

Residence Time of Polaprezinc (Zinc L-Carnosine Complex) in the Rat Stomach and Adhesiveness to Ulcerous Sites

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ABSTRACT—Polaprezinc, an insoluble zinc complex of L-carnosine, exhibits anti-ulcer effects by acting directly on mucosal lesions. The disposition of polaprezinc in the stomach was studied to clarify the usefulness of its structure as an insoluble complex. The time courses of ^{14}C -radioactivity in the gastric contents and gastric tissues were parallel to those of ^{65}Zn after oral administration of a mixture of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc (^{14}C -, ^{65}Zn -polaprezinc) to rats. The gastric contents of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc were greater than those of ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$. Mean residence times (MRT) of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc in the stomach were almost the same (ca. 2 hr), and they were double those of ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$. In gastric tissues, the area under the concentration curves ($\text{AUC}_{0-8\text{ hr}}$) of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc were 1.7 times greater than those of ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$, respectively. After administration of ^{14}C -, ^{65}Zn -polaprezinc to rats with acetic acid-induced ulcers, ^{14}C and ^{65}Zn -radioactivities in the ulcerous sites were very similar and greater than those of ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid. In conclusion, polaprezinc is retained in the stomach longer and adheres to the ulcerous sites more than zinc or L-carnosine. The characteristics of this compound may arise from its insolubility and contribute to its strong pharmacological action.

Keywords: Polaprezinc (zinc L-carnosine complex), Mean residence time (MRT), Ulcer, Adhesiveness

Polaprezinc is a zinc complex of carnosine (β -alanyl-L-histidine) (1) that exhibits marked anti-ulcer activity against various experimental models of gastric lesions and duodenal ulcers by acting directly on the gastric mucosa (2–4). In the pharmacological actions of the components of polaprezinc, zinc has membrane-stabilizing activity (5, 6), anti-oxidative activity (7) and can promote wound-healing (8); and on the other hand, L-carnosine has healing-promoting (9) and immunoregulatory actions (10).

Yoneta et al. (11) reported that the efficacy of polaprezinc was more potent than those of L-carnosine, zinc and mixtures of both these agents on indomethacin induced gastric lesions and acetic acid induced gastric ulcers. Furthermore, the pharmacological duration of polaprezinc was longer than that of the mixture (11). It was caused by an increment in its adhesiveness to ulcerous sites, because of its insoluble complex structure (12, 13).

In the present study, the disposition of polaprezinc in the stomach was compared with its component compounds L-carnosine and zinc sulfate to clarify the usefulness of this structure as an insoluble complex. In addition, the adhesiveness of polaprezinc to ulcerous sites in

rats with acetic acid-induced ulcers were studied.

MATERIALS AND METHODS

Chemicals

Unlabeled polaprezinc and L-carnosine were synthesized by Hamari Chemicals (Osaka). [^{14}C -Histidine]-polaprezinc (^{14}C -polaprezinc) and ^{65}Zn -polaprezinc were synthesized by Nemoto Tokushu Kagaku (Tokyo) and Amersham International plc. (Buckinghamshire, UK), as shown in Fig. 1. ^{14}C -Polaprezinc had specific radioactivities of 92.5 and 162 kBq/mg and a radiochemical purity of 96.6%; ^{65}Zn -polaprezinc had specific radioactivities of 0.458, 1.865 and 2.105 MBq/mg. [^{14}C -Histidine]-L-carnosine (^{14}C -L-carnosine) was synthesized by Nemoto Tokushu Kagaku, and had a specific radioactivity of 808.6 kBq/mg and a radiochemical purity of 96.7%. $^{65}\text{ZnSO}_4$ (Zn-labeled zinc sulfate) was purchased from NEN Research Products (Boston, MA, USA), and had a specific radioactivity of 20.2 GBq/mg.

