Annu. Rev. Pharmacol. Toxicol. 1991. 31:457–67

SYSTEMIC DELIVERY OF POLYPEPTIDE DRUGS THROUGH OCULAR ROUTE

George C. Y. Chiou

Institute of Ocular Pharmacology and Department of Medical Pharmacology and Toxicology, Texas A&M University College of Medicine, College Station, Texas 77843

KEY WORDS: polypeptides, ocular route, systemic absorptions, absorption enhancers, insulin/glucagon

INTRODUCTION

Recent advances in biotechnology have made it possible to mass produce natural polypeptides for clinical uses and nonnatural polypeptides as novel drugs. The major routes of polypeptide administration are parenteral injections as after oral administration polypeptides are degraded by proteases and aminopeptidases in the gastrointestinal tract (1–6). The disadvantages of repeated injections in the treatment of chronic diseases are well known, and numerous attempts have been made to find alternate routes of polypeptide administration (1–15). Although rectal (7), vaginal (8), buccal (9), nasal (10–12), tracheal (13), and transdermal (14, 15) routes have been tried, none of them is accepted enthusiastically for various reasons, such as cultural resistance and inconvenience for rectal and vaginal administration; inaccurate dosing for nasal and trachael routes; and slow, inefficient absorption across the skin.

Systemic delivery of polypeptide drugs through the ocular route has several advantages: (*a*) It is simple and easy to administer the drug as eyedrops; (*b*) Various formulations can be prepared which prolong drug action and/or reduce drug concentration so that consistent drug action can be achieved

0362-1642/91/0415-0457$02.00

without producing side effects; (*c*) The administered dose can be very accurate as the eye can hold only one drop of ophthalmic solution regardless of how much volume is instilled to the eye; (*d*) The systemic absorption is extremely fast—as fast or faster than subcutaneous and/or intramuscular injections; (*e*) The absorbed polypeptides bypass the portal circulation to the liver, thus avoiding the first-pass metabolism by the liver; and (*f*) It is more economical than injections as it does not require syringes and needles.

Because systemic delivery of polypeptides depends on their diffusion across the mucous membrane in conjunctiva and in the nasolacrimal system (16, 17), polypeptide absorption through the eyes is not without limitations. These limitations include: (*a*) The molecular weights of polypeptides cannot exceed around 10,000, otherwise a permeation enhancer must be used; (*b*) The absolute quantity of polypeptide to be put in each eye cannot exceed 2.5 mg because the eye can tolerate no more than 25 μl of 10% ophthalmic solution; (*c*) The eyes are very sensitive to irritants, thus any eyedrops to be instilled must be devoid of any local irritation and/or side effects (*d*) The long-term use of polypeptides could induce allergic responses, although the probability is small when small peptides are used; and (*e*) Many people are not used to taking drugs through eyes, thus, extensive public education might be needed.

SYSTEMIC ABSORPTION OF POLYPEPTIDES THROUGH EYES

Systemic absorption of polypeptides through animal eyes was tested primarily with albino rabbits using radioactive polypeptides (18–25). Blood samples were taken at certain time points to determine blood concentrations of polypeptides. Biological responses were also monitored to correlate polypeptide absorption and the biological responses induced, e.g. increase of blood glucose by glucagon, decrease of blood glucose by insulin, increase of blood pressure by vasopressin, and analgesic effects by enkephalins and endorphins. The amount of polypeptides absorbed systemically was estimated by comparing the blood concentrations of rabbits given polypeptides through the eyes with controls given intravenous injections where absorption was complete (100%).

Small polypeptides such as thyrotropin releasing hormone (TRH with MW of 300), luteinizing hormone releasing hormone (LHRH with MW of 1200), enkaphalins (with MW of around 630), and glucagon (with MW of 3500) were very well absorbed systemically through the eyes (19, 21). The systemic absorption of these small polypeptides was almost complete (99% absorbed) in 2 hr, except for LHRH whose absorption was slower than the other three and did not reach the plateau even 12 hr after drug instillation (19). The

amount of these polypeptides absorbed into the eyeball itself was almost negligible—only 0.05%, 0.30%, 0.28%, and 0.18% for TRH, enkephaline, LHRH, and glucagon, respectively (19, 21).

