A Possible Mechanism for the Gastric Mucosal Protection by Oren-Gedoku-To (OGT), a Traditional Herbal Medicine

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Abstract—We investigated the involvement of sulfhydryl-compounds in the cytoprotective effect of Oren-gedoku-to (OGT) against ethanol-induced gastric lesions and potential difference (PD) reduction in comparison with those of sucralfate and glutathione in rats. Pretreatment with indomethacin (IND) had little influence on the cytoprotective effects of OGT and glutathione, but attenuated the effect of sucralfate. Pretreatment with N-ethylmaleimide (NEM) blocked the cytoprotective effects of these three drugs. Pretreatment with iodoacetamide diminished the cytoprotection of OGT and glutathione, but had little effect on that of sucralfate. The cytoprotective activities of OGT, glutathione and sucralfate were little affected by pretreatment with 6,6'-dithiodinicotinic acid. The inhibitory effects of OGT and glutathione against the PD reduction were completely blocked by pretreatment with NEM, while they were not influenced by pretreatment with IND. On the other hand, the inhibitory effect of sucralfate disappeared both by IND and NEM. These results suggest that the cytoprotective effect of OGT may be mediated by endogenous sulfhydryl compounds, but not by endogenous prostaglandins in the gastric mucosa.

We reported previously that Oren-gedoku-to (OGT) prevented the development of experimentally induced acute ulcers (1). Though OGT had a moderate antisecretory activity (2), the main mechanism of its antiulcer effect seemed to be related to an increase of mucosal barrier resistance because OGT inhibited ethanol-induced potential difference (PD) reduction at the doses that protected gastric mucosa from ethanol injury (1, 3). Furthermore, we found that the cytoprotective effect of OGT might be ascribed to the potentiations of its component herb drugs (4).

Recently, it has been shown that endogenous sulfhydryl compounds, in addition to endogenous prostaglandins (PGs), might play an important role in the defense mechanisms of gastric mucosa (5). N-Ethylmaleimide (NEM) and iodoacetamide (IDA) have been used as selective sulfhydryl alkylating reagents to determine the role of sulfhydryl radicals (6), because these two drugs, unlike 6,6'-dithiodinicotinic acid (DTA), are capable of reacting with certain accessible sulfhydryl groups (7, 8). Indomethacin (IND) is known to produce a deficiency of endogenous PGs in the gastric mucosa (9).

In the present study, we examined the possible involvement of endogenous sulfhydryl compounds and endogenous PGs in the protective effect of OGT against ethanol-induced gastric lesions and PD reductions using sulfhydryl blockers or IND in comparison with those of sucralfate and glutathione in rats.

Materials and Methods

Animals and drugs: Male Wistar rats (ST, substrain from Shizuoka Lab. Co., Ltd.), weighing 170–230 g, were used. The animals were fasted overnight, but allowed free access to water up to the beginning of the experiment. OGT (yield: 17.5%) was extracted with hot water from Coptidis rhizoma, Scutellariae radix, Phellodendri cortex and Gardeniae fructus combined in the ratio of 1.5:3:1.5:2, and the aqueous extract was concentrated and spray-dried in a hot air stream as de-
scribed previously (4). The drugs used are as follows: ethanol (Wako), sucralfate (Chugai), reduced form glutathione (Wako), N-ethylmaleimide (Wako), iodoacetamide (Wako), 6,6'-dithiodinicotinic acid (Wako), indomethacin (Sigma). OGT, sucralfate, glutathione, NEM, IDA and DTA were dissolved in saline, and IND was suspended in a 5% gum arabic solution. Each drug was given in a volume of 5 ml/kg of body weight.

**Ethanol-induced gastric lesions:** Gastric mucosal lesions were produced according to the method of Robert et al. (10). Each drug or saline vehicle was given to individual rats orally 30 min prior to oral administration of 1 ml of 99.5% ethanol. One hr after ethanol treatment, the animals were sacrificed. The stomach was removed and inflated by injecting 10 ml of 1% formalin to the gastric lumen for 10 min. Subsequently, the stomach was incised along the greater curvature and examined for lesions. The length (mm) of each lesion was measured under a dissecting microscope (×10) with a square grid and the sum per stomach was used as the lesion index. IND (5 mg/kg) was given subcutaneously (s.c.) 2 hr before ethanol treatment. NEM (10 mg/kg) and IDA (20 mg/kg) were given s.c. 1 hr before ethanol administration. DTA (50 mg/kg) was administered orally 50 min before ethanol administration.

