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Antiulcer effect of diltiazem in rats

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AIM: To study the effects of diltiazem (Dil) on several experimental gastric ulcers in rats. **METHODS**: The gastric mucosa damage was induced by indometacin (Ind), restraint, pyloric ligation, and absolute ethanol in rats. Dil 5, 25, 50 mg·kg^{-1} ig bid for 5 times. The number of gastric ulcers, the secretion of gastric juice, hydrochloric acid (HCl), and pepsin A were detected. The production of malondialdehyde (Mal) and the activity of superoxide dismutase (SOD) in gastric mucose were examined. **RESULTS**: Dil 5, 25, 50 mg·kg^{-1} ig protected the gastric mucosa against the damages in a dose-dependent manner. Dil inhibited the secretion of gastric juice, HCl, pepsin A, and Mal production of the gastric mucosa, but increased the activity of SOD in the gastric mucosa. The production of Mal was decreased from 9.3 ± 3.7 to 6.5 ± 1.9 μmol/g wet weight ($P<0.05$) and the activity of SOD was increased from 6.1 ± 5.6 to 12.8±2.8 kU/g protein ($P<0.01$) by Dil 50 mg·kg^{-1}. **CONCLUSION**: The inhibition of gastric secretion and lipid peroxidation induced by oxygen free radicals of gastric mucosa was related to the antiulcer effect of Dil in rats.

KEY WORDS diltiazem; stomach ulcer; gastric acid; pepsin A; malondialdehyde; superoxide dismutase; free radicals

The calcium channel blockers inhibit the entry of calcium ions into the cells with resultant inhibition of calcium effects on gastric function. Calcium channel blockers can inhibit the contractility of gastric muscle as well as basal and stimulated gastric acid secretion, and protect rats against experimental gastric lesions[1-4]. However, there are contradictory results in the literature on the effect of calcium channel blockers on gastric mucosal lesions[5]. Our current study was to determine the effects of diltiazem (Dil) on several experimental gastric ulcers in rats, and to examine the mechanisms responsible for such effects.

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MATERIALS AND METHODS

Dil (Sigma) and indometacin (Ind, The Third Pharmaceutical Factory of Beijing) were suspended in 1 % carboxymethyl cellulose (CMC) solution. Superoxide dismutase (SOD) and tetraethoxypropane (TEP) were from Sigma. Dimethyl sulfoxide (Me_2SO) was the product of Shanghai Sulfuric Acid Factory. Sodium diethyldithio-carbamate (DDC) was the product of Chemical Reagent General Factory of Shanghai. Luminol was from Merck.

Wistar rats of either sex ($n=206$) weighing 210 ±s 14 g were provided by Experimental Animal Center of Zhejiang Medical University.

Experimental gastric ulcer[6] Rats were randomly divided into control and Dil groups. Dil 5, 25, 50 mg·kg^{-1} were given ig bid (at 8:00 and 14:00) for 5 times. The control rats were treated similarly but with 1 % CMC. The rats were fasted for 36 h before the induction of ulcer, but allowed free access to water.

1 Ind Rats ($n=28$) were divided into 4 groups. One hour after the last ig Dil, Ind 30 mg·kg^{-1} was injected sc. Six hours after Ind injection, the rats were killed by stunning. The stomach was examined for mucosal damage.

2 Restraint Rats ($n=28$) were divided into 4 groups. One hour after the last ig Dil, rats were placed in a stress cage and immersed to the level of the xiphoid process in water at 23±1 C for 18 h. The

stomach was examined.

3 Pylorus ligation Rats ($n=32$) were divided into 4 groups. One hour after the last ig Dil, under ether anesthesia the abdomen was incised and the pylorus was ligated. The stomach was examined after 18 h.

4 Ethanol [7] Rats ($n=18$) were divided into 3 groups. One hour after the last ig Dil, absolute ethanol (1 mL) was given ig. The stomach was examined 1 h after ig ethanol.