Obviously, the absorption profile obtained with total radioactivity of polypeptides may be misleading because the polypeptides are rapidly metabolized in the blood circulation. To determine the true consequences of intact polypeptides in the systemic circulation, radioimmunoassay (RIA) and/or HPLC must be used. With RIA, polypeptides were absorbed rapidly and reached peak concentrations within 30 min after drug instillation. The concentration of polypeptides in the blood fell rapidly to original base in 2 hr (25, 26).

Polypeptides having higher molecular weights, such as β-endorphin (MW ca 5000) and insulin (MW ca 6000), were also absorbed into systemic circulation from eyes (22–24). However, the amount of insulin absorbed was small (only 4–5% absorbed) as compared to small polypeptides (almost 100% absorbed) (19, 21, 23, 24). To improve the absorption, the systemic delivery of insulin was studied under various pHs, ranging from 5 to 8 and in the presence of aminopeptidase inhibitors (23). Neither modification was able to improve the absorption, however. The absorption was markedly improved only with the simultaneous use of permeation enhancers, as presented in the following section (23, 24).

IMPROVEMENT OF SYSTEMIC ABSORPTION WITH PERMEATION ENHANCERS

The numerous permeation enhancers available are shown in Table 1 (27–29). However, most are unsuitable for use in enhancing systemic absorption through the eyes because of local irritation and limited potency. BL-9 and Brij-78 are the most promising because they can substantially enhance insulin absorption (more than fourfold increase at 0.5% concentration) without producing local irritation (Table 2; 22–26). Although fusidic acid has been added into insulin nasal spray, its absorption-enhancing potency is not impressive. Further, it is likely to cause slight local irritation to the eyes at concentrations between 0.25–0.5%. Saponin and polyethylene 20-oleorylether are potent agents for enhancing insulin permeation across the mucous membrane, but both cause eye irritation at the 0.5% level.

Chow & Hsieh described a permeation enhancer, belonging to the class of macrocyclic ketanes and lactanes, that can markedly enhance nasal absorption of insulin without producing local irritation (30). The actual chemical structure of the compound is not revealed as it is under the process of patent application (D. Hsieh, personal communication).

Table 1 Classification of permeation enhancers[1]

1. *Anionic Surfactants*

   Deoxycholic acid
   Glycocholic acid
   Taurocholic acid

2. *Cationic Surfactants*

   Decamethonium bromide

3. *Nonionic Surfactants*

   A. Polyoxyethylene ethers
   Polyoxyethylene 10-cetylether
   Polyoxyethylene 20-cetylether
   Polyoxyethylene 4-laurylether
   Polyoxyethylene 9-laurylether (BL-9)
   Polyoxyethylene 10-laurylether
   Polyoxyethylene 23-laurylether
   Polyoxyethylene 20-oleylether
   Polyoxyethylene 10-stearylether
   Polyoxyethylene 20-stearylether (Brij 78)

   B. Polyoxyethylene sorbitan
   Polyoxyethylene monolaurate

   C. Sorbitan
   Sorbitan monolaurate
   Sorbitan monooleate
   Sorbitan trioleate

4. *Saponin*

5. *Fusidic Acid*

   Taurodihydrofusidic acid

6. *Glycyrrhizic Acid*

7. *Chelating Agent*

   EDTA

8. *Fatty Acids*

   Oleic acid
   Monoolein

[1] See refs. 27–29

When insulin eyedrops were instilled into rabbits' eyes and the concentrations of insulin in the blood were measured using RIA, 1% insulin eyedrops reached a concentration of 68 μU/ml within 30 min and dropped quickly back to the basal level by 90 min (26). Addition of BL-9 markedly enhanced the insulin absorption. With 1% insulin plus 0.5% BL-9, the concentration of insulin in the blood increased from 78 μU/ml (with 1% insulin alone) to 270 μU/ml within 30 min with a T 1/2 of approximately 100 min. When 1% BL-9 was added into 1% insulin, the peak concentration of insulin in the blood did not increase further, but concentrations remained high for a longer period of time with the T 1/2 extended to 200 min. The concentration of insulin in the blood reached 140 μU/ml with 0.5% insulin plus 1% BL-9 as compared with 68 μU/ml with 1% insulin alone. These results indicate that there is a dose-dependent enhancement of insulin absorption by the ocular route with BL-9 up to 0.5%. Further increase of BL-9 concentration prolongs the duration of insulin action but not the peak concentration in the blood (26). Similar results were obtained with another permeation enhancer, Brij-78 (26). To possibly increase the tolerability, a mixture of BL-9 and Brij-78 could be used at half the previously studied concentration (0.25% instead of 0.5%) of each permeation enhancer.

