J. Pharm. Pharmacol. 1989, 41: 770–774
Received January 15, 1989

Rectal Absorption of E-2078 (Dynorphin Analogue Peptide) in Rats

NAOKAZU MURAHASHI, AKIRA KATO, NORITOSHI KOYAMA, SUMIO WATANABE, TERUAKI YUZURIHA AND YASUO MIYAKE

Tsukuba Research Laboratories, Eisai Co. Ltd., Tokodai 5-1-3, Tsukuba-shi, Ibaraki 300-26, Japan

Abstract—The plasma levels in rats of a dynorphin analogue peptide (E-2078) after rectal administration have been studied. The bioavailabilities of E-2078 after rectal administration, subcutaneous injection, intramuscular injection, and oral administration were 21·6, 67·8, 67·1, 0·7%, respectively. The effect of dose, pH, osmolarity and viscosity on the rectal absorption of E-2078 were studied. A sigmoid relationship between the dose and the AUC was observed on rectal administration, while there was a linear relationship on intramuscular administration. The AUC was increased in acidic solution and highly viscous solution. The osmolarity did not affect the absorption of E-2078. In microscopic studies, E-2078 caused little or no damage to the rectal mucosa in rats.

A previously synthesized dynorphin (1) analogue, E-2078 (2) (Tachibana et al 1987), has a specific affinity for the opioid κ receptor (Nakazawa et al 1987).

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Iys-Trp-Asp-Asn-Glu (1)

CH_3-Tyr-Gly-Gly-Phe-Leu-Arg-CH_3-
1 2 3 4 5 6 7
Arg-D-Leu-NHC_2H_5 (2)
8

E-2078 is about 3 times more potent than morphine in the mouse tail pinch and tail flick tests, and has little tendency to produce tolerance and physical dependence (Nakazawa et al 1987).

Many peptide drugs have been developed for therapy, but absorption of peptides after oral administration is generally poor, owing to their decomposition in the gastrointestinal tract. E-2078, which is also a peptide, must be administered by injection. However, injections cause discomfort, allergic reactions, and tissue lesions (Gunn 1964; Saunders et al 1965) and are best avoided in self treatment.

The possibility that the nasal, rectal and vaginal routes may be useful for peptides having low bioavailability after oral administration, has received attention. For example, enkephalins (Su et al 1985), progesterone (Anik et al 1984; Okada et al 1982), and insulin (Nakanishi et al 1986), have been shown to be more effectively absorbed via the intranasal, vaginal and rectal routes than by oral administration.

Therefore we have compared the pharmacokinetics and the bioavailability of E-2078 in rats after its administration by several injection routes, and by the oral and rectal routes. We also studied the rectal absorption of E-2078, and the effects of dose, pH, osmolarity, and viscosity on rectal absorption, and the histology of the rectal mucosa of rats after rectal administration of the peptide.

Materials and Methods

Materials

E-2078 (MW: 1035) was synthesized in our laboratories.

Correspondence to: N. Murahashi, Tsukuba Research Laboratories, Eisai Co. Ltd., Tokodai 5-1-3, Tsukuba-shi, Ibaraki 300-26, Japan.

Other chemicals were obtained commercially and were of analytical grade.

Preparation of solutions for administration

E-2078 (3 mg) was dissolved in 0·5 mL of physiological saline and used for intravenous, subcutanous, intramuscular and rectal administration. E-2078 (30 mg) was dissolved in 0·5 mL of physiological saline for oral administration. For the dose-dependence study on intramuscular and rectal administration, E-2078 was dissolved in physiological saline to make solutions from 0·6 to 20 mg mL^{-1}. For the study of pH on rectal administration E-2078 (3 mg) was dissolved in 0·5 mL of isotonic phosphate buffer solution prepared from 1/15 M disodium hydrogenphosphate and 1/15 M potassium dihydrogen phosphate. The buffer solutions used were from pH 5 to 8. To examine the osmolarity-effect, E-2078 (3 mg) was dissolved in 0·5 mL of water with or without sodium chloride. The osmotic pressure of the solutions were from 0 to 2000 mOsm kgH_2O^{-1}. To examine the viscosity effect, E-2078 (3 mg) was dissolved in 0·5 mL of 2% moderately substituted and highly substituted hydroxypropylcellulose solution. The viscosities of the solutions were 250 and 980 cP, respectively.

