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Peptide and protein drugs: II. Non-parenteral routes of delivery

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Summary

In this second part of a two-part review, the absorption and delivery of peptide and protein drugs via non-parenteral routes are discussed. Approaches considered include the absorption enhancers, iontophoretic methods, the use of absorption inhibitors and prodrugs.

Introduction

Possible non-parenteral administration routes for delivery of peptide and protein drugs include the nasal, buccal, rectal, vaginal, transdermal, ocular and oral routes. In the absence of an absorption enhancer, these routes are generally much less efficacious than parenteral administration. For example, the oral administration of LHRH required a dose 3000-times higher than that of the parenteral route. The effective doses of LHRH via the other routes, relative to the parenteral route, were 100-times (nasal), 400-times (rectal) and 600-times (vaginal), higher (Sandow and Petri, 1985).

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Oral Delivery

Bioavailability

The oral route is by far the most popular route of drug administration and a number of studies have been carried out in attempts to deliver peptide and protein drugs effectively into the circulation. Four problems mentioned earlier must be overcome for efficient delivery of the drugs:
(i) acid-catalyzed degradation in the stomach;
(ii) proteolytic breakdown in the gastrointestinal tract;
(iii) poor permeability across the gastrointestinal mucosa; and

(iv) first-pass metabolism during transfer across the absorption barriers and in the liver.

Table 1 shows the absorption of orally administered peptide and protein drugs and their molecular weights.

Captopril and enalapril have relatively higher bioavailabilities (Table 1) due to their low molecular weights and their ability to inhibit tissue carboxylpeptidase. Cyclosporin is a cyclic peptide with a number of methylated amino acid residues and is resistant to enzymic hydrolysis, hence its good bioavailability.

Some protease inhibitors and absorption enhancers have been co-administered with peptide drugs to enhance their oral absorption. A good example is oral arginine-vasopressin. To produce a 50% reduction in urine flow, in the rat, an oral dose of about 3500 pmol was required. When the drug was co-administered with aprotinin, a protease inhibitor, the oral dose was reduced to 1000 pmol (Saffran et al., 1988). For most peptide and protein drugs, despite the use of many kinds of enhancers, bioavailability is still inadequate. For instance, the bioavailability of human recombi-

TABLE 1

Gastrointestinal absorption of peptides and protein drugs

Compound	Route	% Absorbed	M.W.	Animal model	Reference
Bovine IgG	oral	2	150 000	rat	Hemmings and Williams (1978)
Bovine serum albumin	oral	< 2	67 000	rat	Warshaw et al. (1974)
Human haemoglobin	oral	1.2	64 000	rat	Seifert et al. (1984)
Interferons	oral	< 1	25 000	rat	Paulesu et al. (1988)
Insulin	oral	< 2	5 700	rat	Peters and Sibbons (1984)
Luliberin (decapeptide)	oral	< 2	1 362	rat	Amoss et al. (1972), Humphrey et al. (1973)
Empedopeptin	oral	nil	1 250	mouse	Konishi et al. (1984)
Leuproline	oral	< 2	1 208	rat	Okada et al. (1982)
Cyclosporin	oral	34	1 203	human	Wood et al. (1983)
1-Deamino-8 D-arginine-vasopressin	oral	< 2	1 007	dog	Lundin and Vilhardt (1986)
Cyclo-(Pro-Phe-D-Trp-Lys-Thr-Phe)	oral	1.5	806	rat	Bell et al. (1984)
Pepstatinyl glycine	oral	< 2	740	rat	Grant et al. (1982)
Tetragastrin	oral	nil	483	dog	Jennewein et al. (1974)
Talampicillin (pro-drug)	oral	> 50	482	human	Jones et al. (1978)
Lisinopril		25–50	405	human	Ulm et al. (1982)
TRH analogs	oral	12	400	dog	Yokohama et al. (1984)
TRH	oral	12	400	dog	Yokohama et al. (1984)
Dietary tetra-peptides	oral	5	400	human	Adibi and Morse (1977)
Enalapril (pro-drug)	oral	> 50	377	human	Ulm et al. (1982)
Ampicillin	oral	25–50	349	human	Jones et al. (1978)
Captopril	oral	> 50	217	human	Ulm et al. (1982)
Alafosfalin	oral	25–50	196	human	Allen et al. (1979)
		25–50		rat	

nant interferon-2 was found to be less than 1% and was increased to only 2% by the absorption enhancers investigated (Paulesu et al., 1988).

