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Systemic delivery of therapeutic peptides and proteins

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Summary

Peptide/protein drugs are increasingly becoming a very important class of therapeutical agents as a result of our gaining more understanding of their role in physiology and pathology as well as the rapid advances in the field of biotechnology/genetic engineering. These drugs are easily degraded by proteolytic enzymes in the gastrointestinal tract and thus are generally not suitable for oral administration. Currently, they are mostly delivered by parenteral administration. Because they are extremely short-acting, repeated injections are often required. To minimize the health hazard by constant injection, there is an urgent need to search for non-parenteral routes of administration as well as to develop formulations with controlled delivery features. Routes of administration that have been investigated include nasal, ocular, rectal, buccal and transdermal. The mode of delivery can also be very important. In the case of luteinizing hormone-releasing hormone analogs, opposite pharmacological effects could be observed depending on whether they are delivered in a pulsatile pattern or in a continuous manner. Self-regulating systems which will release drug based on the needs of the body (feedback mechanism) have also been developed for some peptide/protein drugs, like insulin. Special considerations are required for the formulation, pharmacokinetic and analytical aspects of peptide/protein drugs. Further work is needed on these aspects and on the routes of administration for commercial viability of therapeutic peptides/proteins.

Introduction

Management of illness through medication is about to enter an era of rapid growth in the area of pharmaceuticals that are of peptide and protein origin (Lee, 1987). The growing interest can be ascribed to the increased understanding of their role in physiology and therapy as well as the established capability of producing a large quantity by sophisticated biotechnological processes. Ailments that might be treated more effectively include cancer, autoimmune diseases, memory impairment, mental disorders, hypertension and certain cardiovascular and metabolic diseases (Table 1) (Lee, 1987).

One typical example is the discovery of tissue plasminogen activator (t-PA) which is a macromolecule consisting of 567 amino acid residues and has a molecular weight of 59,050 Da. t-PA is produced normally by the body, as a thrombolytic agent, to clear debris from the bloodstream by causing the dissolution of blood clots. It can be used in the treatment of certain heart attacks and strokes, for which it has only recently received

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TABLE 1

Some representative peptide and protein drugs and their potential functions and biomedical applications

Peptide/protein drugs	Functions/applications
Cardiovascular-active peptides and proteins	
Angiotension II antagonist	lowering blood pressure
Antriopeptins	regulating cardiovascular function and electrolyte and fluid balance
Bradykinin	improving peripheral circulation
Calcitonin gene-related factor	vasodilator
Captopril	heart failure management
Tissue plasminogen activator	dissolution of blood clots
CNS-active peptides and proteins	
Cholecystokinin (CCK-8 or CCK-32)	suppressing appetite
Delta sleep-inducing peptide (DSIP)	improving sleep that is disturbed
β-Endorphin	relieving pain
Melanocyte inhibiting factor-I	improving the mood of depressed patients
Melanocyte stimulating hormone	improving attention span
Neuropeptide Y	controlling feeding and drinking behavior
Nerve growth factor	stimulating nerve growth and repair
GI-active peptides and proteins	
Gastrin antagonist	reducing secretion of gastric acid
Neurotension	inhibiting secretion of gastric juice
Pancreatic enzymes	digestive supplement
Somatostatin	reducing bleeding of gastric ulcers
Immunomodulating peptides and proteins	
Bursin	selective B-cell differentiating hormone
Colony stimulating factor	stimulating granulocyte differentiation
Cyclosporine	inhibiting functions of T-lymphocytes
Enkephalins	stimulating lymphocyte blastogenesis
Interferon	enhancing activity of killer cells
Muramyl dipeptide	stimulating non-specific resistance to bacterial infections
Thymopoietin	selective T-cell differentiating hormone
Tumor necrosis factor	controlling polymorphonuclear functions
Metabolism-modulating peptides and proteins	
Human growth hormone	treating hypopituitary dwarfism
Gonadotropins	inducing ovulation, spermatogenesis and cryptorchidism
Insulin	treating diabetes mellitus
Luteinizing hormone-releasing hormone (LHRH)	inducing ovulation in women with hypothalmic amenorrhea
Oxytocin	maintaining labor
Thyrotropin releasing hormone (TRH)	prolonging infertility and lactation in women who are breastfeeding
Vasopressins	treating diabetes insipidus

Source: expanded from Chien (1987) and Lee (1987).

FDA approval for marketing (Activase, Genentech) (F-D-C Reports, 1987). Results from recent clinical trials have demonstrated the efficacy of t-PA when it is delivered by parenteral administration within a few hours from the onset of a heart attack. Another protein drug found benefi-

TABLE 2

Polypeptide growth factors with varying degree of characterization and growth-regulating agents

Detailed characterization	Initial characterization
Epidermal growth factor (EGF)	B-cell growth factor
Insulin-like growth factors I&II (IGF-I&-II)	Bone-derived growth factor
Inter-leukin-2 (T-cell growth factor) (IL-2)	Chondrocyte growth factor
Nerve growth factor (NGF)	Endothelial-derived growth factors
Platelet-derived growth factor (PDGF)	Macrophage-derived growth factor
Transforming growth factor (Type 1 or α) (TGF)	Neurotrophic growth factors
	Transforming growth factor (γ)
Partial characterization	Growth-regulating agents
Cartilage-derived growth factor	Growth hormone
Colony-stimulating factors (CSFs)	Insulin
Endothelial-cell growth factors (ECGFs)	Placental lactogen
Erythropoietin	Plasminogen activators
Eye-derived growth factors (EDGF)	Prolactin
Fibroblast-derived growth factor (FDGF)	Relaxin
Fibroblast growth factors (FGFs)	Thrombin
Glial growth factor (GGF)	Transferrin
Osteosarcoma-derived growth factor (ODGF)	Vasopressin
Thymosin	
Transforming growth factor (Type II or β) (TGF)	

Source: James and Bradshaw (1984)

cial for the treatment of heart attack, during animal studies, is the enzyme called superoxide dismutase (SOD).

A large family of hormone-like regulating agents called polypeptide growth factors (PGFs) (Table 2) are being characterized (James and Bradshaw, 1984). PGFs are important in the stimulation of growth and maintaining the viability of a broad variety of cell types. Epidermal growth factor (EGF), a PGF which has been characterized in detail, is a polypeptide affecting the growth and/or the differentiating functions of a wide variety of tissues (Stoscheck and King, 1986a). Interest in EGF has been growing, since it has been shown that EGF and its receptor may play a role in carcinogenesis (Stoscheck and King, 1986b). EGF may also be useful therapeutically for burn and wound healing, cataract surgery and other ophthalmic applications. Interleukin-2 (IL-2) is also a well characterized PGF and is a lymphokine that affects a number of immune systems and their immunological responses; it will likely be used to treat cancer either alone or in conjunction with other lymphokines and chemotherapies.

Erythropoietin, a PGF with partial characterization, is a circulating glycoprotein hormone which is produced naturally by the kidney and is known to stimulate the production of red blood cells. In a recent study (Eschbach et al., 1987), recombinant human erythropoietin was administered to anemic patients with end-stage renal disease who were undergoing hemodialysis. The results suggested that it is effective in eliminating the need for transfusion and restoring the hematocrit to normal levels.

Atrial natriuretic factor (ANF) is a peptide hormone naturally secreted by the heart (Atlas, 1986). It is a natural diuretic and lowers blood pressure. ANF is a potential drug for the treatment of hypertension and a number of biotechnology companies are active in developing its biosynthesis. Other cardiovascularly active peptide/protein drugs of potential interest include bradykinin and related kinins (Table 1) (Regoli and Barabe, 1980).

Contribution of biotechnology to the advance of peptide/protein drugs

Biotechnology has played a key role in the development of peptide/protein drugs (Elander,

1985; Harford, 1985; Dibner, 1986; Sadee, 1986; Baum, 1987; Itoh, 1987). A new series of peptide- and protein-based pharmaceuticals have now arrived with the advent of recombinant DNA and hybridoma techniques and also with the recent progress in large-scale fermentation and purification processes (Jones, 1987). Product-oriented recombinant DNA research was initiated by a number of small biotechnology companies in the late seventies, and many of the giant pharmaceutical companies and other industries have followed this lead by either establishing their own in-house biotechnology research programs or by entering into joint ventures with the fledgling biotechnology companies (Beck and Pope, 1984). Economic forecasts predict a bright future for the new industry (Harford, 1985) (Table 3). The United States and Japan are expected to be the top competitors with the highest potential for success, but a number of European countries have also recently taken considerable efforts to commercialize biotechnology (Dibner, 1986).

With the advancements in biotechnology, several therapeutic proteins have been successfully produced through recombinant DNA technology such as human growth hormone, human insulin, α-interferon and hepatitis B vaccine. These proteins are now commercially available. Human growth hormone (hGH), or somatotropin, is useful for the treatment of hypopituitary dwarfism or short stature. The hGH product produced by recombinant DNA technique provides an effective and safe alternative to that prepared from human pituitary glands.

Rationale for development of novel drug delivery systems for peptide / protein drugs

A growing number of peptide/protein drugs have been produced by recombinant DNA processes. These new biological products are the exact chemical replicas of the natural products. Although they are highly potent and specific in their physiological functions, most of them are difficult to administer clinically. While they are generally not therapeutically active by oral administration, they have an extremely short biological half-life when administered parenterally and repeated injections are often needed. Further bio-

TABLE 3

Genetic engineering and biotechnology — an economic forecast

Market sectors	Economic growth ($ million)		
	1980	1986	1990
Pharmaceuticals	28	600	> 8000
Diagnostics	8	72	> 3000
Vaccines	–	60	1600
Total market sales	36	732	> 12600

Source: Modified from Harford (1985)

medical applications and marketing of these therapeutic peptides/proteins requires parallel development of viable delivery systems to improve their systemic bioavailability.

Several of these, notably growth hormone (GH), luteinizing hormone-releasing hormone (LHRH), interferon, cyclosporin and t-PA are either commercially available or close to it. As most of these are not effective by oral administration, they must be administered parenterally. Daily multiple injections are required and this therapeutic regimen is highly risky to administer without close medical supervision and also often difficult for most patients to accept. Thus, commercial success of peptide/protein drugs for medication will depend on the development of non-parenteral routes of administration such as nasal, rectal, buccal, pulmonary, vaginal, ophthalmic and transdermal (Lee, 1987) or on the successful development of other novel approaches, such as implantable delivery systems, self-regulating delivery systems etc. to overcome the drawbacks associated with parenteral administration. However, each of the transmucosal routes mentioned above may add additional biological barriers on top of the constraints already imposed by the physical and chemical properties of peptide/protein drugs. The limitations inherent in these routes can be very different in terms of protease activity, tissue permeability and other drug-loss processes (Robinson, 1987).

Structural aspects of peptides / proteins

Proteins are the most abundant components of cells. They exist as enzymes, antibodies, hormones,

transport mediators, and even the structural components for the skeleton of the cell itself (Zubay, 1983). Even though all the proteins are constructed from the same 20 amino acids, proteins are the most functionally diverse of all biological substances (Smith et al., 1983). Basically, they are macromolecules with molecular weights ranging from approximately 5,000 to several millions. Each protein molecule is a polymer with α-amino acids linked together in sequential manner by peptide bonds, which are peptide linkage formed by the α-carboxyl and α-amino groups of the adjacent amino acid residues.

The resulting polymers are called peptides. The term polypeptides refers to the peptides which contain about 8 or more amino acids whereas oligopeptides are those peptides which have less than 8 amino acids. An amino acid unit in a polypeptide is called a residue. Polypeptides that contain from about 50 to as many as 2500 amino acids are called proteins. Proteins with two or more polypeptide chains are known as oligomeric proteins; their component chains are called subunits or protomers.

