Vinegar is a Dietary Mild Irritant to the Rat Gastric Mucosa

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Abstract—Exposure of the rat stomach to acetic acid (0.3–3%) caused a concentration-dependent reduction of transmucosal potential difference (PD) and increase of luminal pH (gastric alkaline response). These concentrations of acetic acid, when given topically to the stomach, significantly prevented development of gastric lesions induced by subsequent exposure to absolute ethanol, the inhibition being 42.3%, 95.8% and 70.4% at concentrations of 0.3%, 1% and 3%, respectively. Gastric alkaline response and protection of ethanol-induced gastric lesions caused by 1% acetic acid were significantly attenuated by pretreatment of the animals with indomethacin (5 mg/kg, s.c.). Although other related carboxylic acids at 1% concentration such as citric acid (52 mM), maleic acid (86 mM) and formic acid (217 mM) affected both PD and luminal pH in varying degrees, these agents, except for 1% maleic acid, failed to prevent gastric lesions in response to absolute ethanol. Similar to 1% acetic acid (167 mM), gastric alkaline response and adaptive cytoprotection induced by 1% maleic acid were significantly antagonized by pretreatment with indomethacin. Formic acid also induced a significant gastric alkaline response, but this effect was not affected by indomethacin. These results suggest that dilute acetic acid such as vinegar (approximately 3% acetic acid) acts as a mild irritant to the stomach, and induces alkaline response and adaptive cytoprotection, mediated by endogenous prostaglandins. Other related carboxylic acids may have similar effects, but those depend upon the concentrations used.

The medicinal properties of vinegar (approximately 3% acetic acid) which is generally used as a condiment of food are part of the traditions of folk medicine. However, there are little pharmacological evidence to show that vinegar favors gastric diseases. Recent studies have demonstrated that a mild irritant such as 20% ethanol or 5% NaCl, when topically applied to the stomach, prevents gastric necrosis induced by subsequent exposure to a strong irritant such as absolute ethanol or 25% NaCl (1–3). Although the mechanisms involved in this protection remain unknown, this phenomenon is considered to be one of adaptive responses of the stomach against mild injury and termed "adaptive cytoprotection" (3, 4). The previous studies from our laboratory showed that gastric alkaline response (an increase of luminal pH) occurs in the stomach with a concomitant reduction of PD after exposure to those mild irritants and may play a role in repair process of injury by preventing further development of the damage (5–7). Furthermore, endogenous prostaglandins are involved in the mechanisms of gastric alkaline response and adaptive cytoprotection seen in the stomach exposed to mild irritants, since these phenomena disappeared in the presence of prostaglandin biosynthesis inhibitors (3, 7–9). Therefore, it is of interest to examine whether dilute acetic acid may act as a mild irritant to the stomach and induce gastric alkaline response and adaptive cytoprotection. In the present study, we determined the effects of low concentrations of acetic acid on PD, luminal pH and ethanol-induced

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gastric lesions, and we compared these with the effects of other related carboxylic acids in rats.

Materials and Methods
Male Donryu rats (200–230 g), kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access to water for 24 hr prior to the experiments.

Operative procedures: The simultaneous determination of PD and pH, and the gastric perfusion system were described in previous papers (5, 6). Briefly, the abdomen of anesthetized rats with 25% urethane (Nakarai, 0.5 ml per 100 g of body weight) was opened through the midline incision, and the stomach and duodenum exposed. The esophagus was tied without disturbing the vagus nerves. An acute gastric fistula prepared by placing a polyethylene tube in the forestomach led to a three way tap and was used for intragastric instillation and removal of solutions and for continuous intraluminal perfusion with saline (154 mM NaCl). The pyloroduodenal junction was exposed, and two catheters were passed into the stomach through a slit in the duodenum. One catheter containing saturated KCl in 4% agar gel served as a detecting electrode for PD determination, and its tip was positioned in the secretory portion of the stomach. The circuit for PD recording was completed by using another agar bridge placed in the abdominal cavity. The second gastric catheter which led to a pH glass electrode of flow type (Horiba Model 6901–25T) was used for determining changes in pH of fluids emerging from the pylorus, and changes in pH were monitored on a Hitachi two channel recorder (Model 056) simultaneously with those in PD. The stomach was perfused with saline which was gassed with 100% O₂, heated at 37°C and kept in a reservoir, at a flow rate of 1 ml/min before and after exposure to test drugs.

