Effect of Cimetidine on Gastric Mucosal Barrier in Dogs

JIN-ICHI KAMEYAMA, HIDENOBU MIYAKAWA, IWAO SASAKI
and TOSHIO SATO

*The First Department of Surgery, Tohoku University School
of Medicine, Sendai 980*

KAMEYAMA, J., MIYAKAWA, H., SASAKI, I. and SATO, T. *Effect of Cimetidine on Gastric Mucosal Barrier in Dogs*. Tohoku J. exp. Med., 1983, 141 (1), 91-98 — The effect of cimetidine on the gastric mucosal barrier (GMB) was investigated in adult mongrel dogs with a Heidenhain pouch. A linear correlation was found between the net flux of Na^+ and H^+ , and transmucosal electrical potential difference (PD). The PD was thus considered to be a good indicator for the degree of impairment of the GMB which can be determined easily with high reproducibility. When irrigated with HCl alone, there was a slight, though not significant, upward tendency of the PD following intravenous cimetidine. The destruction of the GMB during perfusion with HCl plus sodium taurocholate resulted in a marked reduction of the PD. This response of the PD was significantly reduced by the injection of cimetidine. These results indicate that cimetidine protects the GMB from impairments by bile acid. ——— gastric mucosal barrier; PD; ionic net flux; cimetidine; bile acid

Histamine H_2 -receptor antagonist, cimetidine, has been demonstrated to have a potent inhibitory action on gastric secretion (Burland et al. 1975; McCloy et al. 1979). However, few studies (Gurll et al. 1977; Rees et al. 1977) have been reported to date concerning its influence on the gastric mucosal barrier (GMB), especially in the presence of bile acids. The present experimental study was undertaken to investigate the GMB in terms of net flux of Na^+ and H^+ (ΔNa^+ and ΔH^+), and transmucosal electrical potential difference (PD). Especially, the effect of cimetidine on the GMB, either intact or impaired by bile acid, was examined using PD as a parameter.

MATERIALS AND METHODS

Twenty-three healthy mongrel dogs weighing between 14 and 29 kg were prepared with a Heidenhain pouch drained by a Gregory cannula. After a 4-week recovery period, each animal was subjected to the following procedures, as previously described in details (Davenport et al. 1964; Momono et al. 1980). Briefly, an extracorporeal circulation circuit was connected to the Heidenhain pouch (Fig. 1) and measurements were made with respect to ΔNa^+ , ΔH^+ , and PD during the irrigation of the pouch with various test solutions. pH and osmolarity were adjusted to 1.0 and 308 mosmol, by changing mannitol concentration, respectively. The concentration of Na^+ was measured by means of a flame photometer (Model: flame photometer 30, Nihon Buncō, Tokyo), while

Received for publication, January 25, 1983.

Fig. 1. Schematic illustration of the experimental apparatus employed for irrigating the Heidenhain pouch with various test solutions.

that of H^+ was determined by titration with 0.1 N NaOH up to an end point of pH 7.0 using a pH titrator (Model: Stoma-1, Toa Denpa, Tokyo). The PD generated was led from the Heidenhain pouch and foreleg skin with calomel half-cells via a salt bridge (consisting of 3 M KCl in 3% agar contained in a polyethylene tube) and recorded continuously with a high impedance electrometer (Model; EPR-200A Toa Denpa, Tokyo). The PD values were read at every 3 min interval. After being deprived of food, but not of water for 18 hr prior to each experiment, the dogs were trained to Pavlov stand quietly, partially supported by cloth slings. The animals were tested once or twice a week.

Experiment 1. This experiment was designed for studying the correlations between net flux of Na^+ and H^+ , and PD. For this purpose, Na^+ , H^+ , and PD were measured during perfusion of the Heidenhain pouch with three different types of solutions, i.e., 1/10 N HCl, 15/100 N HCl, and 1/10 N HCl plus 20 mM sodium taurocholate* (DIFCO Laboratories, Detroit, Michigan, USA) (taurocholate). The perfusion was made for 2 periods of 15 min each. The sum of PD read at 3 min intervals for 15 min was expressed as $\Sigma PD/15$ min.

