Antisecretory and Antiulcer Effects of Ebselen, a Seleno-Organic Compound, in Rats

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ABSTRACT—The effects of ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-on), a metal-containing organic compound, on gastric secretion and gastric ulceration were examined in rats. Intraduodenal ebselen (30 to 300 mg/kg) significantly and dose-dependently inhibited gastric secretion in pylorus-ligated rats. Both aspirin- and water-immersion restraint stress-induced ulcers were significantly prevented by oral administration of ebselen at doses equivalent to the antisecretory doses. These results indicate that the antisecretory effect of ebselen underlies its antiulcer effect in these models.

Keywords: Ebselen, Gastric ulcer, Gastric acid secretion

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-on) is a seleno-organic compound with anti-inflammatory and gastric protective actions (1–6). Ebselen has been shown to be an inhibitor of 5-lipoxygenase in vitro, and it also blocks biological oxidation (2, 7, 8). Beil et al. (9) have recently shown that ebselen is a potent inhibitor of hog gastric H⁺/K⁺-ATPase. However, it has not been determined whether ebselen has an antisecretory action in vivo. In this study, we examined the antisecretory effect of ebselen in rats, and we also studied whether this compound affects the formation of experimentally induced stomach ulcers.

Male Donryu rats (Japan Charles River, Kanagawa) were used in this experiment. They were fasted overnight, but allowed free access to water, except in the stress ulcer experiment.

Pylorus-ligation was performed according to the method of Shay et al. (10). Under light ether anesthesia, rats (130–160 g) were laparotomized, and the pylorus was ligated. Gastric juice was collected for 4 hr after ligation, and it was analyzed for volume and acidity. Ebselen (A. Nattermann & Cie., Cologne, Germany) was suspended in 0.5% carboxymethylcellulose (CMC) solution and injected into the duodenum immediately after pylorus ligation.

For the experiments with aspirin-induced ulcer, rats (160–200 g) were treated orally with 300 mg/kg aspirin (Ebisu, Osaka). Five hours after the administration of aspirin, under light ether anesthesia, the stomach was resected and inflated by injecting 10 ml of 2% buffered-formalin into the lumen side to fix the stomach at the inflated size. The stomach was then incised along the greater curvature, and the extent of the gastric mucosal lesions was evaluated. The length (mm) of each necrotic lesion was measured and summed per stomach. The sum was used as an ulcer index.

The experimental procedure for water-immersion restraint stress has been described previously (11). In brief, nonfasted rats (270–300 g) were each placed in an individual restraint cage and immersed vertically to the level of the xiphoid process in a water bath maintained at 21°C for 7 hr. Gastric mucosal lesions were evaluated as described above. Ebselen, suspended in 0.5% CMC solution, was given orally 30 min before aspirin or stress-exposure.

Statistical significance was analyzed by Dunnett’s multiple comparison test for unpaired data.

As summarized in Table 1, intraduodenal administration of ebselen at doses from 10 to 300 mg/kg inhibited gastric acid secretion in pylorus-ligated rats, as evidenced by the dose-related reductions in the volume, acidity and output of gastric acid. A statistically significant decrease in acid output was achieved at doses over 30 mg/kg. Complete suppression of acid output was observed at the highest dose (300 mg/kg, i.d.).

Figure 1 shows the prophylactic effect of ebselen on water-immersion restraint stress- and aspirin-induced gastric ulceration in rats. Oral ebselen at doses from 30 to 300 mg/kg dose-dependently inhibited gastric ulcer for-
Table 1. Effect of ebselen on gastric acid secretion in pylorus-ligated rats

| Dose (mg/kg) | Volume (ml/100 g b.w.) | Acidity (mEq/l) | Acid output (μEq/100 g b.w.) |
|-------------|------------------------|-----------------|-----------------------------|
| Vehicle     | 4.5±0.4                | 123.3±2.1       | 564.4±49.4                  |
| 10          | 4.8±0.4 (−7)           | 121.6±3.1 (1)   | 585.0±52.3 (−4)             |
| 30          | 3.1±0.3** (31)         | 125.3±3.4 (−2)  | 388.0±45.8** (31)           |
| 100         | 1.1±0.2** (76)         | 82.9±6.0** (33) | 93.9±17.6** (83)            |
| 300         | 0.5±0.1** (89)         | 56.6±5.2** (54) | 25.9±4.3** (95)             |

Ebselen was administered intraduodenally immediately after pylorus ligation. The injection volume was constant at 5 ml/kg b.w., and an equal volume of vehicle was given to the control group. Gastric juice was collected for 4 hr after ligation and analyzed for acidity with an automatic titrator (HSM-1OA, Toa Electronics, Tokyo). Acid output was calculated as the product of acidity multiplied by the volume and expressed in terms of μEq/100 g b.w. All data indicate means±S.E. (N=10). **P<0.01 vs. vehicle control group (Dunnett’s multiple comparison). Figures in the parentheses indicate the percent inhibition.

