Enhancement of Nasal Delivery of a Renin Inhibitor in the Rat Using Emulsion Formulations

Tugrul T. Kararli,[1,3] Thomas E. Needham,[2] Grant Schoenhard,[1] David A. Baron,[1] R. Eric Schmidt,[1] Barbara Katz,[1] and Bayani Belonio[1]

Received March 6, 1991; accepted December 19, 1991

Nasal absorption of *O*-(*N*-morpholino-carbonyl-3-L-phenylaspartyl-L-leucinamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (I), a renin inhibitor, was evaluated in two rat nasal models, one involving surgery and the other requiring no surgical intervention. Oleic acid/monoolein emulsion formulations were tested along with a control PEG 400 solution. The percent absolute bioavailability of the compound was enhanced from 3–6% (PEG 400 solution) to 15–27% when the emulsion formulations were used. The different nasal model techniques (with and without surgery) did not produce any statistical difference in the absolute bioavailability values for I. Emulsion formulations did not produce appreciable damage as assessed morphologically. It is suggested that emulsion formulations containing membrane adjuvants such as oleic acid and monoolein can be used to enhance the nasal delivery of low-bioavailable, lipid-soluble drugs.

KEY WORDS: renin inhibitor; peptide nasal delivery; emulsions; oleic acid; monoolein.

INTRODUCTION

The surge of new peptide and protein drugs demands the development of new delivery routes and formulation technologies. Oral delivery of such molecules, although most desirable, is often not feasible because of degradation and/or limited absorption in the intestine (1,2). The nasal route represents an alternative to oral drug delivery (3) where first-pass liver metabolism is not an issue (4). The nasal mucosa is highly vascularized (3,5), leading to greater and faster absorption (2,6). Further, with the nasal route, formulation additives and/or absorption promoters seem to have a pronounced effect on the mucosal transport of drugs (7–16); however, the potential for these formulations and/or adjuvants to cause mucosal damage and irritation is a concern.

Bile salts have been shown to cause a significant increase in the nasal delivery of small and large peptide drugs (2,7,8,13,15,16). Although in some studies morphological examination did not reveal significant mucosal damage (2,7), the chronic use of a nasal formulations containing bile salts may induce membrane damage. Bile salt/oleic acid mixed micellar solutions were shown to be more effective than a simple bile salt solution in enhancing the absorption of insulin from the nasal mucosa (9). The use of lipids in mixed micellar solutions may reduce the mucosal damage caused by bile salt micelles alone (17).

Compound I [*O*-(*N*-morpholino-carbonyl-3-L-phenylaspartyl-L-leucinamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane] is a potent inhibitor specific for primate renin (IC_{50} for human renin is 5×10^{-8} *M*) (18) (Scheme I). It is a modified tripeptide with very low water solubility. Emulsion formulations containing oil components that are considered to be membrane adjuvants (oleic acid and mono- and diglycerides) were formulated and tested by Kararli *et al.* to increase the oral bioavailability of I in rats (19). These emulsion formulations, when compared to the aqueous suspensions, increased the absolute bioavailability of Compound I significantly.

In this study, the nasal bioavailability of I was studied using emulsion formulations containing oleic acid, monoolein, and sodium taurocholate (or Tween 80). Emulsions were formulated based on the greater solubilization of the compound in the oils as well as the predicted effect of these oils on the permeability of the compound. The formulations were evaluated in rat nasal models involving surgery and no surgery.

MATERIALS AND METHODS

Materials

Compound I was synthesized by G. D. Searle Co. (Skokie, IL). Oleic acid (99%), monoolein (99%), and sodium taurocholate (NaTC) were obtained from Sigma Chemical Co. (St. Louis, MO). Tween 80 was obtained from J. T. Baker Co. (Phillipsburg, NJ).