Experimental protocols: Approximately 1 hr after both PD and pH had stabilized, the perfusion system was interrupted, and the solution in the stomach was withdrawn. The stomach was then exposed for 10 min to 3 ml of 0.1–3% acetic acid (Nakarai), 1% maleic acid (Nakarai), 1% citric acid (Nakarai) or 1% formic acid (Nakarai), by instillation through the three way tap. After application of these drugs, the stomach was rinsed gently with saline, another 3 ml of saline instilled, and the perfusion system resumed. Monitoring the pH was also interrupted for 10 min while the stomach was exposed to various agents, whereas gastric PD was continuously measured throughout a 2 hr experimental period. In half the animals treated with either of above agents at a concentration of 1%, indomethacin (Sigma), suspended in saline with a trace of Tween 80 (Nakarai), was given subcutaneously in a dose of 5 mg/kg 1 hr before exposure to those agents. In some cases, 16,16-dimethyl prostaglandin E₂ (16-dmPGE₂) (Ono), dissolved in absolute ethanol and diluted with saline, was given subcutaneously in a dose of 3 μg/kg 30 min before exposure to 1% acetic acid in rats pretreated with indomethacin. The doses and time intervals of indomethacin and 16-dmPGE₂ were chosen according to a previous paper (7).

In the second study, gastric lesions were induced in conscious rats by oral administration of 1 ml of absolute ethanol. One ml of acetic acid (0.1–3%) or other carboxylic acids (1%) was orally given 30 min before ethanol treatment. In some cases, indomethacin (5 mg/kg) was given subcutaneously 1 hr before administration of ethanol. The animals were killed 1 hr after ethanol treatment, and the stomachs removed, inflated by injecting 12 ml of 2% formalin, put into 2% formalin for 10 min to fix both the inner and outer layers of the gastric wall, and then opened along the greater curvature. The stomach was examined for lesions under a dissecting microscope (×10), and the length (mm) of each lesion was measured, summed, and used as a lesion index. The person measuring the lesions did not know the treatment given to the animals.

Statistics: Data are presented as the mean±S.E.M. from 6 to 10 rats per each group. The means were compared using the unpaired Student’s t-test, and values of P<0.05 were regarded as significant.

Results
Changes in PD and luminal pH after
exposure to acetic acid: The effects of various concentrations of acetic acid on gastric PD and luminal pH are shown in Fig. 1. There was a concentration-dependent reduction of PD from the basal value, the maximal reduction being 85% and 95% after application of 1% and 3% acetic acid for 10 min, respectively. In the mucosa exposed to 0.1% and 0.3% acetic acid, the PD rapidly returned to the basal values within 30 min after removal of acetic acid from the stomach. However, the PD returned gradually and only partially within 1 hr in the stomach exposed to acetic acid at a concentration of 1% or greater.

After exposure of the stomach to acetic acid at a concentration over 0.3%, the luminal pH increased significantly and kept the elevated values during a remaining test period. Although 0.1% acetic acid also induced a slight reduction of PD and increase of luminal pH, those effects were not significantly different from control values.

Effects of various carboxylic acids on PD and pH: Since the responses of PD and pH caused by 1% acetic acid were apparent, the effects of other related carboxylic acids on PD and pH were examined at a concentration of 1%. As shown in Fig. 2, application

![Figure 1](image-url)

**Fig. 1.** Effects of topical application of low concentrations (0.1–3%) of acetic acid on the PD and luminal pH in anesthetized rats. The stomach was exposed for 10 min to 3 ml of acetic acid in various concentrations and was perfused with saline before and after the exposure. Data represent the mean ± S.E.M. of values read every 10 min from 6 rats. *Significantly different from controls, at *P* < 0.05.
of 1% formic acid produced a rapid reduction in PD and an increase of pH, the magnitude of these responses being similar to those seen after exposure to 1% acetic acid. Although a significant reduction in PD was observed in response to 1% maleic acid, this change was less than that induced by 1% acetic acid. Similar to 1% acetic acid, a significant gastric alkaline response occurred in the stomach exposed to 1% maleic acid. However, application of 1% citric acid to the stomach had no significant effect on either PD and pH.

Effect of indomethacin on gastric alkaline response caused by acetic acid or other carboxylic acids: The increase of luminal pH seen after exposure to 1% acetic acid was significantly attenuated by pretreatment with indomethacin (5 mg/kg, s.c.) (Fig. 3). Gastric alkaline response caused by 1% maleic acid was also significantly antagonized by indomethacin, but that induced by 1% formic acid was not significantly affected by this agent. Administration of indomethacin itself had no effect on both PD and pH (not shown). On the other hand, subcutaneous administration of 16-dmPGE₂ (3 µg/kg) to the rats given indomethacin completely restored the gastric alkaline response in the stomach exposed to 1% acetic acid, without
any effect on the PD reduction (Fig. 4).

