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Role of active oxygen species in diethyldithiocarbamate-induced gastric ulcer in the rat

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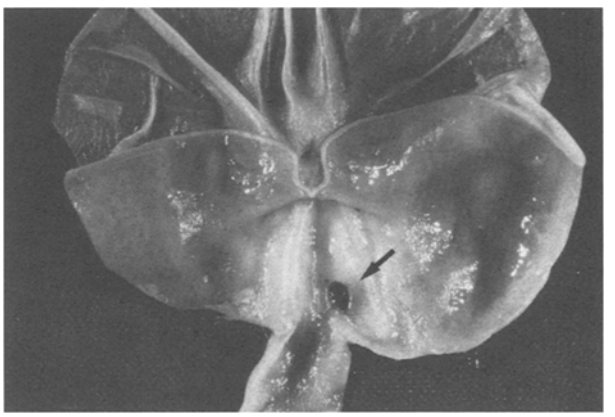
Summary. Diethyldithiocarbamate, an inhibitor of Cu,Zn-superoxide dismutase, was recently found to be ulcerogenic in the rat stomach, and active oxygen species were found to be responsible for its ulcerogenicity. To clarify which active oxygen species play a role in ulcerogenesis, the effects of various scavengers and iron-chelators were studied. As superoxide dismutase and catalase reduced the ulcerogenesis induced by diethyldithiocarbamate, the superoxide radical and hydrogen peroxide were considered to play a pathogenic role in this ulcer model.
Key words. Diethyldithiocarbamate; gastric ulcer; superoxide dismutase; catalase.

Recent studies have indicated that active oxygen species contribute to mucosal injury in the alimentary tract, and as superoxide dismutase (SOD) prevents this mucosal injury, its role in the alimentary tract has received much attention[1,2]. We previously reported that diethyldithiocarbamate (DDC), an inhibitor of Cu,Zn-SOD, showed ulcerogenicity in rat stomach, and that the effect was due to active oxygen species[3–6]. In this study, we investigated which active oxygen species played a role in the induction of antral ulcers by DDC in the rat.

Materials and methods

The experimental ulceration method used in this study has already been described in detail[6]. Briefly, rats, each weighing 200–230 g, previously fasted for 24 h, were anesthetized with ether. After pylorus ligation, DDC 800 mg/kg was injected subcutaneously and 1 ml of 0.1 N HCl was administered orally. Seven hours later, a large ulcer was observed in the antrum along the lesser curvature, penetrating into the muscular layer (fig.). The rats were killed and the stomach was filled with 10% formalin. The ulcer index was measured under a dissecting microscope with a square-grid eyepiece and expressed as the area of the antral ulcer (mm^2).

Study 1: In a preliminary study, the effect of the concomitant administration of HCl was investigated. Two groups, one treated with 0.1 N HCl and another without HCl, were studied. Both groups were subjected to the same procedures except for HCl administration.



Photograph of a stomach with antral ulcer (arrow) induced by diethyldithiocarbamate, pylorus ligation and 0.1 N HCl.

Study 2: Eleven different pretreatment regimens were assigned to groups of animals as follows:
1) superoxide dismutase (SOD) (Sigma Chemical Co.) 60,000 U/kg; 2) catalase (Cat) (Sigma Chemical Co.) 500,000 U/kg; 3) SOD plus Cat; 4) mannitol (Wako Pure Chemical Industry) 400 mg/kg, three times; 5) dimethyl sulfoxide (DMSO) (Wako Pure Chemical Industry) 20 mg/kg; 6) DMSO 40 mg/kg; 7) 5% DMSO 1 ml; 8) diethylenetriaminepentacetic acid (DETAPAC) (Wako Pure Chemical Industry) 250 mg/kg; 9) deferoxamine (DFO) (Ciba-Geigy) 30 mg/kg; 10) DFO 60 mg/kg; 11) DFO 100 mg/kg.

SOD, Cat, DMSO, DETAPAC and DFO were administered subcutaneously 20 min before the experiment. Five percent DMSO was administered orally 60 min before the experiment. Mannitol was administered subcutaneously 30 min before, and 2 h and 4 h after the experiment.

