International Journal of Pharmaceutics, 46 (1988) 9–17
Elsevier

IJP 01550

Intravenous and subcutaneous pharmacokinetics and rectal bioavailability of human epidermal growth factor in the presence of absorption promoter in rats

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(Received 2 December 1987)
(Modified version received 1 February 1988)
(Accepted 2 February 1988)

Key words: Human epidermal growth factor; Intravenous administration; Subcutaneous administration; Pharmacokinetics; Rectal absorption; Absorption promoter; Sodium caprate; Sodium carboxymethyl cellulose

Summary

Human epidermal growth factor (hEGF), produced in *E. coli* with genetic technology, was rectally administered to rats and the immunoreactive plasma concentrations of hEGF were determined periodically. Enzyme immunoassay for hEGF was developed in the present study. To evaluate the enhanced rectal bioavailability of hEGF with the aid of an absorption promoter in a quantitative manner, intravenous and subcutaneous administrations were also performed. When hEGF was administered i.v. at a dose of more than 100 μg/kg, plasma hEGF disappeared biexponentially with half lives of 1–4 min for the α-phase and 14–24 min for the β-phase. However, when administered at a dose of 50 μg/kg, plasma hEGF disappeared monoexponentially with a half life of 1.5 min. The extraordinary dose-dependent pharmacokinetics of hEGF was observed as follows; values of area under the plasma concentration–time curve (AUC) after i.v. administration of hEGF at doses of 50, 100, 200, 500 and 1,000 μg/kg were 0.27, 3,0, 15.7, 70.5 and 129 μg min ml^{-1}, respectively. The AUC values of hEGF administered s.c. were lower than those after i.v. administration, probably due to the degradation of hEGF at the s.c. injection site. However, hEGF administered s.c. as a solution containing 0.2% sodium carboxymethyl cellulose (CMC Na) showed a 100% bioavailability. When hEGF was administered rectally as an aqueous solution (pH 7.9 isotonic Tris-HCl buffer), no absorption was observed irrespective of the presence of the absorption promoter such as sodium caprate in the dosing solution. On the other hand, a marked enhanced rectal absorption by absorption promoter was observed when hEGF was administered as a solution containing sodium caprate and CMC Na. The bioavailability of hEGF administered rectally at a dose of 100 μg/ml/kg was 68.2% in the presence of 100 mM sodium caprate and 1% CMC Na in the dosing solution. Some discussions with respect to the role of CMC Na are also presented.

Introduction

Human epidermal growth factor (hEGF) as well as mouse epidermal growth factor (mEGF) have been known to have various biological functions such as stimulation of cell proliferation (Carpenter, 1978) and inhibition of gastric acid secretion (Gregory, 1975). Physical, chemical, and biological properties of hEGF have been well characterized (Carpenter and Cohen, 1979; Hollenberg, 1979; Das, 1982).

The concentration of endogenous hEGF in hu-

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man tissues including biological fluids have been determined by many investigators (Gregory et al., 1977; Hirata and Orth, 1979; Hirata et al., 1980 and 1982; Uchihashi et al., 1982; De Leon et al., 1986). With respect to the pharmacokinetics of exogenously administered hEGF and mEGF, some literature is available. Covelli et al. (1972) reported that the epidermis and the epithelial cells of the cornea concentrate ^{131}I-labeled mEGF when administered i.p. Elder et al. (1978) reported that the half-life of hEGF administered i.v. to 5 healthy conscious female dogs at doses of 10 μg or 20 μg was one to 4 minutes. Panaretto et al. (1982) reported the rapid plasma clearance of immunoreactive mEGF in sheep after s.c. injection and i.v. infusion of mEGF at various doses and various periods.

With respect to the gastrointestinal absorption of mEGF, Thornburg et al. (1984) investigated the processing of orally administered mEGF in suckling rats. They reported that mEGF was absorbed from the intestine of suckling rats and was concentrated in the skin.

In the present study, the possibility of rectal absorption of EGF with the aid of an absorption promoter was examined in rats using highly purified hEGF, because some peptides are absorbed in the presence of absorption promoters. I.v. and s.c. administration of hEGF were also performed in order to examine the pharmacokinetic behavior and to evaluate the bioavailability in rectal absorption.

Materials and Methods

Materials

hEGF (mol. wt, 6216), used in the present study was produced in *Escherichia coli* with genetic technology by Wakunaga Pharm. Co. Ltd., Hiroshima (Oka et al., 1985). The purity of hEGF was more than 99.9% (Amagase et al., 1985). Antiserum for hEGF used for the determination of hEGF in plasma was commercially available (Wakunaga Pharm. Co., Ltd.). Anti-hEGF IgG was purified from anti-hEGF antiserum with Protein A-Cellulofine (Seikagaku Kogyo, Tokyo, Japan). The conjugate of Fab′, which was prepared from IgG with pepsin and β-D-galactosidase was synthesized by using N,N'-*o*-phenylenedimaleimide according to the method of Ruan et al. (1984). All other reagents used were of analytical grade and were used without further purification.