**Effects of acetic acid and other carboxylic acids on ethanol-induced gastric lesions:** Oral administration of 1 ml of absolute

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**Fig. 3.** Effect of subcutaneously administered indomethacin (5 mg/kg) on gastric alkaline response (an increase of luminal pH) in the stomach exposed to 1% acetic acid, 1% citric acid, 1% maleic acid or 1% formic acid in anesthetized rats. Indomethacin was given 1 hr before exposure of the stomach to above agents. Data represent the mean±S.E.M. of values observed immediately before and 30 min after the exposure from 6 rats. * *: Significantly different from values in Before or in the corresponding control groups, respectively, at P < 0.05.

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**Fig. 4.** Representative figures showing the effects of indomethacin (5 mg/kg, s.c.) and 16-dmPGE$_2$ (3 μg/kg, s.c.) on gastric alkaline response in the stomach exposed for 10 min to 3 ml of 1% acetic acid in anesthetized rats. Note that the increase of luminal pH induced by 1% acetic acid was inhibited by indomethacin and restored by 16-dmPGE$_2$, respectively, without any effect on the PD reduction. A: control rat, B: indomethacin-treated rat, C: indomethacin plus 16-dmPGE$_2$-treated rat.
ethanol produced multiple streak lesions in the corpus region of the stomach, the lesion index being 71.0±8.0 mm (Table 1). Acetic acid, given orally 30 min before ethanol treatment, prevented the development of gastric lesions caused by ethanol in a concentration-dependent manner, the inhibition being 8.5%, 42.3%, 95.8% and 70.4% at a concentration of 0.1%, 0.3%, 1% and 3%, respectively. Pretreatment with 1% maleic acid also showed a significant inhibition against ethanol-induced gastric lesions (36.3%), but other carboxylic acids such as 1% citric acid and 1% formic acid had insignificant effect on the formation of lesions in response to ethanol. The inhibitory effect of 1% acetic acid or 1% maleic acid on ethanol-induced gastric lesions was significantly mitigated by prior administration of indomethacin, dilute acetic acid may act to the gastric mucosa as a mild irritant to result in adaptive cytoprotection mediated by prostaglandins.

Previous studies in our laboratory (4, 7, 9) showed that the gastric alkaline response (an increase of luminal pH) induced by 1 M NaCl or 20 mM taurocholic acid in the rat stomach is a net result of both a diffusion of HCO₃⁻ and acid inhibition caused by endogenous prostaglandins. Gastric alkaline response seen after exposure to 1 M NaCl was significantly inhibited by pretreatment with indomethacin, aspirin or prednisolone. Additionally, the inhibitory effects of those

Table 1. Effects of acetic acid and other carboxylic acids on gastric lesions induced by absolute ethanol in rats

| Treatment          | Dose (%) | No. of rats | Lesion index (mm) | Inhibition (%) |
|--------------------|----------|-------------|-------------------|---------------|
| Control            | —        | 10          | 70.1±8.0          | —             |
| Acetic acid        | 0.1      | 10          | 65.0±7.2          | 8.5           |
|                    | 0.3      | 10          | 41.0±4.5*        | 42.3          |
|                    | 1.0      | 10          | 3.0±0.9*         | 95.8          |
|                    | 3.0      | 10          | 21.0±4.5*        | 70.4          |
| Citric acid        | 1.0      | 10          | 55.0±8.0         | 22.5          |
| Maleic acid        | 1.0      | 10          | 45.2±5.0*        | 36.3          |
| Formic acid        | 1.0      | 10          | 83.2±9.8         | 18.1          |
| Indomethacin pretreatment |        |             | 81.0±7.8         | —             |
| Control            | —        | 10          | 42.0±5.0*        | 48.1          |
| Acetic acid        | 1.0      | 10          | 66.3±8.2         | 18.1          |

Data are presented as the mean±S.E.M. Acetic acid or other carboxylic acids were given orally 30 min before oral administration of 1 ml of absolute ethanol. Indomethacin was given subcutaneously in a dose of 5 mg/kg 1 hr before ethanol treatment. *Significantly different from controls, at P<0.05.

