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The cytoprotective effect of zinc L-carnosine on ethanol-induced gastric gland damage in rabbits

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Abstract—The effects of zinc L-carnosine on the damaging actions of ethanol were examined in rabbit isolated gastric glands. Ethanol (8%, v/v) incubation produced a 50% viability of the gland populations and released a significant amount (38%) of the total lactate dehydrogenase (an index of membrane injury) of the glands. Zinc L-carnosine pre-incubation for 15 min markedly prevented these actions of ethanol; however, L-carnosine by itself did not have these effects. These findings indicate that zinc ion but not carnosine in the zinc L-carnosine molecule possesses cytoprotective action against ethanol-induced gastric gland damage in rabbits.

Zinc compounds such as zinc chloride, sulphate and acexamate are known for their activity against various types of gastric ulceration in rats (Cho et al 1976; Cho & Ogle 1977, 1978; Ogle & Cho 1978; Glavin 1985; Escolar et al 1987; Li 1990). Recently, zinc L-carnosine, another new zinc compound, was reported to possess a direct protective action on isolated gastric cells in the same type of animals (Arakawa et al 1990). However, no study has been reported regarding the direct protective action on isolated gland preparations in other species. Such gland preparations are isolated systems which still preserve the normal physiological cell interactions of the intact organ. Thus, the present study made use of this preparation to examine the direct cytoprotective action of zinc L-carnosine on rabbit gastric glands in-vitro.

Materials and methods

General. Male New Zealand White rabbits, 2·5–3 kg (13–15 weeks old) were kept in a temperature- (22 ± 1°C) and humidity (65–70%)-controlled room and starved but with free access to water for 24 h before experimentation.

Isolation of gastric cells. Animals were anaesthetized with a single intravenous dose of pentobarbitone (40 mg kg^{-1}). The abdominal cavity was opened and the stomach was rapidly removed. The organ was cut along the greater curvature and immediately washed in an oxygenated phosphate-buffered solution (PBS). The different mucus on the gastric mucosa was gently removed with a glass slide. The glandular mucosa was subsequently scraped off and incubated in a Falcon tube containing 10 mL PBS with collagenase (1 mg mL^{-1}). The gland suspension was incubated at 37°C with constant shaking for 50 min. After sedimentation, the supernatant with a small portion of digested glands was decanted and washed twice with 20 mL PBS containing bovine albumin solution. The gland pellets were then collected after centrifugation at 200 *g* for 2 min. The residue in the first digestion was further digested with 10 mL PBS with collagenase for another 20 min. When the second digestion was completed, the whole preparation was washed and centrifuged as in the first digestion. The gland pellets were finally collected and pooled with the glands obtained in the first digestion. The final gland concentration was adjusted to 20×10^4 glands mL^{-1}, by counting the gland number in a haemocytometer which could hold 10 μL of the gland solution. The examination was performed under a light microscope (200 × magnification). The gland preparation was subsequently used for studying drug action on the glands.

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Drug administration and viability test. Zinc L-carnosine (a gift from Central Research Laboratories, Zeria Pharmaceutical Co. Ltd, Saitama, Japan) and L-carnosine were dissolved in PBS at the final concentrations of 10^{-7}, 10^{-6} or 10^{-5} M. After 15 min of drug pre-incubation, ethanol 8%, (v/v) was added and incubation was continued for another 30 min. The whole experimental procedure was undertaken at 37°C with constant shaking. At the end of the incubation, 50 μL of the gland suspension was then mixed with an equal volume of 0·4% (w/v) trypan blue. Ten μL of this mixture was examined for gland viability in a haemocytometer under a microscope. The number of dead glands (more than one-third of the total number of cells in a gland stained with blue signified a dead gland) was counted in each sample and expressed as % of dead glands in the sample. The degree of gland damage was also assessed by the percentage of lactate dehydrogenase (LDH) released from the total LDH level (determined after sonication) in a sample during the 45 min incubation period (including 15 min drug pre-incubation plus 30 min ethanol incubation). The LDH activity was measured by monitoring the rate at which the substrate pyruvate was reduced to lactate. The reduction was coupled with the oxidation of NADH which was measured spectrophotometrically (Cary 219 spectrophotometer) in terms of reduced absorbance at 340 nm. Serum samples were also collected from the rabbits to determine the direct effects of zinc L-carnosine, L-carnosine or ethanol on serum LDH activity.

Statistical analysis. The data were analysed by a two-tailed Student's *t*-test and by analysis of variance.