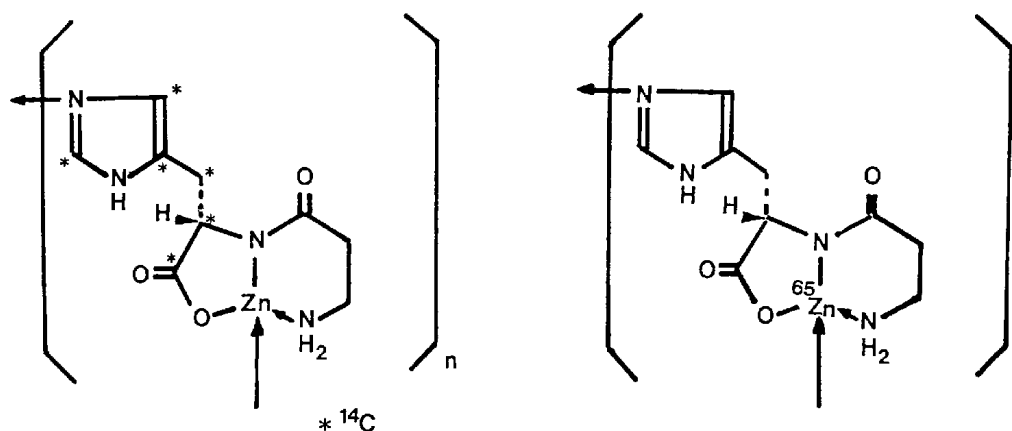


Fig. 1. Chemical structures of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc.

Animals

Male SPF rats of the Sprague-Dawley strain (7–8 weeks of age, body weight: approximately 250 g) were used. Rats were purchased from Charles River Japan (Hino) and were acclimatized to our facilities for 1 week before use. Rats with acetic acid-induced ulcers were prepared according to the method described by Okabe and Pfeiffer (14). The animals were given water and solid laboratory food *ad libitum*. Food (Charles River CRF-1) was purchased from Oriental Yeast (Tokyo), and the zinc content was $52 \mu\text{g/g}$ of diet.

The animals were housed in an animal room maintained at $23 \pm 2^\circ\text{C}$ and $55 \pm 10\%$ relative humidity.

Method of administration

A mixture of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc (^{14}C -, ^{65}Zn -polaprezinc), and ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ were administered orally to normal rats at doses of $171.6 \mu\text{mol}/5 \text{ ml}/\text{kg}$ (calculated as $50 \text{ mg}/\text{kg}$ of polaprezinc). ^{14}C -Polaprezinc and ^{65}Zn -polaprezinc were mixed and suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) solution with an agate mortar, and then the specific radioactivity was adjusted by addition of non-radioactive polaprezinc; the radioactivity administered was approximately $4.5 \text{ MBq}/5 \text{ ml}/\text{kg}$ for ^{14}C -polaprezinc and approximately $6.3 \text{ MBq}/5 \text{ ml}/\text{kg}$ for ^{65}Zn -polaprezinc. ^{14}C -L-Carnosine or $^{65}\text{ZnSO}_4$ was dissolved in 0.5% CMC-Na, and the radioactivity administered was $4.5 \text{ MBq}/5 \text{ ml}/\text{kg}$ of ^{14}C -L-carnosine or $6.3 \text{ MBq}/5 \text{ ml}/\text{kg}$ of $^{65}\text{ZnSO}_4$. Non-labeled polaprezinc was suspended in 0.5% CMC-Na at a dose of $171.6 \mu\text{mol}/5 \text{ ml}/\text{kg}$, and phenol red was dissolved into distilled water at a dose of $20 \text{ mg}/5 \text{ ml}/\text{kg}$.

For rats with acetic acid-induced ulcers, ^{14}C -, ^{65}Zn -polaprezinc was suspended in distilled water with an agate mortar. ^{14}C -Polaprezinc and ^{65}Zn -polaprezinc were dis-

solved in a small amount of 0.1 N HCl and added into distilled water (^{14}C -, ^{65}Zn -polaprezinc dissolved in acid).

^{14}C -, ^{65}Zn -Polaprezinc and ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid were administered orally to rats with acetic acid-induced ulcers at doses of $10.3 \mu\text{mol}/5 \text{ ml}/\text{kg}$ (calculated as $3 \text{ mg}/\text{kg}$ of polaprezinc). Rats were fasted for 16 hr prior to dosing but were allowed free access to water.

Measurement of radioactivity

The ^{14}C -radioactivity in each sample was measured in a liquid scintillation spectrophotometer (TRI CARB Type 4640; Packard, Chicago, IL, USA), and the ^{65}Zn -radioactivity was measured in an auto-gamma counter (Type 5780, Packard). Since the half life of ^{65}Zn is 245 days, the specific radioactivity of ^{65}Zn -polaprezinc or $^{65}\text{ZnSO}_4$ (A) was calculated according to the following equation:

$$A = A_0 \cdot e^{-0.693 \cdot T/t_{1/2}} \quad (\text{Eqn. 1})$$

A_0 : The specific radioactivity at $T=0$

$t_{1/2}$: Half life of ^{65}Zn (245 days)

T : Time elapsed (days)

The ^{14}C -radioactivity mixed with ^{65}Zn was converted to an absolute count of ^{14}C according to the calibration curve for quenching by the double tracer method as described previously (15).