Groups of non-pretreated rats were treated with OGT (10, 25, 50, 100, 250 and 500 mg/kg), sucralfate (50, 100 and 500 mg/kg), glutathione (50, 100, 250 and 500 mg/kg) or vehicle. Groups of NEM-pretreated rats were treated with OGT (100, 250 and 500 mg/kg), sucralfate (100, 500 and 1000 mg/kg), glutathione (100, 250, 500 and 1000 mg/kg) or vehicle. Groups of IDA-pretreated rats were treated with OGT (100, 250 and 500 mg/kg), sucralfate (50, 100 and 500 mg/kg), glutathione (100, 250 and 500 mg/kg) or vehicle. Groups of DTA-pretreated rats were treated with OGT (50, 100 and 250 mg/kg), sucralfate (50, 100 and 250 mg/kg), glutathione (50, 100 and 250 mg/kg) or vehicle. Groups of IND-pretreated rats were treated with OGT (25, 50, 100 and 250 mg/kg), sucralfate (100, 500 and 1000 mg/kg), glutathione (50, 100, 250 and 500 mg/kg) or vehicle. At least three doses of a drug, each tested in a group of 5 to 13 animals, were used to obtain a dose-response (% inhibition of gastric lesions) relationship.

**Gastric PD:** The experimental procedure was essentially the same as that described by Nagashima et al. (11). Under anesthesia with urethane (1.25 g/kg, i.p.), the trachea of each rat was cannulated. The abdomen was opened and the stomach exposed. The esophagus was ligated without disturbing the vagus nerves. One catheter filled with 3% agar in saturated KCl was inserted into the stomach through an incision in the duodenum and served as the intragastric electrode. A temporary gastric fistula was prepared using a polyethylene tube in the forestomach. The fistula that led to a three-way tap was used for intragastric instillation and for removal of gastric contents. A second catheter filled with 3% agar and saturated KCl was inserted into the peritoneal cavity and served as the indifferent electrode. The intragastric and intraperitoneal electrodes were both placed in separate beakers containing saturated KCl solution in which balanced Ag-AgCl electrodes were positioned. The whole interior of the stomach was gently rinsed with warm saline 3–4 times, and then 4 ml of saline was instilled into the stomach. The changes in PD were continuously monitored using a recorder connected to the millivoltmeter. PD recordings were taken at 3-min intervals. The gastric PD measurements were done at the corpus portion where the tip of the detecting electrode was placed. Each drug was intragastrically administered prior to treatment with 50% ethanol. IND (5 mg/kg) and NEM (10 mg/kg) were administered subcutaneously 1.5 hr and 0.5 hr before ethanol treatment, respectively.

**Statistical analysis:** ED50 values and 95% confidence limits were determined by Finney's probit analysis (12). The data were analyzed by two-way analysis of variance and the differences between treatment groups compared by the Dunnett's t-test or Duncan's multiple range test.

**Results**

Effects of OGT, sucralfate and glutathione on ethanol-induced gastric lesions in the absence or presence of NEM, IDA, DTA and IND, respectively: OGT, sucralfate and glu-
thione prevented the formation of ethanol-induced lesions in a dose-related manner. ED50 values for OGT (10–500 mg/kg), sucralfate (50–1000 mg/kg) and glutathione (50–1000 mg/kg) on ethanol-induced gastric lesions in the absence or presence of NEM, IDA, DTA and IND, respectively, are summarized in Table 1. ED50 values for OGT, sucralfate and glutathione (the doses that produced 50% inhibition of ethanol-induced gastric lesions) were 54.8 (95% confidence limits: 44.8–66.4), 212 (177–260) and 135 (114–158) mg/kg, respectively. The ED50 values and 95% confidence limits for OGT were shifted to 473 (382–657), 237 (197–285), 71.5 (51.9–89.7) and 81.7 (62.1–110) mg/kg after NEM, IDA, DTA and IND, respectively; the protective doses of OGT against ethanol in the presence of NEM, IDA, DTA and IND were increased 8.6-, 4.3-, 1.3- and 1.5-fold, respectively, above the ethanol alone model. The administration of sucralfate at 100 and 500 mg/kg had no inhibitory effect after pretreatment with IND (data not shown); and at the dose of 1000 mg/kg, it inhibited only 50% of the gastric lesions. The ED50 values and 95% confidence limits for glutathione were shifted to 714 (570–970), 503 (433–625), 92.6 (81.6–105) and 149 (121–182) mg/kg after NEM, IDA, DTA and IND, respectively; the protective doses of glutathione against ethanol in the presence of NEM, IDA, DTA and IND were increased 5.3-, 3.7-, 0.7- and 1.1-fold, respectively, above the ethanol alone model.