Barriers

Proteolytic activity

Ingested peptides and proteins undergo attack by pepsin in the stomach, followed by hydrolysis mainly by the action of brush border peptidases. The small peptides (dipeptide or tripeptide) produced undergo further hydrolysis by cytoplasmic or brush border intestinal enzymes. Peptidases found in the intestinal mucosa include leucine aminopeptidase (EC 3.4.11.1), aminopeptidase (aminopolypeptidases, EC 3.4.11.2), aminotripeptidase (EC 3.4.11.4), angiotensinase (EC 3.4.99.3), glycylglycinedipeptidase, deaminopeptidase (EC 3.4.14), serine carboxypeptidase (EC 3.4.16), dipeptidase (with lower specificity), tripeptidase, prolidase (Ganapathy et al., 1984) and carboxypeptidase A (Appel, 1974). Besides peptidases, there are also some proteinases in the intestine.

Pore radius of intestinal mucosa

The pore radius of the intestinal mucosa is a limiting factor for peptide transport. The equivalent pore radius of the intestinal mucosa in the rat was found to be about 4 Å (Lindemann and Solomon, 1962), a value confirmed by Smyth and Wright (1966). Amino acids, dipeptides, and tripeptides are therefore able to penetrate the intestinal wall but large peptides are hindered. The pore radius of the mucosa was found to be reversibly enlarged by absorption enhancers such as sodium cholate (Hirai et al., 1981a; Ziv et al., 1987).

Active transport of peptides

A number of studies have demonstrated that peptides are absorbed by active transport via specific peptide-mediated carrier systems (Rubino et al., 1971; Addison et al., 1972, 1975; Sigrist-Nelson, 1975; Berteloot et al., 1981, 1982; Ganapathy et al., 1981a,b, 1984; Cheeseman and Johnston, 1982). Peptides absorbed by carrier-mediated transport systems are obviously subject to competition by structurally similar peptides. A proton gradient is generated and maintained by an Na^+-H^+ exchanger in the brush-border membrane in response to an inward Na^+ gradient (Ganapathy and Radhakrishan, 1980). An Na^+-K^+-ATPase system actively pumps Na^+ out of the cell. This process lowers the intracellular concentration of Na^+ and consequently results in an inward proton gradient across the brush-border membrane (Ganapathy and Leibach, 1985). More recently, the intestinal transport of the ACE inhibitor, captopril, has been shown to be mediated by an active carrier system (Hu and Amidon, 1988). Transport of captopril was shown to be sodium-dependent and two structurally similar peptides, glycylproline and acetylproline, were shown to interfere with its absorption. Harmaline, a specific inhibitor of ATPase, reduced the uptake of captopril by the intestinal route (Zhou and Li Wan Po, 1991a). The antihypertensive peptide agent, lisinopril, has also been found to be transported by an active absorption mechanism (Friedman et al., 1989). Similar active carrier-mediated systems do not appear to have been identified in other tissues such as the rectal, buccal, nasal and ocular tissues.

Oral protein delivery systems investigated include spheres coated with cross-linked polymeric coatings and biodegradable microspheres. Saffran and his group (1987), for example, reported promising results using cross-linked coatings for administering insulin and other peptides orally. Peptide drugs were coated with polymers cross-linked with azoaromatic groups to form an impervious protective film resistant against enzymatic hydrolysis in the upper gastrointestinal tract. When the coated drug reaches the large intestine, the endogenous microflora degrades the polymer film and the drug is released into the lumen of the colon for absorption. In their study, a significant drop in blood glucose level was observed.