Emulsion Preparation

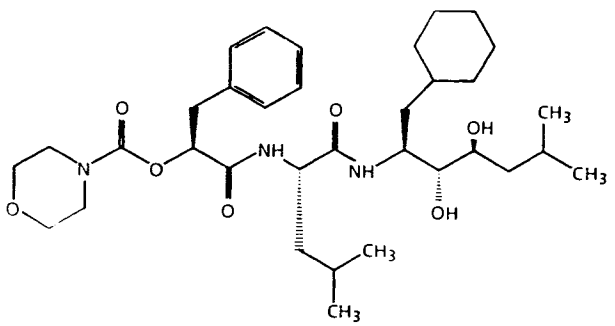
Both I and monoolein (12%, w/w) were dissolved in oleic acid (20%, w/w). NaTC (0.5%, w/w) was dissolved in water (67.5%, w/w). The aqueous and oil solutions were then mixed. The mixture was sonicated using a tissue disrupter (Tekmar Sonic Disrupter, Tekmar Co., Cincinnati, OH) for 2 to 3 min. The emulsions with Tween 80 (3%) were prepared in the same way, but pH 7.0 phosphate buffer (10 m*M*) was used as the aqueous solution to prepare stable emulsions with small droplet sizes. The average particle size of the emulsions prepared with NaTC was measured to be 0.26 μm (photon correlation spectroscopy was used; see Ref. 19). The droplet size of the Tween 80 containing emulsion was not measured with photon correlation spectroscopy. However, under light microscopy the droplet size of this emulsion appeared similar to that prepared with NaTC.

Nasal Studies

Male Sprague–Dawley rats (~300 g) were employed for the two nasal model techniques. The animals were fasted for 18–24 hr prior to the experiments. Nembutal (Abbott Laboratories, 50 mg/kg, i.p.) was used to induce anesthesia. The nasapalatine tract which connects the nasal cavity with the mouth was sealed with glue to prevent drainage of the drug solution from the nasal cavity into the mouth. In both nasal methods, the formulations were introduced into the nostril of the animals using a 5- to 200-μl pipette. Great care was taken

[1] G. D. Searle & Co., 4901 Searle Parkway, Skokie, Illinois 60077.
[2] University of Rhode Island, Drug Delivery R&D Laboratory, Department of Pharmaceutics, Kingston, Rhode Island 02881-0809.
[3] To whom correspondence should be addressed.

0724-8741/92/0800-1024$06.50/0



Scheme I. Compound I

not to puncture the nasal mucosa when delivering the dose with a pipette.

Nonsurgical Method. The dose was delivered into only one nostril to ensure sufficient breathing by the animals.

Surgical Method. The surgical method was similar to that used by Hussain *et al.* (20). In brief, the rats were anesthetized and an incision was made in the neck area. A polyethylene tube (PE 260, Intramedic, Clay Adams, New York) was inserted into the trachea. This aided the breathing of the animals. Also, a sealed polyethylene tube was inserted into the esophagus toward the nasal cavity to prevent the leakage of the formulations from the nasal cavity.

In the surgical method, the nostril openings of the animals were closed with glue after administration of the dose to prevent leakage of the formulations from the nose.

The percent absolute bioavailability (%AB) values were calculated using the AUC (total area under the plasma concentration–time curve) of the nasal and iv doses as shown in Eq. (1)

$$\%AB = \frac{AUC^{nasal} \times Dose^{iv}}{AUC^{iv} \times Dose^{nasal}} \quad (1)$$

The AUC calculations were extended to infinite time by using the biological half-life of the compound calculated from the iv data (19). The statistical evaluation used a *t* test.

Morphological Studies

Oleic acid/monoolein/NaTC emulsion or saline were introduced (100 μl) into one nostril of each of two animals in both the surgical and the nonsurgical methods and sacrificed 4 hr after the dose delivery. The nostril of the animals that received the dose was perfused immediately with 2.3% glutaraldehyde/2.0% paraformaldehyde in 0.1 *M* cacodylate buffer (pH 7.4). The nasal turbinate, maxilloturbinate, and endoturbinate were dissected out and placed in the same fixative for at least 24 hr at 4°C. The olfactory mucosa was rinsed 3× in the same buffer and stripped, minced, dehydrated in graded ethanols (25–100%) and propylene oxide, and embedded in epoxy resins. Thick sections for light microscopy (1 μm) were stained with 1% toluidene blue. Thin sections (500 Å) for electron microscopy were stained with uranyl acetate and lead citrate and examined in a Zeiss EM10CA (Thornwood, NY) at 60 kV.

Plasma Analysis

The plasma concentrations of Compound I were measured by the HPLC method described earlier (19).

RESULTS AND DISCUSSION

The solubility of I in water is approximately 1 μg/ml (19). Because of this low solubility in water, a nonaqueous solvent PEG 400 was used as the control solvent.