Effect of polaprezinc on gastric emptying

Normal rats were given phenol red at 1 hr after oral administration of polaprezinc or 0.5% CMC-Na (control). The animals were killed under ether anesthesia at 2 hr after administration of phenol red. The stomach was removed and cut open, and then it was washed with 20 ml of ice cold saline to collect the gastric contents. After centrifugation (1000 rpm, 2 min), 3 ml of the supernatant was added to 0.5 ml of 5 N NaOH, the samples were meas-

ured at 560 nm in a spectrophotometer (U-2000; Hitachi, Tokyo).

Plasma and stomach concentrations and gastric contents of radioactivity

Normal rats were divided into 3 groups and given ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine or $^{65}\text{ZnSO}_4$ orally. At 0.5, 1, 2, 4 and 8 hr after administration, blood samples were withdrawn using heparinized syringes from the abdominal aorta of rats under ether anesthesia and centrifuged (3000 rpm, 10 min) to separate the plasma.

The animals were subsequently killed, and the stomach was isolated with both ends clipped by clamps. The stomach was cut and washed with cold physiological saline to collect the gastric contents. After the gastric tissue was weighed, ^{65}Zn -radioactivity was determined directly, and the tissue was solubilized in 2 ml of Soluene-350 (Packard) to determine ^{14}C -radioactivity.

Adhesiveness and formation of polaprezinc at ulcerous sites

Rats with acetic acid-induced ulcers were killed under ether anesthesia at 15, 30 and 60 min after oral administration of ^{14}C -, ^{65}Zn -polaprezinc or ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid.

The stomach was removed and cut open, and the ulcerous and non-ulcerous parts were isolated and washed with 20 ml of saline. Subsequently, each part was washed in 20 ml of 0.1 N HCl, and the radioactivities of the HCl solution (acid soluble fraction) were measured.

Data analyses

The gastric emptying (G.E.) of phenol red (PR) was calculated using Eqn. 2:

$$\text{G.E.}(\%) = 100 \times (\text{amount of dose} - \text{recovered}) / \text{amount of dose} \quad (\text{Eqn. 2})$$

The area under the concentration curve (AUC) was calculated by the trapezoidal method.

The statistical moments (the mean residence time in the stomach: $\text{MRT}_{\text{stomach}}$ and the variance of residence time: VRT) were calculated from the radioactivity in gastric contents (A_g) using Eqns. 3 and 4 (16):

$$\text{MRT}_{\text{stomach}} = \int_0^{\infty} t \cdot A_g dt / \int_0^{\infty} A_g dt \quad (\text{Eqn. 3})$$

$$\text{VRT} = \int_0^{\infty} (t - \text{MRT})^2 \cdot A_g dt / \int_0^{\infty} A_g dt \quad (\text{Eqn. 4})$$

The values of $\text{MRT}_{\text{stomach}}$ of solids and liquids were calculated from the elimination half life reported by Marcus and Lengemann (17) using Eqn. 5 (18):

$$\text{MRT}_{\text{stomach}} = t_{1/2} / \ln 2 \quad (\text{Eqn. 5})$$

where $t_{1/2}$ is the elimination of half life.

Student's *t*-test was utilized to determine the significance of differences.

RESULTS

Effect of polaprezinc on gastric emptying

The gastric emptying of phenol red in control rats was $82.40 \pm 2.85\%$ (mean \pm S.D., $n=3$), and polaprezinc did not inhibit that at a dose of $171.6 \mu\text{mol/kg}$ ($83.14 \pm 1.42\%$).