The coated particles may take the form of nanocapsules. Such nanocapsules have been used for delivering cytostatic drugs. In in vivo cancer models, the nanocapsules have been shown to prolong the absorption of lipophilic drugs through

the intestinal mucosa. Recently, polyalkylcyanoacrylate nanocapsules were used as potential insulin carriers, and a significant and prolonged hypoglycaemia was observed (Demge et al., 1986, 1987). After subcutaneous administration of nanocapsules, a 60% decrease in blood glucose level was induced. This low glucose level was maintained for 20 h. The same drop in glucose level was observed on subcutaneous administration of the control formulation but this effect completely disappeared 8 h after the injection. After intragastric administration of encapsulated insulin, a 50% decrease in glucose level was found from the second to the ninth day and control values were reached from the 13th day. In their more recent work (Demge et al., 1988), a 50–60% decrease in glycemia was observed by the second day after oral administration of insulin nanocapsules (12.5, 25, and 50 IU/kg). This effect was maintained for 6 and 20 days with 12.5 and 50 IU/kg, respectively. In normal rats, hyperglycaemia induced by an oral glucose load was reduced by 50% with the same dose of oral insulin nanocapsules (Demge et al., 1988).

Nasal Delivery

The nasal delivery of peptides and proteins has been reviewed by several authors (Sandow and Patri, 1985; Harris, 1986; Su, 1986).

Bioavailability

Systemic delivery of protein and peptide drugs by the nasal route appears to be efficient when compared to other routes. Administration of LHRH and its agonists via the nasal route may require a 100-fold increase in dose to achieve a comparable level to that of the parenteral route. Nevertheless, this is about 4–6-times more efficient than the rectal and vaginal routes. Oral bioavailability of LHRH is extremely low, requiring a 30-times higher dose than that required by the nasal route (Sandow and Petri, 1985). Some polypeptide drugs, such as oxytocin (Patocon®, Sytocinon®, a nine-amino-acid peptide), vasopressin (Postacton®), desmopressin (Minirin®, DDAVP or 1-deamino-8-D-arginine-vasopressin)

TABLE 2

Nasal bioavailability and molecular weights of some peptide and protein drugs

Drugs	Bioavailability (%)	Relative absorption with enhancer (%)	Number of amino acids	Reference
TRH	45	–	3	Mitsuma and Nogimori (1984)
Enkephalin analogues	59	94 (glycocholate)	5	Su et al. (1985)
Somatostatin analogue	73	–	6	Davies et al. (1983)
Oxytocin	1	–	9	Stander et al. (1963), Su et al. (1986)
Vasopressin analogues	5–10	–	9	Harris (1986)
LHRH agonists	1–5	40–50 (glycocholate)	9–10	Sandow and Petrie (1985), Anik (1984b)
ACTH(1–18)	12	–	18	Sandow and Petrie (1985)
Secretin	10	–	27	Ohwaki et al. (1985)
Glucagon	< 1	70–90 (glycocholate)	29	Su et al. (1985), Pontiroli et al. (1983, 1985)
GHRH	< 1	2–20 (vehicle [a])	40–44	Su et al. (1985), Evans et al. (1985)
Calcitonin	< 1	15–20 (glycocholate)	32	Hanson et al. (1986)
Insulin	< 1	10–30 (glycocholate)	51	Su et al. (1985)
Met-hGH	< 1	7– 8 (glycocholate)	191	Daugherty et al. (1988)

[a] The vehicle for GHRH was 1 mM hydrochloric acid, 1 mM ascorbic acid, and 154 mM bacteriostatic sodium chloride containing 10% mannitol and 25% human serum albumin.

and LHRH analogue (Buserelin) are now routinely given by the nasal route (Harris, 1986).

