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Disposition of Polaprezinc (Zinc L-Carnosine Complex) in Rat Gastrointestinal Tract and Effect of Cimetidine on its Adhesion to Gastric Tissues

SHIGERU FURUTA, SEIJI TOYAMA, MASAHIRO MIWA AND HIROSHI SANO

Central Research Laboratories, Zeria Pharmaceutical Co. Ltd., 2512-1 Oshikiri, Konan-machi, Osato-gun, Saitama, 360-01 Japan

Abstract

The disposition of polaprezinc in the rat gastrointestinal tract was studied by a double tracer method using [^{14}C]- and [^{65}Zn]polaprezinc. At 0·5 h after oral administration of [^{14}C]-, [^{65}Zn]polaprezinc to rats, the ^{14}C-radioactivity in the gastric contents was comparable with the ^{65}Zn-radioactivity. However, a significant difference was observed in the time course of changes in gastric contents between ^{14}C- and ^{65}Zn-radioactivity over 1 h after administration, indicating that polaprezinc existed in complex form at 0·5 h after administration and was dissociated to L-carnosine and zinc in the gastrointestinal tract as a function of time.

The adhesion of zinc to stomach mucosa after oral administration of polaprezinc to rats was significantly increased by treatment with cimetidine.

These results suggest that the adhesion of zinc to gastric tissues is increased by inhibiting the dissociation of polaprezinc, and that H_2-receptor antagonists, such as cimetidine, may increase anti-ulcer effects of polaprezinc.

Polaprezinc (Z-103) is a complex compound of doubly deprotonated L-carnosine (β-alanyl-L-histidine) and zinc in a 1 : 1 molar ratio (Matsukura et al 1990). Polaprezinc exhibits marked anti-ulcer lesions and duodenal ulcer by acting directly on the gastric mucosa (Matsukura et al 1990; Seiki et al 1990). The pharmacological actions of polaprezinc are considered to be due to protection of the gastric mucosa membrane, stabilizing activity, antioxidative activity and promotion of wound-healing (Ito et al 1992). Studies of the absorption, distribution, metabolism and excretion of [U-^{14}C]histidine-polaprezinc and [^{65}Zn]polaprezinc have been reported (Sano et al 1991; Toyama et al 1991; Furuta et al 1992). In these studies, the metabolic disposition of [^{14}C]polaprezinc was found to be significantly different from that of [^{65}Zn]polaprezinc in rats. Polaprezinc is practically insoluble in water and organic solvents (Matsukura et al 1990), suggesting that its structure as an insoluble complex is important for its pharmacological actions (Aita et al 1992; Seiki et al 1992a, b). However, the absorption of polaprezinc in particular its disposition in the gastrointestinal tract had not been sufficiently investigated.

In the present study, the disposition in the rat gastrointestinal tract after oral administration of polaprezinc was studied. We also investigated the effects of cimetidine on the adhesion of polaprezinc to gastric tissues.

Materials and Methods

Chemicals

Polaprezinc was synthesized by Hamari Chemicals Co. Ltd (Osaka, Japan). [U-^{14}C]histidine-polaprezinc ([^{14}C]polaprezinc) and [^{65}Zn]polaprezinc were synthesized by Nemoto Tokushu Kagaku (Tokyo, Japan) and Amersham (Buckinghamshire, UK) (see Fig. 1). [^{14}C]Polaprezinc had a specific radioactivity of 92·5 kBq mg^{-1} and a radiochemical purity of 96·6%. [^{65}Zn]Polaprezinc had a specific radioactivity of 2·105 MBq mg^{-1}. Cimetidine was purchased from Sigma (St Louis, MO).

Correspondence: S. Furuta, Central Research Laboratories, Zeria Pharmaceutical Co. Ltd., 2512-1 Oshikiri, Konan-machi, Osato-gun, Saitama, Japan 360-01.

Animals

Male, specific pathogen-free (SPF) Sprague-Dawley rats (7–8 weeks of age, 250 g) were purchased from Charles River Japan Co. Ltd, and had free access to water and solid laboratory food.

Food (Charles River CRF-1) was purchased from Oriental Yeast Co. Ltd., and its zinc content was 52 μg g^{-1} of diet.

The animals were housed in an animal room maintained at a constant temperature of 23 ± 2°C and 55 ± 10% r.h., and were acclimatized for more than one week before experiments.