The polypeptide chain of a protein is folded into a specific three-dimensional structure, which is referred to as the conformation of the protein. The functional groups on amino acids and the sequence of amino acids in the polypeptide chain determine the specific 3-dimensional folding in the polypeptide chain (Zubay, 1983). Hydrophobic residues, i.e., amino acids with aliphatic hydrocarbon groups (such as alanine, leucine etc.) or with aromatic rings (such as phenylalanine and tryptophan), tend to inhabit the interior of the protein molecule. On the other hand, hydrophilic residues, i.e., amino acids with charged and/or polar groups (such as aspartic acid, lysine etc.), tend to lie on the surface in contact with water.

Based on the conformation, proteins can be classified into two major classes: fibrous and globular. Fibrous proteins consist of polypeptide chains which are arranged in parallel position along a single axis to yield long fibers or sheets. They are insoluble in water and form the basic structural elements in the connective tissue, e.g., collagen, keratin and elastin (Lehninger, 1975). The second class is the globular proteins which include the peptide/protein pharmaceuticals. In globular proteins, the polypeptide chains are tightly folded into compact spherical or globular shapes. Most globular proteins are soluble in aqueous systems.

Proteins actually have several levels of structure and the general term 'conformation' only refers to these structures in combination (Lehninger, 1975). A protein molecule has a primary structure, which refers to the covalent backbone of the polypeptide chain and the sequence of its amino acid residues; a secondary structure, which refers to a regular, recurring arrangement in space of the polypeptide chain along one dimension; a tertiary structure, which refers to how the polypeptide chain is bent or folded in 3 dimensions to form the compact, tightly folded structure of globular proteins; and a quaternary structure, which refers to how individual polypeptide chains of a protein having two or more chains are arranged in relation to each other.

Potential routes for peptide/protein administration

Parenteral administration of peptide/protein drugs

General considerations

Because of their susceptibility to the strong acidic environment and the proteolytic enzymes in the gastrointestinal tract, the oral bioavailability of most peptides and proteins is very low. Also, peptides and proteins are high-molecular-weight substances and thus do not easily cross the intestinal mucosa. For systemic delivery of peptide and protein drugs, parenteral administration is currently required in order to achieve their therapeutic activities. However, most peptide/protein drugs cannot accomplish their full range of therapeutic benefits when administered by parenteral route, as limited by the nature of their extremely short-acting biological functions. Thus, research programs have been initiated, over the years, to search for a viable nonparenteral route for the effective systemic delivery of peptide/protein drugs. Another alternative for minimizing the drawbacks of parenteral administration is to develop long-acting parenteral preparations for

peptide/protein drugs as has been done in the case of insulin and heparin.

For parenteral administration, major routes are intravenous (i.v.), intramuscular (i.m.) and subcutaneous (s.c.). A judicious choice of these routes must be made. For instance, because of the limitation in the muscle mass volume, optimal blood levels of antibody that are needed for treating the diseases of primary immune deficiency cannot be achieved with the administration of γ-globulin by the i.m. route (Buckley, 1982). By i.v. administration, on the other hand, γ-globulin therapy can achieve the optimal blood levels of antibody in patients with broad spectrum of antibody deficiencies. However, i.m. administration of γ-globulin has found benefits for the long-term prevention of hepatitis.

I.v. administration is the only currently available means of delivering protein and peptide drugs in situations where the drug is excessively metabolized and/or bound at the site of i.m. or s.c. injection. Besides the usual complications often resulting from i.v. administration like thrombophlebitis, tissue necrosis etc., additional complications associated with the systemic delivery of peptide/protein drugs are due to immunogenicity. While amino acids and small peptides are themselves not immunogenic, macromolecular proteins are often recognized as "non-self" by the body which then responds with the production of a specific antibody. The most potent immunogens are the proteins with molecular weights greater than 100,000 (Martis, 1986). In addition to molecular size, chemical complexity is also important; for example, the aromatic amino acid components in the protein molecule contribute more to its immunogenicity than the non-aromatic amino acid residues. Impurities, such as contaminating bacterial protein etc., represent another source of immunogenic substances. Insulin preparations could be an interesting example. Prior to 1973, these preparations could contain potentially antigenic impurities which could include a significant amount of proinsulin, the biosynthetic precursor of insulin, and also its incompletely converted products as well as other pancreatic hormones (Gilman et al., 1980). Now, 'purified' insulins contain no more than 10 parts per million of proinsulin, and 'purified' porcine insulin has been considered the least immunogenic of the non-human insulin preparations available.

Insulin (Maberly et al., 1982), interferon (Wills et al., 1984), and γ-globulins (Good, 1982) have been reportedly metabolized and/or bound at the i.m. injection sites and as a result, the systemic bioavailability of these protein drugs following i.m administration is less than that obtained after i.v. injection. With the use of proper adjuvants and electric current, systemic absorption of peptides from the i.m. site can be facilitated; tissue plasminogen activator represents such an example. The absorption of t-PA was facilitated by using hydroxylamine or by electric stimulation of muscle and therapeutic blood levels were achieved promptly, accompanied by coronary thrombolysis (Sobel et al., 1985). However, at the present time, t-PA is administered either i.v. or delivered directly into the coronary arteries. Lysis of coronary clots can be achieved with blood levels of t-PA as low as 77 ng/ml. For s.c administration, insulin represents the best example of a protein drug that is administered s.c. on a chronic basis for diabetes treatment.

Controlled delivery of peptide/protein drugs from subdermal polymeric implants

The sustained release of peptide and protein drugs from s.c. implanted polymeric devices was first reported by Davis (Davis, 1972, 1974). He used cross-linked polyacrylamide and polyvinylpyrrolidone gels and demonstrated a prolonged release of immunoglobulin, luteinizing hormone, bovine serum albumin, insulin and prostaglandin. Subsequent studies by Langer and Folkman (1976, 1978) using hydroxyethylmethacrylate (Hydron) polymer and ethylene-vinyl acetate copolymer demonstrated that large molecules with molecular weights up to 2×10^6 Da can be released steadily from the implanted polymeric device over periods longer than 3 months. However, the reproducibility of release kinetics was poor in these studies. Later, the reproducibility of drug release was improved following the use of low-temperature solvent casting method (Rhine et al., 1980; Hsieh et al., 1983). Since this procedure was time-consuming and required the use of organic solvent, an

alternative sintering technique was developed for the preparation of polymer matrices to control the release of macromolecules (Cohen et al., 1984; Siegel et al., 1984). The method consists of mixing drug and polymer powders below the glass transition temperature of the polymer and compressing the mixture at a temperature above the glass transition point. It has also been reported that delivery of macromolecules from polymeric devices can be modulated using magnetism (Hsieh et al., 1981; Hsieh and Langer, 1982; Langer et al., 1985; Kost and Langer, 1986).

The biocompatibility of subdermal polymeric implants is an important concern since these implants may reside in the body for an extended period of time (Leung et al., 1987). In general, the biocompatibility of a given polymeric material is described in terms of the acute and chronic local inflammatory responses and the subsequent fibrous capsule that may form following implantation of the polymeric material. In addition to the inflammatory responses, the biocompatibility of implants can also be measured in terms of sensitivity reactions and infections. These consist of an initial adhesion of macrophages on the tissue/implant interface and then phagocytosis of the polymer by macrophages and giant cells.

Polymers which are attractive from a biocompatibility point of view are the biodegradable polymers as their degradation products are biocompatible. Also, they need not be surgically implanted or removed. Bioerodible polymers that have been investigated for peptide/protein delivery include poly (D,L-lactide-coglycolide) (PLGA), cross-linked serum albumin and poly (lactic acid). PLGA is a linear polyester that hydrolyzes by an acid- or base-catalyzed reaction to form the final breakdown products like D-lactic, L-lactic, and glycolic acids. L-Lactic and glycolic acids will be metabolized by the Krebs cycle while D-lactic acid will be excreted intact. These polymers undergo bulk rather than surface erosion and thus their permeability changes with time, rendering the drug release rate neither constant nor predictable. In view of this drawback, hydrophobic polymers that undergo surface erosion, such as poly (ortho-ester) and polyanhydride are being investigated as alternatives (Lee, 1988).

Currently, ethylene-vinyl acetate copolymer (EVAc) is a non-degradable polymer extensively used in the preparation of polymer matrices to control the delivery of polypeptides. EVAc is biocompatible and has already been used in some non-parenteral controlled-release drug delivery systems approved by the FDA for human applications (Langer et al., 1985), one such example being the Ocusert system for ocular drug delivery. The particle size of the polypeptide and its loading dose can be varied to control its rate of release from EVAc polymer matrix. It has been suggested (Langer et al., 1985) that incorporation of the powdered polypeptide during polymer matrix casting creates a series of interconnecting channels through which water can diffuse into the matrix to dissolve the macromolecule, which is then released through the porous matrix. At higher loadings, polypeptide particles are more likely to touch each other and thus create an efficient or less tortuous channel network which would facilitate the movement of macromolecules out of the matrix. On the other hand, at low loadings, most polypeptide particles will be completely surrounded by polymer and will thus get trapped in the matrix. Only the particles on the surface of the matrix could be released and thus not all the polypeptide impregnated will be released from the polymer.

In addition to being delivered in controlled manner from EVAc matrices (Brown et al., 1986a and b), insulin has also been reportedly released from porous poly (ϵ-caprolactone) matrices (Bechard et al., 1987) and from poly(lactic acid) microbeads and pellets (Kwong et al., 1986). The feasibility of using the biocompatible silicone elastomers for the controlled delivery of proteinaceous macromolecules has also been demonstrated (Hsieh et al., 1985).

Specific examples of parenteral administration of peptide/protein drugs

LHRH and analogs. LHRH is a naturally occurring decapeptide hormone with a molecular weight of 1182 Da. Numerous analogs of this hormone with agonistic or antagonistic activity have been synthesized, e.g., Nafarelin, in which the glycine at the 6th position of native LHRH has been replaced by D-naphthyl alanine, is a

superagonist (Anik, 1986). LHRH is a neurohormone of hypothalamic origin whose function is to stimulate the synthesis and release of gonadotropins from the gonadotropes in the anterior pituitary. However, the biological activity of LHRH depends on the mode by which the hormone is administered. Pulsatile administration of LHRH, which mimics the natural secretory pattern, causes a sustained secretion of gonadotropins (Leyendecker et al., 1980; Crowley and McArthur, 1980). On the other hand, the long-acting synthetic analogs of LHRH have been shown to desensitize the pituitary gland and thus inhibit gonadotrophin release when administered as a single daily subcutaneous injection (Belchetz et al., 1978; Hoffman and Crowley, 1982; Skarin et al., 1982; Cutler et al., 1985). This fact is now being exploited clinically for contraceptive purposes.

LHRH and its analogs are poorly absorbed by oral intake. Thus, parenteral administration with specialized delivery systems are required and work has been initiated in this direction (Kent et al., 1982; Sanders et al., 1982; Vickery et al., 1984). One approach has been the use of an injectable gel system (Vickery et al., 1984) to extend the duration of the action of LHRH agonist. The gel formulation was prepared by suspending the LHRH agonist in sesame oil and then gelling it with aluminium monostearate by slowly heating it to 125 °C with stirring. A thixotropic gel is formed when the substance is cooled to room temperature, which could be injected subcutaneously using a standard syringe. This, by itself, is not a very efficient delivery system; but when used together with a mixed zinc tannate salt of a potent LHRH analog, the gel can exert an action that is very prolonged in duration. Using an implantable cholesterol matrix pellet, the subcutaneous controlled delivery of LHRH agonist analogs has been achieved (Kent et al., 1980; Vickery et al., 1984). On the other hand, LHRH analogs have a very low polymer solubility and diffusivity through silicone elastomer, presumably because of their hydrophilic nature and high molecular weight, and therefore a silicone device is not a suitable delivery system for their controlled delivery. Since LHRH agonists are required only at very low doses, they are particularly suitable to be delivered by bioerodible polymeric delivery systems. The in vivo performance of nafarelin acetate, which was either dispersed in PLGA polymer microspheres (Sanders et al., 1984) or encapsulated in PLGA microcapsules, has been evaluated (Kent et al., 1984). It should be pointed out that PLGA has also been used earlier for the delivery of other drugs and even some clinical trials have been carried out (Beck et al., 1981; Lewis et al., 1982).