Animal studies

Male Wistar rats (200–300 g) were fasted for 16 h before the experiment, but water was freely available. Rats were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (Nembutal sodium solution, Abbott Laboratories) at a dose of 50 mg kg^{-1} and their body temperatures were maintained above 36°C.

On intravenous, subcutaneous and intramuscular administration, the drug solution was administered by bolus injection into the femoral vein, under the skin surface of the back, or into the thigh muscle, respectively. For rectal administration, a microenema (0·5 mL kg^{-1}) was administered into the anus through a polyethylene tube inserted to a depth of 2 cm after which the anus was closed with surgical adhesive (Aronalpha, Sankyo Co. Ltd).

Blood samples were taken from the jugular vein with syringes (containing sodium citrate solution) at appropriate times. Blood samples were cooled on ice, and the plasma was

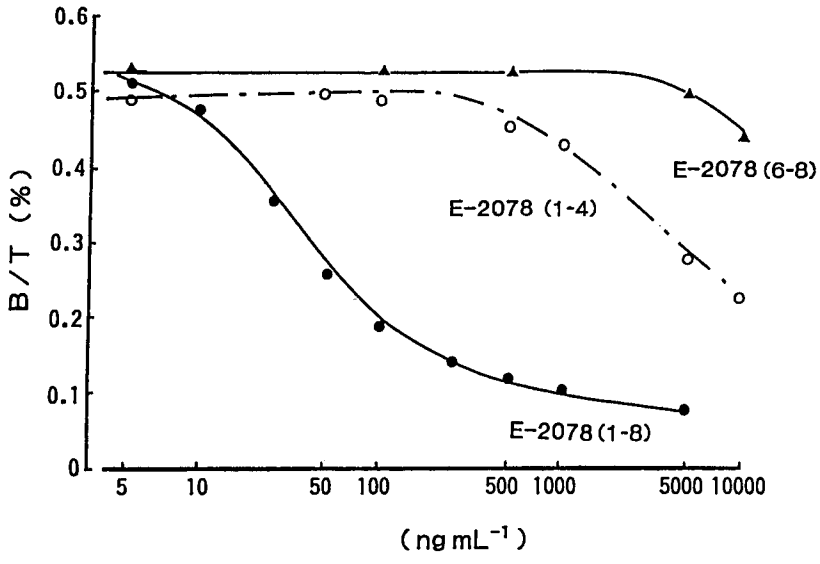

FIG. 1. Standard curve for the measurement of E-2078. Ordinate: binding/total, abscissa: concentration of E-2078 and E-2078 metabolites. E-2078(6–8): Arg-CH_3Arg-D-Leu-NHC_2H_5. E-2078(1–4): CH_3-Tyr-Gly-Gly-Phe. E-2078(1–8): CH_3-Tyr-Gly-Gly-Phe-Leu-Arg-CH_3Arg-D-Leu-NHC_2H_5.

separated by centrifugation within 30 min and then stored at −20°C until analysis.

Determination of E-2078 level in rat plasma

To determine the plasma levels after E-2078 administration to rats, a radioimmunoassay was developed. Antiserum against E-2078 was obtained from immunized rabbits. E-2078 (10 mg) conjugated with bovine serum albumin in 1 mg of 0·15 M NaCl was mixed with 1 mL of Freund's incomplete adjuvant (Difco Laboratories) to form a water-in-oil emulsion. Two mL of emulsion was injected into the hind footpads and the thigh of a rabbit weighing about 2 kg. The administration of the emulsion was repeated 3, 5 and 10 weeks after the first immunization. Two weeks later, the serum was obtained from blood taken from the ear vein.