Method of administration

[^{14}C]Polaprezinc and [^{65}Zn]polaprezinc were mixed and suspended in 0·5%-sodium carboxymethyl cellulose (CMC-Na) solution using an agate mortar, and the specific radioactivity was adjusted by dilution with non-radioactive polaprezinc. The radioactivity administered was approximately 4·5 MBq/5 mL kg^{-1} for [^{14}C]polaprezinc and approximately 6·3 MBq/5 mL kg^{-1} for [^{65}Zn]polaprezinc. Rats were fasted for 16 h before dosing but were allowed free access to water.

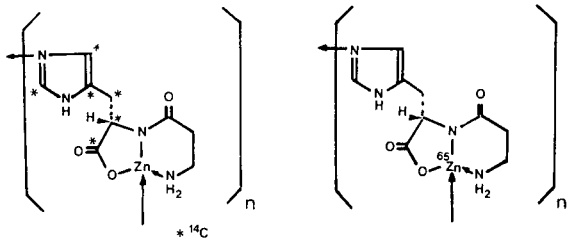

FIG. 1. Chemical structure of [^{14}C]polaprezinc and [^{65}Zn]-polaprezinc.

Measurement of radioactivity

The ^{14}C-radioactivity in each sample was measured in a liquid scintillation spectrophotometer (TRI CARB Type 4640, Packard) (Chicago, IL). Samples were measured by a double tracer method using ^{14}C- and ^{65}Zn-radioactivity (Sano et al 1991). In this case, the ^{14}C-radioactivity mixed with ^{65}Zn was converted to an absolute count of ^{14}C according to the calibration curve for quenching based on the spill-over method (Viotti & Nucca 1975), which is generally employed for the measurement of the radioactivity of the ^{14}C- and ^{3}H-double tracers.

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 (A) was calculated according to:

$$A = A_0 \cdot e^{-0.693 \cdot T/t\frac{1}{2}} \quad (1)$$

where A_0 is the specific radioactivity at $t = 0$, $t\frac{1}{2}$ is the half-life of ^{65}Zn (245 days) and T is the time elapsed (days).

IR absorption spectra

Polaprezinc (0·1 g) was dissolved in 5 mL 0·2 M HCl, and the resultant solution was evaporated to dryness under reduced pressure at 40°C. The residue was dried for an additional 2 h under reduced pressure in a desiccator with silica gel and NaOH before using it as a sample. L-Carnosine (0·1 g) and zinc chloride (0·05 g) were mixed and dissolved in 5 mL purified water and processed thereafter in the same way as polaprezinc to obtain residues. The IR absorption spectra of these samples were determined using the IR (potassium bromide disc) method described in the General Tests, Processes and Apparatus section of the Japanese Pharmacopoeia (Pharmacopoeia of Japan (XI)) using an IR spectrophotometer (Japan Spectroscopic IR-700).

The dissolution profiles of polaprezinc in acidic solution

An excess sample of polaprezinc was placed in 30 mL 0·01 M HCl and incubated at 37°C with shaking (100 oscillations min^{-1}).

At 0·5, 1, 2, 3 and 5 h, 3 mL aliquots of the supernatants were collected and filtered using a filter unit (0·45 μm). The zinc concentrations were then measured by an atomic absorption spectrophotometer (Model 180-60, Hitachi (Tokyo)); L-carnosine was measured by HPLC.

Polaprezinc in plasma and gastric contents

Polaprezinc in 0·5% CMC-Na solution was orally administered to rats at the dose of 50 mg/5 mL kg^{-1}. At 0·5, 1, 4, 8, 24 and 120 h after administration, blood samples were withdrawn using heparinized syringes, from the abdominal aorta of rats under ether anaesthesia and centrifuged to separate plasma. The animals were subsequently killed, and the stomach and both small and large intestine were isolated with both ends clipped by clamps. The stomach, caecum and large intestine (from the outlet from caecum to the anus) were cut carefully, then gastric contents from each region were collected. The small intestine, from the pylorus to the caecum, was divided into four equal portions defined as the duodenum, jejunum, jejunum-ileum and ileum according to the method of Marcus & Lengeman (1962). After collecting their contents, each segment was washed with ice-cold physiological saline, and washings were combined with each respective collected contents for use as samples.