Table 2 lists the reported bioavailabilities and molecular weights of peptide or protein drugs following nasal administration.

Intranasal delivery of insulin has been extensively studied (Hirai et al., 1981a,b; Moses et al., 1983; Mishima et al., 1987; Aungst and Rogers, 1988; Aungst et al., 1988). Its transnasal permeability and intranasal absorption were found to be significantly enhanced by different types of enhancers, including bile acids (Yokosuka et al., 1977; Hirai et al., 1981a,b; Moses et al., 1983, 1984; Mishima et al., 1987; Aungst and Rogers, 1988; Aungst et al., 1988), fatty acids (Mishima et al., 1987), esters (Hirai et al., 1981a), ethers (Hirai et al., 1981a), glycosides (Hirai et al., 1981a) and lipopeptides (Hirai et al., 1981a). These enhancers have also been used to promote the uptake of other peptide or protein drugs by the intranasal route, for example, laureth-9 for hGH (Daugherty et al., 1988), glycocholate for glucagon (Pontiroli et al., 1983, 1985) and sodium lauryl sulphate for calcitonin (Hanson et al., 1986) (Table 2). Another enhancer used for insulin is STDHF (sodium taurodihydrofusidate), which has a relatively low membrane lytic activity (Longenecker et al., 1987; Deurloo et al., 1989).

Intranasal delivery of interferon for a localised effect is another area of investigation. For example, a number of studies have demonstrated the prophylactic antiviral efficacy of recombinant human IFN-β. The incidence and severity of respiratory virus infections including those causing the common cold were significantly reduced by intranasal administration of interferon-β (Higgins et al., 1983; Turner et al., 1986). Recombinant IFN-β also has intranasal prophylactic efficacy in protecting volunteers from infection after intranasal challenge with the rhinovirus (Higgins et al., 1986).

Intranasal delivery has also been investigated for the administration of vaccines (Table 3).

Intranasal delivery of peptide or protein drugs is attractive for several reasons; the nasal mucosa appears to have less proteolytic activity than the gastrointestinal tract (Parr, 1983; Zhou and Li Wan Po, 1990), first-pass hepatic metabolism is by-passed and absorption into the systemic circulation can occur relatively rapidly. Intranasal administration may result in more consistent absorption for drugs, such as propranolol, which have a variable oral bioavailability (Hussain et al., 1979). The surface area of the highly vascularized nasal mucosa is extensive due to subdivisions of the nasal passages into turbinates, sinuses, and numerous microvilli on mucosal cells (Parr, 1983). Proposed mechanisms by which drugs are absorbed through the nasal mucosa include passive diffusion, facilitated and active transport (Parr, 1983) and paracellular transport through intracellular channels with water influx (Morimoto et al., 1985). Possible routes of absorption through the nose have been reviewed by Chien and Chang (1987). For example, amino acids, including arginine, glutamic acid, glycine, proline, serine and γ-aminobutyric acid, have been shown to be absorbed from the nasal mucosa to blood vessels via active transport systems. The absorption route for peptide drugs, such as penicillins, has been found to be across the nasal mucosa into the bloodstream. Proteins including egg albumin and serum albumin, however, were transported from the nasal mucosa to the lymphatic system (Chien and Chang, 1987).

TABLE 3

Vaccines administered by intranasal route

Vaccines	Reference
Influenza Virus Vaccine	Beare et al. (1968), Waldman et al. (1969)
Parainfluenza Virus Vaccine	Wigley et al. (1970)
Measles Vaccine	Sabin et al. (1983)
Poliovaccine	Ogra and Karzon (1969)
Rhinovirus Type 13 Vaccine	Kumlien et al. (1985)
Respiratory Syncytial Virus Vaccine	Wright et al. (1976)