Animal study

Male Sprague–Dawley rats weighing 180–200 g were used. Following an overnight fast, rats were anesthetized by an i.p. injection of sodium pentobarbital (Nembutal solution, Abbott Laboratories) at a dose of 30 mg/kg and were kept supine on a surface controlled at 37 °C to maintain their body temperature above 36 °C during the experiment.

hEGF was dissolved in an isotonic pH 7.4, 0.05 M Tris-HCl buffer solution containing Tween 80 (0.01%) to make final concentrations of 50, 100, 200, 500, or 1000 μg/ml. For s.c. administrations, sodium carboxymethyl cellulose (CMC Na) or methylcellulose (MC) was also added to the dosing solution. After mixing all ingredients, pH and osmolarity of the solution were adjusted to pH 7.4 with 1 N NaOH and 280 mOsm/kg with NaCl (Osmostat, OM-6020, Daiichi Kagaku Co. Ltd., Japan). Tween 80 was used to avoid the adsorption of hEGF to the glass containers or syringes. The solution was administered i.v. or s.c. at a dosing volume of 1 ml/kg. Bolus i.v. administration was via the tail vein and s.c. administration was under the abdominal skin. For rectal administration of hEGF, drug solutions containing various amounts of sodium caprate were prepared with pH 7.9, 0.05 M Tris-HCl buffer containing various concentrations of CMC Na or MC. Their osmolarities were adjusted to 280 ± 10 mOsm/kg H_2O with NaCl. Rectal absorption experiments were performed using a rectal loop technique as reported previously (Murakami et al., 1983). Dosing volume of the drug solution was 1 ml/kg. Blood was collected from a jugular vein at appropriate time intervals and immediately centrifuged to obtain plasma samples. Plasma samples were stored at -30 °C until analysis.

Analytical methods

An aliquot (100 μl each) of purified anti-hEGF IgG solution (10 μg/ml in 0.1 M sodium carbonate

buffer, pH 9.8) was put into the Immulon microtiter plates (96-well plates, Dynatech Laboratories Inc., Alexandria, VA, USA). After standing for 2 h at room temperature, solutions were discarded and each plate was washed with 0.5 ml of 0.01 M phosphate buffer (pH 7.4) containing 0.15 M NaCl, 0.1% NaN_3, 0.1% bovine serum albumin (fraction V), 0.005% Tween 20 and 1 mM $MgCl_2$ (EIA buffer). Then, 0.1 ml of standard hEGF solution or plasma sample diluted appropriately with EIA buffer were transferred onto these IgG-coated plates. Plasma samples were diluted at least 10 fold with EIA buffer to minimize the influences of plasma protein on enzyme reaction. After addition of 0.05 ml of Fab′-β-D-galactosidase (0.2×10^{-3} units), the plates were incubated for 4 h at 37 °C. After washing the plates with 0.5 ml of EIA buffer solution again, the plates were incubated with 0.05 ml of EIA buffer for 20 min at 37 °C, and then the enzyme reaction was started by addition of 0.05 ml of EIA buffer containing 3×10^{-4} M 4-methylumbelliferyl-β-D-galactoside (4-MUF-β-D-Gal). After standing for 20 min at an ambient temperature (about 25 °C), the reaction was terminated by adding 0.1 ml of 0.5 M glycine-NaOH (pH 10.3). The fluorescence intensity of released 4-MUF was measured with a micro fluoro reader (Dynatech Laboratories, Inc.) at emission and excitation wavelengths of 360 and 450 nm, respectively. A linear calibration curve was obtained in the concentration range of hEGF from 10 to 10,000 pg/ml as shown in Fig. 1. Every determination was carried out at least in triplicate. Specificity of the present assay was also tested by using different pleiotypic polypeptides, such as insulin, somatostatin or mEGF. However, no cross-reaction was found. The practical detection limit of this assay was 0.1 ng hEGF/ml plasma. And the value of coefficient variance was less than 5% ($n = 3$) in each point.

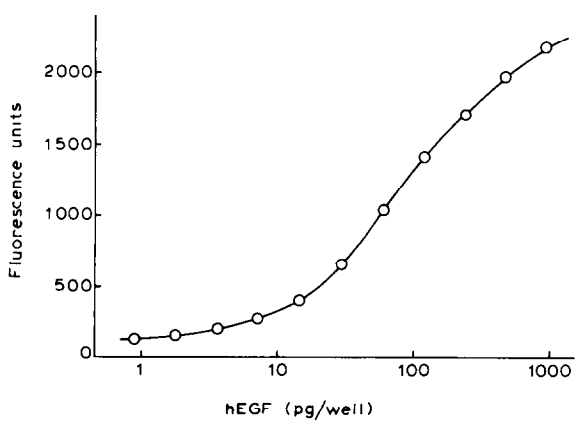

Fig. 1. Calibration curve of highly purified hEGF by enzyme immunoassay. The fluorescence intensity of released 4-MUF was determined at emission and excitation wavelengths of 360 and 450 nm, respectively. Each point represents the mean of at least 3 trials. See Materials and Methods for details.

