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Biliary and urinary excretions of immunoreactive human epidermal growth factor after intravenous administration in rats

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Summary

The biliary and urinary excretions of immunoreactive human epidermal growth factor (hEGF) after intravenous administration were determined in rats. At intravenous doses of 50, 75, 100 and 500 μg/kg of hEGF, the same maximal biliary concentrations of immunoreactive hEGF were observed. The maximal concentration of immunoreactive hEGF in bile (about 20 ng/ml) sustained for 2 h after injection at a dose of 500 μg/kg. Above findings indicate the presence of saturable biliary secretion system. However, the extent of total biliary excretion of the dose was less than 0.12% in all cases. On the other hand, no saturable urinary excretion of immunoreactive hEGF was observed. The extent of total urinary excretion of the dose was less than 1.5%. It was concluded that biliary and urinary excretions would not contribute to the marked dose-dependent pharmacokinetics of immunoreactive hEGF observed after intravenous administration in rats.

Introduction

Human epidermal growth factor (hEGF) and/or mouse EGF is known to show various biological actions on living animals as well as on isolated cells such as stimulation of cell proliferation and inhibition of gastric acid secretion (Carpenter, 1978; Carpenter and Cohen, 1979; Gregory, 1975). Endogenous hEGF has been detected in various human biological fluids (Gregory et al., 1977; Hirata and Orth, 1979; Hirata et al., 1980, 1982; Uchihashi et al., 1982). Its receptors also have been detected in a variety of human tissues (Damjanov et al., 1986). However, the pharmacokinetics of exogenous hEGF in human as well as the physiological role of specific binding of hEGF in each human tissue still remain to be clarified.

In a previous report, we demonstrated an extraordinary dose-dependent pharmacokinetics of immunoreactive hEGF in rats (Murakami et al., 1988). The marked dose-dependency may be derived from the saturation of the tissue distribution and/or of the elimination process. With regard to the tissue distribution of hEGF, Yanai et al. (1987)

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reported the existence of specific binding sites for hEGF in several rat tissues such as liver, kidney, small intestines, stomach, lung and heart. However, the contribution of hEGF excretion to the plasma dose-dependent pharmacokinetics is yet undetermined, although urinary excretion (Elder et al., 1978), biliary excretion (Hiraire et al., 1983) and luminal secretion (Elder et al., 1978) have been reported as excretion routes of hEGF at a supraphysiologic dose.

The present study describes the effect of a dose of hEGF on the extent of biliary and urinary excretions of immunoreactive hEGF after intravenous administration in rats.

Materials and Methods

Materials

hEGF used in the present study was produced by Wakunaga Pharm. Co. with genetic technology. Reagents used for the determination of immunoreactive hEGF in bile and urine were also the same as reported previously (Murakami et al., 1988). 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. Rats were anesthetized with an intraperitoneal 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, 75, 100 or 500 μg/ml. The solution was administered from the tail vein at a dosing volume of 1 ml/kg.

Bile collection after i.v. injection of hEGF. A midline abdominal incision was made in rats under pentobarbital anesthesia. The common bile duct was cannulated with polyethylene tubing (PE 10). The opening of urethra of the anesthetized rat was closed with a drop of surgical glue (Aron-α A, Sankyo, Japan) prior to the intravenous injection of hEGF to prevent leakage of urine. Bile was collected for 2 or 4 h (at a dose of 500 μg/kg only) at appropriate time intervals after intravenous injection of hEGF. To obtain a constant bile flow during the experiment, 1 ml of saline solution was intramusculary injected every 1 h after the initiation of the bile collection. The collected bile was weighed and stored at −30 °C until analysis.

Urine collection after i.v. injection of hEGF. Two or 4 h (at a dose of 500 μg/kg only) after the intravenous injection of hEGF, the urinary bladder was removed. Urine was collected by washing the bladder with a sufficient amount of ice-cold water. Urine and the washings were combined and water was added to make up 50 ml. The urine sample was stored at −30 °C until analysis.

Analytical methods

Bile or urine sample was diluted appropriately, but at least by more than 10-fold with a 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 $MgCl_2$. The concentration of immunoreactive hEGF was determined by the enzyme immunoassay developed for hEGF as reported previously (Murakami et al., 1988). The practical detection limit of hEGF in the assay was 0.1 ng/ml.