Proteolytic barrier

Enzymes within the nasal tissues acting as barriers to peptide absorption include leucine aminopeptidase which can significantly reduce the

bioavailability of peptide/protein drugs (Chien and Chang, 1987). Methionine enkephalin (Dodda Kashi and Lee, 1986b), leucine enkephalin (Dodda Kashi and Lee, 1986b), substance P (Lee, 1988), insulin and proinsulin (Lee, 1988) are among the peptide or protein drugs which have been found to be hydrolyzed by nasal proteases. Polypeptide drugs such as methionine enkephalin and substance P have been found to be more readily hydrolysed by nasal homogenates than drugs such as insulin and proinsulin (Table 4). Because insulin was found to be mainly degraded by leucine aminopeptidase (Smith et al., 1958), this would suggest that a number of as yet unidentified proteolytic enzymes other than leucine aminopeptidase are involved in the hydrolysis of methionine enkephalin and substance P.

The data listed in Table 4 summarise the susceptibilities of different peptide and protein drugs to nasal proteases. The relative resistance of leucine enkephalin to nasal proteases is worth noting. Chemical modification for peptide drugs is therefore likely to be a useful approach to enhance their absorption. It may also explain why some larger peptide drugs such as secretin have higher bioavailabilities than smaller peptide drugs like oxytocin and LHRH agonists (Table 2).

Although the nasal proteolytic barrier can significantly reduce the bioavailability of peptide and protein drugs, the level of aminopeptidase present may be much lower in nasal tissues than in gastrointestinal tissues (Zhou and Li Wan Po, 1990, 1991b).

Microspheres present a potential controlled release system for nasal peptide drug delivery (Illum, 1986). The microspheres are drug-loaded degradable starch microspheres (DSM) with a diameter of 45 μm. In a more recent study by Bjork and Edman (1988), insulin (0.75 and 1.7 IU/kg)-DSM preparations administered nasally as a dry powder resulted in a dose-dependent decrease in blood glucose in rats. The bioavailability of the nasal insulin was found to be 30%, whereas the effect of DSM alone or soluble insulin on its own produced no effect. Degradable starch microspheres therefore offer a possible avenue for further investigation on the nasal administration of peptide or protein drugs.

Rectal Delivery

Bioavailability

Rectal delivery of peptide and protein drugs is another very active area of research. Rectal delivery offers many advantages including:

(a) Avoidance of drug dilution prior to reaching the systemic circulation and of drug contact with digestive fluids and consequential degradation.

(b) The rate of drug absorption via the rectum is not influenced by ingestion of food or the rate of gastric emptying.

(c) Reduction in first-pass metabolism.

(d) Rapid systemic absorption by administering the drug rectally in a suitable solution form.

(e) Safe and convenient, especially for infants and children.

(f) Possibility of administering a large dose volume.

(g) Drug absorption can be interrupted in case of accidental overdose or drug reaction.

The extent of breakdown of polypeptides is low in the lower digestive tract relative to the small intestine and the stomach, due to low enzymatic activity and neutral pH. However, the nor-

TABLE 4

Sensitivity of some peptide or protein drugs to nasal proteolytic activity

Peptide/protein drug	Half-life (min)	Reference
Methionine enkephalin	16.3 ± 1.4	Dodda Kashi and Lee (1986b)
Leucine enkephalin	162.0 ± 26.9	Dodda Kashi and Lee (1986b)
Substance P	11.6 ± 0.9	Lee (1988)
Insulin	29.2 ± 2.9	Lee (1988)
Proinsulin	86.2 ± 5.6	Lee (1988)

TABLE 5

Bioavailability of some peptide and protein drugs administered by the rectal route