Effects of cimetidine on the adhesion of polaprezinc to gastric tissues

Non-labelled polaprezinc (50 mg kg^{-1}) was orally administered at 30 min after intraperitoneal administration of cimetidine (25 mg kg^{-1}) to rats. Blood samples were collected from the abdominal aorta at 0·25, 0·5, 0·75, 1, 2, 4, 6, 8, 11 and 24 h after administration of polaprezinc, and centrifuged to separate plasma. The stomach, clipped with clamps, was isolated at 0·5, 1, 2, 4, 6 and 8 h after administration and cut open along the greater curvature to collect the contents. The stomach was washed with ice-cold physiological saline and the washing was combined with the contents. Gastric mucosa was scraped with slide glasses, and mucosa and muscular layer were separately weighed. Plasma, gastric contents, mucosa and muscular layer were washed using perchloric acid and hydrogen peroxide in Kjeldal's flasks, and zinc concentration of each sample was determined using an atomic absorption spectrophotometer (180-60, Hitachi).

Data analysis

The area under the drug concentration–time curve (AUC) was calculated by the trapezoidal method. For data analysis, Student's *t*-test was used.

Results

IR absorption spectra of polaprezinc

The IR absorption spectra of polaprezinc and of polaprezinc dissolved in 0·2 M HCl, concentrated and dried, are shown in Fig. 2A. The IR absorption spectrum of polaprezinc was changed after dissolution in HCl and drying, the most significant change being the loss of the sharp peak (3280 cm^{-1}) of primary amine of L-carnosine bound to zinc, resulting in a broad peak of the ammonium salt. The IR absorption spectrum of an equimolar mixture of L-carnosine and zinc chloride dissolved in purified water and dried was very similar to that of polaprezinc dissolved in HCl (Fig. 2B).

Dissolution profile of polaprezinc in acidic solution

Polaprezinc was incubated in 0·01 M HCl, and the concentrations of zinc and L-carnosine in supernatant were measured. The dissolution profiles of zinc and L-carnosine were very similar and each concentration increased with

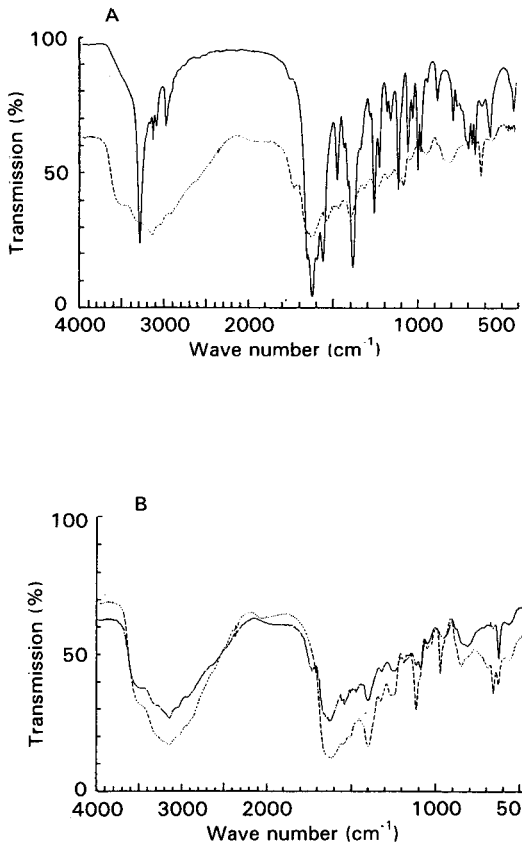

FIG. 2. IR absorption spectra of intact and acid-treated polaprezinc. A. intact (——) and acid-treated (------) polaprezinc. B. acid-treated polaprezinc (——) and a mixture of L-carnosine and zinc chloride (------).

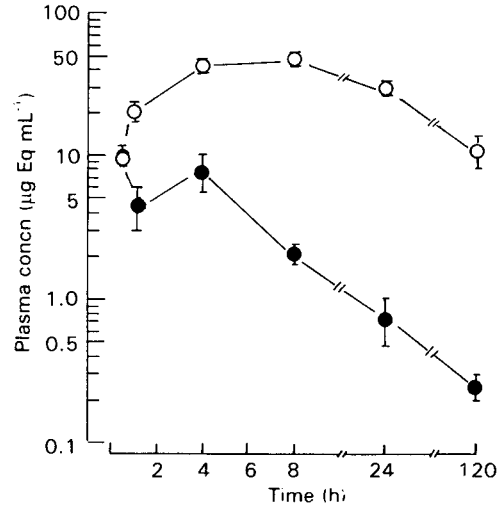

FIG. 3. Time courses of changes in total radioactivity in plasma after oral administration of [^{14}C], [^{65}Zn]polaprezinc to rats. Dose was 50 mg kg^{-1}. Each point represents the means ± s.d. (n = 3). ○ [^{14}C]Polaprezinc, ● [^{65}Zn]polaprezinc.