Results

Biliary excretion of immunoreactive hEGF after i.v. administration

The concentrations of immunoreactive hEGF in bile collected at an appropriate period of time are summarized in Table 1. The maximal biliary concentration of immunoreactive hEGF was about 20 ng/ml in each case. There were no significant difference in the maximal concentration among 4 different dosing levels. When hEGF was administered at a dose of 500 μg/kg, the maximal value of about 20 ng/ml in bile continued for 2 h after injection. The rate of biliary excretion normalized with body weight of the rat and the cumulative amount of immunoreactive hEGF excreted in bile are shown in Figs. 1 and 2, respectively.

TABLE 1

Biliary concentration of immunoreactive hEGF after intravenous administration at various doses in rats

Each value represents the mean ± S.E.M. N = number of trials. No significant difference in the maximal biliary concentration of immunoreactive hEGF among 4 different dosing levels.

Sampling time (min)	50 μg/kg (ng/ml)	75 μg/kg (ng/ml)	100 μg/kg (ng/ml)	500 μg/kg (ng/ml)
0– 10	2.91 ± 1.34	3.49 ± 0.95	4.99 ± 0.81	22.47 ± 3.45
10– 20	18.05 ± 3.18	21.10 ± 0.47	17.54 ± 3.69	25.05 ± 3.56
20– 30	16.88 ± 0.87	14.43 ± 1.31	22.94 ± 3.86	21.76 ± 2.16
30– 45	13.44 ± 3.36	11.84 ± 3.18	11.84 ± 1.51	22.25 ± 0.55
45– 60	10.86 ± 3.89	9.20 ± 3.60	7.76 ± 1.69	21.48 ± 1.29
60– 90	9.10 ± 3.10	7.32 ± 1.77	5.90 ± 0.67	21.48 ± 2.27
90–120	6.56 ± 2.16	4.88 ± 1.40	3.55 ± 0.52	22.66 ± 2.18
120–180				14.84 ± 2.87
180–240				9.61 ± 3.72
N	4	3	5	3

TABLE 2

Biliary and urinary excretion of immunoreactive hEGF after intravenous administration in rats

Each value represents the mean ± S.E.M. of 3–5 trials. ND = not detected. Biliary and urinary excretions were determined for 2 h except 500 [a] after injection.

Dose (μg/kg)	Biliary excretion (%)	Urinary excretion (%)
50	0.103 ± 0.016	ND
75	0.111 ± 0.007	0.857 ± 0.337
100	0.079 ± 0.006 [c]	0.877 ± 0.180
500	0.030 ± 0.004 [b,c,d]	
500 [a]	0.052 ± 0.002 [b,c,d]	1.455 ± 0.568

[a] Determined for 4 h.
[b] Significantly different from 50 μg/kg, $P < 0.05$.
[c] Significantly different from 75 μg/kg, $P < 0.05$.
[d] Significantly different from 100 μg/kg, $P < 0.05$.

Total biliary excretion of immunoreactive hEGF 2 or 4 h after injection are summarized in Table 2. A significant decrease in percent of the biliary excretion of hEGF to the dose was observed with an increase of dose, although the extents were very low in all cases. Those findings suggest the existence of saturation in the biliary excretion of immunoreactive hEGF.