Results and Discussion

Bolus i.v. administration of hEGF

Bolus i.v. administration of hEGF was made at doses of 50, 100, 200, 500, and 1000 μg/kg to rats, and the immunoreactive plasma concentration was determined periodically. The plasma concentration–time profile is shown in Fig. 2. A marked dose-dependent elimination of hEGF from plasma was observed. A biphasic elimination was observed at doses of more than 100 μg/kg, whereas monophasic elimination was observed at a dose of 50 μg/kg. The pharmacokinetic parameters calculated by a least-squares regression analysis following a one-compartment model for the dose of 50 μg/kg and a two compartment model for higher doses (> 100 μg/kg) are presented in Table 1. The half life of hEGF obtained at low dose (50 μg/kg) in the present study was consistent with previous results (Elder et al., 1978). However, the half lives of hEGF in the β-phase increased with an increase of dose (Table 1). Plotting the value of area under the plasma concentration vs time curve ($AUC_{0-\infty}$) and the value of total plasma clearance, calculated by dividing the dose by the AUC value as a function of the dose reveals an extraordinary dose dependency (Fig. 3). For example, the AUC value increased 5-fold when the dose of hEGF was increased from 100 μg/kg to 200 μg/kg, whereas the AUC value at a dose of 1000 μg/kg was twice that of 500 μg/kg (Fig. 3). The decrease in the total plasma clearance with an increase of the dose is due to the decreases in both the elimination constant (k_{10}) and the distribution volume of the central compartment (V_d). The

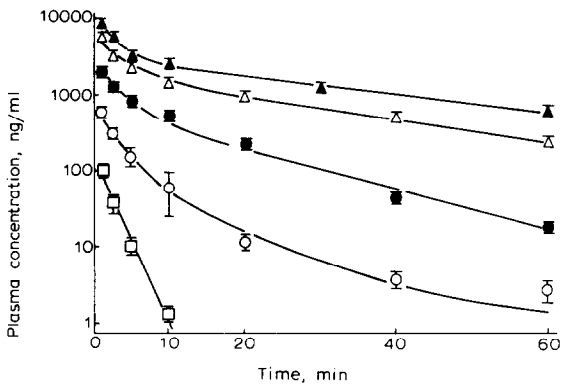

Fig. 2. Plasma concentrations of hEGF after i.v. administration in rats. Dose of hEGF: (□———□), 50 μg/kg; (○———○), 100 μg/kg; (●———●), 200 μg/kg; (△———△), 500 μg/kg; (▲———▲), 1000 μg/kg. The error bars represent the S.E.M. with $n = 3$.

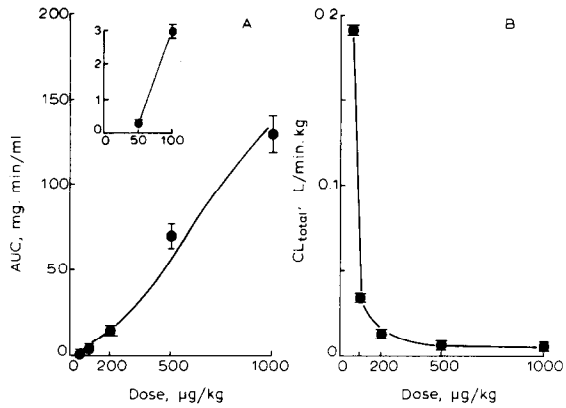

Fig. 3. Plotting of the area under the plasma concentration–time curve (A) and the total plasma clearance (B) as a function of the dose of human epidermal growth factor administered i.v. in rats. The error bars represent the S.E.M. with $n = 3$.

present results also suggest that the total plasma clearance of hEGF becomes constant at doses of more than 500 μg/kg.

Urinary excretion (Elder et al., 1978), biliary excretion (Hilaire et al., 1983) and luminal secretion (Elder et al., 1978) have been reported as excretion routes of hEGF administered at a supraphysiologic dose. Halaire et al. (1983) reported that 10 min after injection of ^{131}I-mEGF equivalent to about 1 μg mEGF/kg into the femoral vein of rats, 99.0 ± 7.0% of the injected radioactivity was found in the liver, and that the uptake of radioactivity by the liver was decreased to 24% when a 100-fold excess of unlabeled mEGF (the dose is equivalent to about 100 μg/kg) was coadministered, suggesting the saturability of the liver uptake. They also demonstrated that a rapid, spontaneous covalent linkage formed between