Insulin. Insulin, a protein hormone with 51 amino acid residues and a molecular weight of 5808 Da, is the drug of choice for diabetes mellitus, a disease that affects about two million people in the United States alone. Insulin consists of two polypeptide chains linked by disulfide bridges. It is easily inactivated by the gastrointestinal enzymes if taken orally and is thus generally given by s.c. administration. However, extensive research has been done to evaluate other non-parenteral routes such as nasal, ocular, buccal, rectal and transdermal as will be discussed in later sections. An extensive review of the chemistry and biochemistry of insulin was published by Klostermeyer and Humbel (1966). An interesting historical discussion on the development of insulin in collaboration with Eli Lilly Co. has been recently completed by Swann (1986). Human insulin (Humulin by Genentech–Eli Lilly) produced from *E. coli* by genetic engineering (Johnson, 1983) is the first therapeutic recombinant product approved by FDA in 1982 (Giddins et al., 1987). Details of production and comparison with other insulins have been reported (Gueriguian, 1981; Johnson, 1983; Bollon, 1984; Selam et al., 1987). The optimum technique for systemic delivery of insulin has been a subject of much debate (Schade and Eaton, 1985; Thomas, 1986).

Long-acting insulin injectables. Early approaches in the development of long-acting insulin preparations included the complexation of insulin with protamine, a water-soluble, strongly basic, simple protein isolated from the sperm of the mature testes of fish. Later approaches did not use protein-like protamine or globin, but instead used a zinc-insulin complex. In a suitable buffer medium, insulin can be precipitated by zinc ion (as zinc chloride) to yield water-insoluble zinc

insulin complex, which is in either crystalline or amorphous form. The crystallinity of the complex can be controlled by controlling the pH of the buffer medium (Halebian and McCrone, 1969).

The zinc–insulin complex has been used to formulate 3 long-acting insulin preparations with varying duration of normoglycemic activities: ultralente, lente and semilente insulin, which are still popularly used today for the treatment of diabetes. These preparations differ with respect to their onset, duration and intensity of action following s.c. administration. The amorphous zinc insulin complex precipitated at pH 6–8 (semilente insulin) has a rapid onset (0.5–1.0 h) and a moderately long duration of action (12–16 h) as compared to regular insulin (8 h). On the other hand, a cloudy suspension of crystalline zinc insulin complex precipitated at pH 5–6 (ultralente insulin) has a much longer onset (4–8 h) and also a rather long duration of action (36 h). A mixture of crystalline (7 parts) and amorphous (3 parts) insulins (lente insulin) is intermediate in onset (1.0–1.5 h) and has intermediate duration of action (24 h) (Hallas-Moller et al., 1952; Patersen et al., 1959). Attempts have also been made to prolong the normoglycemic action of insulin by entrapping it in liposome–collagen gel matrix for s.c. administration (Weiner et al., 1985).

New injection devices for insulin delivery. A new jet-type injector (Precijet-50) was recently developed (Lindmayer et al., 1986; Halle et al., 1986). The features of this device include small size, simplicity of design and the capability of mixing two types of insulin, which were not available in the earlier jet injection technology (Hingson and Hughes, 1947; Weller and Linder, 1966; Cohen et al., 1972). A sprinkler needle for insulin injection has also been recently described (Kuhl et al., 1986). Another recent development has been the development of an insulin-injection 'pen' (Novopen) which is a pocket-sized apparatus that resembles a fountain pen. When fitted with a disposable needle and unit-dose ampule of insulin, it becomes a portable, self-contained insulin syringe (Haycock, 1986).

Infusion pumps for insulin delivery. Continuous s.c. insulin infusion (CSII) devices or pumps have been in use for almost a decade now (Pickup et al., 1978; Tamborlane et al., 1979; Nathan et al., 1982). However, a possible increase in mortality (Teutsch et al., 1984), morbidity due to mechanical failure (Kitabchi et al., 1983) or lack of data on long-term safety of the treatment (Leichter et al., 1985) indicate that further long-term assessment of the CSII is required. I.p. delivery of insulin through a steam-sterilized micropump has

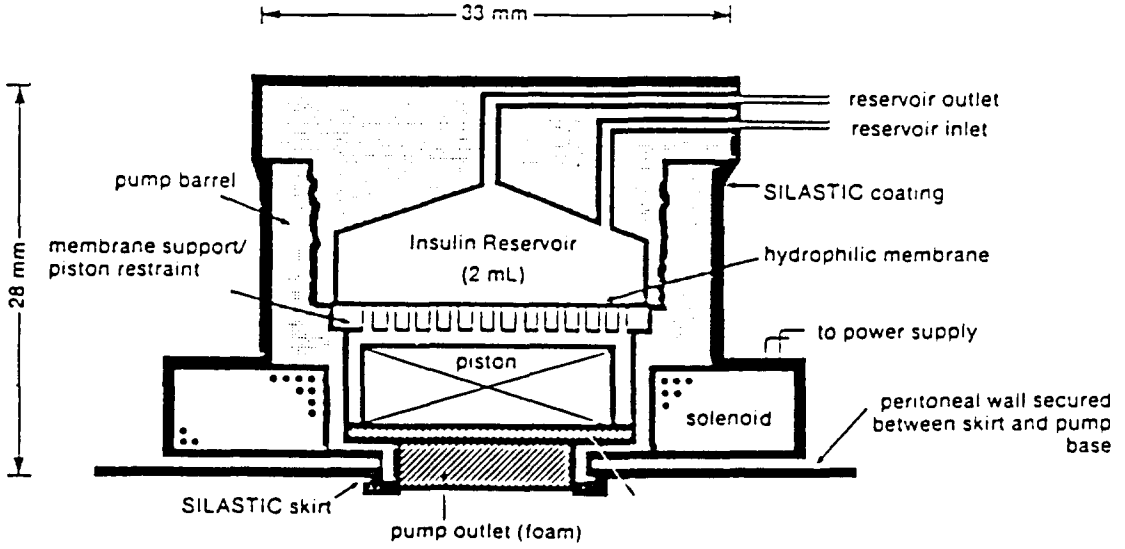

Fig. 1. Schematic illustration of the controlled release micropump (CRM) with a solid piston (Sefton et al., 1984).

been investigated in dogs by implantation (Sefton et al., 1979, 1984). The controlled release micropump (CRM) used is an open-loop control system characterized by operation at two levels: basal delivery for between meals and augmented delivery for short periods after meals. The rate of delivery is adjusted to meet the insulin requirement of the respective meal. With adequate supply of insulin to the pump, the concentration difference between the reservoir and the surrounding fluid results in diffusion through the pump (Fig. 1). It provides the basal delivery of insulin when no external power source is used. Augmented delivery is achieved by repeated compression of the foam membrane by the coated mild steel piston. The piston is the core of the solenoid and compression is effected when current is applied to the solenoid cell. Development of a piezoelectric controlled micropump (P-CRM) has been recently reported (Watler and Sefton, 1987).

Self-regulating delivery systems. An interesting approach was reported on the use of an artificial β-cell having a glucose-sensitive membrane for feedback control of insulin delivery (Horbett et al., 1983, 1984). The membrane is fabricated from a glucose oxidase-entrapped hydrogel polymer containing pendant amine groups. As glucose diffuses into the polymer, glucose oxidase catalyzes its conversion to gluconic acid, thereby lowering the microenvironment pH within the membrane. The reduced pH results in increased ionization of the pendant amine groups. The electrostatic repulsion between ionized amine groups increases the degree of swelling and thus increases the permeability of the hydrogel to insulin contained in the reservoir. Ultimately, then, the membrane permeability to insulin is a function of glucose concentration external to the membrane and insulin delivery is accelerated by the increase in glucose level (Albin et al., 1986). Another potential method to achieve the self-regulating delivery of insulin is to use a biochemical approach based on the principle of competitive and complementary binding behavior of concanavalin (Con) A with glucose and glycosylated insulin (G-insulin) (Jeong et al., 1984, 1986; Kim et al., 1984; Sato et al., 1984; Seminoff and Kim, 1986). A schematic representation of this system is given in Fig. 2. As the glucose level

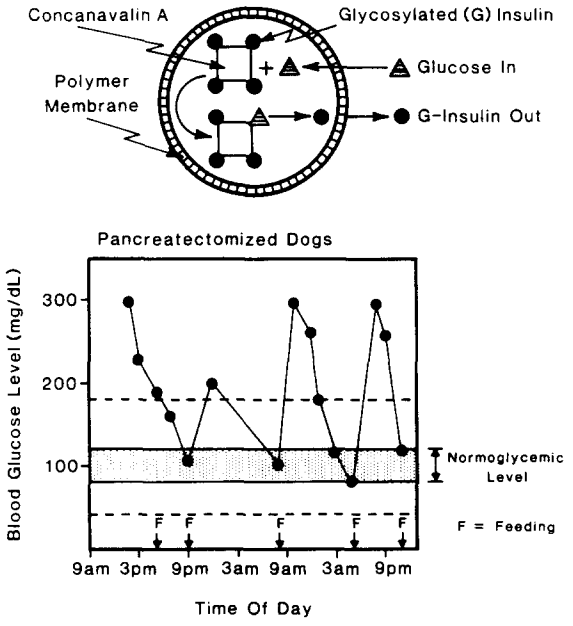

Fig. 2. Schematic representation of a self-regulating insulin delivery system and blood glucose levels of a pancreatectomized dog implanted with this delivery system (Replotted from Jeong et al., 1986).

increases, the influx of glucose to the pouch increases, displacing G-insulin from the Con A substrate. Increasingly displaced G-insulin in the pouch results in efflux of G-insulin to the body. Fig. 2 also shows the blood glucose levels of a pancreatectomized dog having a G-insulin delivery pouch implanted.

Heparin. Heparin, an anticoagulant drug, is used in the treatment of thrombosis and embolism. Heparin exerts its anticoagulating effect by preventing the conversion of fibrinogen to fibrin via the action of proteolytic enzyme called thrombin. Besides its anticoagulant activities, heparin also exerts other pharmacological functions: one of them is its anti-inflammatory function which takes place in the connective tissue as a result of its local interaction with toxic substances.

For the prevention of the thromboembolic phenomenon in man, a continuous i.v. administration of heparin is required. However, the conventional delivery systems currently available cannot maintain a continuous infusion of heparin on a long-term basis, since complications, such as hem-

orrhage, may develop. The development of a pump called Infusaid has permitted the continuous, i.v. administration of heparin in the ambulatory patients.

Heparin is a polysaccharide and being a macromolecule, its delivery could encounter some barriers similar to those needed to be overcome by peptide/protein drugs. Early work on the prevention of surface thrombosis involved the development of a graphite–benzalkonium–heparin (GBH) surface (Whiffen and Beeckler, 1966), which was developed by soaking a colloidal graphite in a benzalkonium sulfate solution and then in a sterile heparin solution immediately prior to implantation (Ebert and Kim, 1984). Another method reported by same authors for developing heparinized polymers is the simple dispersion of heparin throughout the polymer matrix. A novel approach to the controlled release of heparin from polymer matrices was developed by Ebert et al. (1980). Heparin can adversely interact with platelets, resulting in aggregation; while prostaglandins are known to prevent platelet aggregation. When heparin and prostaglandins were simultaneously dispersed in polyhydroxyethyl methacrylate, medicated polymers were formed in which the release of heparin and prostaglandin is controlled over a duration of approximately 10 h. A disadvantage with the ionically bound or physically dispersed heparin/polymer systems is that heparin is continually depleted with time, thereby limiting the effective anticoagulation duration of such systems. Numerous investigations have been initiated to covalently bind heparin molecules to a polymer surface to provide a long-acting heparinized material (Ebert and Kim, 1984).