Systemic blood levels of I following administration of PEG 400 (2- and 5-mg/kg dose) solutions in the rat surgical technique are given in Fig. 1. The resulting percent absolute bioavailability values are provided in Table I. The plasma concentrations of Compound I following the PEG 400 solution showed a dose-proportional increase in the plasma concentrations. The T_{max} for the PEG 400 formulation shifted from approximately 20 to 60 min when the dose was increased from 2 to 5 mg/kg.

The systemic plasma concentrations of I are shown in Fig. 1 following delivery of the emulsion formulations. The results indicated that the plasma levels of Compound I following delivery with the emulsion formulation were much higher than those of the PEG 400 solution. Further, in Fig. 2, there is still significant absorption of I in the rats at the last time point (4 hr), as compared to the decrease in plasma levels seen at the same time after administration of PEG 400 solutions. As a result of higher plasma levels and extended absorption, the percent absolute bioavailability values for I from the oleic acid/monoolein/NaTC emulsion were significantly higher than the percent absolute bioavailability values found after administration of the PEG 400 solution (Table I). The above results indicate that there is prolonged interaction between the emulsions and the nasal mucosa despite the fact that the half-life of nasal clearance is less than 30 min (21). Such extended absorption was also observed in the nasal absorption of human growth hormone using degradable starch microspheres (22).

In the delivery of a NaTC-containing emulsion, the nonsurgical rat nasal technique gave higher plasma levels and percent absolute bioavailability values than the surgical method (Table I, Fig. 2). In the surgical model, the plasma levels of I reached plateau levels at 120 and 240 min. However, the mean plasma levels (except at 120 min, $P = 0.02$) and absolute bioavailability values for the two methods were

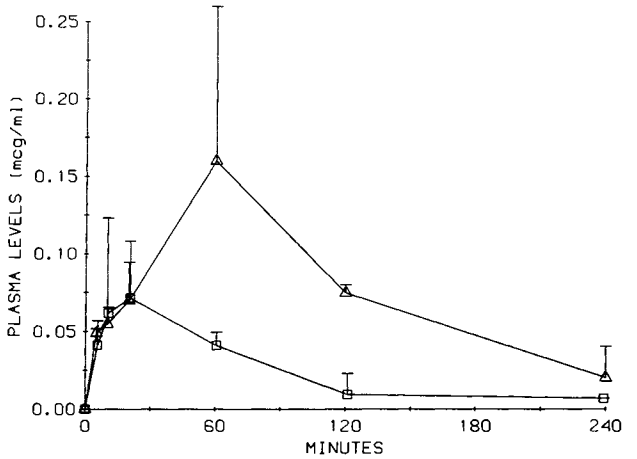


Fig. 1. Systemic plasma concentrations of Compound I in the rats following nasal delivery of 2 mg/kg (□) and 5 mg/kg (△) doses in PEG 400 solution. The animal method involves surgery. The bars indicate standard deviations.

Table I. Percent Absolute Bioavailability (%AB) of Compound I in the Rat[a]

Formulation	Dose volume (ml/kg)	Dose (mg/kg)	No. of animals	%AB (mean ± SD)
PEG 400	0.22	2	2	5.41 ± 2.11
PEG 400	0.37	5	2	3.73 ± 0.18
Emulsion[b,c]	0.31	5	6	20.21 ± 3.77*
Emulsion[b,d]	0.31	5	6	15.02 ± 5.35*
Emulsion[c,e]	0.32	5	8	26.60 ± 10.00*

[a] Note that the above emulsion volumes were based on the total dose and solubility of the compound in the lipid phase.
[b] Emulsion was prepared with NaTC.
[c] Nonsurgical rat model was used.
[d] Surgical rat model was used.
[e] Emulsion was prepared with Tween 80.
* Significantly different from PEG 400 solutions at $P < 0.006$ using Student's t test.

not found to be statistically different. In a recent study, nasal absorption of propranonol (aqueous solution) was also evaluated in the same surgical and nonsurgical animals models used in this study (23). In that study, the plasma levels of propranolol after the delivery in the two models were found to be very close.

In another oleic acid/monoolein emulsion, NaTC was replaced with a nonionic surfactant, Tween 80. This formulation was evaluated using the rat nonsurgical method. The resulting plasma levels and percent absolute bioavailability values were comparable to those found after administration of the NaTC containing emulsion (Fig. 2, Table I).