Radioactivity of gastric contents and tissue

The radioactivities of gastric contents after administration of ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ are shown in Fig. 2. The amounts of ^{65}Zn -polaprezinc were slightly higher than those of ^{14}C -polaprezinc in the gastric contents from 0.5 hr to 4 hr after administration of ^{14}C -, ^{65}Zn -polaprezinc, but the difference was not significant. The amount of ^{14}C -polaprezinc was similar to ^{14}C -L-carnosine at 0.5 hr. However, the content of ^{14}C -polaprezinc was 150–250 times higher than that of ^{14}C -L-carnosine at 2 and 4 hr, but were almost comparable at 8 hr after administration. The amount of ^{65}Zn -polaprezinc was the same as that of $^{65}\text{ZnSO}_4$ up to 2 hr. ^{65}Zn -polaprezinc content at 4 hr was about 40 times higher than that of $^{65}\text{ZnSO}_4$, but the two were comparable at 8 hr after administration.

The $\text{MRT}_{\text{stomach}}$ after oral administration of ^{14}C -, ^{65}Zn -

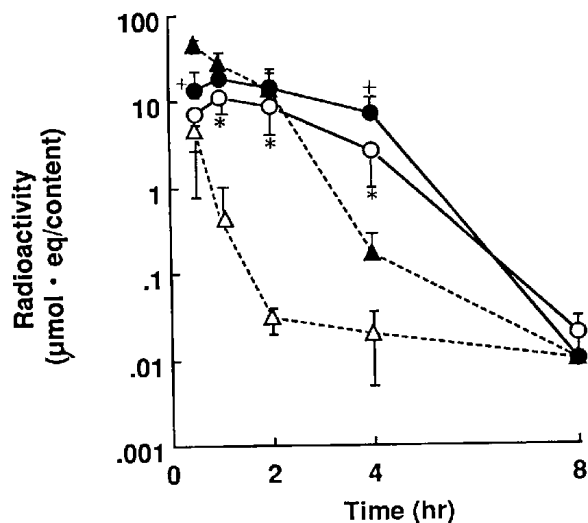


Fig. 2. Time courses of total radioactivity in gastric contents after oral administrations of ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ to rats. The dose of each agent was $171.6 \mu\text{mol/kg}$. Each data point is the mean \pm S.D. ($n=3$). * $P < 0.05$: statistically significant compared with ^{14}C -L-carnosine. † $P < 0.05$: statistically significant compared with $^{65}\text{ZnSO}_4$. ○: ^{14}C -polaprezinc, ●: ^{65}Zn -polaprezinc, △: ^{14}C -L-carnosine, ▲: $^{65}\text{ZnSO}_4$.

Table 1. Mean residence time in stomach contents (MRT_{stomach}) after oral administrations of ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ to rats (dose: $171.6 \mu\text{mol/kg}$)

	MRT_{stomach} (hr)	VRT (hr ²)
^{14}C -polaprezinc	2.07	1.52
^{65}Zn -polaprezinc	2.28	1.74
^{14}C -L-carnosine	0.95	4.32
$^{65}\text{ZnSO}_4$	1.17	0.47

polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ were calculated from the radioactivities in gastric contents (Table 1). The MRT_{stomach} s of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc showed almost the same values (ca. 2 hr). The MRT_{stomach} values of ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ were about 1 hr, half of those of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc.

Radioactivity in the gastric tissues and the pharmacokinetic parameters are shown in Fig. 3 and Table 2, respectively. ^{14}C - and ^{65}Zn -radioactivities in gastric tissues were very similar up to 4 hr after administration of ^{14}C -, ^{65}Zn -polaprezinc. However, the concentration of ^{14}C -polaprezinc was about 6 times higher than that of ^{65}Zn -polaprezinc at 8 hr after administration. The concentration of ^{14}C -polaprezinc was almost the same as that of

