Effects of Topical Application of KT1-32 on Transmucosal Potential Difference and Acid secretion in the Rat Stomach

Koji TAKEUCHI, Tetsuo TANAKA, Syuichi WAKABAYASHI
Seiichiro MOCHIZUKI, Akira TOMIYAMA and Susumu OKABE

Department of Applied Pharmacology, Kyoto Pharmaceutical University,
Misasagi, Yamashina, Kyoto 607, Japan

1Research Laboratory, Kotobuki Seiyaku Co., Ltd., Nagano 389-06, Japan

Accepted July 17, 1990

Abstract—Effects of intragastric application of azuletil sodium (KT1-32), a novel antiulcer drug, on transmucosal potential difference (PD) and acid secretion were investigated in the rat stomach. The stomach was mounted on a Lucite chamber and perfused with saline before and after exposure to KT1-32 for 10 min. KT1-32 (3–30 mg/kg) produced an elevation of PD in a dose-dependent manner with a rise of the luminal pH. The increased PD response caused by KT1-32 (10 mg/kg) persisted after removal of the agent from the stomach, but this PD generating effect was significantly mitigated by pretreatment with omeprazole (60 mg/kg, i.p.). KT1-32 raised PD under basal conditions, but did not significantly affect the reduced PD response caused by 30% ethanol. In addition, topical application of KT1-32 significantly reduced acid secretion caused by histamine (4 mg/kg/hr, i.v.) and carbachol (20 μg/kg/hr, i.v.). In the in vitro study, KT1-32 at 3.9×10^{-4} M showed 50% inhibition of the H/K ATPase activity prepared from the hog gastric mucosa. These results suggest that KT1-32 exerts locally antisecretory and PD generating effects. The latter may be accounted for by the antisecretory action, which is probably related to the H/K ATPase inhibition.

KT1-32 [sodium 3-ethyl-7-isopropyl-1-azulenesulfonate 1/3 hydrate], a more stable azulene derivative than sodium guaiazulene-3-sulfonate (GAS), exhibits antiulcer activity against a variety of experimental gastric ulcer models in the rat, including ethanol-induced lesions (1, 2). Although the previous study suggested that the mucosal protection by KT1-32 may be related to the inhibition of endothelial injury (2) and the thromboxane A2 antagonistic action (3), the mechanisms still remain unclear.

Transmucosal potential difference (PD) has been used as an index for the mucosal integrity of the stomach (4–6). Recently, we found that KT1-32, only when given intragastrically, produced an increase of PD with concomitant elevation of luminal pH (acid inhibition) in the rat stomach. Since the gastroprotective action of KT1-32 against ethanol was observed after oral administration but not the parenteral one (unpublished data: S. Okabe et al.), it may be possible that this agent exerts some influences locally to enhance the mucosal resistance against irritants.

In the present study, we demonstrate the effect of topical application of KT1-32 on transmucosal PD of the rat stomach and characterize the PD generating effect of this agent based on the mucosal resistance to ethanol and the inhibition of acid secretion.

Materials and Methods

Male Sprague Dawley rats (250–300 g; Charles River), kept in individual cages with raised mesh bottoms. were deprived of food but allowed free access to tap water for 18 hr prior to the experiment. Unless otherwise specified, all studies were carried out using 4 to 6 rats under anesthetized conditions induced by urethane (1.25 g/kg, i.p.).