Table 2 Effects of various permeation enhancers on systemic absorption of insulin through eyes[a]

Permeation enhancers	Concentration of enhancers	Insulin absorption enhanced	Local irritation to eyes[b]
Saponin	0.5%	4.0X	+
	1%	7.0X	+
Fusidic acid	0.25%	2.3X	±
	0.5%	2.7X	±
	1%	3.9X	+
	2%	7.5X	+
Polyethylene 9-laurylether (BL-9)	0.25%	2.6X	−
	0.5%	4.5X	−
	1%	6.0X	±
	2%	7.6X	+
Polyethylene 20-Stearylether (Brij 78)	0.5%	6.8X	−
	1%	6.3X	±
Polyethylene 20-oleorylether (Brij 99)	0.5%	4.0X	±

[a] See refs. 22–26.

[b] (−), No irritation; (±), Borderline slight irritation; (+), Positive irritation

BIOLOGICAL RESPONSES INDUCED WITH POLYPEPTIDE EYEDROPS

Each polypeptide induces a particular set of biological responses after absorption into the systemic circulation through the ocular route. Table 3 lists the therapeutic indications for some small polypeptides that can be administered through the eyes. Among these small polypeptides, the hypoglycemic action of insulin eyedrops (18, 22–25) and the hyperglycemic effect of glucagon eyedrops (20, 26) are discussed as representative examples.

The reduction of rabbit blood glucose by insulin eyedrops was first demonstrated by Christie & Hanzal in 1931 (18). They demonstrated a dose-response relationship of blood sugar reduction with 10, 20, 30, and 40 units of insulin instilled into rabbit eyes which lowered the blood glucose by 15, 27,

Table 3 Clinically useful small polypeptides which can be administered through ocular route

Polypeptides	MW	Therapeutic indications
TRH	360	Diagnosis of thyroid cancer, differential diagnosis of hyperthyroidism, treatment of thyroid carcinoma with ^{131}I.
Leu-enkephalin	630	Analgesic
Met-enkephalin	650	AIDS
Oxytocin	1010	Induce uterine contraction, migraine
Vasopressin	1080	Diabetes insipidus
LHRH	1200	Induce ovulation to promote pregnancy
Vasoactive intestinal peptide (VIP)	3325	Release insulin from β-cells
Calcitonin	3420	Hypercalcemia, Paget's disease
Glucagon	3490	Hypoglycemic crisis
ACTH	4540	Antiinflammation, antiallergic, decongestant, etc
β-endorphin	4000	Analgesic
Insulin	6000	Diabetes mellitus

37, and 68 mg%, respectively. However, application of this method to humans was soon abandoned because the body weight of a human is 20–25 times higher than that of a rabbit, and it was not felt possible to give sufficient insulin through the eyes. Further, insulin was incorrectly assumed to be absorbed through the conjunctiva at the cul de sac rather than through the nasolacrimal system (18).

In human patients, the dose of insulin is approximately 20 units or 1 mg, which could be administered through eyes without any problem if insulin could be completely absorbed. Unfortunately, since the absorption of insulin is not complete, the amount of insulin to be instilled into human eyes would need to be 20–25 times higher than that instilled into rabbit eyes, namely approximately 20–25 mg or 400–500 units. To achieve this goal, permeation enhancers such as BL-9 and Brij-78 must be added to increase the systemic absorption of insulin through the eyes. In rabbits, 25 μl of topical 1% insulin (equivalent to 0.25 mg or 5 units) did not affect the blood glucose (23, 24). However, 1% insulin plus 0.5% or BL-9 or Brij-78 did lower the blood sugar significantly (22–26).