Several emulsion formulations of I, including those used in the present study, were administered orally to rats by Kararli *et al.* to evaluate their bioavailability (19). These emulsion formulations enhanced the oral bioavailability of I from 0.3% (water suspension) to 5%. However, because of a very high first-pass liver metabolism for the compound, bioavailability enhancements greater than 5% could not be achieved. The enhancement of the absolute bioavailability of I up to 27% in the nasal delivery studies with the emulsion formulations could reflect both the increased permeability of the nasal mucosa and the absence of first-pass liver metabolism.

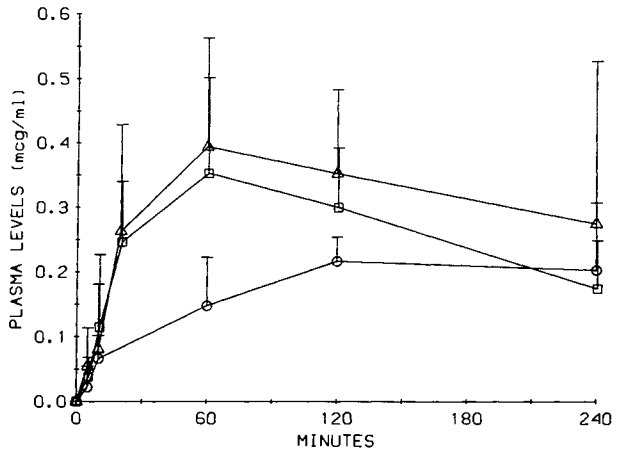


Fig. 2. Systemic plasma concentrations following nasal delivery of Compound I in an oleic acid/monoolein/NaTC emulsion formulation at the 5 mg/kg dose, in the rat nasal models involving no surgery (□) and surgery (○). The symbol (△) represents the results using an oleic acid/monoolein/Tween 80 emulsion at the same dose using the nonsurgical nasal technique. The bars indicate standard deviations.

The rationale for the use of emulsion formulations in these studies was (i) to increase the solubility of the compound and (ii) to alter membrane permeability for the compound. The solubility of I in both oleic acid and PEG 400 is in excess of 100 mg/ml. The higher plasma levels and extended bioavailabilities obtained from the emulsion formulations compared to the PEG 400 solutions seem to reflect favorable transport of Compound I from the emulsion formulation.

The mechanism of drug transport from oil vehicles was discussed by Kararli *et al.* (19). Oleic acid and monoolein can decrease membrane fluidity by directly interacting with the membrane and consequently increase membrane permeability for drugs (1,24–26). In addition to this effect, the interaction of these oils with the membrane can also lead to cotransport of lipids and drugs (see Ref. 19 for discussion). Therefore, the physical state of the oils at the absorption site may be important in the transport process.

The physical state of oils in the oleic acid/monoolein/NaTC (or Tween 80) emulsion can be predicted (27). A monoolein–oleic acid mixture at the molar ratio 1:2 in aqueous solutions (with and without 10 m*M* sodium taurodeoxycholate) forms an inverse micellar phase, L2 phase, when swelled in an aqueous medium (27). When the monoolein content increases, the system converts into a hexagonal liquid crystalline (H-LC) and then a cubic phase (CP). Therefore, the ternary system of oleic acid/monoolein/NaTC (or Tween 80) used in the present emulsions is probably in the dispersed L2 phase. The other two phases along with the mixed micellar phase may also be present. The enhancement of Compound I absorption in this study may be related to the favorable interaction of these lipid phases with the nasal membrane. In the study by Temgamnuay *et al.* a linoleic acid emulsion did not appear to enhance the nasal transport of insulin (9). However, insulin was in the water phase of the emulsion, suggesting the importance of formulating the drug in the lipid phase.