Determination of luminal pH and potential
difference: Simultaneous measurements of both pH and PD were performed using a Lucite chamber, according to a previously published method (7). Briefly, the stomach was exposed through a midline incision and delivered onto the abdominal surface by gentle traction on the spleen, and both the esophagus and pylorus were ligated. The stomach was drawn through the center hole with the forceps applied only to the fore stomach. The stomach was then opened along the greater curvature from the middle part of the forestomach to the area where the epiploic artery is terminated, and the edges were pinned out by gently stretching the glandular mucosa. The plastic rim was then applied and tightened down on the mucosa. Under these conditions, the mucosa was perfused at a flow rate of 1 ml/min with saline that was heated at 37°C. The exit tube was connected to a pH glass electrode of the flow type (Horiba Model, 6901-25T) to determine the pH of the gastric effluent. PD was measured using two agar bridges, one is placed in the chamber, and the other in the abdominal cavity. Changes in PD were continuously monitored using a Hitachi two channel recorder (Model 056) simultaneously with those of pH. After both pH and PD had stabilized, KT1-32 (3–10 mg/kg) was applied into the chamber in a volume of 2 ml for 10 min, and the mucosa was perfused with saline before and after exposure to the drug. In some cases, omeprazole (60 mg/kg) was given i.p. 30 min before the application of KT1-32 (10 mg/kg). In a separate study, the mucosa was exposed for 5 min to 30% ethanol (2 ml), and both pH and PD were monitored for 30 min thereafter. In this experiment, KT1-32 (10, 30 mg/kg) was applied for 10 min 20 min before ethanol treatment.

Measurement of gastric acid secretion: Acid secretion was measured using a pH-stat method in the lumen-perfused stomach as previously described (8). Briefly, the stomach was perfused at a flow rate of 1 ml/min with saline that was gassed with 100% O₂, heated at 37°C and kept in a reservoir. Acid output was measured by titrating the perfusate to the endpoint of pH 7.0 using the pH-stat method (Hiranuma Comtite-7) and by adding 100 mM NaOH. Acid secretion was stimulated by i.v. infusion of histamine (4 mg/kg/hr) or carbachol (20 μg/kg/hr) at a rate of 1.2 ml/hr. After acid output had reached the plateau level, KT1-32 (3–30 mg/kg) was applied into the stomach for 10 min, and acid secretion was measured for 100 min.

Studies on purified H/K ATPase activity: The membrane enriched in H/K ATPase was purified by differential and density gradient centrifugation from a hog gastric mucosal homogenate, essentially according to Beil et al. (9). The membrane preparation obtained was stored at −70°C until use. The measurement of H/K ATPase activity was performed according to the modified method reported by Hongo et al. (10). Briefly, the assay medium contained 2 mM MgCl₂, 32.5 mM Tris/HCl buffer (pH 7.4), 40 μg membrane protein with or without 5 mM KCl in a final volume of 2 ml. The reaction was started by addition of ATP (final concentration 2 mM) and stopped after 15 min incubation at 37°C with 1 ml of 15% trichloroacetic acid. Inorganic phosphate (Pᵢ) liberated from ATP was measured according to the method of Fiske and Subbarow (11). Reaction rates were 24.23±0.28 μmol Pᵢ/mg protein/hr in the absence and 118.10±5.81 μmol Pᵢ/mg protein/hr in the presence of 5 mM KCl (N=3). KT1-32 (1 x10⁻⁵ to 3 x10⁻³ M) was added in the incubation medium. The experiments were performed in duplicate, and the % inhibition was calculated under the condition that the K-stimulated reaction rates in the absence of KT1-32 were set to 100%.

Preparation of drugs: The drugs used were urethane (Tokyo Kasei), KT1-32 (Kotobuki Seiyaku Co.), omeprazole (synthesized at the labs of Kotobuki Seiyaku Co.), histamine-2HCl (Nacalai Tesque) and carbachol (Sigma). Omeprazole was suspended in 0.5% carboxymethylcellulose solution, while other agents were dissolved in saline. All agents were prepared immediately before use and given in a volume of 0.5 ml/100 g body wt. in the case of i.p. administration, in a volume of 2 ml/stomach in the case of topical application, or in a volume of 1.2 ml/hr in the case of i.v. infusion.

Statistics: Data are presented as the mean±S.E. from 3 to 6 rats per group. The statistical analysis was performed using a two-tailed Dunnett’s multiple comparison test (12), and
the values of $P<0.05$ were regarded as significant.