Measurement of gastric lesions[6] Rats were sacrificed at the end of the experiment. The stomach of each rat was removed, filled with 5 mL of 1 % formalin solution and put into 1 % formalin solution for 10 min to fix the outer layer of stomach wall. The stomach was then incised along the greater curvature, spread on a pad and examined macroscopically. The inhibitory rate:

$$R_i=(U_c-U_t)/U_c\times 100\ \%$$

where U_c and U_t were the number of ulcers in control and tested rats, respectively.

Measurement of gastric juice[8] Gastric content was titrated with NaOH 10 μmol·L^{-1} using phenolphthalein as an indicator. The rate of secretion:

$$R_s(\text{mmol}\cdot\text{h}^{-1})=T_s/P_t$$

where T_s was the total acid output, and P_t is the tested period.

Pepsin assay The pepsin activity was determined by Anson's method, expressed as μg tyrosine·min^{-1}.

Measurement of lipid peroxide[9] **and activity of superoxide dismutase (SOD)**[10] **of gastric mucosa** Thirty Ind-treated rats were divided into 3 groups. Lipid peroxide content of the gastric mucosa was measured using the thiobarbituric acid method, expressed in terms of Mal.

Forty rats were divided into 5 groups (Control; Ind; Ind + Dil; DDC, DDC + Dil). SOD activity of gastric mucosa was measured with the alkaline Me_2SO luminol chemiluminescence method.

Statistical analysis The significance of differences was assessed by t test.

RESULTS

In the rats treated with Dil, the number of ulcers was declined dose-dependently (Tab 1).

In rats with pylorus-ligated ulcer, Dil decreased gastric juice secretion and acitvity of pepsin (Tab 1).

Dil declined lipid peroxide in gastric mucosa of rats with Ind-induced ulcer. A single dose of DDC 1 g·kg^{-1} sc inhibited gastric mucosal SOD activity in rats. The inhibition of SOD activity may return to normal level after treatment with Dil (Tab 2).

Tab 1. Effect of ig diltiazem 5 − 50 mg·kg^{-1} bid for 5 times on gastric ulcers, volume of gastric juice, secretion of gastric acid and activity of pepsin. Number of rats in parentheses. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control (1 % CMC).

Diltiazem/mg·kg^{-1}		0	5	25	50
Indometacin	Number of ulcers	15.4±5.8 (7)	13.4±5.3 (7)^a	8.9±4.6 (7)^b	6.0±2.9 (7)^c
	Inhibitory rate (%)	—	13	42	61
Restraint	Number of ulcers	12.3±3.2 (7)	8.6±3.2 (7)^a	5.4±2.6 (7)^c	3.8±3.0 (7)^c
	Inhibitory rate (%)	—	30	56	69
Pyloric ligation	Number of ulcers	17.8±1.7 (8)	13.9±2.7 (8)^c	7.6±2.4 (8)^c	4.6±1.4 (8)^c
	Inhibitory rate (%)	—	22	57	74
Absolute ethanol	Number of ulcers	8.3±1.4 (6)	4.2±3.3 (6)^b	2.8±1.5 (6)^c	—
	Inhibitory rate (%)	—	49	66	—
Pyloric ligation	Gastric juice/mL·h^{-1}	10.4±2.7 (7)	3.9±3.0 (8)^c	4.7±2.9 (8)^c	4.1±3.0 (7)^c
	Gastric acid/mL·h^{-1}	20.2±7.9 (7)	6.3±3.1 (8)^c	6.8±4.7 (8)^c	4.1±3.0 (7)^c
	Pepsin/tyrosine μg·min^{-1}	159.8±86.2 (7)	51.6±18.6 (8)^b	59.8±33.7 (8)^c	54.3±9.2 (7)^c

Tab 2. Effect of diltiazem (Dil) 50 mg·kg^{-1} ig on MDA and SOD. Number of rats in parentheses. $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$ *vs* control. $^{c}P<0.05$, $^{d}P<0.01$ *vs* Ind or DDC.