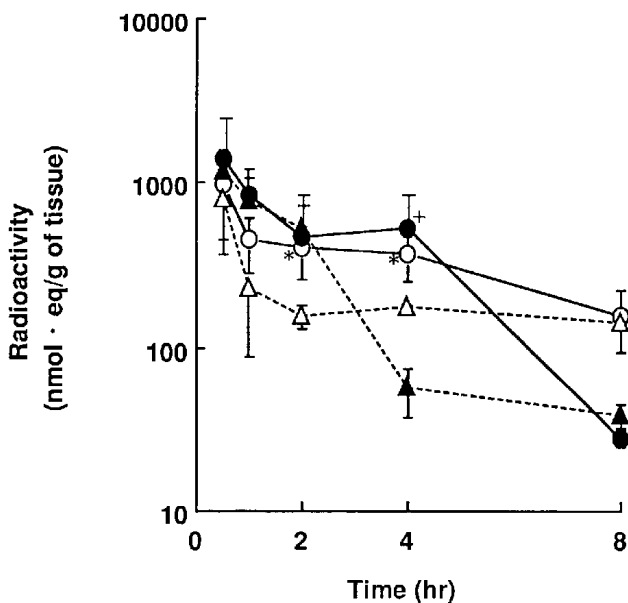


Fig. 3. Time courses of total radioactivity in gastric tissues after oral administrations of ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ to rats. The dose of each agent was $171.6 \mu\text{mol/kg}$. Each data point is the mean \pm S.D. ($n=3$). * $P < 0.05$: statistically significant compared with ^{14}C -L-carnosine. + $P < 0.05$: statistically significant compared with $^{65}\text{ZnSO}_4$. ○: ^{14}C -polaprezinc, ●: ^{65}Zn -polaprezinc, △: ^{14}C -L-carnosine, ▲: $^{65}\text{ZnSO}_4$.

Table 2. Pharmacokinetic parameters of ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ after oral administration to rats (dose: $171.6 \mu\text{mol/kg}$)

	t_{max} (hr)	C_{max} (nmol · eq/ml)	$AUC_{0-8 \text{ hr}}$ (nmol · eq · hr/ml)
Stomach			
^{14}C -polaprezinc	0.5	985.52	2826.27
^{65}Zn -polaprezinc	0.5	1391.94	3681.48
^{14}C -L-carnosine	0.5	806.02	1614.29
$^{65}\text{ZnSO}_4$	0.5	1175.06	2199.67
Plasma			
^{14}C -polaprezinc	8	76.06	379.55
^{65}Zn -polaprezinc	4	24.23	121.91
^{14}C -L-carnosine	1	200.49	988.02
$^{65}\text{ZnSO}_4$	1	33.46	145.21

^{14}C -L-carnosine at 0.5 hr. However, ^{14}C -polaprezinc concentrations were about double those of ^{14}C -L-carnosine at 1 and 4 hr, but these concentrations were comparable at 8 hr after administration. The concentrations of ^{65}Zn -polaprezinc were the same as those of $^{65}\text{ZnSO}_4$ up to 2 hr. However, ^{65}Zn -polaprezinc concentration was about 10 times that of $^{65}\text{ZnSO}_4$ at 4 hr, and it was comparable at 8 hr after administration. The $AUC_{0-8 \text{ hr}}$ values of ^{14}C -polaprezinc and ^{65}Zn -polaprezinc were about 1.7 times higher than those of ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$, respectively.

Plasma concentration of radioactivity

The time courses of the plasma concentrations of radioactivity after administration of ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ to rats at a dose of $171.6 \mu\text{mol/kg}$ are shown in Fig. 4, and the pharmacokinetic parameters calculated from the plasma concentrations are shown in Table 2. The plasma concentration of ^{14}C -polaprezinc increased with time after administration of ^{14}C -, ^{65}Zn -polaprezinc, reaching $76.04 \text{ nmol} \cdot \text{eq/ml}$ at 8 hr after administration. On the other hand, the plasma concentration of ^{65}Zn -polaprezinc peaked at 4 hr (C_{max} : $24.23 \text{ nmol} \cdot \text{eq/ml}$), and it decreased to $5.14 \text{ nmol} \cdot \text{eq/ml}$ at 8 hr after administration. The plasma concentration of ^{14}C -L-carnosine reached a C_{max} of $200.49 \text{ nmol} \cdot \text{eq/ml}$ at 1 hr, and it decreased to $68.88 \text{ nmol} \cdot \text{eq/ml}$ at 8 hr after administration. The $^{65}\text{ZnSO}_4$ concentrations peaked at 1 hr (C_{max} : $33.46 \text{ nmol} \cdot \text{eq/ml}$), and it decreased to $6.57 \text{ nmol} \cdot \text{eq/ml}$ at 8 hr after administration. The $AUC_{0-8 \text{ hr}}$ of ^{14}C -L-carnosine was 2.6 times greater than that of ^{14}C -polaprezinc. However, the $AUC_{0-8 \text{ hr}}$ of $^{65}\text{ZnSO}_4$ was almost equivalent to that of ^{65}Zn -polaprezinc.