Vasopressin. Vasopressin or antidiuretic hormone (ADH) is a nonapeptide hormone with a molecular weight of 1084 Da and is secreted by the posterior pituitary glands. Its physiological role is to maintain serum osmolality within a narrow range. It acts on the renal cells that are responsible for the reabsorption of free water from the glomerular filtrate. Deficiency of ADH causes an inability to produce concentrated urine, a condition called diabetes insipidus. Vasopressin has also been indicated in the emergency treatment of bleeding from esophagogastric varices.

Using a small-diameter microporous polypropylene (Accurel) tubing, which was covered with collodion, a long-lasting and constant (in vitro) release of vasopressin was achieved in serum solutions for periods up to 50 days (Boer, 1983). The in vivo potency of this preparation was also shown in the adult vasopressin-deficient Brattleboro rat following s.c. implantation. Normal levels of urine production and osmolality were achieved and maintained for at least 1 month. This successful result led to further investigations of the Accurel/Collodion device (Kruisbrink and Boer, 1984, 1986). It was found that the device appears to be biocompatible and can be used as an implant in adult rats.

Non-parenteral administration of peptide/protein drugs

The non-parenteral administration routes for delivery of peptide/protein drugs include the nasal, buccal, rectal, vaginal, transdermal, ocular, oral and pulmonary routes. In the absence of an absorption-promoting adjuvant, these routes are generally much less efficacious than parenteral administration. Incomplete absorption is probably due to a combination of poor membrane permeability and metabolism at the absorption site. It has been reported (Lee, 1988) that protease activities in the homogenates of the nasal, buccal, rectal and vaginal mucosae of the albino rabbit are substantial and comparable to those in the ileal homogenate. A recent study (Aungst et al., 1988) compared the insulin absorption from various non-injection sites of administration. Rectal insulin was found to be more efficacious than nasal, buccal and sublingual insulin, when administered without an absorption-promoting adjuvant. The efficacy for each route, however, was low compared to i.m. administration. Sodium glycocholate, an absorption-promoting adjuvant, increased insulin efficacy by each route and the rank order was nasal > rectal > buccal > sublingual, with nasal and rectal insulin being roughly half as efficacious as i.m. insulin. A detailed discussion of these non-parenteral administration routes is given in the following sections.

Nasal delivery

While the intranasal route of administration has been commonly used for the delivery of topically effective drugs to alleviate the histaminic symptoms in the nasal cavity resulting from local infection and/or inflammations, there is a growing interest in using this route for the systemic delivery of systemically effective drugs. Currently, a host of drugs have been evaluated for the feasibility of nasal delivery (Table 4) and within the next few years, many drugs are likely to be formulated in nasal preparations and made available in the prescription and/or over-the-counter market (Nudelman, 1987). The existence of an extensive microcirculation network underneath the nasal mucosa allows the effective systemic absorption of protein drugs, which are susceptible to gastrointestinal degradation when administered orally. Furthermore, the fact that the drug molecules absorbed nasally can directly enter the systemic circulation before passing through the hepatic circulation should potentially benefit certain types of drugs which are otherwise subject to extensive hepatic "first pass" elimination. Extensive reviews of the field have recently been completed (Chien, 1985; Chien and Chang, 1988).

The nose is primarily an olfactory organ, but it also plays a role in the clearance of dust, allergens and bacteria. The nasal mucosa consists of ciliated, columnar epithelium; the cilia help to remove foreign substances by transporting them posteriorly towards the nasopharynx, where they are swallowed into the stomach. The cilia also transport some materials anteriorly for removal by nose blowing or wiping (Su, 1986). Therefore, any nasal drug delivery must be critically evaluated for its possible effect on the nasal mucociliary functions. Any potential physical damage to the nasal epithelium must also be investigated. In one study (Su et al., 1985), histopathological examination of the rat mucosa, following nasal delivery of enkephalins, was conducted and mild mucosal necrosis was observed. However, the degree of irritation was considered to be slight in view of the 5-h contact time.

The nasal mucosa is bathed by secretions which contain proteolytic enzymes. This enzymatic barrier can significantly reduce the bioavailability of peptide/protein drugs. The aminopeptidase present in the nasal mucosa are under investigation for their enzymatic activity and effect on hydrolysis of peptides (Su, 1986). Since bile salts enhance nasal absorption, it has been proposed that the effect of sodium glycocholate in enhancing nasal insulin absorption is due to inhibition of proteolytic activity (Hirai et al., 1981a). But a recent investigation (Lee and Kashi, 1987) concluded that aminopeptidase inhibition by bile salts is not necessarily a suitable predictor of their enhancing effect.

Evaluations of the potential of posterior pituitary gland extracts for the treatment of diabetes insipidus have led to the commercialization of synthetic lypressin ('Diapid', by Sandoz) and de-

TABLE 4

Biopharmaceutics of nasal delivery of peptides/protein drugs

Peptide/Protein Drugs	Number of amino acid residues	Time of peak level (min)	Relative bioavailability (%) *	Testing model
Thyrotropin-releasing hormone (TRH)	3	5–15	10–20	Rats, humans
Enkephalin analogues	5	5–10	70–90	Rats, humans
Oxytocin	9	5–10	30–40	Humans
Vasopressin analogues	9	10–20	6–12	Humans
LHRH agonists and antagonists	9–10	10–30	2–5	Monkeys, humans
Glucagon	29	5–10	70–90	Humans
Growth hormone releasing factor (GhRF)	40–44	20–40	2–20	Rats, dogs, humans
Insulin	51	5–10	10–30	Rats, dogs, humans

Source: Modified from Su (1986)

* Relative to the i.v., s.c., or i.m. dose; based on data from different references

smopressin ('DDAVP', by Ferring AB) in nasal dosage forms (Su, 1986). Furthermore, a number of proteins have been studied and found to be systemically bioavailable by nasal route (Table 4) (Solbach and Wiegelmann, 1973; Hirai et al., 1978; Evans et al., 1983; Su et al., 1985). Several investigations have been carried out to evaluate the feasibility of using the nasal route as a simple and practical way for the systemic delivery of LHRH analogs and also to determine their efficacy as contraceptive agents (Zatuchni et al., 1976; Illig et al., 1980a and b; Koch, 1981; Anik et al., 1984). Buserlin, for instance, is a synthetic nonapeptide with a biological activity that is 50–70 times more potent than the natural LHRH. Although a much higher dose has been found necessary for nasal delivery than by the parenteral administration, such synthetic LHRH analogs have been observed to be therapeutically effective when administered intranasally (Koch, 1981). Nasal delivery of calcitonin using a polyacrylic acid gel base has also been investigated (Morimoto et al., 1985)

Intranasal administration of insulin. Insulin has been extensively studied for intranasal delivery (Pontiroli et al., 1982; Moses et al., 1984; Flier et al., 1985; Salzman et al., 1985). The transnasal permeability and nasal absorption of insulin were found to be enhanced by using permeation enhancers such as the bile salts (e.g., glyco- and deoxycholate) or other surfactants. By using these adjuvants, the therapeutically effective plasma levels of insulin necessary for its normoglycemic effects have been achieved (Hirai et al., 1981a and b; Moses et al., 1983). Gordon et al. (1985) reported 2.4 mM as the minimum concentration of sodium deoxycholate required to enhance the transnasal permeation of insulin. By varying the concentrations of sodium deoxycholate coadministered, they demonstrated that therapeutically useful amounts of insulin can be absorbed nasally in healthy human volunteers. The nasal absorption of insulin was found to correlate positively with the hydrophobicity of the bile salts (as inferred from their HPLC retention factors, k'), with the rank order of deoxycholate > chenodeoxycholate > cholate > ursodeoxycholate (Gordon et al., 1985) (Fig. 3). Recently, sodium taurodihydrofusidate, a novel detergent-like adjuvant, has also

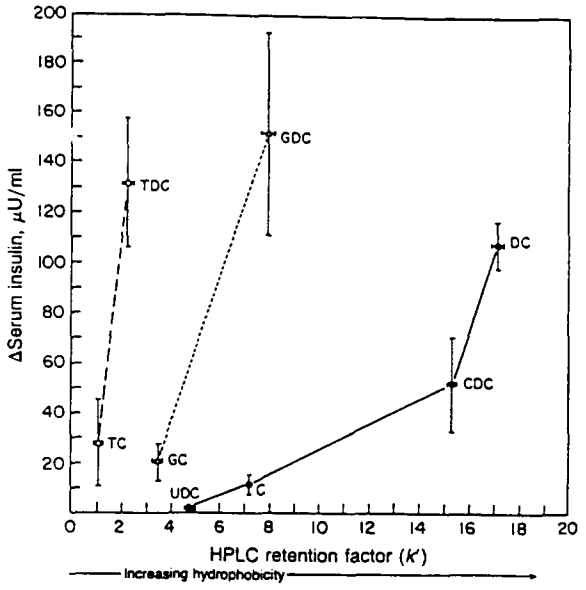

Fig. 3. Dependence of serum insulin levels (Δ) following the intranasal administration of insulin with bile salts on the hydrophobicity of bile salt, which is expressed as HPLC retention factor (k'). Insulin was administered at a dose of 0.5 U/kg b. wt. Deoxycholate (DC), chenodeoxycholate (CDC), cholate (C), ursodeoxycholate (UDC), glycodeoxycholate (GDC), glycocholate (GC), taurodeoxycholate (TDC) and taurocholate (TC) were administered at a final concentration of 1% (w/v) (Gordon et al., 1985).

been demonstrated as an excellent enhancer for the systemic delivery of insulin by intranasal administration (Longenecker, 1987) and clinical trials demonstrated that insulin is absorbed readily into the systemic circulation when delivered intranasally in formulations containing this enhancer (Foster and Lee, 1988).

Intranasal administration of interferon. Interferon is a protein released by cells following exposure to a virus, that enables other cells to resist viral infection. It is now recognized that a given cell type can produce several types of interferon (Hiscott et al., 1984). The current typology of classes α, β and γ has replaced the previous designation of leucocyte, fibroblast and immune interferons, respectively. The availability of recombinant DNA technology has led to the isolation of human interferon genes, their cloning in bacteria, the production in commercially viable quantities of recombinant human interferon by

fermentation and its purification by means of monoclonal antibodies (Pestka, 1983). Extensive evaluations of interferon efficacy against viral diseases and cancer are currently under way.

Nasal delivery of interferons has been investigated. Merigan et al. (1973) studied the inhibition of respiratory virus infection by intranasal administration of human leucocyte interferon. By applying interferon intranasally, using a high daily dose (14,000,000 units) and combining one day of prophylaxis with 3 days of treatment, they observed a statistically significant decrease in the severity of symptoms and in the frequency of virus shedding following the challenge of 16 volunteers with rhinovirus 4. Several investigators have suggested that nasal epithelial cells can be made antiviral in vivo by intranasal administration of human leucocyte interferon. A significant antiviral activity could be developed, depending upon the method of delivering interferon to the nasal mucosa and the extent of interferon contact with the nasal epithelial cells. Scott et al. (1982) reported that repeated nasal spraying with a purified human leukocyte interferon (2×10^6 U) has reduced the incidence and the severity of colds in the volunteers when challenged with human rhinovirus 9. Hayden et al. (1983) discussed the human tolerance and histopathologic effects of long-term intranasal administration of interferon-α_2.

For intranasal delivery of drugs, nasal sprays or inhalers are the delivery methods of choice as they achieve a better absorption as compared to nasal drops, probably because they reach the nasal mucosa in a more diffuse form and the drugs delivered are distributed to a greater area of mucosa surface. Also, they were observed to produce far less pathologic changes than nasal drops (Su, 1986; Hardy et al., 1985). Some new generations of nasal delivery systems have recently been introduced such as the metered-dose nebulizer which is operated by mechanical actuation, and the metered-dose aerosol, which is operated by pressurized actuation (Chien, 1985; Petri et al., 1985). The selection of a delivery system will depend on the physicochemical nature of the peptide to be delivered.