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The present results clearly demonstrate that ebselen, a seleno-organic compound, has antisecretory activity. In pylorus-ligated rats, intraduodenal administration of ebselen (30 to 300 mg/kg) resulted in a significant and dose-related reduction in acid output. Beil et al. (9) have shown that this seleno-organic compound is a potent inhibitor of both hog gastric H+/K+-ATPase activity (IC50=0.15 μM) and H+/K+-ATPase-mediated proton transport in intact hog gastric vesicles (IC50=0.7 μM). The H+/K+-ATPase inhibitory activity of this compound was comparable to that of omeprazole (IC50=0.47 μM) (12). Furthermore, Beil et al. (9) also demonstrated that ebselen markedly inhibited hydrochloric acid production stimulated by histamine or dibutyryl cAMP in isolated guinea pig parietal cells (IC50=12 μM), indicating that ebselen reduces acid production by interfering with the gastric proton pump, the H+/K+-ATPase. In our preliminary experiment, we found that ebselen inhibited hog gastric H+/K+-ATPase activity (IC50=0.06 μM) and that it inhibited both phosphorylation (IC50=0.25 μM) and dephosphorylation (IC50=0.09 μM) of the enzyme (Y. Tabuchi et al., unpublished data). It is therefore most likely that the antisecretory action of ebselen observed in vivo reflects substantial inhibition of gastric H+/K+-ATPase activity. It has been reported that omeprazole, a gastric H+/K+-ATPase inhibitor, prevents gastric acid secretion in rats (ED50=3.5 mg/kg) (13). In our experiment, the ED50 value for inhibition of acid secretion by intraduodenal ebselen was estimated to be about 50 mg/kg. Therefore, the antisecretory activity of ebselen seems to be weaker than...
that of omeprazole in vivo. However, this inhibitory effect of ebselen was comparable to the anti-inflammatory activity of the compound against cobra-venom factor-induced edema (ED$_{50}$ = 56 mg/kg, p.o.) (3).

It is well established that gastric acid plays a crucial role in the pathogenesis of gastric lesions. Water-immersion restraint stress- and aspirin-induced gastric lesions are typical ulcer models in which aggressive factors are preferentially involved, as antisecretory drugs including H$_2$-antagonists, cholinolytics and proton pump inhibitors are very effective in preventing ulcer formation (13–15). Our data also showed that ebselen was effective in preventing gastric mucosal ulceration induced by either aspirin or water-immersion restraint stress (30 to 300 mg/kg). It is conceivable that the anti-ulcer effect of ebselen is due to its inhibitory action on gastric acid secretion.

A non-antisecretory dose of ebselen (10 mg/kg) showed a tendency to prevent gastric mucosal lesions induced by aspirin, indicating that this type of ulcer, rather than the stress-induced ulcer model, is more susceptible to ebselen. It should however be noted that the development of gastric ulcers induced by aspirin or stress took 5 and 7 hr, respectively. Moreover, non-fasted rodents were used in the stress ulcer experiment, in contrast to fasted-rats in the aspirin ulcer experiment. Pharmacokinetic differences such as absorption rate, serum concentration, and half-life may therefore explain the discrepancy between the effectiveness of ebselen in these experimental models.

It has been postulated that peptidoleukotrienes and peroxides participate in the pathogenesis of peptic ulcers, because of the significant prophylactic effects of peptido-leukotriene-antagonists, 5-lipoxygenase inhibitors and radical scavengers. Ebselen inhibits 5-lipoxygenase and also acts as an antioxidant (2, 7, 8). These beneficial effects of ebselen could be involved in its antiulcer activity. In addition, it has been demonstrated that ebselen is a cytoprotective agent and effectively prevents gastric mucosal injury induced by necrotizing agents (5) and that this compound inhibits diclofenac-induced gastric damage (6).

In summary, the present study indicates that ebselen has potent antiulcer activity in rats, mainly as a result of its inhibitory effect on gastric secretion.

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