Drug	Bioavailability (%)	Relative absorption with enhancer (%)	Number of amino acids	Reference
Tetragastrin	5–16	–	4	Jennewein et al. (1974)
Pentagastrin	2–10	18 (5-methoxy SA [a])	5	Yoshioka et al. (1982)
Gastrin	11–15	33 (5-methoxy SA [a])	17	Yoshioka et al. (1982)
Calcitonin	0.8	26 (POE 9 laury [b])	32	Morimoto et al. (1985)
Insulin	< 1	27.5 (enamine derivative [c])	51	Yagi et al. (1983)
Epidermal growth factor	0	68.2 (caprate in CMC Na [d])	55	Murakami et al. (1988)
Growth hormone	0.2	7–9.5 (salicylate)	191	Moore et al. (1986)

[a] Salicylate.
[b] Polyoxyethylene 9-lauryl ether.
[c] DL-Phenylalanine ethyl acetoacetate.
[d] Sodium carboxylmethylcellulose.

mal adult's lower intestine has been shown to be relatively impermeable to the uptake of macromolecules (Warshaw et al., 1974, 1977; Taniguchi et al., 1980) such as bovine serum albumin, heparin and other protein drugs with high molecular weights.

Reports on peptide and protein drugs delivered by the rectal route include insulin (Ziv et al.,

TABLE 6

Proteolytic activities in different experimental animal tissues using peptides and proteins as substrate

Substrate	Tissue							Reference
	Duo [a]	Ileal	Rectal	Vaginal	Buccal	Nasal	Dermal	
Met-enkephalin [e] ($t_{1/2}$, min)		15.1	11.3	22.2	12.0	16.3		Dodda Kashi and Lee (1986b)
Leu-enkephalin [e] ($t_{1/2}$, min)		226.5	114.3	183.7	153.2	162.0		Dodda Kashi and Lee (1986b)
Substance P [e] ($t_{1/2}$, min)		5.8	5.9	10.9	8.7	11.6		Lee (1988)
Insulin [e] ($t_{1/2}$, min)		98.1	71.6	106.0	318.4	29.2		Lee (1988)
Proinsulin [e] ($t_{1/2}$, min)		55.7	122.0	163.2	528.3	86.2		Lee (1988)
dDAVP [b,e] ($t_{1/2}$, min)	9.0							Lundin et al. (1989)
AVP [c,e] ($t_{1/2}$, min)	3.1							Lundin et al. (1989)
LNA [d,f] (% hydrolysis)	100.0		53.0		50.2	60.4	59.7	Zhou and Li Wan Po (1990)

[a] Duodenal tissue homogenate.
[b] [Mercaptopropionic acid 1, D-arginine 8]-vasopressin.
[c] Nonapeptide vasopressin.
[d] L-Leucine-β-naphthylamine hydrochloride. Unit used for this substrate is expressed as hydrolysis percentage by the proteolytic activities in the homogenates and the enzymic activity of intestinal tissue homogenate is taken as 100%.
[e] Tissue homogenates used from rabbits.
[f] Tissue homogenates used from rats.

1981; Kim et al., 1983; Morimoto et al., 1983; Aungst and Rogers, 1988; Aungst et al., 1988), calcitonin (Morimoto et al., 1984, 1985), gastrin, pentagastrin (Yoshioka et al., 1982), human growth hormone (Moore et al., 1986) and vasopressin (Saffran et al., 1988). Table 5 shows the rectal bioavailability of some peptide or protein drugs.

Proteolytic barrier

Rectal tissues are known to contain a number of proteolytic enzymes, including enkephalinase (Grimaud et al., 1989), protease (Burtin et al., 1984), cathepsin B, cathepsin H, collagenase-like peptidase (Durdey et al., 1985), protease-like peptidase (Gelister et al., 1986a), plasminogen activators (Gelister et al., 1986b; Harvey et al., 1988) and urokinase (Gelister et al., 1987). The data in Table 6 show that the activity of proteolytic enzymes in rectal tissue is similar to that in nasal tissue.