In conclusion, it appears that the mucosal membrane of the nose offers a practical route of administration for peptide delivery. The advantages include rapid absorption, fast onset of action, ease of administration and good local tolerance as compared to parenteral injection. The biopharmaceutics for some peptide/protein drugs following intranasal administration is summarized in Table 4. For certain peptide/protein drugs, e.g. insulin, the listed biopharmaceutical responses could be achieved only with the help of enhancers.

Ocular delivery

As in the case of the nasal mucosa, the ocular membrane can also be used for the systemic delivery of therapeutic peptides and proteins (Lee, 1985, 1987; Lee and Robinson, 1986). Systemic delivery via the ocular route relies on overflow of the eye drops instilled and nasolacrimal duct absorption. These attempts, however, have met with only a very limited success. However, the ocular route is useful for local delivery of peptides and proteins for the treatment of ocular diseases that affect the anterior segment tissues of the eye (Table 5).

TABLE 5

Peptides and proteins which are potentially useful in ophthalmology

Peptides and proteins that may affect aqueous humor dynamics
Atrial natriuretic factor
Calcitonin gene-related factor
Luteinizing hormone-releasing hormone
Neurotensin
Vasoactive intestinal peptide
Vasopressin
Peptides and proteins that have immuno-modulating activities
Cyclosporine
Interferons
Peptides and proteins that are associated with inflammation
Substance P
Enkephalins
Peptides and proteins that affect wound healing
Epidermal growth factor
Eye-derived growth factor
Fibronectin
Insulin-like growth factor
Mesodermal growth factor

Source: Modified from Lee (1987).

During the permeation of the corneal epithelium, the molecules of peptides and proteins encounter several difficulties arising from their unfavorable hydrophilicity and large molecular size, their susceptibility to metabolism by peptidases in various ocular tissues, and some physiological and vehicle-related factors which could affect the ocular absorption of drugs. Some of the general approaches which have been found useful in enhancing the ocular absorption of drugs, such as the use of nanoparticles, liposomes, gels, inserts, latex systems, bioadhesives, surfactants etc. (Lee et al., 1985; Hui and Robinson, 1985) may also be utilized for the ocular delivery of peptide/protein drugs as well. In addition to the permeation barrier, the enzymatic metabolism barrier, created by the existence of peptidases in the ocular tissues, is also very important in determining the systemic bioavailability of peptides and proteins when delivered ocularly (Pert et al., 1976; Stratford and Lee, 1985a and b; Inagaki and Lee, 1987) e.g., enkephalins were found to be readily hydrolyzed in the albino rabbit's eyes (Lee et al., 1986; Kashi and Lee, 1986a and 1986b). One solution to this problem is to administer the analogs of an active peptide that are resistant to metabolism by the peptidase without compromising its intrinsic pharmacological activity, e.g., [D-Ala^2]-Met-enkephalinamide is resistant to aminopeptidase (Pert et al., 1976). A recent work by Lee et al. (1986) demonstrated that only less than 0.4% of inulin (with molecular weight of 5000), in a topically instilled solution, is absorbed into the anterior segment tissues of the albino rabbit eye. The polypeptide antibiotics, like cyclosporine, tyrothricin, gramicidin, tryocidine, bacitracin, and polymyxins, have often been considered as the likely candidates for achieving some local effects in the eye (Siddiqui and Chien, 1987).

Further work needs to be done in the ocular delivery of peptide/protein drugs, since literature reports have been found to be rather scarce. However, this route of administration is not likely to become popular for systemic delivery since the bioavailability is extremely low. Also, ocular tissues are extremely sensitive to foreign substances and patient acceptance could be rather low.

Buccal delivery

Buccal tablets, sublingual tablets and lozenges have been introduced several decades ago and are commonly used for delivering drugs to the oral mucosa for either local or systemic medication. These drugs are absorbed quickly into the reticulated vein which lies under the oral mucosa and enter the systemic circulation directly, by-passing

TABLE 6

Buccal delivery of proteins

Protein	Testing model	Response/bioavailability	Reference
α-Amylase	Guinea pigs Rabbits	Antibody production as demonstrated by anaphylactic shock in guinea pigs and precipitin reaction in rabbits.	Wieriks (1964)
Albumin	Guinea pigs Norwegian pigs	Penetrated the gingival epithelium as demonstrated by autoradiography.	Tolo (1971)
Dextran *	Rabbits	Oral mucosa penetration as demonstrated in vitro by appearance of radioactivity on receptor side of diffusion chamber.	Tolo & Jonsen (1975)
Insulin	Beagle dogs	Bioavailability of 0.5% was obtained as compared to i.m. administration (two-phased buccal tablet with oleaginous core used).	Ishida et al. (1981)
		Bioavailability was enhanced to 0.75% by using "Witepsol" as core base.	Nagai & Machida (1985)

* Included here as it is a macromolecule similar in molecular dimensions to many proteins.

the liver. Several studies on the penetration of macromolecules through oral epithelia have been reported (Wieriks, 1964; Tolo, 1971; Tolo and Jonsen, 1975) (Table 6). Merkle et al. (1985) developed a self-adhesive buccal patch and reported that it is feasible to deliver peptide hormones, like protirelin (a thyrotropin-releasing hormone) and buserelin (a synthetic LHRH derivative), through the buccal mucosa. At approximately the same time, a mucosal adhesive delivery system was also developed for the buccal delivery of insulin (Ishida et al., 1981; Nagai, 1985; Nagai and Machida, 1985; Nagai, 1986). It was found that insulin cannot be effectively absorbed from a simple disk-shaped dosage form, prepared by direct compression of insulin in a mixture of hydroxypropylcellulose (HPC) and Carbomer (Carbopol 934) (CM). But, some buccal absorption was achieved by preparing a dome-shaped two-phase mucosal adhesive device (Nagai and Machida, 1985), with an adhesive peripheral layer and an oleaginous core (Fig. 4). The core was prepared by dispersing insulin and sodium glycocholate in a cocoa butter base, while the adhesive peripheral layer was made from a blend of HPC and CM. This mucosal adhesive device adhered tightly to the oral mucosa of the dogs in a gel-like swollen state, and its shape was maintained for longer than 6 h. The systemic bioavailability of insulin, in comparison with i.m. administration, was found to be only 0.5%. The systemic bioavailability was improved by the use of permeation enhancers, e.g., sodium glycocholate. Even though the total bioavailability was still very low, the effective plasma concentration of insulin was achieved and the blood glucose level was substantially reduced (Fig. 5). Because of anatomical and physiological differences among various oral mucosal tissues, the location for buccal delivery should be carefully chosen. A methodology to compare permeabilities of different oral mucosal sites has been recently reported (Veillard et al., 1987).

Oral delivery

The problems which make the oral route unsuitable for the systemic delivery of peptide/protein drugs are the potential degradation by strong acidic environment and proteolytic enzymes in the gastrointestinal tract. Also, the macromolecular peptide/protein drugs have a very low permeability across the gastrointestinal mucosa. For an orally administered peptide to reach its site of action, it must be able to resist any chemical and enzymatic degradation in the gut lumen, and then, after penetration of the mucosal membrane, to escape the 'first pass' metabolism and clearance by the gut mucosa and liver (Humphrey, 1986) (Fig. 6). Only a very small fraction of an oral insulin dose becomes available for absorption through the gastrointestinal membrane (Crane et

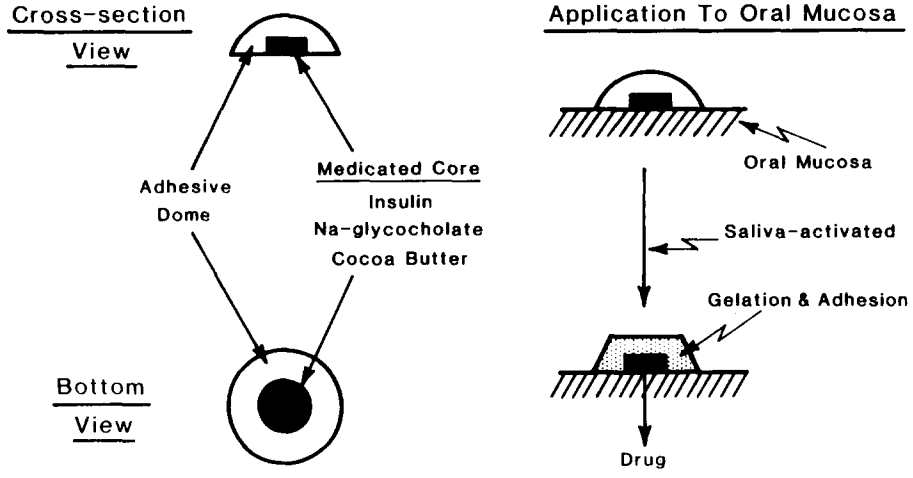

Fig. 4. Mucosal adhesive dosage form for buccal delivery of insulin (replotted from Nagai and Machida, 1985).

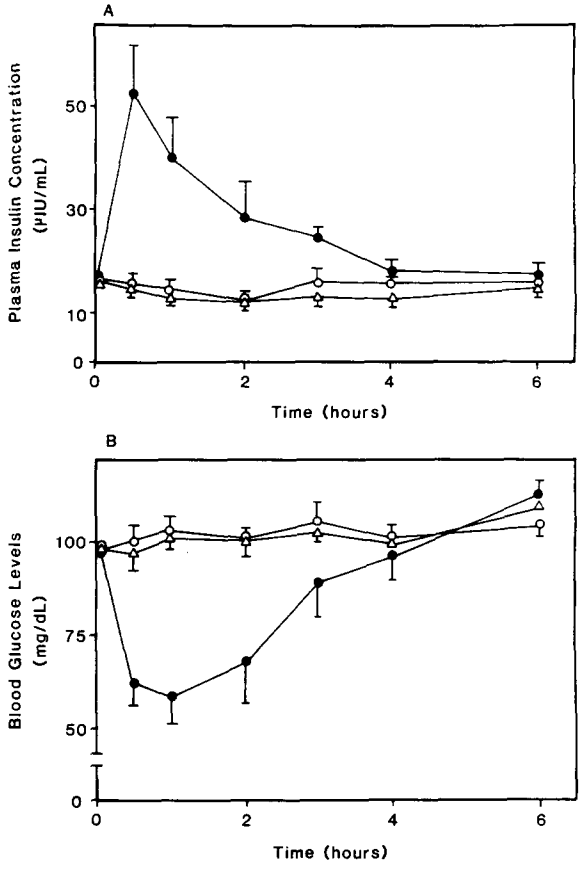

Fig. 5. Change in the plasma insulin levels (A), and blood glucose levels (B), after the buccal administration of insulin from a mucosal adhesive delivery system applied to beagle dogs: Control (○); insulin 10 mg (△); insulin 10 mg + sodium glycocholate (●) (replotted from Nagai and Machida, 1985).

al., 1968; Gilman et al., 1980). The oral bioavailability of peptide/protein drugs is thus very low, generally less than 2%. The absorption of insulin from the intestine was shown to be feasible if it is injected directly into the ascending colon with sodium deoxycholate (Kidron et al., 1982). A 50% reduction in blood glucose could be obtained by this approach. On the other hand, a similar injection into the ileum did not result in any lowering in the blood glucose levels unless insulin is injected with a trypsin inhibitor, because of the rapid digestion of insulin by the proteolytic enzymes present in the ileum. However, oral delivery is feasible for certain peptides, e.g., arginine and lysine vasopressin (AVP and LVP) and a synthetic analogue, 1-desamino-8-D-arginine vasopressin (DDAVP). They could be administered orally to rats for a rapid antidiuretic response (Saffran et al., 1988). The oral activities of AVP and LVP could be enhanced by the simultaneous administration of aprotonin, a natural inhibitor of trypsin; however, the effect of aprotonin on the oral activity of DDAVP was inconsistent.