Both oleic acid and monoolein are the end products of hydrolysis in the intestine following a fatty meal. This argues for the safety of these oils when given orally as emulsion formulations. The overall morphology of the nasal mucosa of control and surgically and nonsurgically delivered drug emulsion groups was not significantly different as assessed by light and electron microscopy. The olfactory epithelia in each group showed no gross disruption of cell-to-cell continuity or focal loss of epithelial cells (Figs. 3A–C). Occasional regions of emulsion exposed epithelia showed evidence of cellular damage such as cytoplasmic vacuolization (Fig. 3C). Focal areas of red blood cells and cell debris were noted in the nasal lumina of all three groups but were not associated with obvious underlying mucosal injury. By electron microscopy, the apical junctional complexes of the olfactory epithelium were shown to be intact (Fig. 4). No significant differences between control and experimental groups in other regions of the nasal cavity were found. In the above mor-

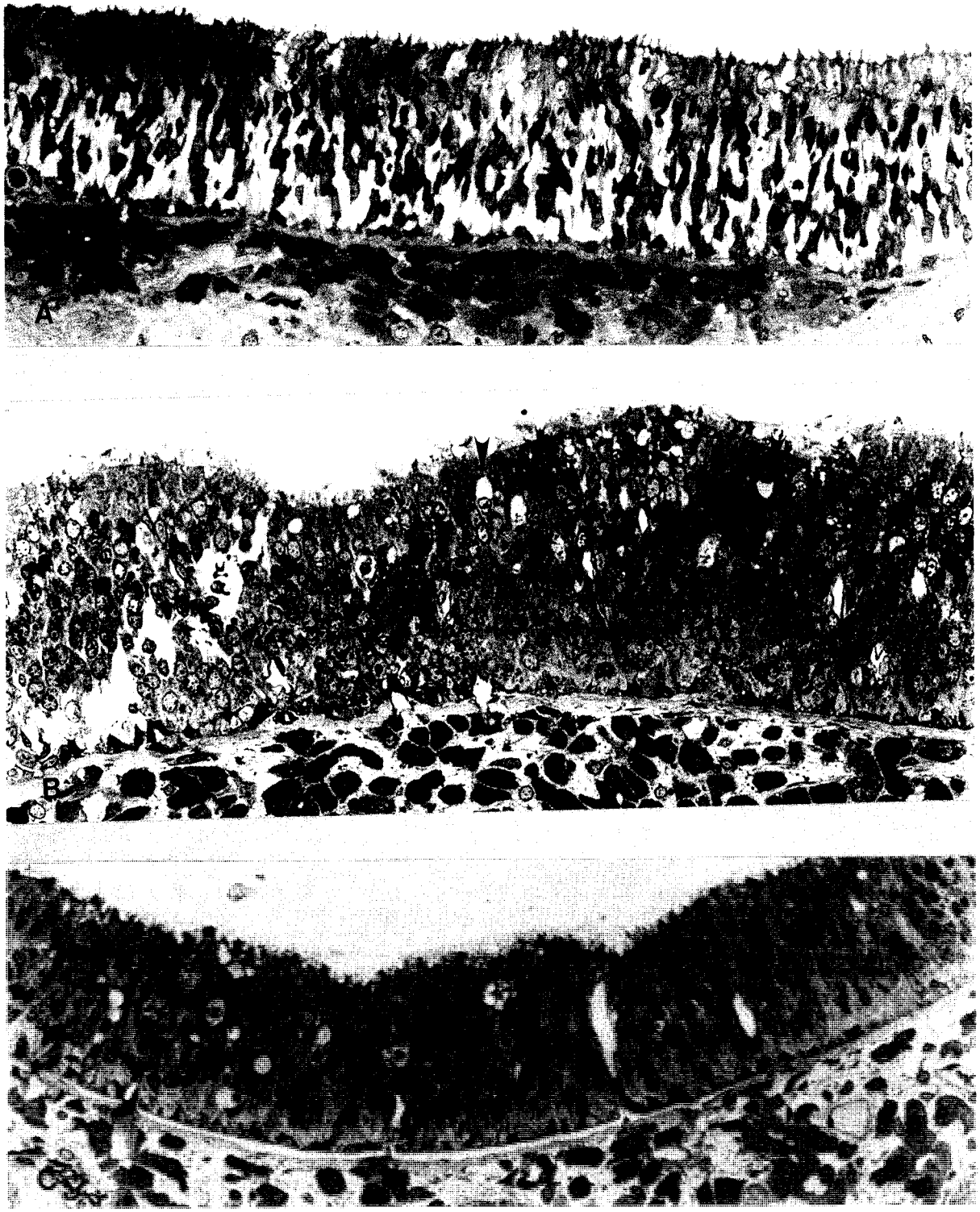


Fig. 3. Light photomicrographs of olfactory mucosa. (A) Control mucosa. (B) Surgically administered drug/emulsion. (C) non-surgically administered drug/emulsion. Arrowheads, cytoplasmic vacuolization. ×350: reduced to 90% for reproduction.

phological studies, only NaTC-containing emulsions were studied. The safety of Tween 80 in the oral route has been established (28). Although the morphology of the nasal mucosa after application of the Tween 80-containing emulsions was not examined, it is suggested that this emulsion would not harm the nasal musosa any more than the NaTC-containing emulsion.