Table 1. Dissolution profiles of polaprezinc in acidic solution (0·01 M HCl).

Time (h)	L-Carnosine (μmol mL^{-1})	Zinc (μmol mL^{-1})
0·5	1·50	1·40
1·0	1·77	1·78
2·0	3·33	3·58
3·0	4·05	4·28
5·0	4·29	4·38

time reaching constant values at 3 h (Table 1). The dissolution rates of L-carnosine and of zinc in supernatant at 0·5 and 1 h were calculated as about 35 and 40%, respectively, of their concentrations at 5 h which were presumed to represent maximum dissolution (100%).

Disposition of polaprezinc in gastrointestinal tract

The dispositions of polaprezinc in the gastrointestinal tract after oral administration to rats were examined. Radioactivities due to [^{14}C]-, [^{65}Zn]polaprezinc of contents in each gastrointestinal tract segment are shown for individual animals as percentages of dose administered (Table 2).

The percentages of ^{14}C- and ^{65}Zn-radioactivity were very similar in all segments at 0·5 h after administration. At 1 and 4 h after administration, high ^{65}Zn-radioactivity was observed between the jejunum and caecum, with ^{14}C- and ^{65}Zn-radioactivities showing quite different patterns. Total radioactivity decreased over 8 h after administration; in particular, total ^{14}C-radioactivity was only about 5% of the dose in all gastrointestinal tract segments.

Table 2. Time courses of changes in total radioactivity in the gastrointestinal tract after oral administration of ^{14}C-, ^{65}Zn-polaprezinc (50 mg kg^{-1}) to rats (n = 3 at each time).

	Percentage of original dose							
	[^{14}C]Polaprezinc				[^{65}Zn]Polaprezinc			
	0.5 h	1 h	4 h	8 h	0.5 h	1 h	4 h	8 h
Stomach	29·57 ± 17·81	43·49 ± 7·74	3·00 ± 3·09	0·18 ± 0·13	27·21 ± 13·99	24·20 ± 27·25	5·21 ± 6·77	0·01 ± 0·01
Duodenum	1·10 ± 0·60	1·26 ± 0·61	0·77 ± 0·17	0·51 ± 0·18	3·65 ± 2·71	1·15 ± 1·04	0·50 ± 0·11	0·04 ± 0·02
Jejunum	4·94 ± 5·10	0·34 ± 0·24	0·68 ± 0·37	0·58 ± 0·02	7·36 ± 4·76	1·22 ± 0·67	1·08 ± 0·66	0·05 ± 0·00
Jejunum-ileum	6·64 ± 3·04	0·55 ± 0·36	0·90 ± 0·22	0·64 ± 0·13	10·35 ± 2·27	5·98 ± 5·23	3·15 ± 1·39	0·06 ± 0·02
Ileum	3·62 ± 2·35	1·83 ± 1·74	1·13 ± 0·05	0·61 ± 0·17	3·94 ± 2·02	15·85 ± 3·56	14·65 ± 5·16	0·23 ± 0·22
Caecum	0·16 ± 0·04	2·95 ± 4·14	2·82 ± 1·07	4·36 ± 3·74	0·13 ± 0·02	24·31 ± 21·67	28·16 ± 4·30	11·62 ± 3·72
Large intestine	0·14 ± 0·05	0·28 ± 0·24	0·07 ± 0·02	0·15 ± 0·11	0·14 ± 0·08	0·20 ± 0·26	0·28 ± 0·22	0·86 ± 0·83

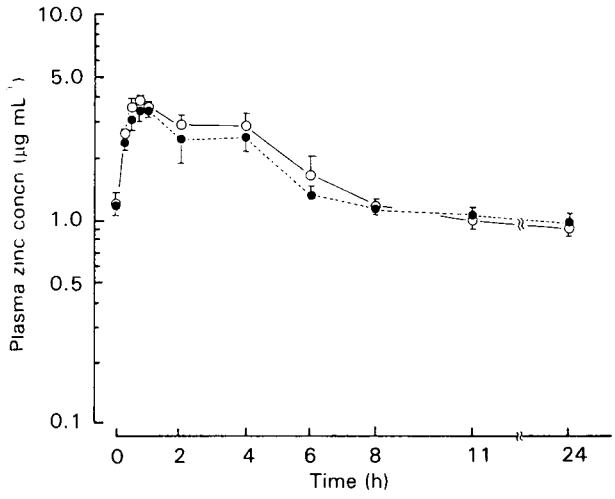

FIG. 4. Effects of cimetidine on plasma concentrations of zinc after oral administration of polaprezinc to rats. Dose: polaprezinc 50 mg kg^{-1} p.o.; cimetidine 25 mg kg^{-1}, i.p. ○ Polaprezinc alone; ● with cimetidine.