The following approaches have been evaluated as the potential means to enhance the gastrointestinal absorption of insulin and other protein drugs:

Entrapment in liposomes. The feasibility of using liposomes as a potential delivery system for the oral delivery of insulin has been extensively studied (Woodley, 1986). Stefanov et al. (1980) reported the oral delivery of insulin using liposomes prepared from phosphatidyl choline (PC) and cholesterol (CH). They did not see any changes in blood glucose levels in normal animals, but obtained a significant reduction with diabetic rats, with the maximum effect being seen 3 h after administration. Moufti et al. (1980) were able to produce a 50% reduction in blood glucose levels by administering insulin-containing liposome to normal rats. Patel et al. (1982) published the results of an extensive study on the delivery of liposome-entrapped insulin to dogs via a catheter in the duodenum. Arrieta-Molero et al. (1982) suggested that oral administration of insulin entrapped in liposomes is effective in reducing the blood glucose level of diabetic animals; however, the stability and effectiveness of insulin-containing liposomes have been found to be rather unpredictable. Dobre et al. (1983) demonstrated a lowering of blood glucose levels in normal rats following the oral administration of insulin entrapped in PC/CH liposomes. However, negative results (Kawada et al., 1981; Weingarten et al., 1981) have also been reported. So, the feasibility of oral delivery of insulin by liposomal entrapment needs further work and standardization of liposome composition and stability. The feasibility of oral delivery of heparin by liposomal entrapment has also been evaluated (Ueno et al., 1982) and anticoagulation activity of liposome-encapsulated heparin has recently been investigated (Kim et al., 1986).

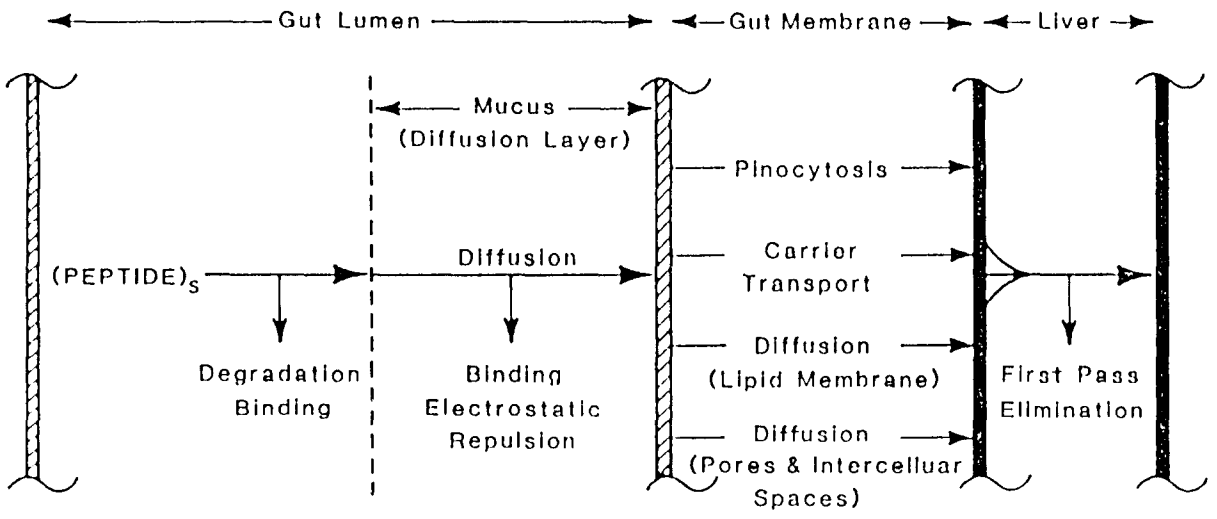

Fig. 6. Barriers to the oral absorption of peptides and proteins (modified from Mumphrey, 1986).

Encapsulation in azo polymer coating. An interesting approach recently reported for oral administration of insulin and other peptide drugs involves coating the peptides with polymers cross-linked with azoaromatic groups to form an impervious film to protect orally administered peptide/protein drugs from digestion in the stomach and small intestine (Saffran et al., 1986). When the azopolymer-coated peptide/protein drugs reached the large intestine, the microflora reduced the azo bonds, broke the cross links and degraded the polymer film, thereby releasing the drugs into the lumen of the colon for absorption or local action. The ability of the azopolymer coating to protect and deliver orally administered peptide/protein drugs was demonstrated in rats with vasopressin and insulin.

Miscellaneous approaches. Shichiri et al. (1975) used a water/oil/water-type multiple emulsion to deliver insulin orally to rabbits and diabetic rats via an indwelling catheter in the jejunum and observed a reduction in the urinary glucose level in the diabetic rats. Bird et al. (1983) carried out an in vivo study to show that incorporation of enzyme inhibitors, during the encapsulation of insulin in erythrocytes, may reduce its degradation in the human body. Erythrocytes have been suggested as a potential drug carrier to prolong the plasma drug levels following i.v. administration and it may, therefore, be of limited use for oral delivery. It has also been suggested that absorption of insulin may be enhanced by simultaneous administration of the inhibitors for proteolytic enzyme with insulin. Another approach for targeted enteral delivery of insulin was recently reported (Touitou and Rubinstein, 1986). It was developed by encapsulating insulin in small, soft gelatin capsules coated with polyacrylic polymer (Eudragit) having pH-dependent properties. Nanocapsules of isobutyl cyanoacrylate have also been used to encapsulate insulin (Damge et al., 1986). These biodegradable polymeric nanocapsules have been used as the delivery system for insulin to enhance its therapeutic efficacy. They were found to normalize hyperglycemia in diabetic rats when administered intragastrically.

Rectal delivery

The potential use of the rectum for the systemic delivery of drugs is a relatively recent idea, even though the administration of drugs in the rectum, using a suppository dosage form, for local medication, is a very old practice. In contrast to the oral route of administration, the rectal delivery of peptide/protein drugs provides the advantage of reduced proteolytic degradation and greater systemic bioavailability, especially with the coadministration of adjuvants. In the absence of ad-

juvants, the absorption of peptides from the rectum is slow with low bioavailability (Caldwell et al., 1984). In the rectum, the upper venous drainage system (superior hemorrhoidal vein) is connected to the portal system, whereas the lower venous drainage system (inferior and middle hemorrhoidal veins) is connected directly to the systemic circulation by the iliac veins and the vena cava. Thus, an opportunity to reduce the extent of hepatic first-pass elimination exists in the rectum, especially when the drug is administered in the low region of the rectum (De Boer et al., 1980; De Leede et al., 1983). The rectum also has a large number of lymphatic vessels which offer an opportunity to target drug delivery to the lymphatic circulation.

Rectal delivery of insulin. Extensive studies have been conducted on the rectal absorption of insulin (Ichikawa et al., 1980; Yamasaki et al., 1981a and b; Mesiha et al., 1981; Liversidge et al., 1985 and 1986; Nishihata et al., 1986) and on the feasibility of enhancing the rectal permeability of insulin by using adjuvants (Touitou et al., 1978 and 1980; Morimoto et al., 1980, Kamada et al., 1981; Nishihata et al., 1981, 1983, 1983b and c; Kim et al., 1983; Yagi et al., 1983; Morimoto et al., 1983; Touitou and Donbrow, 1983; Nishihata

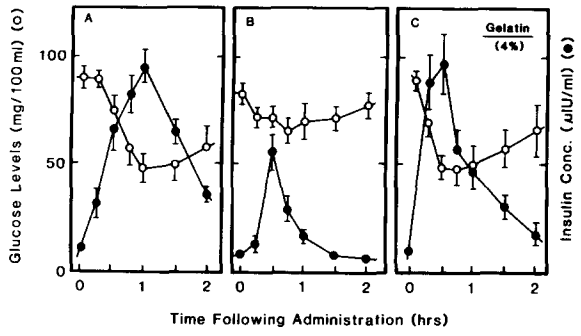

Fig. 7. Plasma concentration profiles of insulin (●) and blood glucose levels (○) in dogs after the administration of insulin by i.m. (5 IU; A) or by a microenema (20 IU; B and C). In (B), 150 mg of sodium 5-methoxysalicylate was coadministered; in (C), 4% gelatin was incorporated in the microenema with sodium 5-methoxysalicylate (replotted from Nishihata et al., 1983a).

TABLE 7

Enhancement of rectal delivery of insulin by adjuvants / formulation

Adjuvant/Formulation	Testing model	Bioavailability */response	Reference
Polyacrylic acid gel	Rats		Morimoto et al. (1983)
Alone		5%	
With 1% oleic acid		10%	
Sodium phenylalanine enamine of ethyl acetoacetate	Beagle dogs (depancreatized)		Nishihata et al. (1985)
Insulin + enamine suppository		19.4%	
Insulin + enamine suppository followed by enamine suppository		38.2%	
Sodium salicylate	Human volunteers	Better than enamine	Nishihata et al. (1986)
Microenema with 4% gelatin	Beagle dogs	Better than enamine	Kim et al. (1983)
	Diabetic rabbits		Nishihata et al. (1983)
Glyceryl esters of acetoacetic acid	Rabbits	Effective and rapid response	Nishihata et al. (1983)
Solid dispersions with sodium salicylate/mannitol	Beagle dogs	Effective and rapid response; high in-vitro dissolution rates	Nishihata et al. (1987)
Sodium deoxycholate/sodium cholate	Rats	50% Reduction in blood glucose levels within one hour	Ziv et al. (1981)
Sodium cholate	Human volunteers	50% Reduction in blood glucose levels within 30 minutes	Raz et al. (1984)

* Compared to i.m. administration.

TABLE 8

Rectal delivery of peptides / proteins

Peptide/protein	Number of amino acid residues	Testing model	Formulation	Bioavailability	Reference
Pentagastrin	5	Rats	Rectal enema	2–10% *	Yoshioka et al. (1982)
			Rectal enema with sodium 5-methoxy salicylate	23–33% *	
Gastrin	17	Rats	Rectal enema	11–25% *	Yoshioka et al. (1982)
			Rectal enema with sodium 5-methoxy salicylate		
Calcitonin	32	Rats	Saline solution	0	Morimoto et al. (1984)
			Polyacrylic acid gel base	2– 3% **	
			Gel base with POE-9-lauryl	30–50% **	Morimoto et al. (1985)
Albumins	Variable	Humans	Radiolabelled albumin in 0.85% saline solution instilled in rectum	Radioactivity appeared in plasma	Dalmark (1968)

* Compared to i.v. administration.

** Compared to i.m. administration.

et al., 1985) (Table 7). The rectal absorption of insulin from a microenema was reported to be significantly promoted after coadministration of sodium 5-methoxysalicylate (SMS) (100 mg or more) (Nishihata et al., 1983a) (Fig. 7), whereas sodium salicylate was found to be less effective as an absorption enhancer. Addition of 4% gelatin was observed to have a synergistic effect on the enhancement of rectal absorption of insulin by SMS. A systemic bioavailability of approximately 25% (compared to i.m. administration) was achieved. Phenylglycine enamines of various β-diketones, e.g. ethylacetoacetate, have also been observed to be effective in promoting the rectal absorption of insulin (Kamada et al., 1981; Kim et al., 1983). Touitou et al. (1978) achieved a hypoglycemia in the rats by administering insulin, via rectal (and vaginal) routes, in a dosage form which contains polyethylene glycols and a surface-active agent. The influence of different suppository bases on the systemic bioavailability of insulin was also reported (Sitnik et al., 1986). Recently, it has been reported that a solid dispersion of insulin with sodium salicylate or mannitol can produce a rapid release of insulin from suppositories. Even at doses as low as 0.5 IU/kg, a significant decrease in plasma glucose concentration was observed in dogs. The addition of lecithin to the suppository base prolonged the effect of salicylate, as an adjuvant in suppositories, due to the slow release of sodium salicylate (Nishihata et al., 1987). Bile salts, sodium deoxycholate or sodium cholate have also been shown to enhance the rectal delivery of insulin in rats (Ziv et al., 1981) and human volunteers (Raz et al., 1984).

