Ulcer Formation in Rat Stomach with Diethyldithiocarbamate

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Summary It has been suggested that superoxide radicals play an important role in the pathogenesis of gastric mucosal lesions after ischemia. To prove a participation of $O_2^-$ in gastric mucosal lesion formation, diethyldithiocarbamate (DDC), an inhibitor of Cu,Zn-superoxide dismutase (SOD), was injected into rats and its ulcerogenicity was examined. Subcutaneous injection of DDC was employed, because of high mortality after intraperitoneal injection of the drug. At 7 h after the injection of the drug, many ulcers appeared in the stomach, with suppression of SOD activities in the gastric mucosa, suggesting the participation of $O_2^-$ in ulcer formation in the stomach.

Key Words: diethyldithiocarbamate, ulcer, rat stomach

Heikkila et al. [1] have reported that diethyldithiocarbamate (DDC), a powerful copper-chelating agent, reduces the superoxide dismutase (SOD) activity in the brain, liver, and erythrocytes. Frank et al. [2] reported that there is a positive association between decreased SOD activity due to DDC treatment and decreased $O_2$ tolerance in young animals normally tolerant to hyperoxia. Therefore, it is convincing that DDC lowers SOD activity and thus increases the superoxide radical ($O_2^-$) level in various organs and tissues.

Recently it has been demonstrated that active oxygen species, $O_2^-$ or $\cdot OH$, may be involved in the pathogenesis of hemorrhagic shock-induced gastrointestinal lesions [3-6].

In this study, we observed the effect of DDC on gastric mucosal lesions and on SOD activity.

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MATERIALS AND METHODS

Male Donryu rats were purchased from Seiwa Animal Farm. The chemicals purchased for this study were DDC, hypoxanthine, hydroxylamine, sulfanilic acid from Nakarai Chemicals, hydroxylamine-O-sulfonate from Merck-Schuchardt Co., xanthine oxidase from Boehringer-Mannheim Co., N-1-naphthyl-ethylene-diamine from Tokyo-Kasei Co., and human erythrocyte SOD from Sigma Chemical Co.

Rats, 200–230 g, were fasted for 48 h but allowed free access to water. They were injected with 0.25 to 2.0 g of DDC/kg and the difference between intraperitoneal injection and subcutaneous injection was assessed in terms of survival. Ulcer formation of the stomach was studied after the subcutaneous injection of DDC. The sum of the length of each mucosal ulcer per rat was used as the ulcer index [7].

After the administration of DDC, rats were sacrificed at 1 h, 3 h, and 7 h, and the stomachs were removed immediately and placed in ice-cold buffer (0.1 M Tris-HCl at pH 7.4). After homogenization the homogenate was then centrifuged at 9,000 × g for 20 min. The supernatant obtained was used for the measurement of SOD activity, which was determined by the method of Ōyanagi [8].

RESULTS

**DDC and survival**

The results of dose-response studies (DDC, 0.2 to 2.0 g/kg) in intraperitoneal and subcutaneous injection groups are shown in Table 1. The calculated LD$_{50}$ values were 0.9 g/kg (intraperitoneal) and 1.3 g/kg (subcutaneous). There was a sharp decline in survival following subcutaneous injection of DDC between the dosage levels of 0.8 g/kg and 2.0 g/kg.

<table>
<thead>
<tr>
<th>DDC dose (g/kg)</th>
<th>Intraperitoneal injection</th>
<th>Subcutaneous injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10/10 (100)</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>0.2</td>
<td>10/10 (100)</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>0.4</td>
<td>10/10 (100)</td>
<td>10/10 (100)</td>
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<tr>
<td>0.8</td>
<td>6/10 (60)</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>1.0</td>
<td>4/10 (40)</td>
<td>8/10 (80)</td>
</tr>
<tr>
<td>1.5</td>
<td>0/10 (0)</td>
<td>2/10 (20)</td>
</tr>
<tr>
<td>2.0</td>
<td>0/10 (0)</td>
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Rats were injected with 0.2 to 2.0 g of DDC/kg and sacrificed 7 h post-injection. Control received saline. Values in parentheses, per cent survival.

Ulcer formation with diethyldithiocarbamate

Ulcer formation in the stomach
At 7 h after a single injection of DDC, many ulcers appeared in the stomach (Fig. 1). Ulcer index was dose-dependent (Table 2). The site of ulcer formed was almost always the fundus.

SOD activity in the gastric mucosa
Changes in SOD activity in the gastric mucosa at early times after DDC injection are shown in Fig. 2. By 1 h after a single subcutaneous injection of DDC (800 mg/kg), the SOD activity reached its lowest level, and by 7 h had returned partially to its initial value.

DISCUSSION

Active oxygen species, $O_2^-$ or $\cdot$OH, have been shown to play a role in the pathogenesis of ischemia-induced injury in a variety of different tissues [9–11].
It has been also demonstrated that $O_2^-$ or $\cdot$OH is involved in ischemic mucosal injury in the cat and rat stomach [5, 6].

According to a recent study, $O_2^-$ in ischemic gastrointestinal tissue is generated by the xanthine oxidase-hypoxanthine system [12]. With ischemia, adenosine triphosphate and adenosine diphosphate levels decreased [13], because of the reduced oxidative phosphorylation and increased adenosine monophosphate level [14]. The increased adenosine monophosphate could be further catabolized to intermediates such as adenosine, inosine, and hypoxanthine. Xanthine oxidase, which is converted from xanthine dehydrogenase with reperfusion after ischemia [5, 15–18], acts aerobically hypoxanthine and produces $O_2^-$. SOD is known to be a scavenger of $O_2^-$. DDC as a copper chelator is known to be an inhibitor of Cu, Zn-SOD [1]. However, DDC has been previously used to inhibit certain other Cu-containing enzymes including aldehyde dehydrogenase in the liver [19] and dopamine-$\beta$-hydroxylase in adrenergic nervous tissue [20]. DDC is highly toxic. In young rats, there is an association between decreased SOD activity after DDC treatment and decreased $O_2^-$ tolerance [2]. It is not easy at present to explain DDC toxicity, but it may be due in part to superoxide radicals produced in many organs and tissues as a result of decreased Cu,Zn-SOD activity.

In the present study, DDC caused gastric ulcers and decreased SOD activity in the gastric mucosa. The mechanism of ulcerogenicity of DDC is not clear because it is now uncertain whether DDC may affect other gastric mucosal defense factors such as prostaglandins, mucosal blood flow, and mucous gel. However, we suggest that superoxide radicals generated by the decrease in SOD may cause gastric mucosal damage.

Fig. 2. Changes in gastric mucosal superoxide dismutase activity after subcutaneous injection of diethyldithiocarbamate. Dose of DDC, 800 mg/kg. Mean ± SE is given.
REFERENCES