Results

Zinc L-carnosine or L-carnosine incubated at the highest concentration, 10^{-5} M, did not affect the serum LDH activity; 8% ethanol, however, significantly reduced the enzyme activity by 24%. This depressive action was not reversed either by zinc L-carnosine or L-carnosine (Table 1). The same concentration of ethanol markedly labilized a significant amount of LDH (37·7% of the total gland LDH level) and produced 50% dead glands of the total gland population, significantly higher than those of the control without ethanol administration (Table 2). L-Carnosine pre-incubation did not affect the damaging actions of ethanol; however, zinc L-carnosine significantly inhibited the release of LDH ($F = 4{\cdot}13$, $P < 0{\cdot}05$) and increased the viability of the whole gland population ($F = 48{\cdot}49$, $P < 0{\cdot}001$).

Discussion

The present study shows the cytoprotective action of zinc L-carnosine on ethanol-induced gastric gland damage in-vitro. This protection was demonstrated by the increase of viability of glands and also by the reduction of LDH release from the gland

Table 1. Effects of ethanol (8%, v/v), zinc L-carnosine (10^{-5} M) and L-carnosine (10^{-5} M) on serum lactic dehydrogenase activity.

Pretreatment	Treatment	Lactate dehydrogenase (unit mL^{-1})
Phosphate buffer	Phosphate buffer	1952·5 ± 10·3
	Ethanol	1490·0 ± 31·1**
Zinc L-carnosine	Phosphate buffer	1947·5 ± 18·9
	Ethanol	1532·5 ± 53·4*
L-Carnosine	Phosphate buffer	1762·5 ± 88·5
	Ethanol	1532·5 ± 64·7*

Values indicate means ± s.e.m. of 5 samples from 3 rabbits. *$P < 0.01$, **$P < 0.001$ when compared with the corresponding phosphate buffer control.

Table 2. Effects of zinc L-carnosine or L-carnosine pretreatment (incubated for 15 min beforehand) on the gastric gland damage induced by ethanol (8%, v/v) in rabbits.

Treatment	Lactate dehydrogenase release (%)	Dead glands (%)
Phosphate buffer without ethanol	25·4 ± 1·7	4·5 ± 0·3
Phosphate buffer with ethanol	37·7 ± 3·1†	50·5 ± 1·9††
Zinc L-carnosine with ethanol		
10^{-7} M	29·6 ± 2·8	35·7 ± 2·3**
10^{-6} M	27·1 ± 1·8*	26·8 ± 1·2**
10^{-5} M	26·6 ± 2·2*	22·7 ± 1·5***
L-Carnosine with ethanol		
10^{-7} M	34·1 ± 2·7	45·8 ± 1·5
10^{-6} M	32·6 ± 2·6	46·7 ± 1·9
10^{-5} M	32·2 ± 2·6	45·5 ± 1·9

Values indicate means ± s.e.m. of 8 samples from 4 rabbits. *$P < 0.05$, **$P < 0.02$, ***$P < 0.001$ when compared with the corresponding controls. †$P < 0.01$, ††$P < 0.001$ when compared with the corresponding controls.

preparation. We also examined whether it was the zinc or the L-carnosine part of the zinc L-carnosine molecule which possessed the protective effect, since both had been demonstrated to provide an anti-ulcer effect in intact animals (Fitzpatrick & Fisher 1982; Cho 1989). It was found that L-carnosine was ineffective against ethanol-induced gland injury, thus indicating that zinc ion, but not L-carnosine, is the active component in the zinc L-carnosine molecule.

It has been shown that treatment with zinc sulphate reduces (Wong et al 1986), and zinc deficiency increases (Oner et al 1981) H^+ back-diffusion and Na^+ leakage from the gastric mucosa in rats, suggesting that zinc ion is important for maintaining the integrity of the cell membrane. Indeed, zinc by its reaction with the thiol groups in the plasma membrane has been reported to maintain the integrity of biomembranes (Chvapil 1973). Recently, zinc L-carnosine was found to be effective for repair of the injury induced by superoxide or singlet molecule oxygen-related free radical reaction (Yoshikawa et al 1988; Yoneta et al 1990). As oxygen free radicals have been demonstrated to play an important role in the formation of gastric lesions in-vivo (Parks et al 1983; Itoh & Guth 1984) and cell damage in-vitro (Hiraishi et al 1987), it is likely that zinc ion could scavenge oxygen free radicals and protect against gland damage. Thus, it is concluded that either the membrane stabilizing action of cell membranes or the decrease of free radical production produced by zinc L-carnosine, could be responsible for the prevention of ethanol-induced gland damage in rabbits.

Our study also demonstrated that zinc L-carnosine did not affect the serum LDH activity. This finding indicates that the reduction of LDH release from the gland preparation by the compound is the result of the direct action on the glands rather than on the depressive action on the enzyme activity measured. It also indicates that, unlike ethanol, zinc L-carnosine is a relatively nontoxic substance which does not affect the normal function of the enzyme system in our experimental conditions.

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