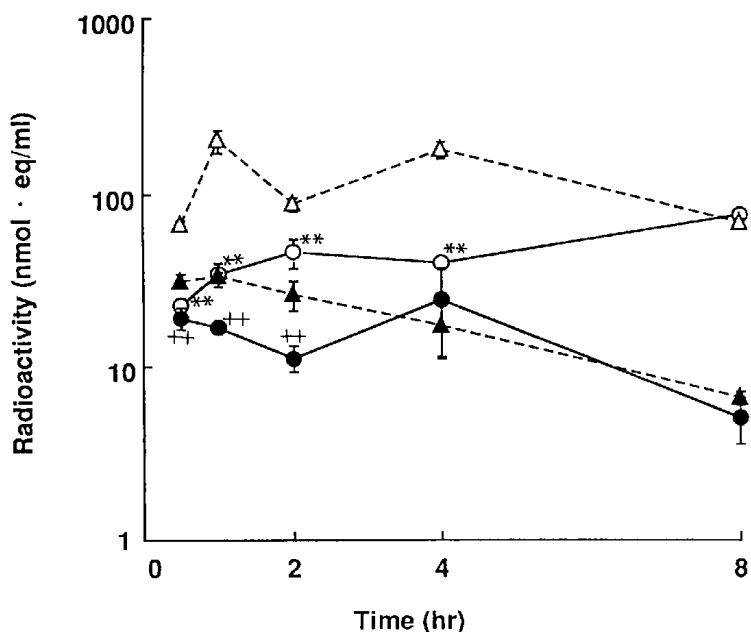


Fig. 4. Time courses of total radioactivity in plasma after oral administration of ^{14}C -, ^{65}Zn -polaprezinc, ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$ to rats. The dose of each agent was $171.6 \mu\text{mol/kg}$. Each data point is the mean \pm S.D. ($n=3$). ** $P < 0.01$: statistically significant compared with ^{14}C -L-carnosine. + $P < 0.01$: statistically significant compared with $^{65}\text{ZnSO}_4$. ○: ^{14}C -polaprezinc, ●: ^{65}Zn -polaprezinc, △: ^{14}C -L-carnosine, ▲: $^{65}\text{ZnSO}_4$.

Adhesiveness and formation of polaprezinc at the ulcerous sites

^{65}Zn -Radioactivities in the acid-soluble fractions of ulcerous and non-ulcerous sites were very similar to ^{14}C -radioactivities at 15 and 30 min after oral administration of ^{14}C -, ^{65}Zn -polaprezinc to the rats with acetic acid-induced ulcers.

^{14}C - and ^{65}Zn -Radioactivities of the ulcerous sites were greater than those of the non-ulcerous sites (Table 3). At

60 min after administration, ^{14}C and ^{65}Zn -radioactivities of ulcerous or non-ulcerous sites were decreased, and ^{65}Zn -radioactivities were greater than ^{14}C -radioactivities. On the other hand, ^{14}C - and ^{65}Zn -radioactivities of ulcerous and non-ulcerous sites after administration of ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid were lower than those of ^{14}C -, ^{65}Zn -polaprezinc; ^{65}Zn -radioactivities were greater than ^{14}C -radioactivities at each time point.

Table 3. Radioactivities of acid-soluble fractions in the stomach after oral administrations of ^{14}C -, ^{65}Zn -polaprezinc or ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid to rats with acetic acid-induced ulcers (dose: $10.3 \mu\text{mol/kg}$)

Time (min)	Ulcerous part			Non-ulcerous part		
	^{65}Zn (nmol · eq/g of tissue)	^{14}C	ratio $^{65}\text{Zn}/^{14}\text{C}$	^{65}Zn (nmol · eq/g of tissue)	^{14}C	ratio $^{65}\text{Zn}/^{14}\text{C}$
^{14}C -, ^{65}Zn -polaprezinc						
15	$39.00 \pm 16.90^+$	$30.38 \pm 12.66^{**}$	1.28	$23.72 \pm 6.34^{++}$	$22.21 \pm 5.66^{**}$	1.07
30	25.28 ± 16.41	24.69 ± 21.97	1.02	14.62 ± 6.48	13.76 ± 7.69	1.06
60	$15.97 \pm 9.69^+$	$5.66 \pm 3.62^*$	2.82	8.83 ± 7.10	2.66 ± 1.90	3.32
^{14}C -, ^{65}Zn -polaprezinc dissolved in acid						
15	19.45 ± 7.14	7.34 ± 2.07	2.65	10.31 ± 5.59	7.28 ± 4.10	1.42
30	18.79 ± 13.69	6.17 ± 5.14	3.04	6.45 ± 6.52	4.69 ± 4.93	1.38
60	4.31 ± 2.72	1.52 ± 0.76	2.84	3.48 ± 1.79	1.28 ± 0.97	2.73

Each value represents the mean \pm S.D. ($n=5$). + $P < 0.05$, ++ $P < 0.01$: statistically significant compared with ^{65}Zn -radioactivities of ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid. * $P < 0.05$, ** $P < 0.01$: statistically significant compared with ^{14}C -radioactivities of ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid.