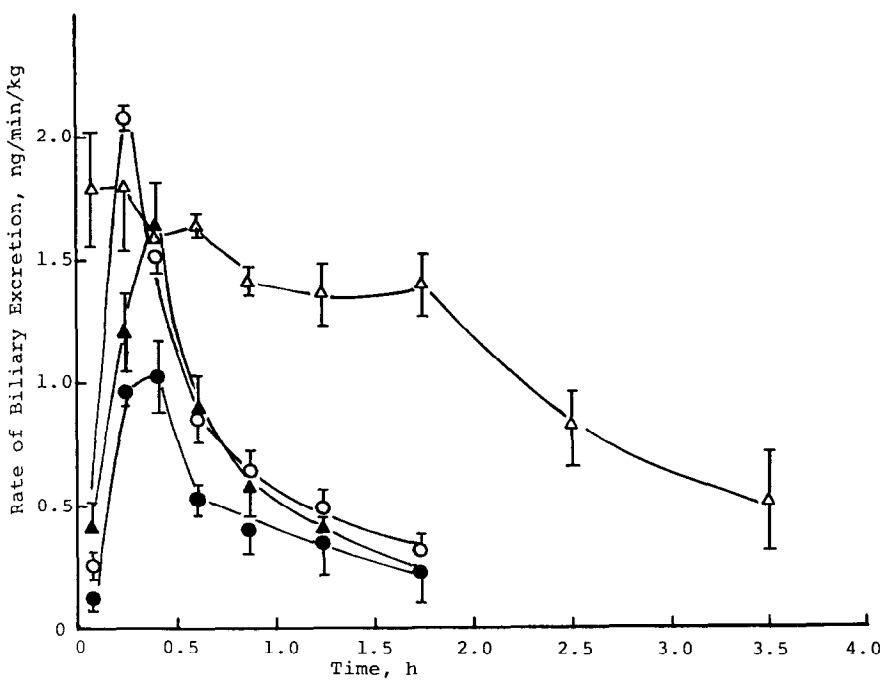

Fig. 1. Biliary excretion rate of immunoreactive hEGF after intravenous administration at various doses in rats. Each value represents the mean ± S.E.M. from 3–5 trials ●, 50 μg/kg; ○, 75 μg/kg; ▲, 100 μg/kg; △, 500 μg/kg.

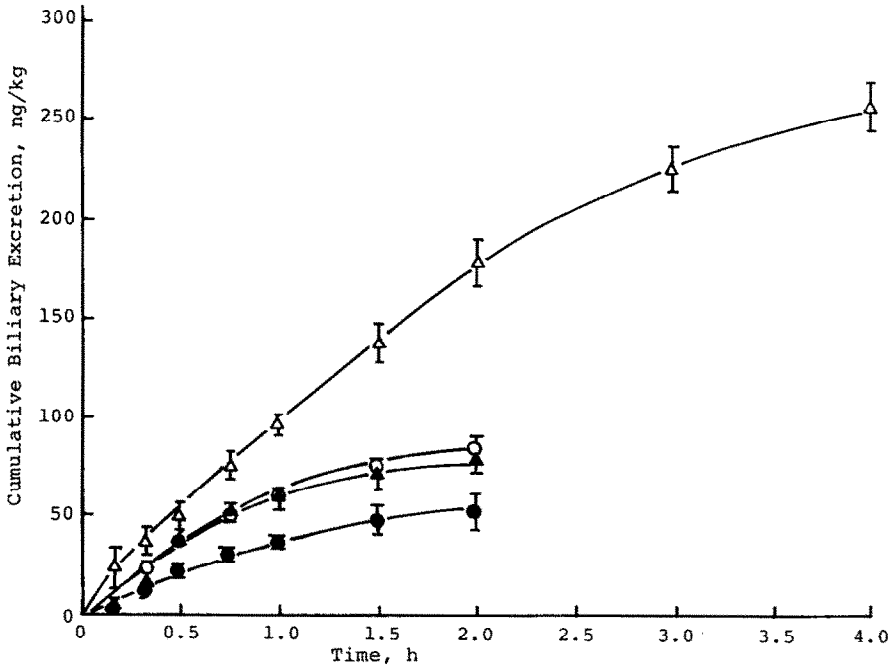

Fig. 2. Cummulative biliary excretion of immunoreactive hEGF after intravenous administration at various doses in rats. Each value represents the mean ± S.E.M of from 3–5 trials. ●, 50 μg/kg; ○, 75 μg/kg; ▲, 100 μg/kg; △, 500 μg/kg.

Urinary excretion of immunoreactive hEGF after i.v. administration

Urinary excretion of immunoreactive hEGF after intravenous administration was also shown in Table 2. At a dose of 50 μg/kg, the concentration of immunoreactive hEGF in the urine was less than the detection limit (2-h collected urine was made up to 500 ml finally). At doses of more than 75 μg/kg, the total percent of urinary excretion was about 0.8–1.5%. However, no significant difference was observed in the urinary excretion ratio among the 3 different dosing levels (> 75 μg/kg).