Effects of OGT, sucralfate and glutathione on ethanol-induced PD reduction by pretreatment with IND and NEM: As shown in Table 2, OGT (100 and 250 mg/kg), sucralfate (500 mg/kg) and glutathione (100 and 250 mg/kg) significantly prevented the PD reduction induced by ethanol. Although the data are not shown, intragastrical administration of OGT, sucralfate and glutathione had no effect on the basal PD. After intragastrical instillation of 2 ml of 50% ethanol, the control rats without and with NEM (10 mg/kg, s.c.) or IND (5 mg/kg, s.c.) pretreatment had a PD reduction of 19.3±2.9 mV (N=10) and 16.4±1.3 mV (N=5) or 17.6±1.9 mV (N=5), re-

### Table 1

| Treatment | OGT (mg/kg) | (95% confidence limits) | sucralfate (mg/kg) | (95% confidence limits) | glutathione (mg/kg) | (95% confidence limits) |
|-----------|-------------|------------------------|-------------------|------------------------|---------------------|------------------------|
| None      | 54.8        | (44.8–66.4)            | 212               | (177–260)              | 135                 | (114–158)              |
| NEM       | 473         | (382–667)              | 1220              | (850–2220)             | 714                 | (570–970)              |
| IDA       | 237         | (197–285)              | 258               | (206–339)              | 503                 | (433–625)              |
| DTA       | 71.5        | (51.9–89.7)            | 287               | (224–395)              | 92.6                | (81.6–105)             |
| IND       | 61.7        | (62.1–110)             | 1000              | <                     | 148                 | (121–182)              |

Gastric lesions were produced by ethanol (99.5%, 1 ml/rat, p.o.) in 24 hr-fasted rats. Each drug was given orally 30 min before administration of ethanol in rats. NEM (10 mg/kg, s.c.), IDA (20 mg/kg, s.c.), DTA (50 mg/kg, p.o.) or IND (5 mg/kg, s.c.) was given at 60, 60, 50 or 120 min before administration of ethanol, respectively. Animals were killed 1 hr after ethanol treatment. ED50 values were calculated according to probit analysis, and confidence limits are shown in parentheses. N=from 5 to 13 rats per each treatment group.
Fig. 1. Typical recording of influence of Oren-gedoku-to (OGT) on ethanol-induced PD reduction. OGT (right) or saline (left) was intragastrically administered prior to treatment with 50% ethanol at the closed circles. (A) Non-pretreated rats, (B) indomethacin (5 mg/kg, s.c.)-pretreated rats and (C) N-ethylmaleimide (10 mg/kg, s.c.)-pretreated rats. These values were not statistically different, indicating that NEM and IND did not affect 50% ethanol-induced PD reduction. In addition, subcutaneous administration of NEM or IND had no significant action on the basal PD (data not shown). Pretreatment with NEM significantly suppressed the inhibitory effect of OGT on ethanol-induced PD reduction, but pretreatment with IND had no effect on the inhibitory activity of OGT compared with OGT treatment alone (Table 2 and Fig. 1). The inhibitory effect of glutathione (100 and 250 mg/kg) on ethanol-induced PD reduction was unaffected by pretreatment with IND, but diminished prior to NEM, except for at 250 mg/kg (Table 2). On the other hand, the inhibitory effect of sucralfate (500 mg/kg) was invalidated both by NEM and by IND.

Discussion

Szabo et al. (5, 13) have proposed that gastric cytoprotection might be mediated through at least two different mechanisms, one concerns PGs and the other, sulfhydryl-containing substances of the mucosa. On the contrary, there are controversial data about the involvement of sulfhydryl compounds (mainly reduced glutathione) in gastric cytoprotective effects (14). However, in our experiments, oral administration of glutathione...
markedly inhibited ethanol-induced lesions, in accordance with the previously reported result of intraperitoneal administration with glutathione (15).