^{125}I-labelled E-2078 with the specific radioactivity of 100 mCi mg^{-1} was prepared by the Iodogen method and the

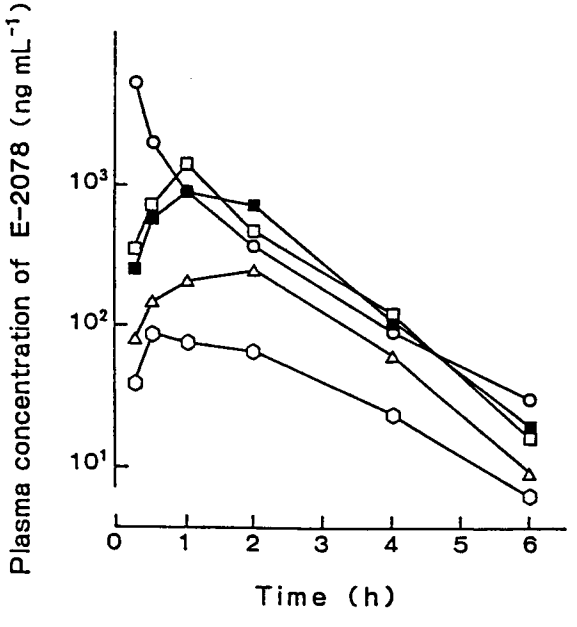

FIG. 2. Plasma E-2078 levels after various routes of administration (○) intravenous 3 mg kg^{-1}, (□) subcutaneous 3 mg kg^{-1}, (■) intramuscular 3 mg kg^{-1}, (△) rectal 3 mg kg^{-1}, (⬡) oral 30 mg kg^{-1}.

Table 1. Pharmacokinetic parameters for E-2078 after intravenous administration of 3 mg kg^{-1} to rats (n=6).

Parameter	A (μg mL^{-1})	B (μg mL^{-1})	α (h^{-1})	β (h^{-1})	$t_{\frac{1}{2}}$ (min)	Vc (mL kg^{-1})
Mean	34·76	2·07	7·57	0·82	56·6	170·8
±s.e.	14·36	0·29	1·80	0·14	7·5	51·4

Vc: the apparent volume of distribution.

Table 2. Comparison of E-2078 bioavailability after various routes of administration.

Route of administration	Dose (mg kg^{-1})	n	AUC (ng h mL^{-1})	Bioavailability (%)
Intravenous	3	6	3628+269	
Subcutaneous	3	5	2458+211	67·8
Intramuscular	3	5	2433+179	67·1
Rectal	3	4	785+269	21·6
Oral	30	4	267+92	0·7

E-2078 was dissolved in saline, and the volume was maintained at 0·5 mL kg^{-1}, AUC: mean±s.e., integrated from 0 to 6 h.

Table 3. Effect of dose on the rectal absorption of E-2078.

Route of administration	n	AUC	Mean Sp AUC	Bioavailability (%)
Intravenous				
3 mg kg^{-1}	6	3628+269	1209	
Rectal				
10 mg kg^{-1}	6	5473+1268	547	45·2
3 mg kg^{-1}	4	785+269	262	21·6
1 mg kg^{-1}	4	64+28	64	5·3
0·3 mg kg^{-1}	5	22+3	73	6·1

E-2078 was dissolved in saline, and the volume was maintained at 0·5 mL kg^{-1} AUC: mean±s.e., integrated from 0 to 6 h. Mean specific AUC: (ng h mL^{-1})/(mg kg^{-1})=mean AUC/administration dose.

separation of the free from the bound form of E-2078 against antibody was with 15% polyethylene glycol. In a 10 mL test tube, 0·1 mL of ^{125}I-labelled E-2078 (10 000 counts min^{-1}) and 0·1 mL of rat plasma sample were mixed with 0·1 mL of antiserum diluted at 1:800. The mixture was incubated at 37°C for 1 h, and then 0·1 mL of bovine γ-globulin (10 mg mL^{-1}) and 0·4 mL of 30% polyethylene glycol were added. After centrifugation at 3000 rev min^{-1} for 15 min, the radioactivity of the precipitate was assayed. In this procedure, the CV was about 6% (n = 6), and 5 to 250 ng mL^{-1} of E-2078 was measurable with no immunological cross-reaction with the metabolites of E-2078 (Fig. 1).