Of the permeation enhancers tested (Table 1; 27–29), only five compounds showed significant potency for enhancing polypeptide absorption (Table 2). Unfortunately, three, saponin, fusidic acid, and Brij-99, are irritable to rabbit eyes. Therefore, only BL-9 and Brij-78 can be used to enhance polypeptide absorption into the systemic circulation.

Hypoglycemia is a life-threatening situation requiring immediate medical attention. It is a common phenomenon occurring on average once a year to diabetes patients who take too large a dose of insulin or forget to take food after an insulin injection. A hypoglycemic episode can be easily corrected by glucose infusion and/or glucagon injection, but if left untreated, may cause irreversible brain damage and even death. On average, one out of 20 episodes results in the patient's death because he/she is alone and unable to obtain medical assistance.

In rabbits, a 0.2% solution of glucagon raised blood glucose effectively within 30 min, just like an i.v. injection of glucagon (20). The blood glucose returned to the original level in 120 min. When higher concentrations of glucagon were used (1% and 5%), the blood glucose concentrations were elevated even more and stayed high for longer periods, 240 min and 360 min, respectively (20).

In human subjects, 2.5%, 5%, and 7.5% solutions of glucagon raised the blood glucose in a dose-dependent manner (31). These results suggest that it may be possible to treat hypoglycemic episodes with glucagon eyedrops.

Potential clinical uses of polypeptide eyedrops are not limited to insulin and glucagon only. For example, diabetes insipidus might be treated with vasopressin eyedrops, pain with leucine-enkephaline, etc (Table 3).

PHARMACOKINETICS OF SYSTEMIC ABSORPTION OF POLYPEPTIDES THROUGH EYES

One source of differences in biological responses to polypeptide drugs between individuals is due to differences in pharmacokinetics (32). However, generalized simulation is possible for a particular patient through the use of predictive models and computer technology. Several powerful computer software systems have been developed to assist in designing optimal dosage regimens: They can be easily used in clinics (33). These pharmacokinetic modelling programs sometimes allow prediction of individualized responses and provide researchers with data modelling capabilities for experimentally generated data (34).

Based on the pharmacological responses and systemic concentrations in blood measured experimentally, a three-compartmental model has been developed for describing the pharmacokinetics of LHRH, leu-enkephalin, glucagon, and insulin plus absorption enhancers when administered through the eyes (35). The three compartments include the precorneal area, the central compartment which includes the blood, and the peripheral tissues or compartment. The precorneal area represents the initial compartment into which the polypeptide eyedrops were instilled as bolus input. Small polypeptides were almost completely absorbed into the central compartment, or blood circulation. The larger molecules such as insulin could be assisted by permeation enhancers to permit better absorption. After reaching the systemic circulation polypeptides would be distributed to other parts of the body represented by the peripheral compartment. This model consists of three linear differential equations with five rate parameters. By varying only one parameter, the rate parameter from the precorneal region to the central compartment and keeping the other four parameters constant, it was possible to obtain an excellent agreement with the measurements of blood polypeptide concentrations at various doses of polypeptides instilled into eyes (35). As this rate constant was a monotonically decreasing function of the initial doses of polypeptide instilled into eyes, the mechanism of polypeptide absorption into systemic circulation could be due to a facilitated diffusion (35).

CONCLUDING REMARKS

We are again witnessing research breakthroughs in the pharmaceutical industry. Sophisticated genetic engineering techniques are developing powerful new drugs requiring innovative drug delivery systems that are entirely different from the traditional drug delivery systems currently in use today.

Although biotechnology products may become extremely important drugs in future years, the majority are polypeptides that require special delivery systems. Since small and medium-size polypeptides are very potent, require

low doses, and are well absorbed from the mucous membrane, their delivery through the eyes may be feasible (19–26).

Traditionally, all drugs administered into the eye are intended to be delivered into the eyeball. However, the major portion of a topically instilled eyedrop does not penetrate the eyeball but leaves the potential absorption site (cornea) unused. In some cases, this produces an undesirable systemic effect when adequate ophthalmic concentrations are used to provide the desired ophthalmic effects. For instance, the majority of the instilled L-timolol enters the systemic circulation as a result of absorption through the nasolacrimal drainage system, and has been reported to produce systemic side effects (17). Therefore, the absorption of drugs used to treat ophthalmic diseases into the systemic circulation has, until now, been considered only from the standpoint of toxicity. Since the drug instilled in the eyes can be well absorbed through nasal mucosa, and the drug solution instilled into the eyes can be better controlled than can nasal drops, sprays, and/or inhalers, it is hoped that potent polypeptide drugs could be delivered into systemic circulation through the eye and the nasolacrimal system.