TABLE 1

Pharmacokinetic parameters of hEGF following i.v. administration in rats

	Dose (μg/kg)				
	50	100	200	500	1000
A (ng/ml)	126.9 ± 17.7	651.4 ± 53.2	1787.1 ± 146.7	7605.3 ± 1245.0	10420.6 ± 365.6
B (ng/ml)		33.2 ± 8.2	198.2 ± 12.6	2245.5 ± 216.7	3226.7 ± 228.2
α (min^{-1})	0.467 ± 0.006	0.281 ± 0.021	0.166 ± 0.001	0.706 ± 0.112	0.583 ± 0.018
β (min^{-1})		0.053 ± 0.006	0.041 ± 0.002	0.038 ± 0.004	0.029 ± 0.001
$t_{1/2}(\beta)$, (min)	1.49 ± 0.02	13.7 ± 1.6	17.1 ± 0.7	18.5 ± 1.7	23.8 ± 0.8 [a]
k_{12} (min^{-1})		0.039 ± 0.001	0.027 ± 0.001	0.414 ± 0.082 [a]	0.344 ± 0.014 [a]
k_{21} (min^{-1})		0.061 ± 0.011	0.053 ± 0.003	0.190 ± 0.019 [a]	0.160 ± 0.008 [a]
k_{10} (min^{-1})		0.231 ± 0.019	0.127 ± 0.002 [a]	0.141 ± 0.017 [a]	0.108 ± 0.012 [a]
V_d (liter/kg)	0.411 ± 0.006	0.148 ± 0.010	0.101 ± 0.007 [a]	0.053 ± 0.007 [a]	0.074 ± 0.002 [a]
AUC (μg · min/ml)	0.272 ± 0.038	2.993 ± 0.211	15.65 ± 0.77	70.50 ± 7.54	129.0 ± 11.3
CL_{total} (liter/min · kg)	0.192 ± 0.030	0.034 ± 0.003	0.013 ± 0.001 [a]	0.007 ± 0.001 [a]	0.008 ± 0.002 [a]

Each value represents the mean ± S.E.M. ($n = 3$). For the dose of 50 μg/kg, the one-compartment model was used, $C = Ae^{-\alpha t}$. For the doses of more than 100 μg/kg, the two-compartment model was used, $C = Ae^{-\alpha t} + Be^{-\beta t}$, as follows:

central $\underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}}$ peripheral

$\downarrow k_{10}$

[a] Significantly different from 100 μg/kg dose, $P < 0.05$.

mEGF and hepatocyte membrane protein receptor, both in vivo and in vitro (Halaire et al., 1983). Based on these findings, possible saturation of EGF receptors may be involved in a dose-dependent decrease in the total plasma clearance of hEGF.

Further investigation is necessary to determine the contribution of urinary or biliary excretion to the total plasma clearance and to clarify the existence of the receptor-mediated elimination process.

Subcutaneous administration of hEGF

The plasma concentration–time profiles are shown in Fig. 4. The AUC value calculated by a trapezoidal rule and the estimated bioavailability are summarized in Table 2. Because of non-linear disposition of hEGF, subcutaneous bioavailability was estimated according to the previous report (Wu et al., 1987) as follows: values of AUC obtained after subcutaneous administration were applied to the AUC-dose curve obtained by i.v. administration. Then, an intravenous dose corresponding to the AUC value will be obtained. The corresponding intravenous dose was regarded as the absorbed amount of hEGF following the s.c. administration. The bioavailability after subcutaneous administration was estimated by dividing the estimated absorbed amount by the s.c. administered dose.

A marked dose-dependency in the plasma concentration–time profile was also observed after subcutaneous administration as shown in Fig. 4. The s.c. bioavailability of hEGF increased with an increase of the dose as listed in Table 2. Absorption and degradation at the injection site will be involved in the subcutaneous bioavailability of polypeptides (Berger et al., 1979). Both degradation of the drug at the injection site and saturation of EGF binding receptor after entry into the circulation could be participated in the dose-dependency of hEGF following subcutaneous administration. Hori et al. (1983) reported that porcine insulin administered s.c. was degraded enzymatically at the injection site. Therefore, the possibility of the enzymatic degradation of hEGF at the s.c. injection site could be considered, since the s.c.