Plasma concentrations of polaprezinc

The time courses of changes in plasma concentrations of radioactivities after oral administration of polaprezinc to rats are shown in Fig. 3. The plasma concentrations of ^{14}C-radioactivity reached their maximum (46·67 μg Eq mL^{-1}) at 8 h after administration and decreased slowing thereafter with a half-life ($t_{\frac{1}{2}}$) of 57·1 h. On the other hand, the plasma concentration of ^{65}Zn-radioactivity reached C_{max} (10·47 μg Eq mL^{-1}) at 0·5 h and decreased with a half-life of 42·6 h. The areas under the plasma concentration-time curves (AUC) of ^{14}C- and ^{65}Zn-radioactivity were 3690·0 and 129·6 μg Eq h mL^{-1}, respectively.

Effects of cimetidine on the disposition and adhesion of zinc to gastric tissues

Plasma concentrations and pharmacokinetic parameters of zinc after oral administration of polaprezinc to cimetidine-treated rats are shown in Fig. 4 and Table 3, respectively.

Plasma concentrations of zinc after oral administration of polaprezinc to cimetidine-treated rats were similar to those in the untreated animals. The values of C_{max} and the areas under the plasma concentrations of zinc–time curves, determined up to 4 and 11 h after administration (AUC_{0-4h} and AUC_{0-11h}) of the cimetidine-treated rats were also comparable with those of unreated animals.

Table 3. Effects of cimetidine on kinetic parameters related to plasma zinc concentrations after oral administration of polaprezinc to rats.

	Control	Cimetidine
T_{max} (h)	0·8	0·8
C_{max} (μg mL^{-1})	3·7	3·3
AUC_{0-4h} [a] (μg h mL^{-1})	11·7	10·3
AUC_{0-24h} [b] (μg h mL^{-1})	22·2	19·9

Dose: cimetidine, 25 mg kg^{-1}, i.p.; polaprezinc, 50 mg kg^{-1}, p.o. Mean (n = 5).
[a]Area under the plasma concentration–time curve determined up to 4 h after administration of polaprezinc. [b]Area under the plasma concentration–time curve determined up to 24 h after administration of polaprezinc.

Amounts of zinc in gastric contents, gastric mucosa and the muscular layer are shown in Table 4.

The amount in gastric contents decreased significantly in the cimetidine-treated rats compared with the untreated rats at 1 h after administration of polaprezinc, but no significant difference was noted from 2 h up to 8 h after administration.

The zinc concentrations in gastric mucosa increased significantly in the cimetidine-treated rats at 0·5 and 1 h after administration, and were also higher in the muscular layer and were higher in the cimetidine-treated animals than in the untreated rats at 0·5 h after administration.

Discussion

In the first experiment, IR absorption spectra of polaprezinc were determined under acidic conditions. The IR absorption spectrum of polaprezinc after dissolution in HCl and evaporation to dryness was apparently different from that of unmodified polaprezinc, with the most marked changes being the loss of the sharp peak (3280 cm^{-1}) of the primary amine of L-carnosine bound to zinc and the appearance of a broad peak of ammonium salt after dissolution in HCl. The IR absorption spectrum of an equimolar mixture of L-carnosine and zinc chloride after dissolution in purified water and evaporation to dryness was very similar to that of polaprezinc dissolved in HCl, indicating that polaprezinc

Table 4. Effects of cimetidine on adhesion of zinc to gastric tissues after oral administration of polaprezinc to rats.