Buccal Delivery

Bioavailability

The bioavailability of peptide drugs such as insulin (Ishida et al., 1981; Nagai, 1985, 1986; Nagai and Machida, 1985), protirelin (TRH analogue) and buserelin (LHRH analogue) (Merkle et al., 1985) by the buccal route is generally poor. Aungst and his co-workers (1988) compared the efficacy of insulin administered by the nasal, rectal, buccal, sublingual and intramuscular routes and the effects of a bile salt absorption promoter. It was found that the rank order of extent of absorption was nasal > rectal > buccal > sublingual. With sodium glycocholate co-administration, rectal and nasal insulin were approximately equipotent, and buccal insulin was slightly less effective. However, it was demonstrated by Robinson (1988) that the buccal route can be used successfully for the delivery of small peptides with a probe tripeptide with a molecular mass of 670 Da. Another peptide drug successfully administered by the sublingual route was captopril for the treatment of acute hypertension (Hauger-Klevene, 1986; Del Castillo et al., 1988).

Proteolytic barrier

Buccal tissue has been found to contain many enzymes including proteolytic enzymes. Table 6 lists the proteolytic activity of buccal tissues relative to others. It can be seen that methionine enkephalin and substance P are highly susceptible to the enzymes while insulin and proinsulin are much less affected.

Ocular Delivery

Ocular delivery of drugs is typically for the treatment of ocular inflammation, corneal wounds and glaucoma. This route has also been investigated for the systemic delivery of peptide and protein drugs.

Bioavailability

Christie and Hanzal (1931) demonstrated that topical ocular administration of insulin produces a sustained lowering of blood glucose in proportion to the dose instilled. The effectiveness of the ocular dose was comparable to that of a dose administered subcutaneously. Since then, many attempts have been made to improve the bioavailability of topical ocular drugs (Lee et al., 1986a). To date only minor improvements have been achieved. Recently, it has been claimed that a painless, simple and practical method has been designed for the systemic delivery of insulin through the ocular route (Chiou and Chuang, 1989) although long-term efficacy and safety have yet to be defined. Saponin (a glycoside) was found to be the best enhancer of insulin absorption via this route, while Tween 20 was the least effective. The efficacy order of seven compounds studied was as follows: saponin > fusidic acid > BL-9 (polyoxyethylene 9-lauryl ether) = EDTA > glycocholate = decamethonium > Tween 20. Bioenhancers are therefore likely to be increasingly important for ocular administration of peptide and protein drugs.

Proteolytic barrier

Topical ocular drug delivery using conventional dosage forms such as solutions, suspensions, and ointments is relatively inefficient with less than 10% of an applied dose being delivered across the cornea into the eye (Lee, 1987). This low absorption is caused by the lipophilicity of the corneal epithelium, dilution of drugs by tears, and binding of drugs to proteins in tears with subsequent loss through the nasal passages (Lee et al., 1986b).

Besides the diffusion barriers, the enzymic barrier also plays a very important role. Many enzymes have been found in ocular tissues (Harding and Crabbe, 1984; Maurice, 1984). The transport of administered peptide and protein drugs across ocular barriers is mainly limited by proteinases such as neutral protease and aminopeptidase. For example, within 5 min of instillation of solutions, over 90% of leucine and methionine enkephalins were found to be hydrolyzed in the corneal epithelium of the rabbit (Lee et al., 1986b). The proteolytic activity in corneal tissues was found to be highest among seven tissues tested using methionine enkephalin as substrate (Table 7) (Dodda Kashi and Lee, 1986a,b).

Therefore, despite the possibility of achieving systemic absorption of peptides from topically applied ophthalmic solutions, much further work is still required to make it a practical reality. The enzymic activity of the ocular tissues will have to be controlled and any absorption enhancers will have to be carefully selected due to the high sensitivity of the eye to irritation by externally applied substances.

