% to 4-2% ± 3-2 and 6-7% ± 2-1%, respectively. In addition, blood glucose levels fell in a concentration-dependent manner. Interestingly, varying the site of administration within the rectum did not alter the availability. In another study, (van Hoogdalem et al., 1989), rectal administration of a vasopressin analogue (desglycinamide arginine vasopressin) with STDHF resulted in greatly increased availability and, in this instance, depended on rate of delivery. If the analogue was delivered as a bolus it amounted to 27% ± 6%, whereas when administered by infusion, 47% ± 12% was achieved. Yoshikawa and co-workers (1984, 1986) have demonstrated the successful enhancement of recombinant interferon-β using suppositories containing lipid–surfactant mixed micelles comprised of linoleic acid (0-5%) and HCO-60 (0-4%, a polyoxyethylene castor oil derivative). In addition to a significant increase in absorption, interferon-β entered the lymph circulation in greater proportion to the blood compartment. The same group have investigated the enhancing effects of another mixed micelle
formulation (mono-olein/sodium taurocholate) with dextran sulphate on bleomycin absorption (Yoshikawa et al., 1981). Once again they demonstrated the preferential uptake into lymph and a significant degree of absorption enhancement.

For some forms of treatment, the rectal route could provide a more acceptable alternative to multiple injections. It is evident from the studies cited that peptide and protein delivery is strongly influenced by numerous formulation factors and does require the judicious use of enhancing agents. However, given that this mode of delivery shares many problems with the oral and nasal routes, it is difficult to envisage widespread use of peptide and protein formulations suitable for rectal delivery. This is especially so within the US and UK markets where patient acceptability is considerably lower than in some other countries, such as France and Italy.

BUCCAL

The buccal route has been used for the delivery of many low molecular weight drugs and has been shown to bypass intestinal and hepatic 'first-pass' metabolism (Hussain, Aungst and Shefter, 1986). It has also been used to deliver oxytocin for the induction of labour (Miller, 1974). However, in the case of oxytocin, the extent of absorption proved erratic and had only limited use in the clinic. Other peptides have been tested, such as insulin (Ishida et al., 1981) and a hydrophobic lauroyl derivative of a tripeptide (Veillard et al., 1987) with varying success. In the insulin study an adhesive patch containing a core of 10% sodium glycocholate in cocoa butter was administered via the buccal mucosa to dogs. However, the percentage insulin absorbed was 0.5% relative to an intramuscular injection.

Aungst and Rogers (1989) and Aungst, Rogers and Shefter (1988) compared various transmucosal absorption enhancers on buccal insulin delivery. They showed that in the absence of promoter, buccal insulin was 3.6% ± 2.8% as effective as an intramuscular dose of insulin. However, several enhancers including laureth-9 (a non-ionic surfactant), steroidal detergents, sodium lauryl sulphate, sodium laurate, palmitoyl carnitine and a lauric acid-propylene glycol vehicle markedly improved buccal absorption. In the case of laureth-9, insulin administered via the buccal cavity was 15.3% ± 4.3% to 31.7% ± 8.4% as effective as the intramuscular dose, depending on the pH of the solution. This work also highlighted the morphological differences between different epithelial barriers, in that some agents which were effective in enhancing nasal or transdermal absorption were not effective when used in the buccal cavity. For example, salicylates, which result in a marked increase in rectal and nasal insulin absorption, have little effect on buccal delivery (Aungst and Rogers, 1988).

One can envisage certain applications for buccal delivery of therapeutic peptides and proteins. Fortunately, the accessibility of the oral mucosa will enable the placement and removal of drug delivery systems. However, given that the difficulty of absorption across the buccal epithelial barrier is similar to that encountered in the GI tract, it is difficult to predict widespread usage.
The eye is readily accessible for drug delivery and is utilized mainly for topical or intra-ocular application. Systemic delivery via this route presents unique challenges related to the effective clearing mechanisms associated with the cornea but nevertheless has been achieved with low molecular weight drugs such as timolol (Chang and Lee, 1987) and oxymetazoline (Duzman et al., 1983). To date, conventional ocular preparations have proved inadequate for the systemic delivery of peptides and proteins (Greaves, 1989).

Lee and co-workers (Stratford et al., 1988) demonstrated the ocular absorption of the pentapeptide enkephalinamide with an availability of 36%. In the same paper, the carbohydrate polymer, inulin, was administered by the ocular route, and in this case an availability of only 3% was achieved. More recently, this group have studied the ocular delivery of insulin to rabbits (Yamamoto et al., 1989). In this study absorption enhancers were used and included polyoxyethylene-9-lauryl ether, sodium glycocholate, sodium taurocholate and sodium deoxycholate. The overall insulin bioavailability from ocular delivery in the presence of these enhancers was 10%, and of this over 80% appearing in the systemic circulation was absorbed via the blood vessels in the nasal mucosa. The authors suggest that the effect of such enhancers on corneal permeability would be modest and transient given the short residence time. Quite how this membrane would be affected after chronic administration is open to debate. Clearly, chronic toxicity studies are necessary to show the extent of long-term damage. Morimoto, Nakai and Morisaka (1987) have also used bile salts, such as sodium taurodeoxycholate and sodium taurocholate, to enhance insulin absorption across rabbit corneas in vitro.