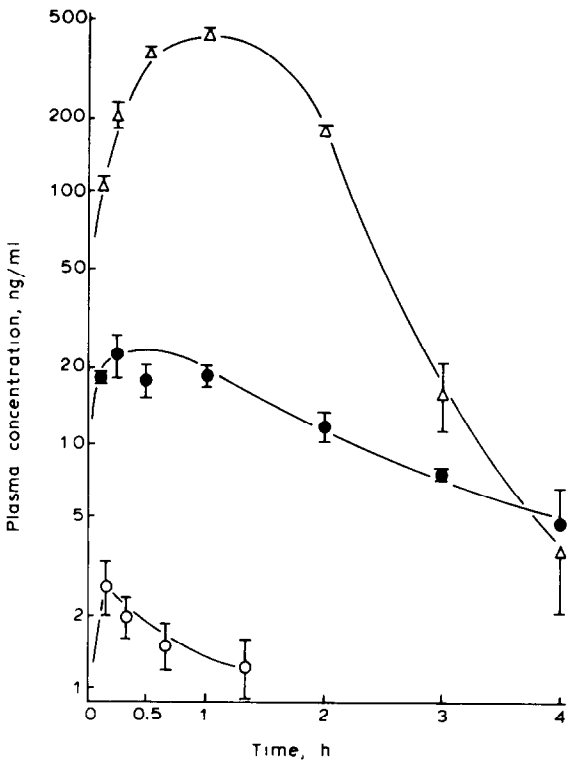

Fig. 4. Plasma concentrations of human epidermal growth factor after subcutaneous administration in rats. Dose of hEGF: (○———○), 100 μg/kg; (●———●), 200 μg/kg; (△———△), 500 μg/kg. hEGF was dissolved in an isotonic pH 7.4 Tris-HCl buffer, and the dosing volume was 0.5 ml/kg. The error bars represent the S.E.M. with $n = 3$.

bioavailability increased with an increase of the dose.

When hEGF (200 μg/kg) was injected s.c. as a CMC Na or MC solution, an interesting effect of the synthetic polymers on the subcutaneous bioavailability of hEGF was observed as shown in Fig. 5 and Table 2. Subcutaneous administration of the solution containing 0.2% or 0.5% CMC Na resulted in a larger AUC value than the solution containing hEGF alone. However, 0.5% MC solution was not the case. The viscosity of 0.5% MC solution was similar to the 0.5% CMC Na solution (0.5% CMC Na, 6.62 cSt; 0.5% MC, 6.09 cSt at 37 °C). The discrepancy in the effect of the two polymers on the hEGF s.c. bioavailability may be explained by considering that CMC Na has both an inhibiting effect on the degradation of hEGF

TABLE 2

$AUC_{0-\infty}$ and bioavailability of hEGF following s.c. administration in rats

Dose of hEGF (μg/kg)	Solution	$AUC_{0-\infty}$ (μg·min/ml)	Bioavailability (%)
50	buffer	n.d.	0
100	buffer	0.18±0.04	ca 50
200	buffer	4.22±0.77	54.5±3.5
500	buffer	43.76±1.39	70.7±1.5
200	0.1% CMC Na	5.00±0.76	64.2±4.2
200	0.2% CMC Na	16.25±1.19 [a,b]	101.2±4.0 [a,b]
200	0.5% CMC Na	14.27±0.26 [a,b]	95.8±3.6 [a,b]
200	0.5% MC	2.06±0.52 [b,c]	42.3±5.4 [b,c]

hEGF was dissolved in a pH 7.4 Tris-HCl buffer containing CMC Na or MC, and was administered at a dosing volume of 0.5 ml/kg.

n.d., not detected. Each value represents the mean±S.E.M. ($n = 3$).

[a] Significantly different from buffer (200 μg/kg), $P < 0.05$.

[b] Significantly different from 1% CMC Na, $P < 0.05$.

[c] Significantly different from 0.5% CMC Na, $P < 0.05$.

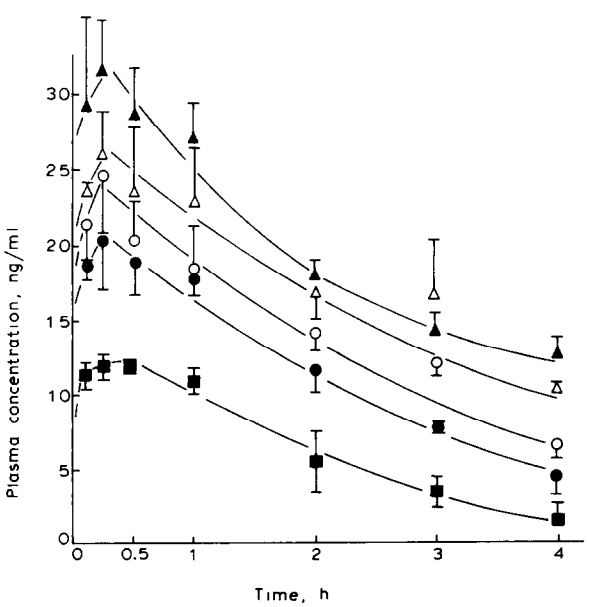

Fig. 5. Effect of CMC Na and MC on the s.c. bioavailability of hEGF in rats. Dose of hEGF was 200 μg/0.5 ml/kg. Dosing solutions: (●———●), pH 7.4 buffer; (○———○), buffer containing 0.1% CMC Na; (▲———▲), buffer containing 0.2% CMC Na; (△———△), buffer containing 0.5% CMC Na; (■———■), buffer containing 0.5% MC. The error bars represent the S.E.M. with $n = 3$.

at the injection site and a decreasing effect on the delivery of hEGF to the blood due to the viscosity, whereas MC showed only the delivery-decreasing effect. Results obtained in 0.5% CMC Na solution show that increase of viscosity resulted in a more decreased delivery rate, whereas degradation of hEGF is still inhibited. The retardation effect of the polymer solution on the drug delivery to blood was also reported by Brunner and Speiser (1976).