DISCUSSION

Polaprezinc, which exerts anti-ulcer effects by acting directly on the ulcerous site, is a zinc complex of L-carnosine, which is practically insoluble in water and other organic solvents but can be dissolved in acidic solution (1). Polaprezinc markedly protected rat gastric mucosa against ethanol-induced damage without affecting the mucosal prostaglandin E₂ level (3). Arakawa et al. (4) reported that polaprezinc was three times more effective on the mucosal damage than zinc sulfate with equivalent amounts of zinc, and L-carnosine did not prevent the damage in a dose equivalent to polaprezinc. Furthermore, polaprezinc, but not the mixture, prevented the gastric lesions induced by absolute ethanol at 4 hr after oral administration (11); the pharmacological effects of polaprezinc were greater than those of its components L-carnosine and zinc sulfate or a mixture of two drugs on indomethacin induced gastric lesions and acetic acid induced gastric ulcers (11). Therefore, the dispositions of polaprezinc in the stomach was compared with its component compounds L-carnosine and zinc sulfate, since the differences of pharmacological activity between polaprezinc and those of the mixtures were observed. In the gastric contents, ¹⁴C-polaprezinc contents were greater than those of ¹⁴C-carnosine at 1–4 hr, and ⁶⁵Zn-polaprezinc content was greater than that of ⁶⁵ZnSO₄ at 4 hr after administration. The differences in radioactivities in the gastric contents were considered to be based on the residence time in the stomach of each drug. One of the pharmacokinetic parameters used to evaluate the disposition of drugs in plasma or tissues is the mean residence time (MRT) calculated by the statistical moment method (16, 18, 19). The MRT is a model-independent parameter calculated from the time courses of the concentrations or amounts of drug, and it is useful for describing the absorption or elimination process. Generally, the residence time of solid forms in the stomach is longer than that of liquid forms (20, 21). Marcus and Lengemann (17) reported the elimination half life of solids and liquids in gastric contents using the ⁹¹Y compound, and MRT_{stomach} values of the liquid and solid forms calculated from the elimination half life were 0.9 and 1.5 hr, respectively. In this study, the MRT_{stomach} values of ¹⁴C-, ⁶⁵Zn-polaprezinc, as an insoluble complex, were about 2 hr and similar to the solid form data reported by Marcus and Lengemann (17). On the other hand, the MRT_{stomach} of ¹⁴C-L-carnosine and ⁶⁵ZnSO₄ in the solution forms were about 1 hr and faster than those of ¹⁴C-, ⁶⁵Zn-polaprezinc. Furthermore, ¹⁴C-polaprezinc or ⁶⁵Zn-polaprezinc concentrations in the stomach were greater than those of ¹⁴C-L-carnosine and ⁶⁵ZnSO₄; therefore, polaprezinc was localized more strongly in the gastric tissues than L-carnosine and zinc sul-

fate. In addition, polaprezinc did not inhibit physiological gastric emptying in rats and mice (22). Thus, it is considered that the increment in pharmacological activity of polaprezinc was caused by its useful structure as an insoluble complex; the residence times in the stomach of L-carnosine and zinc were postponed.

The effects of residence time on drug absorption were reported previously (23, 24). From these reports, we evaluated the absorption properties of ¹⁴C-, ⁶⁵Zn-polaprezinc, ¹⁴C-L-carnosine and ⁶⁵ZnSO₄ in rats. After administration of ¹⁴C-, ⁶⁵Zn-polaprezinc, plasma concentrations of ⁶⁵Zn-radioactivities were different from ¹⁴C-radioactivities. This finding indicated that polaprezinc is dissociated to zinc and L-carnosine on administration, and zinc is metabolized along with the metabolic turnover of the endogenous zinc, while L-carnosine is metabolized and utilized in the synthesis of endogenous high molecular weight substances such as proteins (15, 25, 26).