Group	MDA (μmol/g wet wt)	SOD (kU/g protein)
Control	6.2±3.1 (10)	10.5±4.9 (8)
IND	9.3±3.7 (10)^{b}	11.5±2.5 (8)^{a}
IND+Dil	6.5±1.9 (10)^{c}	14.9±4.8 (8)^{a}
DDC	—	6.1±5.6 (8)^{b}
DDC+Dil	—	12.8±2.8 (8)^{d}

DISCUSSION

The gastric ulcer formation in rats is complex and multifactorial. Pepsin and hydrochloric acid in the gastric juice are the aggressive factors inducing ulcers. Reports about calcium channel blockers influence on gastric acid secretion are controversial[2]. The present study clearly demonstrated that treatment of rats with Dil before Ind sc, restraint, pyloric ligation, ethanol ig decreased the number of gastric ulcer, inhibited the gastric acid and pepsin secretion, dose-dependently. These results showed that the improvement of the gastric lesions by Dil might be due to its antipeptic acitivity and inhibiton of gastric acid secretion.

Oxygen free radical may be involved in gastric mucosal lesions induced by restraint stress and ethanol. SOD may play an important role in the gastric mucosal defense mechanism against oxygen free radical[11-13]. The present results confirmed that lipid peroxidations were lowered in the gastric mucosa of Ind-induced ulcer treated with Dil. Gastric mucosal SOD activity was decreased by DDC, but increased by Dil. These results indicated that the effects of Dil on the experimental gastric ulcer were related to the inhibited production of oxygen free radical and the increased scavenge of oxygen free radicla in gastric mucosa.

The gastric mucosal damage induced by Ind and ethalol was related to the inhibition of synthesis of prostaglandin (PG) in gastric mucosa. The inhibiton of PG synthesized in gastric mucosa may stimulated the synthesis of leukotrienes (LT). Dil inhibited the gastric mucosal injury caused by Ind and ethanol. The antiulcer effect of Dil may be related to the increase of PG synthesis and the inhibition of LT production[3,13].

This study suggested that the antigastric ulcer effects of Dil might be to inhibit gastric juice secretion and pepsin acitivity, decrease lipid peroxidation and increase SOD acitivity in the gastric mucosa.

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地尔硫䓬抗大鼠胃溃疡作用

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目的：研究地尔硫䓬(Dil)抗大鼠实验性胃粘膜损伤的作用。**方法**：胃粘膜损伤由吲哚美辛、束缚应激、结扎幽门和无水乙醇引起，Dil ig，一日2次，共5次。**结果**：Dil 5，25，50 mg·kg^{-1} ig 能抑制大鼠胃粘膜损伤，抑制胃酸分泌及胃蛋白酶分泌和活性，减少胃粘膜中 Mal 含量（自 9.3±3.7 至 6.5±1.9 μmol/g wet wt），提高胃粘膜中 SOD 活性（自 6.1±5.6 至 12.8±2.8 kU/g protein）。**结论**：Dil 抗实验性胃溃疡作用与抑制胃液分泌、减少胃粘膜 Mal 含量和提高 SOD 活力有关。

关键词 地尔硫䓬；胃溃疡；胃酸；胃蛋白酶A；丙二醛；超氧化物歧化酶；自由基

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大鼠颈上神经节培养神经元烟碱电流及美加明的使用依赖性阻滞[1]

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Nicotinic currents of cultured rat superior cervical ganglion neurons and use-dependent block by mecamylamine

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AIM: Comparison of action of nicotinic agonists and antagonists on nicotinic acetylcholine receptor (nAChR) in superior cervical ganglion (SCG) neurons. **METHODS**: Whole-cell recordings were made from cultured neonatal rat SCG neurons. Cholinergic drugs were applied by local pressure perfusion. **RESULTS**: The neurons were activated by nicotinic agonists and peak current were acetylcholine (ACh), 443±183 pA; nicotine, 1175 ± 377 pA; dimethylphenylpiperazinium (DMPP), 2946±358 pA, respectively. The nicotinic responses were blocked by mecamylamine (Mec), hexamethonium and curare, the efficacies were 435±154 pA, 725±320 pA, 887±214 pA, but not by α-bungarotoxin. The block by Mec was use-dependent, ie, it was dependent on repeated presentation of the agonists. The first 6 peak currents were expressed as percentage of the first

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