Komada et al. (1985) reported that some polypeptides such as bacitracin, leupeptin, phosphoramide, and benzyloxycarbonyl-Gly-Pro-Leu-Gly inhibited significantly the degradation of porcine insulin in the s.c. tissues. Further investigation is required to clarify the role of synthetic polymers in the enhancing effect on the s.c. bioavailability of hEGF.

Rectal administration of hEGF

It has been generally accepted that oral administration of protein hormones such as insulin is not effective without any pharmaceutical modifications such as emulsion (Shichiri et al., 1975) and liposome (Patel and Ryman, 1976; Dapergolas and Gregoriadis, 1976). However, the rectal or nasal administration of insulin coadministered with absorption promoters such as surfactants (Shichiri et al., 1978), enamines (Kim et al., 1983) and bile salts (Gordon et al., 1985) decreases the blood glucose levels significantly with an accompanying increase in the plasma insulin levels.

In the present study, rectal administration of hEGF was performed using a rectal loop technique. Sodium caprate was used as an absorption promoter for the rectal absorption of hEGF, since sodium caprate is a potent absorption promoter for the rectal absorption of phenolsulfonphthalein, *P*-aminobenzoic acids and sodium ampicillin (Yata et al., 1983) in rats. Additionally, sodium caprate is now clinically used in a commercial rectal suppository as absorption promoter for sodium ampicillin in Japan (Helpen, Kyoto Pharm. Ind., Co., Kyoto, Japan). However, hEGF was not detected in plasma irrespective of the presence of 100 mM sodium caprate in the rectal dosing solution when hEGF was administered as an isotonic pH 7.9 Tris-HCl buffer solution at a dose of 100

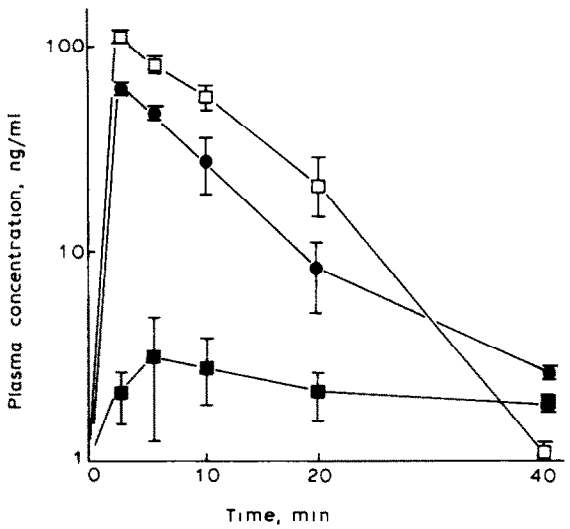

Fig. 6. Enhanced rectal absorption of hEGF by sodium caprate in rats. hEGF was dissolved in pH 7.9 Tris-HCl buffer containing 1% CMC Na. Dose of hEGF was 100 μg/ml/kg. Concentrations of sodium caprate: (■———■), 25 mM; (●———●) 50 mM; (□———□), 100 mM. The error bars represent the S.E.M. with $n = 3$.

μg/kg. On the other hand, a markedly enhanced rectal absorption by sodium caprate was observed when hEGF was administered as a buffer solution containing 1% CMC Na. In Fig. 6, the effect of concentration of sodium caprate in the dosing solution containing CMC Na on the rectal absorption of hEGF is represented. A rapid rectal absorption of hEGF was observed. A higher concentration of sodium caprate resulted in a greater rectal absorption of hEGF. The rectal bioavailability of hEGF was estimated in the same manner as s.c. bioavailability. And the estimated bioavailability of hEGF coadministered with 100 mM sodium caprate and 1% CMC Na was 68.2%. The terminal slopes of plasma hEGF were different among 3 preparations (25 mM, 50 mM and 100 mM of sodium caproate). These phenomena were observed also in the s.c. administration (Fig. 4). These phenomena may be due to the differences in the apparent absorption rate between doses or between preparations of hEGF, but detailed reason is unclear.

The effect of the concentration of CMC Na in the dosing solution on the enhancing effect of sodium caprate was also investigated. Results are summarized in Table 3 in terms of AUC value and bioavailability. These results suggested that an appropriate concentration of CMC Na (1 or 2%) in the dosing solution is required for the absorption-promoting effect of sodium caprate, and also that the existence of a free carboxyl group in CMC Na molecule has an important role in the action, since no absorption of hEGF was observed in the MC solution irrespective of the presence of sodium caprate.