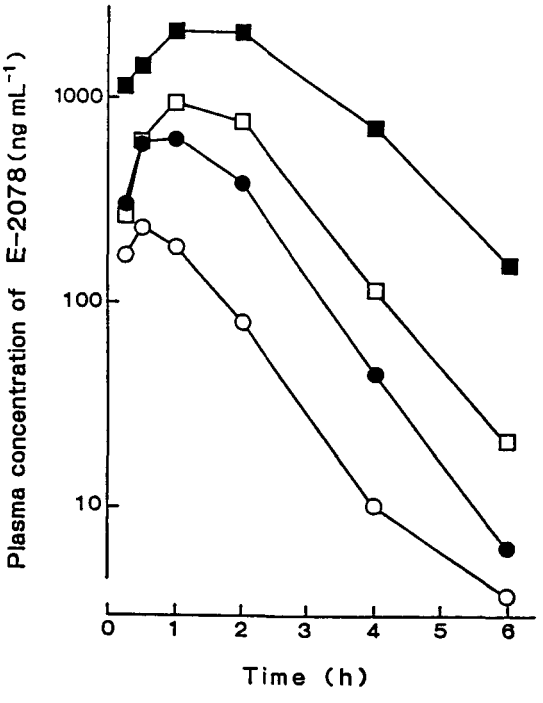

FIG. 3. Plasma E-2078 levels after intramuscular administration. (■) 10 mg kg^{-1}, (□) 3 mg kg^{-1}, (●) 1 mg kg^{-1}, (○) 0·3 mg kg^{-1}.

Pharmacokinetic analysis

The pharmacokinetic parameters of intravenous administration were calculated by MULTI (Yamaoka et al 1981). The amount of absorption into the blood was determined by measuring the area under the concentration versus time curve (AUC). The AUC was calculated by the trapezoidal method using a linear scale.

Histological examination of rectal mucosa

After the rectal absorption studies, the rats were killed, and their rectal tissues were surgically excised, washed with cold saline, and fixed with 10% formaldehyde in isotonic buffer solution (pH 7·2) containing 1/20 M disodium hydrogenphosphate and 1/20 M sodium dihydrogenphosphate. They were then sectioned, stained with haematoxylin-eosin solution and examined under an optical microscope.

Results and Discussion

Comparison of routes of administration

Plasma levels after intravenous, subcutaneous, intramuscular, rectal and oral administration of E-2078 to rats are shown in Fig. 2. The time course of the plasma level changes after intravenous injection was fitted to a two compartment open model by non-linear least square regression analysis. The estimated pharmacokinetic parameters after intravenous injection are listed in Table 1. E-2078 was eliminated from the plasma rapidly, showing a biphasic pattern with half lives of 6·9 min in the α phase, and of 56·6 min in the β phase after intravenous injection. On subcutaneous and intramuscular administration, the T_{max} values were 1·0 h and 1·4 h, respectively, and the C_{max} values were 1485·5 ng mL^{-1} and 1032·0 ng mL^{-1}, respectively. The apparent elimination half-lives after subcutaneous and intramuscular administration were 52·8 min and 46·4 min, respectively, which were

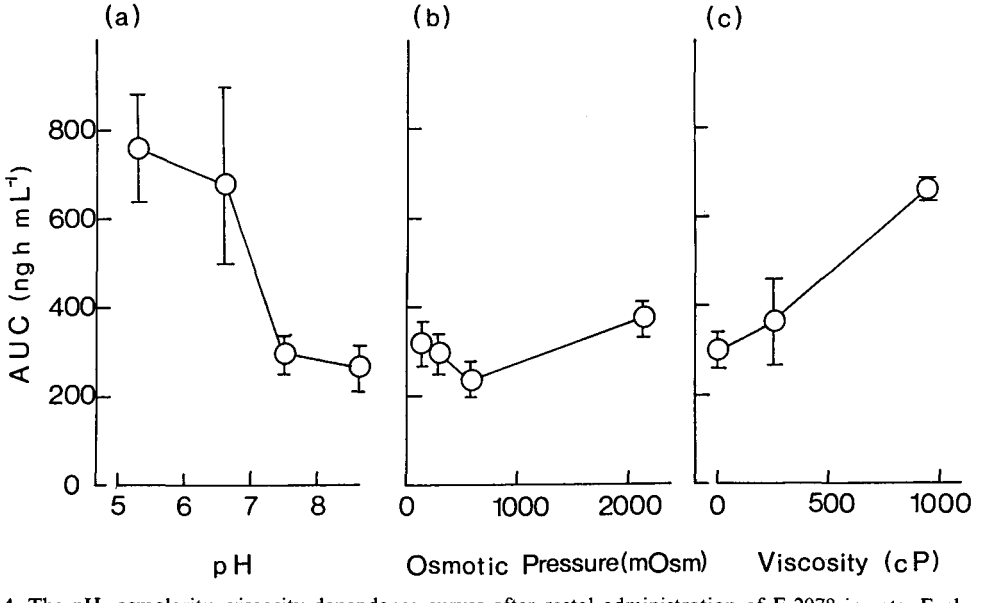

FIG. 4. The pH, osmolarity, viscosity dependence curves after rectal administration of E-2078 in rats. Each point represents the mean ± s.e. of 4–6 rats.