Results are expressed as the mean ± standard error. As this model is very much affected by experimental conditions (temperature, humidity, time of day and so on), it is essential to have a control for each time-point and to make comparisons only with the control group which was studied at the same time and under the same conditions. Statistical analyses were performed by Student's t-test comparing each pretreatment group with its control group. $p < 0.05$ was considered significant.

Results

Study 1: As a result of the concomitant HCl administration, the ulcer index increased and incidence of ulcer reached 100% (table 1).

Study 2: Table 2 shows the effect of drug pretreatment on DDC-induced antral ulcer.

1) SOD and Cat study: Pretreatment with SOD, Cat and SOD plus Cat significantly reduced the ulcer index. Inhibition rates were 65%, 54% and 76%, respectively. 2) DMSO and mannitol study: Pretreatment with DMSO and mannitol showed no effect against the ulcerogenicity of DDC. 3) DETAPAC and DFO study: Pretreatment with DETAPAC and DFO failed to protect against the action of DDC. DFO 100 mg/kg caused a significant increase in ulcer index.

Table 1. Effect of concomitant HCl administration on DDC-induced antral ulcer.

Treatment	Number of rats	Ulcer index (mm^2)	Incidence of lesion*	Incidence of ulcer
Without HCl	10	3.7 ± 1.1	10/10	8/10
With HCl	10	4.7 ± 0.6	10/10	10/10

The data show the mean ± SE. *Lesion means erosion, which is by definition a disruption of the epithelium; and ulcer, which is defined as damage penetrating through the muscular layer.

Table 2. Effects of scavengers and iron-chelators on DDC-induced antral ulcer.

Name of drug	Ulcer index (mm^2) Pretreatment	Control
SOD 60,000 U/kg	2.16 ± 0.56 (7)**	6.16 ± 1.23 (8)
Cat 500,000 U/kg	3.33 ± 0.95 (6)**	7.25 ± 1.00 (6)
SOD + Cat	1.50 ± 0.81 (7)**	6.16 ± 1.23 (8)
Mannitol 400 mg/kg × 3	8.60 ± 2.16 (6)	4.31 ± 0.41 (7)
DMSO 20 mg/kg	4.65 ± 0.40 (6)	4.26 ± 0.49 (7)
DMSO 40 mg/kg	4.88 ± 0.43 (6)	4.31 ± 0.41 (7)
5% DMSO 1 ml p.o.	5.40 ± 0.89 (6)	4.31 ± 0.41 (7)
DETAPAC 250 mg/kg	6.00 ± 0.71 (7)	4.31 ± 0.41 (7)
DFO 30 mg/kg	6.67 ± 1.15 (7)	6.16 ± 1.23 (8)
DFO 60 mg/kg	4.35 ± 0.89 (7)	6.16 ± 1.23 (8)
DFO 100 mg/kg	7.30 ± 1.11 (7)*	4.31 ± 0.41 (7)

The data show the mean ± SE. The number of rats used in each group is shown in parentheses. *$p < 0.05$; **$p < 0.01$ when compared with its own control.

Discussion

DDC is an inhibitor of Cu,Zn-SOD and has been reported to worsen various kinds of tissue damage due to active oxygen species [7–9]. Previous reports showed that DDC was ulcerogenic and that active oxygen species were involved in the pathogenesis of the mucosal injury [3–6]. One of the causes of this ulcerogenicity may be that the reduction of SOD activity lowers the protective power of gastric mucosa against active oxygen species.

In the preliminary study, the effect of concomitant HCl was studied. HCl administration is sometimes used to aggravate the mucosal injury [10–12]. This effect has been reported to be due to a decrease in mucosal blood flow [11] and the profound decrease in intramural pH caused by back diffusion of luminal H^+ [12]. According to this study, DDC by itself showed ulcerogenicity, but DDC treatment without HCl failed to cause ulceration in some rats. As the deep ulcer is the specific feature being studied, concomitant HCl administration was considered to be advantageous, since the ulcer incidence was then 100%. DDC-induced antral ulcer is characterized by its similarity to human gastric ulcer [6], because the mucosal injury is located in the lesser curvature of the antrum and penetrates into the muscular layer [13, 14]. As it has recently been suggested that active oxygen species are responsible for gastric mucosal injury [1], this type of ulcer is a useful model for the elucidation of the etiology of human gastric ulcer. In this study, the effects of various scavengers and iron-chelators were studied to identify which active oxygen species play a pathogenic role in this model.