TABLE 3

$AUC_{0-\infty}$ and bioavailability of hEGF following rectal administration in rats

Solution	Promoter	$AUC_{0-\infty}$ (μg · min/ml)	Bioavailability (%)
buffer	–	n.d.	0
buffer	100 mM $C_{10}Na$	n.d.	0
1% CMC Na	–	n.d.	0
1% CMC Na	25 mM C_{10} Na	0.089 ± 0.043	
1% CMC Na	50 mM $C_{10}Na$	0.700 ± 0.097 [a]	57.5 ± 2.0
1% CMC Na	100 mM $C_{10}Na$	1.335 ± 0.232 [a]	68.2 ± 5.0
0.1% CMC Na	50 mM $C_{10}Na$	n.d.	0
2% CMC Na	50 mM $C_{10}Na$	0.211 ± 0.035 [b]	47.8 ± 0.3 [b]
1% MC	50 mM $C_{10}Na$	n.d.	0

hEGF was dissolved in a pH 7.9 isotonic Tris-HCl buffer containing gel base and/or sodium caprate, and was administered at a dose of 100 μg/ml/kg.

n.d., not detected. Each value represents the mean ± S.E.M. ($n = 3$).

[a] Significantly different from 1% CMC Na (25 mM $C_{10}Na$), $P < 0.05$.

[b] Significantly different from 1% CMC Na (50 mM $C_{10}Na$), $P < 0.05$.

Morimoto et al. (1980) reported that a marked rectal absorption of insulin and hypoglycemic effect were observed without any absorption promoters when insulin was rectally administered as a suspension in a polyacrylic acid aqueous gel (0.1%, pH 6.5). However, in the case of CMC Na, no rectal absorption of hEGF was observed without any absorption promoter, although CMC Na was found to have an enhancing effect on the absorption promoting activity of sodium caprate.

The inhibiting effect of CMC Na on the degradation of hEGF in the rectal tissue as suggested in Fig. 5, the lowering effect on the surface tension of the mucosal surface resulting in an increase of the wetting area of contact (Lamp and Holly, 1972), and the effect on decreasing the delivery due to viscosity may be involved in the action of CMC Na for the rectal absorption of hEGF. However, MC also has a lowering effect on the surface tension (Lamp and Holly, 1972). Thus, in the present study, the mechanism of CMC Na for the accelerating effect on the activity of the absorption promotor is still unclear. Further investigation is necessary to clarify the role of the polymer functional groups.

References

Amagase, H., Ushijima, M., Hashimoto, K., Arakawa, M. and Fuwa, T., Gastric cytoprotection of human epidermal growth factor produced by *Escherichia coli* engineered genetically. *Gastroenterology*, 88 (1985) 1305.

Bergar, M., Halban, P.A., Girardier, L., Seydoux, J., Offord, R.E. and Renold, A.E., Absorption kinetics of subcutaneously injected insulin: evidence for degradation at the injection site. *Diabetologia*, 17 (1979) 97–99.

Brunner, B.H.S. and Speiser, P.P., Release of a drug from homogeneous ointments containing the drug in solution. *J. Pharm. Pharmacol.*, 28 (1976) 23–28.

Carpenter, G., The regulation of cell proliferation: advances in the biology and mechanism of action of epidermal growth factor. *J. Invest. Dermatol.*, 71 (1978) 283–287.

Carpenter, G. and Cohen S., Epidermal growth factor. *Annu. Rev. Biochem.*, 48 (1979) 193–216.

Covelli, I., Rossi, R., Mozzi, R. and Frati, L., Synthesis of bioactive ^{131}I-labeled epidermal growth factor and its distribution in rat tissues. *Eur. J. Biochem.*, 27 (1972) 225–230.

Dapergolas, D. and Gregoriadis, G., Hypoglycemic effect of liposome-entrapped insulin administered intragrastrically into rats, *Lancet*, 2(1976) 824–827.

Das, M., Epidermal growth factor: mechanism of action, *Int. Rev. Cytol.*, 78 (1982) 233–256.

DeLeon, F.D., Vijayakumar, R., Brown, M., Rao, Ch. V., Yussman, M.A. and Schultz, G., Peritoneal fluid volume, estrogen, progesterone, prostaglandin, and epidermal growth factor concentrations in patients with and without endometriosis. *Obstet. Gynecol.*, 68 (1986) 189–194.

Elder, J.B., Kiff, E.S. and Gregory, H., Half-life of urogastrone in conscious dogs. *Gut*, 19 (1978) A436.

Gordon, G.S., Moses, A.C., Silver, R.D., Flier, J.S. and Carey, M.C., Nasal absorption of insulin: enhancement by hydrophobic bile salts. *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 7419–7423.

Gregory, H., Isolation and structure of urogastrone and its relationship to epidermal growth factor. *Nature (Lond.)*, 257 (1975) 325–327.

Gregory, H., Holmes, J.E. and Willeshire, I.R., Urogastrone levels in the urine of normal adult humans. *J. Clin. Endocrinol. Metab.*, 45 (1977) 668–672.

Hilaire, R.J.S., Hradek, G.T. and Jones, A.L., Hepatic sequestration and biliary secretion of epidermal growth factor: evidence for a high-capacity uptake system. *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1983) 3797–3801.