similar to that of intravenous administration. On rectal administration, the plasma levels of E-2078 were lower than that after intravenous administration, but the apparent elimination half life was almost the same as that for intravenous injection. The absorption of E-2078 was retarded, but the reason for this is not clear. However, this could be a result of the high molecular weight permitting less permeation of the membranes. The maximum plasma concentration of E-2078 was 92·1 ng mL^{-1} at 0·5 h after oral administration of 30 mg kg^{-1}. The rectal administration of E-2078 resulted in much higher plasma levels of this peptide than those obtained by oral administration. The AUCs and the bioavailabilities of E-2078 following the various administration routes are summarized in Table 2. The bioavailability on rectal administration was 30 times that on oral administration. In general, the rectal absorption of peptides is poor. For example, the rectal absorption of leuprolide is less than 1% (Okada et al 1982), and that of calcitonin is 0·8% (Morimoto et al 1984). Compared with other peptides, rectal absorption of E-2078 was much better, and probably depended on its stability of E-2078 against the peptides of the mucosa.

Effect of dose, pH, osmolarity, and viscosity

The effect of dose on the rectal absorption is shown in Table 3. There may be some uncertainty in determining the AUC

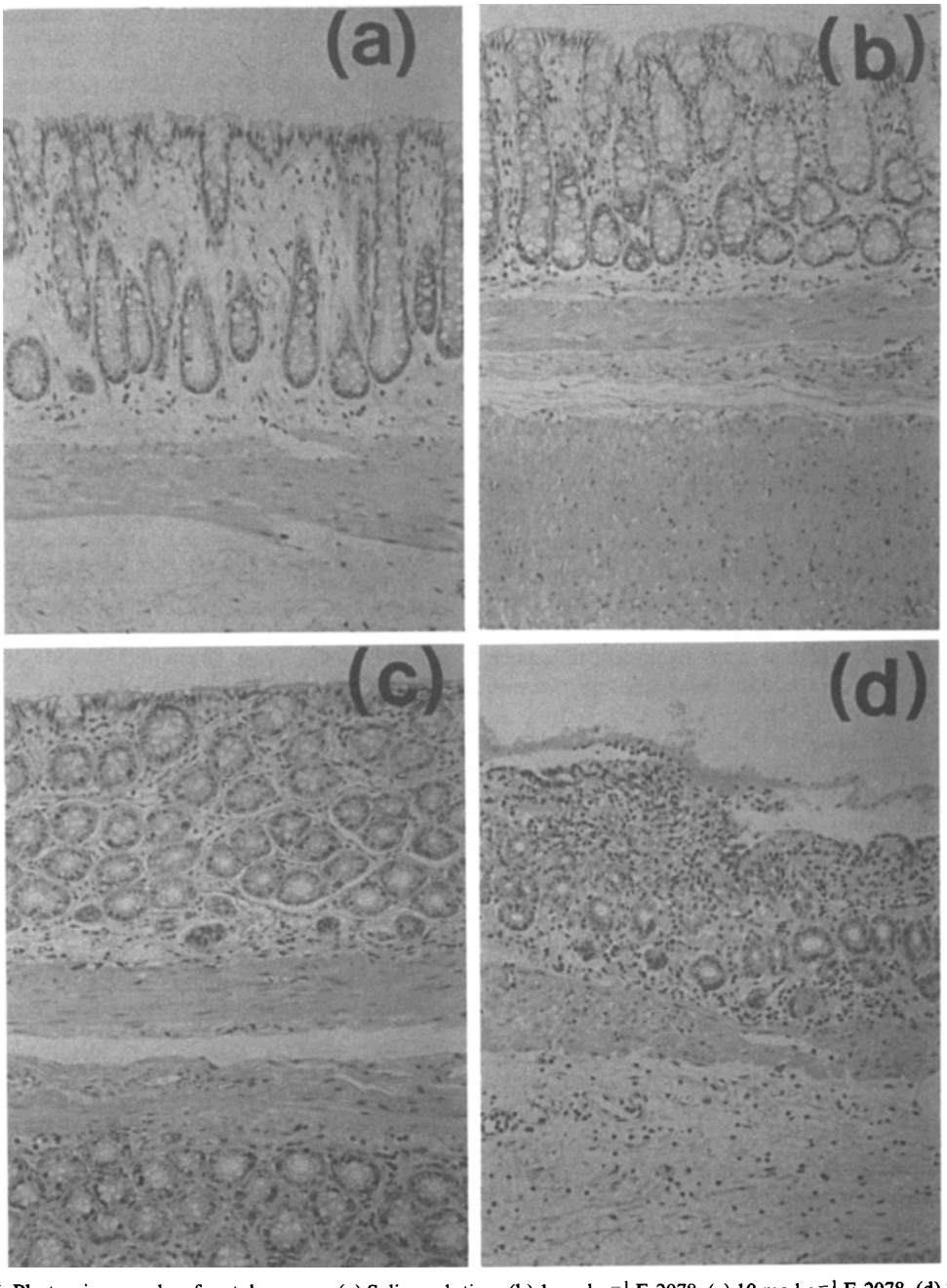

FIG. 5. Photomicrographs of rectal mucosa. (a) Saline solution, (b) 1 mg kg^{-1} E-2078, (c) 10 mg kg^{-1} E-2078, (d) 2 mg kg^{-1} indomethacin.

value at a dose of 0·3 mg kg^{-1}. The plasma concentrations in this dose were low and almost undetectable in the radioimmunoassay. The bioavailability of E-2078 increased with increasing dose, but no linear relationship was observed between the dose and the AUC. Fig. 3 shows the effect of dose on the intramuscular injection of E-2078. The elimination half lives estimated from the terminal phase were almost the same at all doses, and a linear relationship between the dose and the AUC was observed ($r = 0{\cdot}997$). This result indicated that the processes of metabolism and elimination of E-2078 were not affected