Discussion

With respect to the biliary excretion of EGF after intravenous administration of EGF at a supraphysiologic dose, only a few reports are available; Hilaire et al. (1983) reported that about 19% of the injected radioisotope appeared in bile within 90 min after an intraportal injection at a dose of 18–24 μCi of [^{125}I]mouse EGF (the dose corresponding to 110–150 ng) and that approximately one-fifth of the amount was immunoprecipitable with a specific anti-EGF antiserum. Burwen et al. (1984) also reported the presence of immunoprecipitable EGF in bile after injection of [^{125}]EGF into rat portal vein. In the present study, the total percent of biliary excretion of immunoreactive hEGF 2 or 4 h after injection was less than 0.12% in all cases within a dosing range of 50–500 μg/kg (Table 2). The marked discrepancy between the results by Hilaire et al. (1983) and those in the present study may be attributed to the difference in the dose and the dosing route. Based on the present study, a greater value of percent of total biliary excretion at a lower dose may be expected. Additionally, Hilaire et al. (1983) reported that almost 100% of the intraportal dose was taken up by the liver, whereas only 58% of the intravenous dose appeared in the liver. The difference in the amount taken up by the liver between the intraportal and intravenous routes may contribute to the difference in the

percent of the total biliary excretion. Although the percent of total biliary excretion of immunoreactive hEGF after intravenous injection was very low, a marked saturable biliary excretion of immunoreactive hEGF was observed in the present study as shown in Table 1 and Fig. 1. Burwen et al. (1984) reported that a small but significant percentage of endocytosed EGF in the liver is transported by a pathway independent of the lysosomal pathway, resulting in the secretion of intact EGF into bile, whereas almost all of the EGF taken up by the hepatocytes is transported to lysosomes to be degraded. Our results also indicate the presence of an excretion pathway to bile.

The saturable biliary excretion of hEGF observed may involve the following two factors: one is the saturable uptake of circulating hEGF into the liver cells and the other the saturable excretion of cellular hEGF into the bile. Recently, Yachi et al. (1988) studied the binding characteristics of hEGF between highly purified canalicular (CMV) and sinusoidal (SMV) rat liver plasma membrane preparations and reported the greater binding capacity of CMV compared to SMV. A marked saturable biliary excretion of hEGF observed in the present study probably may be derived from the saturable specific binding of hEGF to bile canalicular membranes as reported by Yachi et al. (1988).

It is well known that an endogenous immunoreactive hEGF is detected in human urine at a high concentration depending on the age and sex, and the urinary excretion of hEGF is expressed as a function of creatinine clearance (Gregory et al., 1977; Uchihashi et al., 1982), indicating the excretion by the glomerular filtration. Elder et al. (1978) reported that the total urinary excretion of hEGF was 3–6% after intravenous administration at a dose of 10 or 20 μg in female dogs. Panaretto et al. (1982) reported that approximately 10% of the dose of immunoreactive material was excreted in the urine 1–3 days after 7 h subcutaneous infusions of 5 mg (0.12 ± 0.01 mg mouse EGF/kg) in sheep. In the present study, the extent of urinary excretion of immunoreactive hEGF was much lower than the results by Elder et al. (1978) and by Panaretto et al. (1982), and no significant difference in percent of the total urinary excretion of immunoreactive hEGF was observed among doses of 75, 100 and 500 μg/kg. The discrepancy in the extent of urinary excretion between their results and the present one may be attributable to the differences in the animal species employed. However, detailed reasons are unknown. The linear urinary excretion of immunoreactive hEGF observed may indicate that immunoreactive hEGF is excreted by glomerular filtration. The extent of urinary excretion of immunoreactive hEGF was greater than that of biliary excretion by about 10-fold.

In the present study, a marked saturable excretion was observed in a biliary excretion, whereas urinary excretion was linear. However, the extents of biliary and urinary excretions of immunoreactive hEGF were less than 0.12% and 1.5% of the dose, respectively. Thus, it can be concluded that biliary or urinary excretion of hEGF does not contribute to the marked dose-dependent pharmacokinetics reported in the previous paper (Murakami et al., 1988) and that the marked dose-dependent pharmacokinetics are attributable to the saturable tissue uptake such as liver and kidney.

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