The results of our studies suggest that emulsion formulations with components that are membrane adjuvants, when administered nasally, can provide improved availability for

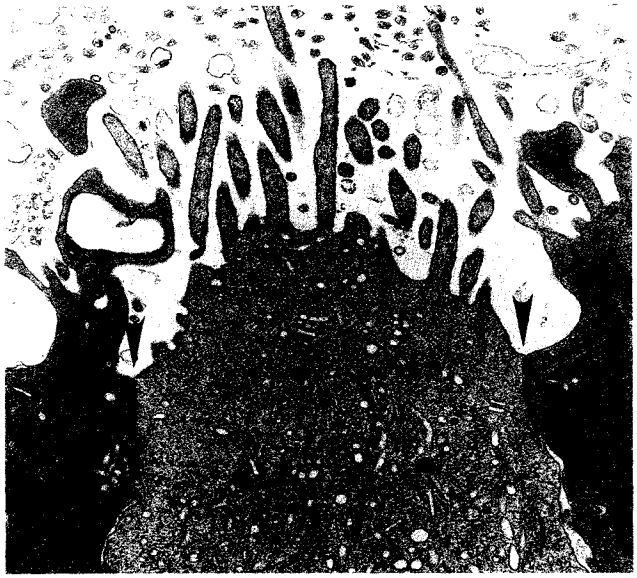


Fig. 4. Electron photomicrograph of olfactory mucosa exposed surgically to drug/emulsion. Tight junctions (arrowheads) are intact. ×14,000.

lipid-soluble drugs possessing a low bioavailability. Further, the rat nasal methods involving surgery and no surgery were statistically equivalent when emulsion formulations were used. A nonsurgical rat nasal method would be preferred in future studies since it is less time-consuming and less stressful to the animal.

ACKNOWLEDGMENTS

The technical contributions of Mr. David Kio and Mrs. Marianne Hidvegi in the morphological studies are gratefully acknowledged.