¹⁴C-L-Carnosine was absorbed better than ¹⁴C-polaprezinc, and the absorption of ⁶⁵ZnSO₄ was slightly better than ⁶⁵Zn-polaprezinc.

Each component of polaprezinc, L-carnosine or zinc, is absorbed after dissociation of polaprezinc in the gastrointestinal tract as a function time (27), and then the absorption rate of polaprezinc is slower than those of L-carnosine or zinc sulfate, because time for dissociation is required in the gastrointestinal tract. It is also well known that the zinc absorption is regulated by homeostatic mechanisms (28–30), and thus, the absorption ratio of ⁶⁵Zn-polaprezinc is small (about 11% of the dose at 50 mg/kg) (15). Therefore, no clear differences in plasma concentration profiles of ⁶⁵Zn-polaprezinc and ⁶⁵ZnSO₄ were observed.

From these results, the disposition of polaprezinc in the stomach and plasma were different from those of L-carnosine or zinc sulfate. No extension of the mean resident time of polaprezinc is caused by its pharmacological action; and thus, it was considered that L-carnosine and zinc sulfate were in the fluid form and remained in the stomach for a short time. On the other hand, polaprezinc remains in the stomach for a relatively long time because of its physico-chemical characteristics as an insoluble complex.

In the second experiment, we examined the formation of polaprezinc adhering to the ulcerous sites in rats with acetic acid-induced ulcers. The ¹⁴C and ⁶⁵Zn-radioactivities of ulcerous or non-ulcerous sites were very similar up to 30 min. However, with ¹⁴C-, ⁶⁵Zn-polaprezinc dissolved in acid, ¹⁴C and ⁶⁵Zn-radioactivities of the ulcerous or non-ulcerous sites were different at all observed time points. Polaprezinc is insoluble in saline, but dissociated to zinc and L-carnosine completely in acidic solution. Furthermore, after dissociation, zinc and L-carnosine could

not be chelated again under the neutral or basic condition. Therefore, polaprezinc could exist as complex form at ulcerous sites at least up to 30 min after administration, because the ^{14}C and ^{65}Zn -radioactivities of the acid-soluble fraction after washing with saline were very similar at the early time points. The ^{14}C and ^{65}Zn -radioactivities in the acid-soluble fraction of ulcerous sites were greater than those of the non-ulcerous parts after administration of ^{14}C -, ^{65}Zn -polaprezinc, thus indicating that gastric mucosal adhesiveness of polaprezinc was specifically localized to ulcerous sites. The ^{14}C and ^{65}Zn -radioactivities of the ulcerous or non-ulcerous sites of ^{14}C -, ^{65}Zn -polaprezinc were greater than those of ^{14}C -, ^{65}Zn -polaprezinc dissolved in acid, indicating that the increment in affinity to the gastric tissues of polaprezinc was caused by its complex formation. In these studies, at 4 hr after administration, ^{14}C -, ^{65}Zn -polaprezinc in tissue and content of the stomach were greater than those of ^{14}C -L-carnosine and $^{65}\text{ZnSO}_4$, respectively.

Furthermore, the amounts of zinc in the ulcer part after administration of polaprezinc were almost the same as those of zinc sulfate up to 1 hr; however, the zinc amounts of polaprezinc were greater than those of zinc sulfate at 3 hr after administration (13). Zinc levels that showed protective effects against gastric lesions were over $20 \mu\text{g/g}$ of tissue (13), and polaprezinc could maintain a high concentration of zinc in the ulcer region for a long time. Histological studies showed that zinc was localized from the covering epithelial cell layer to the gastric lamina propria mucosae in the normal tissue and in the most superficial ulcerous layer and the granulous layer of the ulcerous site (12). As the gastric pH value is about 2 in fasted rats (31), administered polaprezinc would be dissociated to zinc and L-carnosine in the gastrointestinal tract as a function of time. All of the polaprezinc was not dissociated immediately in the stomach, because the amount of acid in the gastric juice is too small to dissolve it. Furthermore, because the adhesion of polaprezinc to gastric tissues was increased by the H_2 -receptor antagonist cimetidine via inhibition of polaprezinc dissociation (27), the pharmacological activity was increased by cimetidine (32). In conclusion, polaprezinc is retained in the stomach longer and adheres to the ulcerous sites more than zinc or L-carnosine. The characteristics of this compound may arise from its insolubility and contribute to its strong pharmacological action.

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