TABLE 7

Resistance of methionine enkephalin to enzymic hydrolysis by different tissue homogenates

Tissues	Half-life of hydrolysis (min)	Reference
Corneal	10.6	Dodda Kashi and Lee (1986a)
Conjunctival	41.9	Dodda Kashi and Lee (1986a)
Nasal	16.3	Dodda Kashi and Lee (1986b)
Buccal	12.0	Dodda Kashi and Lee (1986b)
Rectal	11.3	Dodda Kashi and Lee (1986b)
Ileal	15.1	Dodda Kashi and Lee (1986b)

Transdermal Delivery of Peptide and Protein Drugs

There are several advantages to the transdermal route provided the drug is absorbed in sufficient quantity to exert a systemic effect. Using this route it is possible:

(i) To avoid hepatic first-pass metabolism and gastrointestinal breakdown of drugs;
(ii) To provide controlled administration;
(iii) To reduce frequency of dosing;
(iv) To improve patient compliance; and
(v) To permit relatively abrupt termination of drug effect by removal of the delivery system from the skin surface.

In fact, most polypeptide and protein drugs are polar compounds with high molecular weights, and therefore diffuse poorly across the stratum corneum. However, more recently, a potent neuropeptide, des-enkephalin-β-endorphin (DEβE) was reported to have been successfully delivered transdermally across both intact human skin and cultured human skin cells (Boddé et al., 1988). Skin adhesive hydrogel films appear to provide an effective method for delivery of some peptides (Boddé et al., 1988).

With techniques such as iontophoresis (Siddiqui et al., 1987), however, large hydrophilic peptides may be administered by the dermal route. With iontophoresis, ions and charged molecules are transported across the skin using an electric current. Many studies have reported encouraging results on transdermal transport of insulin using this technique (Rolf, 1987; Siddiqui et al., 1987; Liu et al., 1988; Srinivasan et al., 1989). Thyrotropin-releasing hormone has also been reported as being partially delivered by this technique (Burnette and Marrero, 1986). Some

TABLE 8

Peptide and protein drugs investigated as candidates for iontophoretic delivery

Protein or peptide drugs	Reference
Insulin	Stephen et al. (1984) Siddiqui and Chien (1987) Chien et al. (1987)
Thyrotropin releasing hormone	Burnette and Marrero (1986)
Vasopressin	Marchand and Hagino (1982)
Alamethicin	Jung et al. (1983)
Suzukacillin	Jung et al. (1983)
Trichotoxin	Jung et al. (1983)

candidates for iontophoretic delivery are listed in Table 8.

The rate of transdermal iontophoretic delivery of peptide or protein drugs has been reported to be dependent on the pH, ionic strength and electrolyte concentration of the formulation as well as on the applied voltage.

The skin has a very low proteolytic activity and a low content of soluble protein (Zhou and Li Wan Po, 1990, 1991b). By using L-leucine-β-naphthylamide as a substrate, the activity of leucine aminopeptidase was found to be lowest in rat skin from among the five tissue homogenates used (intestinal, buccal, nasal, rectal and dermal tissues). Low skin permeability is likely to be the main limiting factor for peptide and protein drug absorption by the transdermal route.

Conclusion

An increasing number of peptide and protein drugs are likely to be introduced into therapeutics in the forthcoming years. Their potential usefulness will be much enhanced if constraints imposed by the parenteral route of delivery used can be overcome. The studies and results reviewed so far suggest that use of absorption enhancers, specialised delivery systems and protease inhibitors may improve delivery of those agents into the systemic circulation. It is unlikely that any one of those approaches would by itself enable the controlled delivery of the peptide and protein drugs or that any single system is going to be universally applicable. Each agent would benefit from its own tailored delivery system. Indeed, the literature reviewed also suggests that some portals of entry may be preferable to others for specific peptides. Molecular modification to introduce resistance to peptidases and proteases also adds to the methods available for improving absorption of the peptide and protein drugs. Specialised drug delivery systems have so far yielded limited improvement. Only the nasal route appears to be a useful practical alternative so far. Despite this, we can confidently predict that problems in peptide and protein drug delivery are likely to continue challenging pharmaceutical scientists for many years to come.

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