by the dose in the range 0·3 mg kg^{-1} to 10 mg kg^{-1}. Therefore, the non-linear relationship between the dose and the AUC of E-2078 on rectal administration was not a result of metabolic or elimination processes, and did not depend on the volume administered, because this was adjusted to 0·5 mL kg^{-1}, the capacity of the rectum (Hayashi et al 1985). The reason for the sigmoid curve seen in dose vs AUC is unclear.

The nature of a protein and a peptide in solution varies according to the pH and salinity of the solution, and their absorption depends on the same conditions. For example, the nasal absorption of secretin is affected by pH and osmolarity (Ohwaki et al 1985). It has also been reported that the nasal absorption of peptide is affected by viscosity (Sekine et al 1986). The effects of pH, osmolarity and viscosity on the rectal absorption of E-2078 are shown in Fig. 4a,b,c. In Fig. 4a the absorption of E-2078 through the rectal mucosa was approximately the same at pH 7·5 and 8·5, and showed a tendency to increase with the decrease of pH from 7·5 and 5·0. In general, the rectal absorption is in accordance with the pH-partition coefficient hypothesis. If peptides are also absorbed according to this hypothesis, E-2078 is a weak base. However, this result indicated that E-2078 was well absorbed in acidic conditions. The phenomenon of increased absorption with decreased pH was thought to be caused by changes of the permeability of the lumen in acidic conditions.

Fig. 4b shows the effect of osmolarity on the rectal absorption of E-2078. There was no significant difference and the osmolarity of the administered solution did not affect the absorption of E-2078.

The viscosity dependency curve of the AUC for the rectal administration of E-2078 preparations is shown in Fig. 4c. The result indicates that increasing viscosity tended to enhance rectal absorption. It can be assumed that the highly viscous solution was not diluted easily and remained in the same place, and that therefore the solution retained a high concentration of the drug. The viscosity effect was thus probably caused by the high concentration of the drug in the rectum. It resulted in good absorption of E-2078.