Discussion

The present findings in rats showed that dilute acetic acid (0.3%–3%) prevents gastric lesions induced by absolute ethanol, probably through "adaptive cytoprotection" mediated by endogenous prostaglandins. Acetic acid at concentrations of 0.3–3% produced a PD reduction and gastric alkaline response, when applied topically to the stomach. These responses in PD and luminal pH were previously reported in the stomach after exposure to 20% ethanol, 1 M NaCl and 20 mM taurocholic acid, using the same perfusion system (5–7, 10). These substances are known to act as mild irritants to the stomach and enhance generation of endogenous prostaglandins (1–3, 10). Since the protective action of 1% acetic acid on ethanol-induced gastric lesions was also significantly mitigated by prior administration of indomethacin, dilute acetic acid may act to the gastric mucosa as a mild irritant to result in adaptive cytoprotection mediated by prostaglandins.
antiinflammatory drugs on gastric alkaline response disappeared in the stomach of rats given cimetidine intravenously to inhibit acid secretion. Since gastric alkaline response induced by 1% acetic acid was significantly inhibited by indomethacin, endogenous prostaglandins may be involved in the mechanism of alkalinization in the stomach exposed to acetic acid. Actually, administration of 16-dmPGE₂ all but completely attenuated the inhibitory effect of indomethacin on gastric alkaline response induced by 1% acetic acid and restored the alkalinization to the corresponding control levels. Thus, occurrence of indomethacin-sensitive gastric alkaline response seems to be a reliable indicator for screening drugs which may act as mild irritants to the stomach.

Application of 1% maleic acid similarly caused a lowering PD and an increase of pH, and it prevented ethanol-induced gastric lesions. The latter two phenomena were significantly antagonized by indomethacin, just as seen in the case of 1% acetic acid. Other carboxylic acids such as 1% citric acid and 1% formic acid failed to significantly affect the formation of gastric lesions in response to absolute ethanol. The former had little effect on PD and pH, while application of the latter agent to the stomach produced a marked PD reduction and an increase of luminal pH. However, the increase in pH caused by 1% formic acid was not significantly influenced by pretreatment with indomethacin. In the present study, we used these carboxylic acids at a concentration of 1%, since acetic acid at the same concentration produced apparent effects on either parameter tested herein. The pKa of these substances are 4.76 for acetic acid, 1.94 for maleic acid, 3.13 for citric acid and 3.75 for formic acid. It is unlikely that their different effects on ethanol-induced gastric lesions are based on the values of pKa. However, when the doses of these agents are expressed as millimolar, considerable differences are observed as follows: 1% acetic acid: 167 mM, 1% maleic acid: 86 mM, 1% citric acid: 52 mM, and 1% formic acid: 217 mM. It is known that ethanol (20–30%) or NaCl (5–10%) at a low concentration acts as a mild irritant to the gastric mucosa, while these agents at high concentrations act as strong irritants to cause gross injury in the stomach (1–3, 11). Therefore, carboxylic acids at a concentration of 100–150 mM (1% acetic acid and 1% maleic acid) may act as a mild irritant to the stomach, but those at concentrations over 200 mM (1% formic acid) may act as a strong irritant. Since 1% citric acid did not significantly affect the PD and pH, this concentration of citric acid seems not high enough to irritate the mucosa to result in adaptive cytoprotection. However, we cannot exclude the possibility that other factors related to the structure of these carboxylic acids may in part contribute to the different responses observed in the present study.

One percent acetic acid and 1% maleic acid produced a PD reduction and induced gastric alkaline response and adaptive cytoprotection. Although a reduction of PD caused by 1% acetic acid was not affected by either indomethacin or 16-dmPGE₂, just like what was observed in other cases using 1 M NaCl or taurocholic acid (4, 7, 10), a lowering PD may be an obligatory process for appearance of adaptive responses induced by mild irritants. A PD reduction in these studies would be mainly due to damage in the surface cells. A close relationship between the changes in PD and pH caused by mild irritants indicates that a reduction of PD may be responsible for enhanced formation of prostaglandins (4). Thus, an increased production of prostaglandins occurs initially as an adaptive response of the mucosa to surface cell injury (PD reduction), and it is responsible for subsequent events such as gastric alkaline response and adaptive cytoprotection induced by mild irritants.

Assoaline and Danon (12, 13) reported that hypertonic solutions including NaCl have antisecretory and antilesion activities in various types of gastric lesions and increase prostaglandin output in the stomach of rats. Dilute acetic acid is generally used as a condiment of food in addition to NaCl or other spices. Therefore, the present results together with the previous findings (7, 10, 12) suggest that these condiments of food may act as dietary mild irritants to the stomach and protect the gastric mucosa.
against acid or other ulcerogenic stimuli through adaptive cytoprotection mediated by endogenous prostaglandins. Other carboxylic acids may have similar effects, but those depend on the concentrations used.

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