**Results**

**Effects of KT1-32 on gastric PD and pH:**
The gastric mucosa mounted in a Lucite chamber generated a PD of about $-32$ to $-36$ mV (mucosa negative) and secreted acid to keep the luminal pH at about 3.4–3.8, and these values remained relatively constant during a 2-hr test period. Exposure of the mucosa to saline (154 mM NaCl) had no effect on both pH and PD. However, KT1-32 (3–30 mg/kg), topically applied to the mucosa for 10 min, produced an increase of the PD in a dose-dependent manner (Figs. 1 and 2). At 3 and 10 mg/kg, the PD remained elevated for 30–50 min after removal of the agent from the chamber, while at 30 mg/kg, the PD elevated in response to the agent initially, followed by a decrease, but again increased gradually after the removal of the agent. The luminal pH also showed a dose-dependent increase after exposure of the mucosa to KT1-32; at 10 mg/kg, the pH was increased from 3.9±0.2 to the maximal values of 4.8±0.2 within 10 min after the exposure.

**Effect of omeprazole:** Since omeprazole similarly produced an increase of both pH and PD in the rat stomach (13), the effect of KT1-32 on these parameters was further examined in the rat pretreated with omeprazole (60 mg/kg, i.p.). In the animal without omeprazole treatment, KT1-32 (10 mg/kg) caused an elevation of the PD from $-31.2$ mV to the maximal value of $-41.6$ mV with a concomitant increase of the pH from 3.5 to 4.1 at

![Fig. 1](image-url)  
**Fig. 1.** Representative figures showing the effects of KT1-32 on PD and pH of the gastric mucosa mounted on a Lucite chamber. The mucosa was exposed for 10 min to KT1-32 (3–30 mg/kg) and perfused with saline before and after the exposure.

![Fig. 2](image-url)  
**Fig. 2.** Effects of KT1-32 on PD and pH of the gastric mucosa. For detailed protocols, refer to Fig. 1. Data are presented as the mean±S.E. of values determined every 5 min from 4–5 rats. *Statistically significant difference from the controls, at $P<0.05$.  

---

229 **Effect of KT1-32 on Rat Stomach**
the plateau value (Fig. 3). Both PD and pH were markedly increased after i.p. adminis-

tration of omeprazole and stabilized in the ranges of $-50$ mV and 5.0, respectively. Under these conditions, the mucosa responded to KT1-32 by a slight increase of PD only during the exposure, and the PD remained in the basal range after removal of the agent. In the presence of omeprazole, the pH remained unaltered before and after exposure to KT1-32. When the degree of PD changes caused by KT1-32 was calculated at various time points, it was noted that pretreatment with omeprazole significantly inhibited the PD generating effect of KT1-32 (Fig. 4). The PD induced by KT1-32 was 7.2±0.6 mV during the exposure and 5.6±0.3 mV at 40-

min post exposure in the absence of omepra-

zole, while these values were significantly reduced to 2.8±0.2 mV and 1.6±0.8 mV, respectively, in the presence of omeprazole.

**Effect on ethanol-induced changes:** Exposure of the mucosa for 5 min to 30% ethanol (2 ml) produced a marked reduction of PD and increase of pH: the PD decreased from $-31.0±0.3$ mV to $-10.4±0.3$ mV immediately after the exposure, followed by a gradual restoration toward the basal levels (Figs. 5 and 6). Pretreatment of the mucosa with...

![Fig. 3](image_url)  
**Fig. 3.** Representative figures showing the effects of KT1-32 on PD and pH of the stomach in the absence or presence of omeprazole. The mucosa was exposed for 10 min to KT1-32 (10 mg/kg) and perfused with saline before and after the exposure. Omeprazole (60 mg/kg) was given i.p. 30 min before the treatment with KT1-32.