REFERENCES

1. T. T. Kararli. Gastrointestinal absorption of drugs. *CRC Crit. Rev. Ther. Drug Carrier Syst.* 6:39–86 (1989).
2. K. S. E. Su, K. M. Campanale, L. G. Mendelsohn, G. A. Kerchner, and C. L. Gries. Nasal delivery of polypeptides I: Nasal absorption of enkephalins in rats. *J. Pharm. Sci.* 74:394–398 (1985).
3. Y. W. Chein. Intranasal drug delivery for systemic medications. *CRC Crit. Rev. Ther. Drug Del. Syst.* 4:67–194 (1987).
4. G. C. Visor, E. Bajka, and E. Benjamin. Intranasal delivery of nicardipine in the rat. *J. Pharm. Sci.* 75:44–46 (1986).
5. D. R. Adams and D. K. Hotchkiss. The canine nasal mucosa. *Zbl. Vet. Med. C. Anat. Histol. Embryol.* 12:109–125 (1981).
6. S. Hirai, T. Yashiki, T. Matzuzawa, and H. Mima. Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.* 7:317–325 (1981).
7. J. P. Longenecker. Naslin^R—Transnasal systemic delivery of insulin. In S. S. Davis, Illumlisbeth, and E. Tomlinson (eds.), *Delivery Systems for Peptide Drugs,* NATO ASI Series A, Life Sciences, Vol 125, Plenum Press, New York and London, 1986, pp. 211–220.
8. P. Tengamnuay and A. K. Mitra. Bile salt-fatty acid mixed micelles as nasal absorption promoters of peptides. I. Effects of ionic strength, adjuvant composition, and lipid structure on the nasal absorption of [D-Arg^2]kyotorphin. *Pharm. Res.* 7:127–133 (1990).
9. P. Tengamnuay and A. K. Mitra. Bile salt-fatty acid mixed micelles as nasal absorption promoters of peptides. II. In vivo nasal absorption of insulin in rats and effects of mixed micelles on the morphological integrity of the nasal mucosa. *Pharm. Res.* 7:370–375 (1990).
10. T. Shimamoto. Pharmaceutical aspects. Nasal and depot formulations of leuprolide. *J. Androl.* 8:514–516 (1987).
11. T. Nagai, Y. Nishimoto, N. Nambu, Y. Suzuki, and K. Sakine. Powdered dosage of insulin for nasal administration. *J. Control. Release* 1:15–22 (1984).
12. K. Morimoto, K. Morisaka, and A. Kamada. Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel. *J. Pharm. Pharmacol.* 37:134–136 (1985).
13. S. Hirai, T. Yashiki, and H. Mima. Effect of surfactants on the nasal absorption of insulin in rats. *Int. J. Pharm.* 9:165–172 (1981).
14. S. Hirai, T. Yashiki, and H. Mima. Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.* 9:173–184 (1981).
15. A. C. Moses, G. C. Gordon, M. C. Carey, and J. S. Flier. Insulin administered intranasally as an insulin-bile salt aerosol: Effectiveness and reproducibility in normal and diabetic subjects. *Diabetes* 32:1040–1047 (1983).
16. P. A. Baldwin, C. K. Klingbeil, C. J. Grimm, and J. P. Longenecker. The effect of sodium tauro-24,25-dihydrofusidate on the nasal absorption of human growth hormone in three animal models. *Pharm. Res.* 7:547–552 (1990).
17. F. Feldman and M. Gibaldi. Bile salt induced permeability changes in the isolated rat intestine. *Proc. Soc. Exp. Biol. Med.* 132:1031–1033 (1969).
18. G. J. Hanson, J. S. Baran, M. Clare, K. Williams, M. Babler, S. E. Bittner, M. A. Russell, S. E. Papaioannou, P. Yang, and G. M. Walsh. Orally active renin inhibitors containing a novel aminoglycol dipeptide (leu-val) mimetic. In J. E. Rivier and G. R. Marshall (eds.), *Peptides, Chemistry, Structure and Biology,* Proceedings of the Eleventh American Peptide Symposium, ESCOM, Leiden, 1990, pp. 396–398.
19. T. T. Kararli, T. E. Needham, M. Griffin, G. Schoenhard, L. J. Ferro, and L. Alcorn. Oral delivery of a renin inhibitor compound using emulsion formulations. *Pharm. Res.,* in press (1992).
20. A. Hussain, S. Hirai, and R. Bawarshi. Nasal absorption of propranolol from different dosage forms by rats and dogs. *J. Pharm. Sci.* 69:1411–1413 (1980).
21. A. S. Harris, E. Svensson, Z. G. Wagner, S. Lethagen, and I. M. Nilsson. Effect of viscosity on particle size, deposition, and clearance of nasal delivery systems containing desmopressin. *J. Pharm. Sci.* 77:405–408 (1988).
22. L. Illum, N. F. Farraj, S. S. Davis, B. R. Johansen, and D. T. O'Hagan. Investigation of the nasal absorption of biosynthetic human growth hormone in sheep-use of a bioadhesive microsphere delivery system. *Int. J. Pharm.* 63:207–211 (1990).
23. C. A. Lau-Cam, K. P. Thadikonda, V. Theofanopoulos, and V. D. Romeo. A simplified rat model for studying nasal drug absorption. *Drug. Dev. Ind. Pharm.* 17:1721–1730 (1991).
24. B. D. McKersie, J. H. Crowe, and L. M. Crowe. Free fatty acid affects on leakage, phase properties and fusion of fully hydrated model membranes. *Biochim. Biophys. Acta* 982:156–160 (1989).
25. J. B. Meddings. Lipid permeability of the intestinal microvillus membrane may be modulated by membrane fluidity in the rat. *Biochim. Biophys. Acta* 984:158–166 (1989).
26. G. A. Thomson. Lipid molecular species retailoring and membrane fluidity. *Biochem. Soc. Trans.* 17:286–289 (1988).
27. M. Lindstrom, H. Ljusberg-Wahren, and K. Larsson. Aqueous lipid phases of relevance in intestinal fat digestion and absorption. *Lipids* 16:749–754 (1981).
28. N. R. Artman. Safety of emulsifiers in fats and oils. *J. Am. Oil Chem. Soc.* 52:49–52 (1975).