For those polypeptides with MW higher than 6000, permeation enhancers such as BL-9 and Brij-78 might be used to promote their absorption across the mucous membrane (22–26). With 0.5% of BL-9 and 0.5% Brij-78, the insulin absorption through the eyes into systemic circulation can be enhanced 4·5–6·8-fold. However, for chronic uses of polypeptide eyedrops, the safety of not only the polypeptides themselves but also the additives such as permeation enhancers and preservatives must be thoroughly investigated.

Additionally, longer-lasting formulations might be prepared using ocular inserts, ointments, gels, and conjunctival perfusion; this is impossible to do using intranasal preparations. It is possible that systemic drug delivery through conjunctival administration, particularly for polypeptide drugs, will become an important drug delivery system in the 21st century.

ACKNOWLEDGMENTS

This work was supported in part by research grants EY07511 from the National Eye Institute/NIH and Available University Funds (AUF) from Texas A&M University system.

Literature Cited

1. Aungst, B. J., Rogers, N. J. 1988. Site dependence of absorption-promoting actions of laureth-9, Na salicylate, Na_2EDTA, and aproptinin on a rectal, nasal, and buccal insulin delivery. *Pharm. Res.* 5:305–8
2. Aungst, B. J., Rogers, N. J., Shefter, E. 1988. Comparison of nasal, rectal, buccal, sublingual and intramuscular insulin efficacy and the effects of a bile salt absorption promotor. *J. Pharmacol. Exp. Ther.* 244:23–27
3. Moore, J. A., Pletcher, S. A., Ross, M. J. 1986. Absorption enhancement of growth hormone from the gastrointestinal tract of rats. *Int. J. Pharm.* 34:35–43
4. Tobey, N., Heizer, W., Yeh, R., Huang, T. I., Hoffner, C. 1985. Human intestinal brush border peptidases. *Gastroenterology* 88:913–26