![Fig. 4](image_url)  
**Fig. 4.** Effect of omperazole pretreatment on the PD responses caused by KT1-32 of the gastric mucosa. For detailed protocols, refer to Fig. 3. Values represent the net increase of PD observed at various time points after the exposure to KT1-32 and are presented as the mean±S.E. from 4–5 rats. *Statistically significant difference from the controls, at P<0.05.
KT1-32 (10 mg/kg) significantly elevated the PD but failed to prevent the PD reduction caused by the subsequent exposure to 30% ethanol; the PD was decreased from $-39.1 \pm 0.3$ mV to $-19.8 \pm 0.2$ mV. Although the lowest PD value in the KT1-32-treated group was significantly higher than that in the control group, the magnitude of PD reduction was not significantly different between these two groups; the 4PD was $19.2 \pm 1.0$ mV and $20.3 \pm 0.8$ mV, respectively, in the KT1-32 treated and control groups. At 30 mg/kg, this agent had no effect on the PD response to ethanol, although it caused an increase of PD during the exposure. On the other hand, KT1-32, at either 10 or 30 mg/kg, did not significantly affect the increased pH responses seen after exposure to 30% ethanol. The pH was significantly increased after exposure to KT1-32 but similarly responded to ethanol, resulting in a marked and persistent increase, as observed in the control group.

Fig. 6. Effects of KT1-32 (10, 30 mg/kg) on the PD and pH responses caused by 30% ethanol in the stomach. For detailed protocols, refer to Fig. 5. Data are presented as the mean±S.E. of values determined every 5 min from 4–5 rats. *Statistically significant difference from the controls, at P<0.05.
Fig. 7. Effect of KT1-32 on acid secretion stimulated by histamine (4 mg/kg/hr, i.v.) in anesthetized rats. The mucosa was exposed for 10 min to KT1-32 (3-30 mg/kg) after acid output had reached the plateau levels. Data are presented as the percentage of the values observed before the exposure and represent the mean±S.E. of the values determined every 10 min from 4-6 rats. *Statistically significant difference from the controls, at P<0.05.

Fig. 8. Effects of KT1-32 (30 mg/kg) on acid secretion stimulated by carbachol (20 μg/kg/hr) as well as histamine (4 mg/kg/hr) in anesthetized rats. For detailed protocols, refer to Fig. 7. Data are presented as the mean±S.E. of values determined every 10 min from 4 rats. *Statistically significant difference from the values observed before the exposure to KT1-32, at P<0.05.
Effect of KT1-32 on acid secretion: Gastric acid secretion in anesthetized rats was markedly stimulated by i.v.-infusion of histamine and reached the plateau values of 18.0±2.1 μEq/10 min within 50 min. Exposure of the stomach for 10 min to KT1-32 (3-10 mg/kg) significantly reduced the histamine-stimulated acid secretion in a dose-dependent manner; at 30 mg/kg, the inhibition lasted for 80 min (Fig. 7). Intragastric application of saline had no effect on the acid secretion. KT1-32 (30 mg/kg) also had an antisecretory effect against the carbachol induced acid secretion. As shown in Fig. 8, the exposure of the mucosa for 10 min to this agent significantly inhibited acid secretion stimulated by carbachol; both the degree and duration of inhibition were similar in the cases of carbachol and histamine.

Effect of KT1-32 on H/K ATPase activity: KT1-32 (3×10^{-5}-3×10^{-3} M) inhibited the H/K ATPase activity of the hog gastric mucosa; the inhibition at 3×10^{-4} M was 46.9%, the IC50 being 3.9×10^{-4} M (Fig. 9). In the same experiment, omeprazole potently inhibited the activity, the IC50 being 1.6×10^{-5} M.

Discussion
The present study demonstrated that intragastric application of KT1-32 elevated the transmucosal PD of the stomach with concomitant inhibition of acid secretion. The PD generating effect may be associated with the antisecretory action of KT1-32, because this effect was significantly attenuated by prior administration of omeprazole, an H/K ATPase inhibitor, and because this agent, although at higher concentrations, inhibited the H/K ATPase activity. However, the PD generating effect does not indicate the enhanced resistance of the gastric mucosa against damage, since the stomach responded similarly to ethanol by a marked reduction of the PD in the absence or the presence of KT1-32.