Another factor when considering the applicability of this route to peptide delivery is patient compliance in delivering drugs to the eye. It is unlikely that this route will be used extensively for the purpose of peptide and protein administration. Nevertheless, in a similar way to buccal delivery, the ocular route may be appropriate for specialized applications.

PULMONARY

The pulmonary route has been used for the delivery of many drugs because of the vast surface area of the human lower respiratory tract (140 m²). Several anti-asthmatics, including sodium cromoglycate (Church, 1986; Richards et al., 1986) and corticosteroids (Sotomayer et al., 1984), are routinely administered via the respiratory tract. However, to date, very few examples of peptide and protein delivery have emerged. Recently, Hubbard and co-workers (1989) investigated the use of the epithelial surface of the lower respiratory tract to administer recombinant α1-antitrypsin (AAT), a 45 kDa neutrophil elastase inhibitor. An aerosol of AAT was formulated and the integrity and activity of the protein demonstrated in vitro. The aerosol was administered to sheep and absorption across the alveolar membrane was evidenced by its presence in lymph and blood, demonstrating the potential of using aerosolization to administer therapeutic proteins to the lung. It is
unlikely that the pulmonary route will gain extensive use for peptide and protein delivery. Incorporation into an aerosol dispersion will prove difficult for some peptides and proteins and the use of nebulizers suitable for these drugs is rather irksome for the patient. Nevertheless, the potential has been demonstrated and once again particular niches may be filled by this form of administration.

VAGINAL

The vaginal route of administration has been used for centuries to deliver suppositories, often for local effects. More recently, this route has been investigated for the systemic delivery of drugs and has been advocated for the delivery of peptide and protein drugs. To date, only a few studies have been performed. The successful administration of insulin has been achieved using PEGs and a surface active agent (Touitou, Donbrow and Azaz, 1978), and water–oil–water insulin micelles (Shichira et al., 1978). Morimoto et al. (1982) used polyacrylic acid aqueous gel bases to deliver therapeutic levels of insulin to diabetic rats and rabbits, and organic acids were used to enhance the delivery of the LHRH analogue leuprolide in rats (Okada et al., 1983).

TRANSDERMAL

The skin is an expansive and readily accessible organ in terms of drug delivery. Not surprisingly, it is used for both topical (see Washington and Washington, 1989) and systemic delivery of drugs (Chien, 1987, 1988). This route of delivery affords several advantages over other non-parenteral and parenteral routes. Local drug metabolism, especially for peptide drugs, and hepatic first-pass clearance are reduced (Barry, 1986). Patient compliance is good and pharmacokinetic profiles are often markedly improved (Cleary, 1984). To date, few examples exist whereby peptide drugs have been delivered via this route, and those which have been performed were generally with animal models (Stephen, Petelenz and Jacobsen, 1984; Kari, 1986; Siddiqui, Shi and Chien, 1987; Chien et al., 1988). However, recent studies have revealed exciting opportunities particularly with reference to iontophoretic (electrically induced) delivery (Rolf, 1987; Banga and Chien, 1988a). Meyer et al. (1988) have described the successful transdermal administration of therapeutic doses of an LHRH analogue (leuprolide) in a 13-subject study. On application of an 0.2 mA electrical current to transdermal patches containing the leuprolide, they demonstrated significant elevation of LH blood levels, comparable to those achieved by subcutaneous injection. Moreover, the patches were well tolerated without signs of cutaneous toxicity. Patches without electrical current produced no elevation of LH concentration. The authors concluded that the low levels of electrical current used induced changes in the permeability of the stratum corneum.

Transdermal delivery may prove to be a convenient and well-accepted alternative to parenteral administration, particularly with respect to iontophoretic delivery. However, three areas of research involving a multidiscipli-
plinary approach need to be addressed, viz. cell biology of the skin with respect to the mechanism of absorption, toxicity considerations of using electrical current for chronic application and miniaturization of the patch and electrical components to a realistic size acceptable for human use.

Conclusion

It is essential that scientists within academia and the pharmaceutical industry devise systems to deliver therapeutic peptides and proteins in a reproducible, pharmacologically relevant, physiologically relevant and safe manner. Development of alternative routes of administration and appropriate delivery systems must progress concurrently with the biotechnological production of these novel biological response modifiers. It is naive to believe that the meagre success to date of site-specific delivery systems will reflect future achievements. The potential benefits of peptide and protein drugs are immense, and a continued investment of resources is needed to marry all the relevant considerations.

Regarding non-parenteral routes of administration, several issues demand to be addressed. It is clear that peptides and proteins will need novel delivery systems to protect from proteolytic degradation and to ensure that therapeutic amounts enter the blood compartment. Improvement in the natural absorption characteristics of peptides and proteins will almost certainly be necessary for all but a few very small or highly potent mediators. To date, little is known regarding the mechanism of the enhancers cited in this review. Broad statements about increasing transcellular or paracellular permeability are commonplace, with but tenuous evidence to support the claims. Detailed, systematic studies are needed to determine the reasons for increased penetration so that more rational approaches can be taken that offer the option of chronic administration as needed in the case of insulin and, for some indications, calcitonin. There are obvious benefits for the patient in using peptide and protein drug delivery systems that do not require multiple injections but rather can be administered by an alternative route, such as orally. Successful implementation of this approach will be a major challenge to researchers and clinicians alike in the coming years.

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Delivered of peptide and protein drugs


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