Although not as extensive as the studies on insulin, there are also several articles in the literature reporting the rectal delivery of other peptide/protein drugs, such as pentagastrin and gastrin (Yoshioka et al., 1982), calcitonin (Morimoto et al., 1984 and 1985), human albumin (Dalmark, 1968), etc. (Table 8). Calcitonin, a peptide with 32 amino acid residues and a molecular weight of 3432 Da exhibits a hypocalcemic effect and has been reported to be useful in the clinical treatment of diseases involving hypercalcemia and osteoporosis. The effect of non-ionic surfactants on the rectal absorption of [$Asu^{1,7}$]eel calcitonin, a calcitonin analogue, has been studied in rats. Incorporation of polyoxyethylene-9-lauryl

ether (0.1–5% v/v) in the polyacrylic acid gel base was observed to enhance the rectal absorption of calcitonin.

Transdermal delivery

Because of their hydrophilicity and large molecular size as well as the lipophilic nature of the stratum corneum, peptide/protein drugs are unlikely to penetrate through the skin. This is probably the reason why not much work has been done in this area of biomedical research. However, a few reports do exist in the literature. As early as in 1966, Tregear studied the permeation of macromolecular proteins and polymers through the excised skin from human and animals. More recently, Menasche et al. (1981) studied the percutaneous absorption of elastin peptides through rat skin and its subsequent distribution in the body tissue. It was found that the percutaneously administered elastin peptides penetrate into the dermis and 30–40% of the administered dose can still be found in the skin 48 h later. Resorption from the skin was found to be a slow process, with very little or no radioactivity detectable at any time in the blood. Some water-soluble proteins and protein hydrolysates do find topical applications in either cosmetics or toiletries (Schuster and Domsch, 1984; Alexander, 1986a and 1986b), for example, collagen has been shown to be an effective moisturizing agent and thus has been formulated into skin preparations (Alexander, 1986a). It may be possible to enhance the transdermal permeation of macromolecules by using enhancers such as dimethyl sulfoxide, azone and surfactants and by other physical means such as electrophoresis, streaming hot air flow etc. (Hsieh, 1987).

One advantage of using the transdermal route for the systemic delivery of peptide/protein drugs is that the skin has a very low proteolytic activity. Most of the other non-parenteral routes have a significant proteolytic enzyme barrier which could drastically reduce the systemic bioavailability of peptides and proteins. Thus, the skin could be a promising site for the administration of peptide/protein drugs provided that the permeation of large hydrophilic peptide macromolecules through the skin can be facilitated by an active diffusion mechanism. The technique of iontophoresis, which

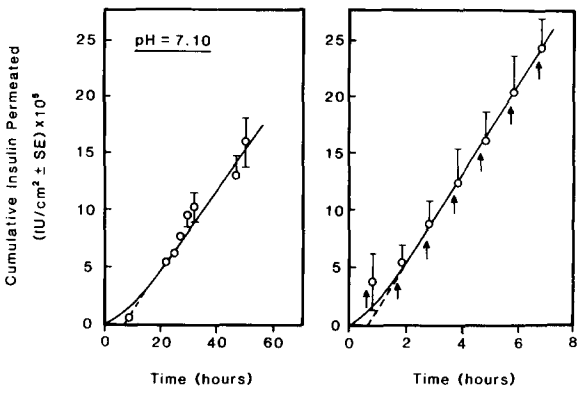

Fig. 8. In vitro skin permeation profiles of insulin, from donor solution at pH 7.10, across hairless rats and the enhancement of skin permeation by DC-iontophoresis treatment at current intensity of 1 mA applied for 5 min on the hour for 7 h (Chien et al., 1988).

delivers ions and charged molecules into the body by the use of electric current, appears to be quite promising in this respect. A comprehensive review of the principles and techniques of iontophoretic drug delivery has been completed very recently (Banga and Chien, 1988). Studies have been initiated to investigate the iontophoretic delivery of insulin (Stephen, 1984; Kari, 1986; Sun et al., 1986; Siddiqui and Chien, 1987; Siddiqui et al. 1987; Rolf, 1987; Chien et al., 1988; Liu et al., 1988; Higuchi, 1988) and encouraging results have been obtained. The in vitro permeation profiles of insulin across the freshly excised hairless rat skin was substantially facilitated by iontophoresis (Fig. 8). In vivo iontophoretic delivery of insulin in diabetic rabbits also provides the pharmacokinetic and pharmacodynamic data to demonstrate the feasibility of transdermal iontophoretic delivery of insulin and its potential advantages (Fig. 9). Recently, transdermal delivery of insulin by electroosmosis has also been reported (Meyer, 1987). Studies were also conducted on the transdermal iontophoretic delivery of thyrotropin-releasing hormone, a tripeptide with a molecular weight of 362 Da which is currently used as an adjunctive agent in the diagnostic assessment of thyroid functions. The data indicated that the flux across the excised dorsal skin of nude mice is greater with iontophoresis than that obtained by passive diffusion alone (Burnette and Marrero, 1986). Also, a

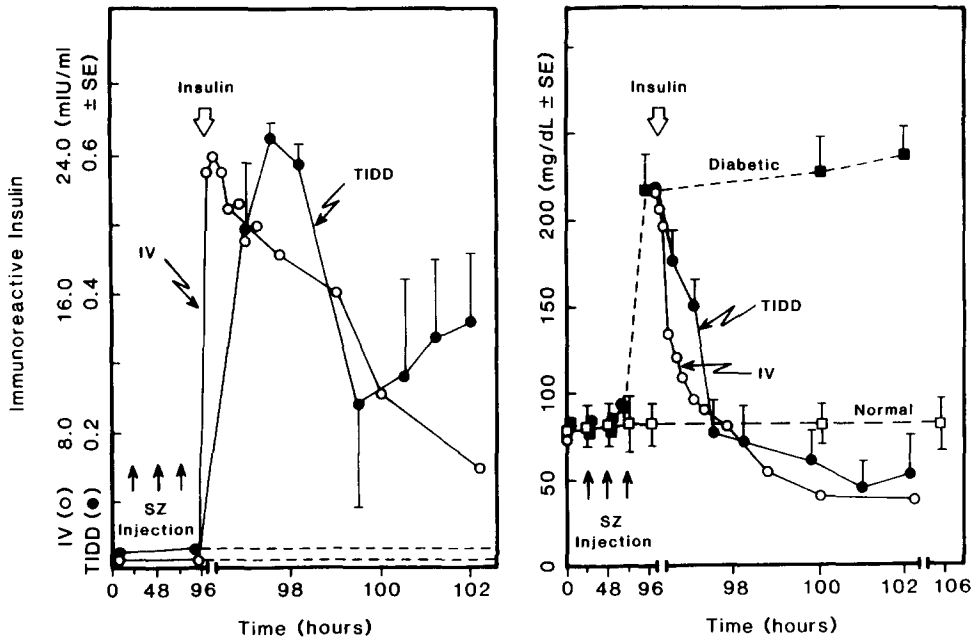

Fig. 9. Comparative plasma profiles of immunoreactive insulin from the transdermal delivery of insulin using direct current (TIDD) and by i.v. administration as well as the correspondent reduction in blood glucose levels in the diabetic rabbits. Equivalent efficacy was achieved by TIDD with significantly lower plasma insulin concentration, which may be attributed to the continuous delivery of short-acting insulin by iontophoresis (Chien et al., 1988).

linear dependence of the flux on the total current density applied was observed. The rate of transdermal iontophoretic delivery of peptide/protein drugs was found to be dependent upon the physicochemical properties of the formulation, e.g. pH, ionic strength and electrolyte concentration, and the electronic variables of the iontophoresis delivery systems, e.g. current intensity, waveform, frequency, on/off ratio and treatment duration (Chien et al., 1988).

An enzyme-responsive transdermal delivery system which is still in the concept stage has also been envisioned for insulin delivery (Check, 1984). An insulin reservoir device which is attached to the skin would generate a minute pulse of electricity to open the skin pores temporarily. While the skin pores are open, the device would take a sample of the blood and process it via a glucose-oxidizing enzyme, by which the device would monitor physiologic indicators and adjust the release of insulin accordingly. A second brief electrical pulse would open the skin pores again to allow the insulin to enter the body.

Pulmonary delivery

The respiratory tract consists of a nasopharyngeal region, a tracheo-bronchial region, and lungs (bronchioles and alveoli). Delivery of medication to the respiratory tract for localized therapy of respiratory diseases is commonly accomplished via the airways because of their enormous area and accessibility, e.g., inhalation aerosols for bronchodilation. Deposition of aerosolized particles is mediated by a variety of mechanisms, depending on the size, shape, density, charge, hygroscopicity of the particles delivered and also on the geometry of airways and other physiological factors such as breathing patterns and air flow dynamics in the respiratory tract (Li et al., 1987).

The pulmonary (or inhalation) route has so far received very little attention for the delivery of peptide and protein drugs. The absorption of peptides/proteins from the respiratory mucosa will probably be somewhat similar to that from the nasal mucosa, but a critical investigation of any histopathological implications needs to be done. Absorption of insulin from the respiratory mucosa

has been reported (Wigley et al., 1971). An aerosol dosage form for insulin delivery by inhalation was subsequently developed by suspending zinc insulin crystals in a propellant system with the aid of a dispersant like oleyl alcohol (Lee and Sciarra, 1976). No chemical incompatibilities between zinc insulin and oleyl alcohol or the propellant system were reported.

Formulation considerations for peptide/protein drugs

The increased chemical and structural complexity of proteins as compared to that of small organic molecules makes formulation of protein/peptide drugs a very challenging and difficult task (Shire, 1987). The design of delivery systems for peptides and proteins and their evaluation depend on their physicochemical and biological properties, including molecular size, biological half life, immunogenicity, conformational stability, dose requirement, site and rate of administration and pharmacokinetics/pharmacodynamics (Lee, 1986). Biological, pharmacological and toxicological issues can also be critically important in the rational design of peptide/protein delivery systems. Potential immunogenicity of any impurities or the peptide/protein drug itself must also be considered. The delivery systems must be extremely precise in the rate of delivery as these drugs are very potent. Also, the delivery pattern is critical for the pharmacological effects of different peptide/protein drugs. Pulsatile release rather than constant release may be required for several peptides and proteins with regulatory functions. The task of formulation development for peptide/protein drugs is faced with a number of problems depending on the route of administration and on the physicochemical properties of peptides and proteins.

Preformulation considerations

Preformulation data must be generated to serve as the basis for the formulation development of dosage form or the design of delivery system to achieve optimum stability and maximum bioavailability. Data on stability, solubility and sensitivity to light, heat, moisture, pH etc. must be generated. The effect of excipients on the physical and chemical stability of peptide/protein drugs should be investigated. Many peptides do not exhibit a strong tendency to crystallize and are often isolated as amorphous powders. A differential scanning calorimetric (DSC) analysis thus may not offer much information because of the lack of thermal transitions associated with the crystallization, phase conversion or melting of a crystal lattice. The lack of crystallinity of many peptides and proteins may thus result in a higher water uptake by these amorphous solids. Also, the moisture content may vary from batch to batch, creating additional problems. Peptide/protein drugs, being amphoteric, usually have a complex solubility–pH profile. Generally, the solubility is minimum at the isoelectric point where the drug is neutral or has no net charge. Intermolecular interactions can lead to precipitation or gelling, resulting in lower solubility. Preformulation studies on t-PA have been reported (Nguyen and Jones, 1987).

Adsorption behavior and its effects on stability

Protein and peptide drugs have a tendency to get adsorbed to a variety of surfaces, including glass and plastic (Hill, 1959; Mizutani and Mizutani, 1978; Mizutani, 1980; Anik and Hwang, 1983; Twardowski et al., 1983; McElnay et al., 1987). Losses are particularly significant at low concentrations and this can result in lower dosage for the patient (Petty and Cunningham, 1974; Hirsch et al., 1977). If the adsorption is due to the ionic interaction of the peptide molecules with the silanol groups on the glass surface, it can be prevented by silylating the glass. Other approaches that have been applied include the use of a carrier protein such as albumin or gelatin (Petty and Cunningham, 1974), surfactants such as sodium lauryl sulphate, amino acids (Mizutani and Mizutani, 1975), sodium chloride (Furberg et al., 1986) etc. A quantitative study of adsorption under various conditions (Mizutani and Mizutani, 1978) and the effect of adsorption on the biological activity of proteins (Mizutani, 1980) has been reported.