The effects of SOD and Cat in preventing this ulcer suggest that the superoxide radical and hydrogen peroxide contribute to its etiology.

The superoxide radical can interact with hydrogen peroxide in the presence of iron (the so-called 'iron-catalyzed Harber-Weiss reaction' or 'Fenton reaction') to generate the hydroxyl radical [15], which is thought to be the most toxic reactant. The effects of SOD and Cat in our studies might have been due to the prevention of hydroxyl radical generation, by the removal of the superoxide radical and hydrogen peroxide. However, though a dosage large enough to scavenge hydroxyl radical was used, pretreatment with DMSO [1, 16] and mannitol [17] failed to protect the gastric mucosa against the toxic action of DDC; in fact, they rather had a tendency to worsen the ulcerogenicity. This suggests that the hydroxyl radical may play only a minor role in gastric mucosal injury induced by DDC.

Secondly, the effects of iron-chelators were investigated. DETAPAC 250 mg/kg has been reported to protect to a large extent against the diabetes induced by alloxan [18], which produces its toxic effect as a result of hydroxyl radical generation. However, in this model DETAPAC has no effect. As DETAPAC can promote or inhibit the

generation of hydroxyl radicals, depending on the ratio of chelator to iron salt concentration, another chelator, deferoxamine, was investigated and was found to be a powerful inhibitor of hydroxyl radical generation at any concentration [19]. Though it was studied in three concentrations, the results were the same as those for DETAPAC, and 100 mg/kg DFO tended to increase the ulcerogenicity of DDC.

On the basis of the present findings, it appears that the superoxide radical and hydrogen peroxide play a more important pathogenetic role in DDC-induced antral ulcer in the rat than does the hydroxyl radical.

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Hypothalamic histamine modulates adaptive behavior of rats at high environmental temperature

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Summary. Histamine content in the rat hypothalamus was lower at 4 °C and higher at 31 °C compared to that at 21 °C. Pretreatment with α-fluoromethylhistidine, a 'suicide' inhibitor of histidine decarboxylase, attenuated both the increased level of hypothalamic histamine and rat adaptive behavior at 31 °C. Increase of histamine content in the hypothalamus appears to be an important factor contributing to rat adaptive behavior to high environmental temperature.
Key words. Histamine; hypothalamus; environmental temperature; adaptive behavior.

To adapt to high environmental temperature, rats decrease food intake and ambulation, and increase water intake [1-3]. These behavioral changes help to maintain their body temperature [1-3]. Administration of histamine into the rat central nervous system has been shown to increase water intake [4, 5], decrease food intake [6], and lower body temperature [7, 8]. Although these findings suggest that brain histamine may contribute to the adaptation of the rat to a heated environment, the function of brain histamine in adaptation to environmental change in temperature is still obscure. The present experiment was aimed at investigating the relationship between hypothalamic histamine and behavioral adaptation to high environmental temperature.

Materials and methods

Subjects. Mature male Wistar King A rats, 270–320 g, were used. They were in a sound-proof room illuminated daily from 08.00–20.00 h (a 12:12 h light-dark cycle) with humidity at 45 ± 5%. Room temperature was maintained at 21 ± 1 °C unless otherwise described. The rats used were reared under these conditions from the prenatal period.

Analytical procedure for histamine and catecholamines in the brain. Rats were sacrificed by decapitation at 19.00 h. The hypothalamus and frontal cortex were isolated and homogenized [9]. The homogenates were centrifuged at 10,000 × g, and the histamine content in the clear deproteinized supernatants was assayed using high-performance liquid chromatography (HPLC) [10]. Catecholamines in the supernatant were also analyzed in a fully automated HPLC-fluorometric system (Model HLC-8030 Catecholamine Analyzer, Tosoh, Japan) using the diphenylethylenediamine condensation method [11]. Samples were taken from 9 rats maintained at 21 ± 1 °C room temperature as the control group, from