Hirata, Y. and Orth, D.N., Epidermal growth factor (urogastrone) in human tissues. *J. Clin. Endoclinol. Metab.*, 48 (1979) 667–672.

Hirata, Y., Moore, G.W., Bertagna, C. and Orth, D.N., Plasma concentrations of immunoreactive human epidermal growth factor (urogastrone) in man. *J. Clin. Endocrinol. Metab.*, 50 (1980) 440–444.

Hirata, Y., Uchihashi, M., Nakajima, H., Fujita, T. and Matsukura, S., Presence of human epidermal growth factor in human cerebrospinal fluid. *J. Clin. Endocrinol. Metab.*, 55 (1982) 1174–1177.

Hollenberg, M.D., Epidermal growth factor – urogastrone, a peptide acquiring hormonal status. *Vitam. Horm.*, 37 (1979) 69–110.

Hori, R., Komada, F. and Okumura, K., Pharmaceutical approach to subcutaneous dosage forms of insulin. *J. Pharm. Sci.*, 72 (1983) 435–439.

Kim, S., Kamada, A., Higuchi, T. and Nishihata, T., Effect of enamine derivatives on the rectal absorption of insulin in dogs and rabbits. *J. Pharm. Pharmacol.*, 35 (1983) 100–103.

Komada, F., Okumura, K. and Hori, R., Fate of porcine and human insulin at the subcutaneous injection site. II. In vitro degradation of insulins in the subcutaneous tissue of the rat. *J. Pharmacobio-Dyn.*, 8 (1985) 33–40.

Lemp, M.A. and Holly, F.J., Ophthalmic polymers as ocular wetting agents. *Ann. Ophthalmol.*, 4 (1972) 15–20.

Morimoto, K., Hama, I., Nakamoto, Y., Takeeda, T., Hirano, E. and Morisaka, K., Pharmaceutical studies of polyacrylic acid aqueous gel bases: absorption of insulin from polyacrylic acid aqueous gel bases following rectal administration in alloxan diabetic rats and rabbits. *J. Pharmacobio-Dyn.*, 3 (1980) 24–32.

Murakami, T., Sasaki, Y., Yamajo, R. and Yata, N., Effect of bile salts on the rectal absorption of sodium ampicillin in rats. *Chem. Pharm. Bull.,* 32 (1984) 1948–1955.

Oka, T., Sakamoto, S., Miyoshi, K., Fuwa, T., Yoda, K., Yamasaki, M., Tamura, G. and Miyake, T., Synthesis and secretion of human epidermal growth factor by *Escherichia coli. Proc. Natl. Acad. Sci. U.S.A.,* 82 (1985) 7212–7216.

Panaretto, B.A., Moore, G.P.M. and Robertson, D.M., Plasma concentrations and urinary excretion of mouse epidermal growth factor associated with the inhibition of food consumption and of wool growth in merino wethers. *J. Endocrinol.,* 94 (1982) 191–202.

Patel, H.M. and Ryman, B.E., Oral administration of insulin by encapsulation within liposomes. *FEBS Lett.,* 62 (1976) 60–63.

Ruan, K-H., Imagawa, M., Hashida, S. and Ishikawa, E., An improved method for the conjugation of Fab′ to β-D-galactosidase from *Escherichia coli. Anal. Lett.,* 17 (B7) (1984) 539–554.

Shichiri, M., Kawamori, R., Yoshida, M., Etani, N., Hoshi, M., Izumi, K., Shigeta, Y. and Abe, H., Short-term treatment of alloxan-diabetic rats with intrajejunal administration of water-in-oil-in-water insulin emulsions. *Diabetes,* 24 (1975) 971–976.

Shichiri, M., Yamasaki, Y., Kawamori, R., Kikuchi, M., Hakui, N., and Abe, H., Increased intestinal absorption of insulin: an insulin suppository. *J. Pharm. Pharmacol.,* 30 (1978) 67–73.

Thornburg, W., Matrisian, L., Magun, B. and Koldovsky, O., Gastrointestinal absorption of epidermal growth factor in suckling rats. *Am. J. Physiol.,* 246 (1984) G80–G85.

Uchihashi, M., Hirata, Y., Fujita, T. and Matsukura, S., Age-related decrease of urinary excretion of human epidermal growth factor (hEGF). *Life Sci.,* 31 (1982) 679–683.

Wu, W.W., Murakami, T., Higashi, Y. and Yata, N., Enhancement of the rectal absorption of sodium ampicillin by *N*-acylamino acids in rats. *J. Pharm. Sci.,* 76 (1987) 508–512.

Yata, N., Higashi, Y., Murakami, T., Yamajo, R., Wu, W.M., Taku, K., Sasaki, Y. and Hideshima, Y., A possible mechanism of absorption promoters. *J. Pharmacobio-Dyn.,* 6 (1983) s-78.