	0 h	0·5 h	1 h	2 h	4 h	8 h
Gastric contents (μg)						
Control	6·1 ± 7·2	1291·0 ± 389·4	1331·1 ± 217·0	900·0 ± 312·5	153·6 ± 300·1	1·3 ± 0·8
Cimetidine	1·2 ± 0·5	806·0 ± 355·0*	753·6 ± 258·7***	764·6 ± 528·6	30·8 ± 30·6	2·4 ± 1·0
Gastric mucosa (μg (g tissue)$^{-1}$)						
Control	13·1 ± 5·3	130·1 ± 24·6	127·1 ± 40·0	52·6 ± 24·1	18·8 ± 11·1	12·1 ± 2·4
Cimetidine	12·4 ± 1·3	229·0 ± 64·0**	258·4 ± 85·1**	111·5 ± 63·8*	22·6 ± 7·8	12·9 ± 1·6
Muscular layer (μg (g tissue)$^{-1}$)						
Control	20·39 ± 1·9	44·2 ± 9·8	60·7 ± 6·7	26·0 ± 12·0	14·9 ± 4·5	17·3 ± 0·9
Cimetidine	15·15 ± 4·1*	64·1 ± 14·2**	44·1 ± 19·0	26·1 ± 17·8	20·0 ± 2·9*	18·1 ± 0·9

Dose: cimetidine, 25 mg kg^{-1} i.p.; polaprezinc, 50 mg kg^{-1} p.o. Mean ± s.d. (n = 5). *$P < 0·1$, **$P < 0·05$, ***$P < 0·01$ (Student's *t*-test).

is dissociated into L-carnosine and zinc under acidic conditions.

Polaprezinc will be dissociated in acidic solution when two protons attack the two ligand positions of polaprezinc. It is difficult to measure the volume of gastric juice in fasted rats. If the gastric juice could be presumed to be equivalent to 0·5 mL 0·01 M HCl (pH 2), 0·73 mg polaprezinc would be dissociated in the stomach. At a dose of polaprezinc of 10 mg/200 g (Sano et al 1991), the dissolution rate of polaprezinc in the stomach was too small. In the present dissolution study, all of the polaprezinc was not dissolved immediately in acidic solution of the same pH value as the gastric juice. To clarify the stability of the complex of polaprezinc in stomach, the disposition in the gastrointestinal tract after oral administration of a mixture of [^{14}C]polaprezinc and [^{65}Zn]polaprezinc to rats was investigated; radioactivities in the gastrointestinal contents were very similar at 0·5 h after oral administration, indicating that the same amount of zinc and L-carnosine were disposed of in parallel. However, in-vivo, polaprezinc administered was not dissociated immediately, and may still be present in its complex form in the stomach at 0·5 h after oral administration. Between 1 and 4 h after administration, ^{65}Zn-radioactivities were 1·6–13·0 times greater than ^{14}C-radioactivity from the jejunum through the caecum. Thus, most of the dose of polaprezinc was dissociated to zinc and L-carnosine in the gastrointestinal tract as a function of time.

Zinc is distributed as an essential metal in the body, the absorption of which is regulated homeostatically (Furuta et al 1994). L-Carnosine and zinc, after dissociation of polaprezinc, were absorbed by different mechanisms, and the rate of absorption of L-carnosine was higher than that of zinc which remained in the gastrointestinal tract.

Polaprezinc is often co-administered with H_2-receptor antagonists for synergistic enhancement of its anti-ulcer activities. H_2-Receptor antagonists increase the gastric pH by inhibition of gastric juice secretion (Burland et al 1975). The adhesion of polaprezinc to gastric tissues may be influenced by H_2-receptor antagonists via inhibition of the dissociation of polaprezinc.

Thus, we also investigated the effects of the H_2-receptor antagonist cimetidine on the adhesion of zinc to gastric tissues after oral administration of polaprezinc to rats.

The zinc concentrations in gastric mucosa and mucosal layer increased significantly in cimetidine-treated as compared with untreated rats. Cimetidine increases gastric pH values from 2 to 4 (Funaki et al 1986), and polaprezinc maintains its complex form at this pH value (pH 4). These results suggest that the adhesion of polaprezinc to gastric tissues increases via inhibition of its dissociation in-vivo. The stability in complex form of polaprezinc is important for adhesion to gastric mucosa. Thus, the anti-ulcer effects of polaprezinc are expected to increase by co-administration with H_2-receptor antagonists in clinical use.

However, plasma concentrations of zinc after oral administration of polaprezinc were decreased slightly by cimetidine. Cimetidine did not influence the plasma concentration of zinc after administration of polaprezinc; the absorption rate of zinc was only 10% of the dose of polaprezinc (Sano et al 1991) and change would be masked by endogenous zinc in plasma (Furuta et al 1991).

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