Histological examination of mucosa

Certain drugs cause damage to the rectal mucosa. For example, non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to cause a slight deficiency of epithelial cells (Nakanishi et al 1984). There was some possibility that the dose and viscosity effects on the absorption of E-2078 were caused by histological changes in the mucosa. Accordingly, histopathological studies were conducted to ascertain the integrity of the mucosa. The results of microscopic observation are shown in Fig. 5. In the control group (a), epithelial cells, the crypts of goblet cells, and principal cells can be distinctly observed. In the groups treated with 10 mg kg^{-1} (b) and 1 mg kg^{-1} (c), the same types of cells were observed as in the control group (a). In the groups treated with indomethacin (d), a deficiency of epithelial cells was observed at the luminal border, but there was no change in the crypt region or the submucosal layer. Although NSAIDs clearly caused significant damage to the mucosa, our results with E-2078 suggested that it was absorbed without physical damage to the rectal mucosa.

In summary, this study has demonstrated that E-2078, a dynorphin analogue, can be absorbed into the systemic circulation without physical damage to the rectal mucosa. The data on rectal absorption indicate that the rectal route is promising for this drug in clinical studies.

Acknowledgements

The authors gratefully thank Dr K. Tukidate, and Mr F. Sagami at the Department of Drug Safety Research in Eisai Co. Ltd. for providing the microscopic specimens of rectal mucosa.

References

Anik, S. T., McRae, G., Nerenberg, C., Worden, A., Foreman, J., Hwang, J., Kushininsky, S., Jones, R. E., Vickery, B. (1984) Nasal absorption of Nafarelin acetate, the decapeptide [D-Nal(2)^6]LHRH, in Rhesus Monkeys I. J. Pharm. Sci. 73: 684–685

Gunn, D. R. (1964) Contracture of the quadriceps muscle. J. Bone Joint Surg. 46B: 492–497

Hayashi, M., Hirasawa, T., Muraoka, T., Shiga, M., Awazu, S. (1985) Comparison of water influx and sieving coefficient in rat jejunal, rectal and nasal absorption of Antipyrine. Chem. Pharm. Bull. 33: 2149–2152

Morimoto, K., Akatsuchi, H., Aikawa, R., Morishita, M., Morisaki, K. (1984) Enhanced rectal absorption of [Asu^{1,7}]-eel Calcitonin in rats using polyacrylic acid aqueous gel base. J. Pharm. Sci. 73: 1366–1368

Nakanishi, K., Ogata, A., Masada, M., Nadai, T. (1984) Effect of nonsteroidal anti-inflammatory drugs on the permeability of the rectal mucosa. Chem. Pharm. Bull. 32: 1956–1966

Nakanishi, K., Masada, M., Nadai, T. (1986) Effect of nonsteroidal anti-inflammatory drugs on the absorption of macromolecular drugs in rat rectum. Ibid. 34: 2628–2631

Nakazawa, T., Mihara, M., Ikeda, M., Kaneko, T., Yamatsu, K., Arakawa, Y., Tachibana, S. (1987) Abstracts, 18th International Narcotics Research Conference; Adelaide p 110

Ohwaki, T., Ando, H., Watanabe, S., Miyake, Y. (1985) Effect of dose, pH and osmolarity on nasal absorption of secretin in rats. J. Pharm. Sci. 74: 550–552

Okada, H., Yamazaki, I., Ogawa, Y., Hirai, S., Yashiki, T., Mima, H. (1982) Vaginal absorption of potent luteinizing hormone releasing hormone analogue (Leuprolide) in rats I: Absorption by various routes and absorption enhancement. Ibid. 71: 1367–1370

Saunders, F. P., Hoefnagel, D., Staples, O. S. (1965) Progressive fibrosis of the quadriceps muscles. J. Bone Joint Surg. 47A: 380–384

Sekine, K., Suzuki, Y., Yamashita, G., Nagai, T. (1986) JPN Patent 61-194034

Su, K. S. E., Champanale, L. G., Mendelsohn, L. G., Kerchner, G. A., Gries, C. L. (1985) Nasal delivery of polypeptides I: Nasal absorption of enkephalins in rats. J. Pharm. Sci. 74: 394–398

Tachibana, S., Yoshino, H., Arakawa, Y., Nakazawa, T., Araki, S., Kaneko, T., Yamatsu, K. (1987) Abstracts, 18th International Narcotics Research Conference; Adelaide p 60

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharm. Dyn. 4: 879–885