Aggregation behavior and its effects on stability

Another potential problem is the self-aggregation of protein molecules, e.g., insulin (Pekar and Frank, 1972; Quinn and Andrale, 1983; Massey

and Sheliga, 1986). This has been prevented by the addition of substances like urea (Sato et al., 1983), dicarboxylic amino acids such as aspartic acid and glutamic acid (Bringer et al., 1981), or other reagents like glycerol (Wigness et al., 1986), EDTA, lysine, Tris buffer or bicarbonate buffer (Quinn and Andrale, 1983). An extensive study of 60 additives and 1125 formulations (Massey and Sheliga, 1986) reported that non-ionic surfactants such as Pluronic F68 (Poloxamer 188), a polyoxyethylene-polyoxypropylene glycol surfactant, appear to be promising stabilizers. Other conclusions drawn by the study were that human insulin aggregates more readily than pig or cow insulin; ionic ingredients and phenolic preservatives accelerate the aggregation of insulin, and zinc insulin is more stable than zinc-free insulin. Also, in the solution, many protein drugs may adopt several different conformations and it could be a potential problem to preserve their pharmacologically active conformation during processing, formulation and sterilization. Another problem can result from the immunogenicity of large protein molecules as discussed earlier.

Other stability considerations

The large size and complexity of protein/peptide drugs makes them amenable to multiple inactivation pathways. These may be chemical in nature (e.g., fragmentation, enzymatic "clipping", deamidation, covalent dimerization, disulfide scrambling, oxidation etc.) or physical (unfolding, non-covalent aggregation, adsorption etc.) or a combination of the two (Jones, 1987). A comprehensive treatment of protein stability has been published (Privalov, 1979). The stability of insulin formulations has been extensively studied (Stephenson and Romans, 1960; Storvick and Henry, 1968; Bingel and Volund, 1972; Federici et al., 1973; Jeffrey et al., 1976; Fisher and Porter, 1981; Lougheed et al., 1983; Grau, 1985; Adams et al., 1987).

Pharmacokinetic considerations for peptide / protein drugs

The pharmacokinetics of peptides and proteins can be handled the same way as for other conventional drugs but the manner of collecting experimental data should be more critical, largely because the half-lives of most peptides and proteins tend to be very short, being in the order of a few minutes or shorter and because of the complex patterns of metabolism. The metabolism by peptidases and proteinases could occur in the vascular endothelium, liver, kidney and/or other non-target tissues and even at the site of administration. Since the doses administered are very small and the metabolites and degradation products may be closely related to the parent drug itself, this can give rise to analytical errors (Hickers, 1986). Also, if the metabolites are biologically active, then the pharmacokinetics of the parent compound alone may not be adequate to determine the dosing requirements.

The pharmacokinetics of insulin has been extensively studied, but the estimates of biological half-life and other pharmacokinetic parameters varied significantly when the rate of disappearance of the hormone after a pulse injection was followed. The variation could be attributed to the time interval chosen for analysis and also to the choice of radiolabelled or unlabelled hormone. One study (Sherwin et al., 1974), however, approached the problem by infusion not to steady-state concentration, but to steady-state blood glucose levels. A 3-compartment model was found adequate to fit all the data from diverse experimental protocols. Compartment 3, which could not be sampled but in which the concentration of insulin could be calculated, was the compartment pertinent to glucose utilization. This compartment probably consisted of the interstitial fluid in the muscle and adipose tissues and was in slow equilibrium with insulin levels.

The relationship between pharmacokinetics and pharmacodynamics for peptide/protein drugs is interesting and complex. This is demonstrated by LHRH and its analogs in which the systemic administration in pulsatile manner or at steady-state pattern achieved opposite pharmacological effects. Also, for many other regulatory agents, such as vasopressin (Koch and Lutz-Bucher, 1985), pulsed delivery may be required for therapeutic effect rather than steady-state levels. This is due to the possibility that the phenomenon of tolerance

or "down-regulation" can result from continuous administration, i.e., the continual presence of the agent at a receptor site can lead to a reduction of activity. Thus, dosing at steady state leads to the desensitization of receptor, while pulsatile dosing mimics the normal physiological rhythm or the circadian pattern.

Basal insulin secretion in healthy subjects also shows a circadian rhythm with a peak time at 1500 h. It has been suggested that a larger amount of insulin is needed during the afternoon and during the night. This may be achieved by a pump programmable in time which can mimic the physiologic circadian baseline of insulin (Reinberg et al., 1988). Another recent study (Hunter et al., 1988) reached similar conclusions using normal and diabetic rodents. Normal mice showed a circadian fluctuation in the basal blood glucose levels with a peak of 112 mg/dl at 14.30 h. Greatest sensitivity to insulin also occurred at 14.30, showing a 60% decrease in blood sugar. From 18.30 to 10.30 h, the insulin produced only a 38% decline in glucose. Diabetic mice showed a circadian variation with phases like that of normal mice, with basal glucose levels peaking at 438 mg/dl between 10.30 and 14.30 h (Hunter et al., 1988).

It appears that the time of administration can also affect the amount of drug absorbed, e.g., it has been reported that salmon calcitonin (SCT), administered intranasally to human volunteers, increases in serum as a function of the drug administration time. Thus, the SCT concentration 10 min after dosing at midnight (00.00 hours) was considerably greater than it was following administration at other times of the day or night (Tarquini et al., 1987).

Analytical considerations for peptide / protein drugs

For some peptide and protein drugs, bioassay has been the only method available for detection and potency determination till today. Bioassays, however, are very time-consuming, labor-intensive and highly variable. They are not suitable for automation and thus cannot be used on a routine basis. Multiple replication and the use of standards, blanks and controls are required for statistical interpretation of the results as variability in the biological response itself can be as high as 50%. In view of the disadvantages of bioassays, there is a recent effort to develop specialized physicochemical assays like spectroscopic, chromatographic and electrophoretic methods and conformationally dependent immunoassays (Malefyt, 1987).

The more common analytical methodology includes high-performance liquid chromatography (HPLC) (Ohta et al., 1984; Adams and Haines Nutt, 1986; Sammons et al., 1986; SubbaRao et al., 1987) and radioimmunoassay (RIA) (Morgan and Lazarow, 1963; Talamo et al., 1969; Ceska et al., 1970; Beardwell, 1971; Yalow, 1973; Talamo and Goodfriend, 1979). HPLC has a very high resolving power and is being increasingly used as an analytical tool to determine the structure and purity of peptides and proteins. However, the low sensitivity of HPLC has limited the direct measurement of peptides in certain instances, e.g., in tissue extracts. Sensitivity can be improved by using derivatizing reagents to enable detection at the picomole level (Meek, 1983). HPLC techniques developed at Lilly can detect proteins that differ by a single amino acid (Chance et al., 1981). For insulin, HPLC has been suggested as being a more precise measurement of potency than the rabbit assay (Johnson, 1983) or the mouse blood glucose assay (Fisher and Smith, 1986). HPLC was found to differentiate among the insulins from cows, pigs and men, and to be both reproducible and stability indicating. For insulin and insulin injections subjected to accelerated stability tests, the HPLC method detects the decomposition that cannot be detected by either the mouse blood glucose assay or the immunochemical assay. It has been suggested that the United States Pharmacopeia should replace the animal response assay for insulin with HPLC assays (Fisher and Smith, 1986).

RIA has the advantage of specificity and sensitivity, but it lacks the resolving power of HPLC. RIA techniques exploit the specific and tight association of antibody with a peptide/protein drug as the antigen, to determine very low concentrations of peptide/protein drugs in a variety of

complex matrices. The ability of a protein antigen to combine with its corresponding antibody is a structurally and conformationally specific interaction. Thus, if the protein or the antibody is conformationally altered, i.e., denatured, a less than optimal protein-antibody interaction would occur. A drop in the immunochemical assay does not necessarily imply a drop in bioactivity since the decomposition products or the conformationally altered protein may still be bioactive. On the other hand, decomposition of the protein may not be reflected in the immunochemical assay as long as the antigenic determinant part of the protein molecule is intact and capable of reacting with the antibody.

Since HPLC assays are becoming popular for peptide/protein analysis, it is important to evaluate that the solvents and/or buffer systems used as the mobile phase do not alter the native conformation of the proteins. One such study for insulin has been reported (Musial et al., 1986). The effects of temperature, buffer composition, pH, ionic strength and solvents on insulin binding were evaluated. Optimum insulin-antibody binding occurred at 22°C and pH 6 with a buffer strength of 0.1 M or less. For all solvents tested in this study, it was found that as the volume or concentration of solvent increased, the amount of insulin binding decreased. Comparison of various solvents tested indicated that ethylene glycol and methanol are the least denaturant, while 1-propanol and acetonitrile are among the most denaturant (Musial et al., 1986).

Fast atom bombardment mass spectrometry (Hemling, 1987) is also very useful in peptide/protein analysis. A radioreceptor assay has also been described for insulin (Baxter, 1986; Sjodin and Vitanen, 1987). Enzyme assays also have very high specificity and sensitivity. A method for determining the particle size of zinc-insulin and its distribution in suspension formulations, based on the measurement of absorbance in the high uv–visible region, has been reported (Beigel et al., 1985).

In view of the complex structure of proteins and the deficiency of various analytical methods in one respect or the other, it is important to utilize several analytical techniques to gain a greater confidence in the data generated. In particular, results obtained by radiolabelled peptide/protein drugs must be verified by some other techniques to prove that the label is associated with the intact, bioactive protein and not with a metabolic fragment or denatured protein.

Protein purification is also a very important aspect in the handling of peptide and protein drugs. In early days, fractional or differential precipitation was widely used. However, the techniques generally used today include electrophoresis, isoelectric focussing, liquid chromatography, etc. (Davis, 1964; Stockell Hartree, 1966, 1985; Reichert, 1971; Farrel, 1975; Parcells, 1984). The electrophoretic method has also been widely used for protein analysis (Kerese, 1984; Walker, 1984).

Regulatory considerations for peptide / protein drugs

Unlike conventional drugs, peptide and protein drugs have primary, secondary and tertiary structures, all of which must be taken into account in order to gain complete control of the identity, strength, quality and potency of the material (Bogdansky, 1987). As a result of this, establishing specific standards for the identity, purity, potency and stability of peptide/protein drugs is a complex procedure; the use of the recombinant DNA (rDNA) techniques or hybridoma manufacturing process to produce peptides and proteins introduces additional complexity (Giddins and Rhodes, 1987).

Biotechnology products are regulated under the statutory authority of 4 federal agencies: the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA) and the United States Department of Agriculture (USDA) (Giddins et al., 1987). The FDA has developed a series of publications to provide guidelines to prospective manufacturers of drugs and biological products by rDNA and hybridoma technology. The United States Pharmacopeial Convention (USPC) will have to develop additional tests and assays for these drugs and products on a case-by-case basis until an extensive

feedback becomes available in the field to allow any generalizations. In the field of biotechnology, the interpretation and enforcement of patent law can be difficult as we may be dealing with patent applications for new organisms. A recent decision of the US Supreme Court seems to suggest that new forms of life can be patented, but the interpretation of the legal language in the ruling can still be ambiguous. Several other reports have also appeared in the literature on the regulatory considerations of peptide/protein drugs (Korwek, 1982; Miller, 1982; Petricciani, 1985; Szkrybalo, 1987).

Conclusion

Peptide/protein drugs are rapidly becoming a very important class of therapeutic agents and are likely to replace many existing drugs in the very near future. The fields of biotechnology/genetic engineering are rapidly developing and many such peptide/protein drugs will be biosynthesized within a short span of time. This poses a challenge to the pharmaceutical industry to urgently develop viable delivery systems for the delivery of these complex drugs. Much work needs to be done on the development of delivery systems for non-parenteral administration in order to make peptide/protein pharmaceuticals a commercial viability.

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