5. Heyman, M., Bonfils, A., Fortier, M., Crain-Denoyelle, A. M., Smets, P., Desjeux, J. F. 1987. Intestinal absorption of RU 41740, an immunomodulating compound extracted from Klebsiella pneumoniae, across a duodenal epithelium and Peyer's patches of the rabbit. *Int. J. Pharm.* 37:33–39
6. Ziv, E., Lior, O., Kidron, M. 1987. Absorption of protein via the intestinal wall. *Biochem. Pharmacol.* 36:1035–39
7. Yamasaki, Y., Shichiri, M., Kawamori, R., Kikuchi, M., Yagi, T., et al. 1981. The effectiveness of rectal administration of insulin suppository on normal and diabetic subjects. *Diabetes Care* 4:454–58
8. Fisher, N. F. 1923. The absorption of insulin from the intestine, vagina and scrota sac. *Am. J. Physiol.* 67:65–71
9. Anders, R., Merkle, H. P., Schurr, W., Ziegler, R. 1983. Buccal absorption of protirelin: An effective way to stimulate thyrotropin and prolactin. *J. Pharm. Sci.* 72:1481–83
10. Moses, A. C., Gordon, G. S., Carey, M. C., Flier, J. S. 1983. Insulin administered intranasally as an insulin-bile salt aerosol, effectiveness and reproducibility in normal and diabetic subjects. *Diabetes* 32:1040–47
11. Hirai, S., Ikenaga, T., Matsuzawa, T. 1978. Nasal absorption of insulin in dogs. *Diabetes* 27:296–99
12. Salzman, R., Manson, J. E., Griffing, G. T., Kimmerle, R., Ruderman, N., et al. 1985. Intranasal aerosolized insulin; mixed-meal studies and long-term use in type I diabetes. *N. Engl. J. Med.* 312: 1078–84
13. Wigley, F. M., Londono, J. H., Wood, S. H., Shipp, J. C., Waldman, R. H. 1971. Insulin across respiratory mucosae by aerosol delivery. *Diabetes* 20:552–56
14. Kari, B. 1986. Control of blood glucose levels of alloxan-diabetic rabbits by iontophoresis of insulin. *Diabetes* 35:217–21
15. Siddiqui, O., Sun, Y., Liu, J. C., Chien, Y. W. 1987. Facilitated transdermal transport of insulin. *J. Pharm. Sci.* 76:341–45
16. Zimmerman, T. J., Kooner, K. S., Kandarakis, A. S., Ziegler, L. P. 1984. Improving the therapeutic index of topically applied ocular drugs. *Arch. Ophthalmol.* 102:551–53
17. Fraunfelder, F. T., Meyer, S. M. 1987. Systemic side effects from ophthalmic timolol and their prevention. *J. Ocul. Pharmacol.* 3:177–84
18. Christie, C. D., Hanzal, R. F. 1931. Insulin absorption by the conjunctival membrane in rabbits. *J. Clin. Invest.* 10:787–93
19. Chiou, G. C. Y., Chuang, C. Y. 1988. Systemic delivery of polypeptides with molecular weights of between 300 and 3500 through the eyes. *J. Ocul. Pharmacol.* 4:165–77
20. Chiou, G. C. Y., Chuang, C. Y. 1988. Treatment of hypoglycemia with glucagon eyedrops. *J. Ocul. Pharmacol.* 4:179–86
21. Chiou, G. C. Y., Chuang, C. Y., Chang, M. S. 1988. Systemic delivery of enkephalin peptide through eyes. *Life Sci.* 43:509–14
22. Chiou, G. C. Y., Chuang, C. Y., Chang, M. S. 1988. Reduction of blood glucose concentration with insulin eyedrops. *Diabetes Care* 11:750–51
23. Chiou, G. C. Y., Chuang, C. Y., Chang, M. S. 1989. Systemic delivery of insulin through eyes to lower the glucose concentration. *J. Ocul. Pharmacol.* 5:81–91
24. Chiou, G. C. Y., Chuang, C. Y. 1989. Improvement of systemic absorption of insulin through eyes with absorption enhancers. *J. Pharm. Sci.* 78:815–18
25. Yamamoto, A., Luo, A. M., Dodda-Kashi, S., Lee, V. H. L. 1989. The ocular route for systemic insulin delivery in the albino rabbit. *J. Pharmacol. Exp. Ther.* 249:249–55
26. Chiou, G. C. Y., Shen, Z. F., Zheng, Y. Q. 1990. Systemic delivery of insulin and glucagon through eyes measured with radioimmunoassay and its effects on blood glucose levels. *J. Ocul. Pharmacol.* In press
27. Hirai, S., Yashiki, T., Mima, H. 1981. Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.* 9:173
28. Monkhouse, D. C., Groves, G. A. 1967. Effect of EDTA on the resistance of *P. aeruginosa* to benzoalkonium chloride. *Aust. J. Pharm.* 9:S70
29. Moses, A. C. 1986. *Nasal Route of Administration. Proc. Land O'Lake, Merrimac, WI.* p. 6
30. Chow, D. S. L., Hsieh, D. 1989. Structure activity relationship of permeation enhancers and their possible mechanisms. *Pharm. Tech. Conf.* In press
31. Chuang, L. M., Wu, H. P., Chiou, G. C. Y. 1989. Treatment of hypoglycemia with glucagon eyedrops. *Pharmacologist* 31:125

32. Gibaldi, M. 1984. *Biopharmaceutics and Clinical Pharmacokinetics* Philadelphia: Lea & Febiger. 3rd ed.
33. Jelliffe, R. W., D-Argenio, D. Z., Schumitz, K. Y. A., Hu, L. C., Lin, M. 1987. The USC PC-Pack programs for managing drug dosage regimen. *IUPHAR Meeting*, Sydney, Aust.
34. Hu, L. C., Hu, C. R. 1974. Identification of rate constant by differential quadrature in partly measureable compartmental model. *Math. Biosci.* 21:71–76
35. Hu, L. C., Chiou, G. C. Y. 1990. A three-compartment model for the systemic delivery of LHRH and enkephalin through the eyes. *J. Theor. Biol.* In press
36. Bezold, C. 1983. *Pharmaceuticals in the Year 2000, Inst. Alternative Future:* 65–80