Exposure of the gastric mucosa for 10 min to KT1-32 dose-dependently increased the PD with an elevation of the luminal pH. The increase of pH in the present system is observed after administration of antisecretory agents or mucosal damaging agents (6, 13). The former is accounted for by acid inhibition, and the latter is due to both diffusion of HCO3 and acid inhibition occurring as the consequence of the barrier disruption. However, since KT1-32 produced an elevation of the PD, it is unlikely that the observed pH response resulted from the barrier disruption caused by this agent. We previously reported the comparative effects of omeprazole and cimetidine on PD and pH in the rat stomach and suggested that the increased PD response accompanied by a rise in pH may be a characteristic phenomenon seen after the blockade of H/K ATPase and occurs because of unmasking of the net Cl transport in the parietal cell (13, 14). Actually, cimetidine increased only pH without much effect on PD. Based on these findings, topically applied KT1-32 acted on the gastric mucosa, similar to omeprazole, resulting in an elevation of the PD and pH. During exposure to 30 mg/kg of KT1-32, the PD showed a sharp elevation, followed by a decrease, and again gradually increased after the exposure. Such a biphasic response was not observed after topical application of lower doses. It might be possible that KT1-32 has an irritative action on the mucosa at higher doses. Even if this occurs, this effect would not be serious, because the reduced PD was restored to over the basal values shortly after removal of the agent from
the stomach.

Exposure of the stomach to KT1-32 not only decreased basal acid secretion (an increase of luminal pH) but inhibited the histamine- or carbachol-stimulated acid secretion as well. These findings disagree with the previous observation that KT1-32 has a minimal effect on acid secretion in the pylorus-ligated rats (1). This discrepancy may be due to the different experimental conditions. In the previous study, KT1-32 was given p.o. 30 min before or given i.d. immediately after pylorus ligation, so that the agent was absorbed in the body without any contact with the gastric mucosa (i.d.) or was emptied in the duodenum after brief contact with the mucosa (p.o.). In this study, since KT1-32 was topically applied to the mucosa mounted on the Lucite chamber, the agent was in good contact with the mucosa without emptying and thereby might be absorbed locally to reach a higher concentration in the mucosa. In fact, this agent given s.c. did not have any influence on PD generation and acid secretion (not shown). Since KT1-32 has no interaction with H2-receptors or muscarinic receptors (unpublished data: S. Wakabayashi et al.), it is unlikely that the antisecretory action occurs because of the blockade by KT1-32 of these receptors on the parietal cells. As expected from the PD responses to KT1-32, this agent showed an inhibition against the H/K ATPase activity in vitro. The IC50 (3.9 x 10^-4 M) of KT1-32 is much higher than that of omeprazole, but this concentration would be expected in the case of topical application. It may be assumed that KT1-32 increased both pH and PD because of the H/K ATPase inhibition, similar to omeprazole (13, 14). This is supported by the fact that the PD response caused by KT1-32 was significantly mitigated by pretreatment of the animals with omeprazole. Although the mechanism underlying the inhibition by KT1-32 of H/K ATPase remains unknown, it might be related to the protein binding capability of this agent (15).

We previously reported that timoprazole failed to affect the PD reduction induced by ethanol and aspirin, despite the fact that this agent produced a marked increase of basal PD values (16). Similarly, KT1-32 did not significantly affect the mucosal PD and pH responses to ethanol, although the basal PD was increased in response to this agent. These findings indicate that the higher PD values do not necessarily mean an increase of the mucosal resistance to irritants. The present study also suggests that the PD generating action does not relate to the preventive effect of KT1-32 on ethanol-induced gastric lesions (1, 2). The mechanism underlying the mucosal protection by KT1-32 may be related to other events such as the prevention of vascular injury (2) or the antagonization of TXA2/PGH2 receptors (3) by this agent.