The present study has shown that OGT was 3.9 times as potent as sucralfate and 2.5 times as potent as glutathione in the ethanol alone model. Furthermore, the inhibitory potency of OGT was greater than those of sucralfate and glutathione even in the presence of all blockers tested, thereby suggesting that OGT gives prominent mucosal protection against necrotizing agents.

The protective dose (ED50 values) of OGT against ethanol damage was increased only 1.5-fold by pretreatment with IND, while that of sucralfate was increased 4.7-fold. The protective effect of sucralfate is considered to be partly due to endogenous PGs (16), and OGT has been reported to be devoid of mild irritant effects to gastric mucosa (1, 3). Consequently, the mucosal protection of OGT is apparently not mediated through endogenous PGs.

The protective effect of OGT was decreased in the presence of NEM and IDA, respectively, whereas that of sucralfate was decreased merely in the presence of the former. Although both sulfhydryl blockers have been shown to attenuate the protective activities of exogenous PGs and polyamines (13, 15), the action sites of NEM are reported to be different from those of IDA (17, 18). These findings probably imply that the action sites of OGT differ from those of sucralfate.

On the other hand, reduced glutathiones, which have been found to be the major component of the endogenous nonprotein sulfhydryl pool (19, 20), are assumed to participate in the gastric protective mechanism in our experiments. The protective doses of glutathione were increased 5.3- and 3.7-fold by pretreatment with NEM and IDA, respectively, but not changed by IND. These results coincide with those by Szelenyi and Brune (21) who reported that the protective effects of exogenous sulfhydryl compounds are mediated through endogenous sulfhydryl compounds, but not through endogenous PGs. This action mode of glutathione was similar to that of OGT, thereby suggesting that endogenous sulfhydryl compounds may be at least partly involved in the protective effect of OGT.

In addition, since the gastric mucosal protection by OGT as well as those by sucralfate and glutathione were not affected by pretreatment with DTA, an extracellular blocker (8), the protective effect of OGT seems to be not associated with extracellular sulfhydryl compounds.

The integrity of the gastric mucosal barrier is affected by many factors such as mucus secretion, acid secretion or gastric blood flow (22-24). The gastric PD, which is an important index of mucosal resistance (25-27), has been thought to be closely related to the cytoprotective functions. The recovery of PD reduction following ethanol administration was reported to be due to the reestablishment of the mucosal integrity (28, 29). The present results have shown that OGT, sucralfate and glutathione exert mucosal protection by maintaining the integrity of the mucosal barrier, since these three drugs prevented ethanol-induced PD reduction. The inhibitory effect of OGT against the PD reduction completely disappeared by NEM. Likewise, glutathione showed an effect similar to that of OGT at 100 mg/kg. Reduced glutathiones are able to scavenge active free radicals (30), which are considered to be the main cause of gastric mucosal necrosis. The above results suggested that the protective mechanism of OGT against ethanol injury was almost similar to that of glutathione. Moreover, OGT is reported to scavenge active free radicals (31).

It is, therefore, probable that OGT protects the gastric mucosa from ethanol injury by scavenging active free radicals. However, the difference between the effect of OGT and that of glutathione was observed at 250 mg/kg. NEM has been observed to alter and inactivate the conformation and the function of various agonist receptors (32, 33). These findings suggest that the conformational and functional maintenance of NEM-sensitive sulfhydryl compounds may play an important role in the maintenance of mucosal barrier integrity afforded by OGT. On the other hand, the protective effect of glutathione at high dose seems to be associated with a mechanism other than the maintenance of the conformation and function of endogenous
sulfhydryl compounds.

As mentioned above, OGT exhibited a unique mucosal protection. We have previously shown that the protective effect of OGT is attributed to the potenatations of the component herb medicines, though the degrees of the inhibitory effects of Coptidis rhizoma and Phellodendri cortex extracts against ethanol damage are superior to those of OGT, Gardeniae fructus and Scutellariae radix extracts (4). However, further studies are required to examine which of the component herb drugs and ingredients are involved in the mechanism of mucosal protection obtained with OGT.

In conclusion, the present study revealed that the mechanism of mucosal protection of OGT against ethanol injury was similar to that of glutathione and different from that of sucralfate. Furthermore, the present study suggests that the main mechanism of mucosal protection by OGT may be at least in part due to reinforcement of mucosal barrier resistance by its interaction with endogenous sulfhydryl compounds present in the gastric mucosa and mucus.

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