Taken together, the present study indicates that KT1-32 given intragastrically acts on the rat stomach to produce both antisecretory and PD generating effects locally. The latter may be attributable to acid inhibition by this agent, probably associated with the inhibition of H/K ATPase activity. Although the relationship of these actions to the antiulcer effect of this agent remains undefined at the present stage of research, the local antisecretory action might contribute to a beneficial effect of KT1-32 and its derivative (GAS) for the relief of subjective symptoms in patients with gastritis (17).

References
1 Okabe, S., Takeuchi, K., Mori, Y., Furukawa, O. and Yamada, Y.: Effects of KT1-32 on acute gastric lesions and duodenal ulcers induced in rats. Folia Pharmacol. Japon. 88, 467-476 (1986) (Abs. in English)
2 Rogers, C., Brown, A. and Szabo, S.: Gastric mucosal protection by new aryl sulfhydryl drugs. Dig. Dis. Sci. 33, 324-329 (1988)
3 Mochizuki, S., Wakabayashi, S., Tomiyama, A., Satake, N. and Shibata, S.: Thromboxane A2 antagonistic action of a new anti-ulcer agent, azuletil sodium (KT1-32). Scand. J. Gastroenterol. 24, Supp. 194-197 (1989)
4 Ivey, K.J.: Effect of bile salts on ionic movement across the human gastric mucosa. Gastroenterology 59, 683-690 (1970)
5 Rutten, M.J. and Ito, S.: Morphology and electrophysiology of guinea pig gastric mucosal repair in vitro. Am. J. Physiol. 244, G171-G183 (1983)
6 Takeuchi, K., Ohno, T. and Okabe, S.: Irritative and protective activity of mild irritants in rat stomach. Dig. Dis. Sci. 32, 889-896 (1987)
7 Takeuchi, K., Ishihara, Y., Okada, M., Niida, H.
and Okabe, S.: A continuous monitoring of mucosal integrity and secretory activity in rat stomach: A preparation using a lucite chamber. Japan. J. Pharmacol. 49, 235–244 (1989)

8 Takeuchi, K., Furukawa, O., Tanaka, H. and Okabe, S.: A new model of duodenal ulcers induced in rats by indomethacin plus histamine. Gastroenterology 90, 636–645 (1986)

9 Beil, W., Hackbarth, I. and Sewing, K-Fr.: Mechanism of gastric antisecretory effect of SCH28080. Br. J. Pharmacol. 88, 19–23 (1986)

10 Hongo, T., Nojima, S. and Setaka, M.: Purification and characterization of (H/K)-ATPase from hog gastric mucosa. Japan. J. Pharmacol. 52, 295–306 (1990)

11 Fiske, C.H. and Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem. 66, 375–400 (1925)

12 Dunnett, C.W.: A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, 1096–1121 (1955)

13 Takeuchi, K., Nishiwaki, H. and Okabe, S.: Effects of topical application of acidified omeprazole on acid secretion and transmucosal potential difference in anesthetized rat stomachs. Japan. J. Pharmacol. 47, 397–408 (1988)

14 Starlinger, M.J., Hollands, M.J., Rowe, P.H., Matthews, J.B. and Silen, W.: Chloride transport of frog gastric fundus: Effects of omeprazole. Am. J. Physiol. 250, G118–G126 (1986)

15 Sato, M., Suzuka, H., Tomiyama, A. and Miyazaki, H.: Studies on the metabolic fate of azuletil sodium (V): Protein bindings and uptake by erythrocytes. Xenobio. Metabol. Dispos. (in press)

16 Takeuchi, K., Nishiwaki, H., Niida, H. and Okabe, S.: Different effects of cytoprotective drugs on ethanol- and aspirin-induced gastric mucosal lesions in the rat. Dig. Dis. Sci. 35, 178–185 (1990)

17 Miyoshi, A., Yoneda, M., Mukaino, S., Suzuki, K., Nishi, S. and Mitsutani, S.: Effects of oral administrations of azunol on patients with gastritis as well as peptic ulcer (1). Japan. Arch. Intern. Med. 7, 912–916 (1960)