Experiment 2. This experiment was aimed to determine the concentration of taurocholate in test solution at which the PD shows submaximal response to destruction of the GMB. The PD was measured during 4 periods of perfusion with 1/10 N HCl plus 1.0, 1.7, 2.8, 5.0, 10, 20 and 35 mM taurocholate.

Experiment 3. This was conducted to investigate the influence of cimetidine on the

Fig. 2. Experimental protocol (Experiment 3).

GMB. The animals were divided into 4 groups, A, B, C and D, and experiments of these groups were performed in 6 periods of 15 min each (Fig. 2). During periods I and II, the pouch was irrigated with 1/10 N HCl alone; these two initial periods were considered as basal phase for each group. For periods III~VI, dogs in group A received perfusion with 1/10 N HCl alone. Group B received $4\text{ }\mu\text{M/kg}$ of cimetidine (Smith Kline-Fujisawa Pharmaceutical Co., Ltd., Tokyo) intravenously immediately before the start of period III, then perfused with 1/10 N HCl for subsequent periods. In group C, 1/10 N HCl plus 2.8 mM taurocholate was used, while in group D, perfusion was carried out in the same manner except that a $4\text{ }\mu\text{M/kg}$ intravenous dose of cimetidine was given just before period III. The PD of these 4 groups was measured at every 3 min interval as well as in experiments 1 and 2. Comparisons were made among these 4 groups on the basis of percentage to basal phase for each group.

Experiment 4. This experiment was designed to explore the influence of cimetidine in an intravenous dose of $4\text{ }\mu\text{M/kg}$ upon gastric secretion. Volume of gastric juice and acid output from the Heidenhain pouch were measured by the stimulation with continuous intravenous tetragastrin administration at $4\text{ }\mu\text{g/kg/hr}$ for 3 hr, while cimetidine was injected intravenously at 1 hr after initiating tetragastrin infusion.

Statistical analyses. All data were expressed as mean $\pm$ s.e. The significance of the differences was assessed by the Student's *t*-test.

RESULTS

Experiment 1

The results of the correlations between Na^+ and H^+ , and PD are shown in Fig. 3. A significant ($p < 0.01$) linear correlation was noted between Na^+ and $\Sigma\text{PD}/15\text{ min}$ and between H^+ and $\Sigma\text{PD}/15\text{ min}$.

Experiment 2

The PD values during perfusion with 1/10 N HCl plus 1.0~35 mM taurocholate are shown in Fig. 4. It is clearly seen that the PD varied depending upon the

Fig. 3. Correlation between ΔNa^+ , ΔH^+ and $\Sigma\text{PD}/15\text{ min}$.

concentration of taurocholate in test solution. A distinct dose-response relationship was noted for PD in the taurocholate concentration of 1.0~5.0 mM, and maximal response of the PD was elicited at 5.0 mM or above. The submaximal response of the PD was seen at the concentration of 2.8 mM.

Fig. 4. Effect of taurocholate concentration on PD.

Experiment 3

(1) *Group A*. The PD remained nearly constant throughout the entire 6 periods of perfusion with 1/10 N HCl, being approximately -60.4 mV on the average (Fig. 5). No significant changes ($p>0.1$) was observed throughout periods I~VI.

(2) *Group B*. The mean PD was -61.6 ± 2.2 mV during basal phase, and appeared to increase slightly to -63.2 ± 2.8 mV at period V with a further increase till the end of period VI (Fig. 5). The differences from basal phase levels were, however, statistically insignificant ($p>0.1$).

(3) *Group C*. The mean PD was -59.7 ± 4.6 mV during basal phase. It declined

Fig. 5. Effect of cimetidine on PD (intact GMB).

Fig. 6. Effect of taurocholate on PD.

abruptly after an addition of taurocholate to test solution and was kept in that level until the end of period VI (Fig. 6). The PD at periods III~VI was significantly ($p<0.01$) lower than that of basal phase.

(4) *Group D.* The mean PD was -57.0 ± 4.5 mV during basal phase, and decreased to an average of -48.4 ± 3.8 mV at period IV and this declining tendency persisted until the end of period VI (Fig. 7). The PD for periods III~VI was significantly lower than that of basal phase ($p<0.05$).

(5) *Comparison of groups A and B.* In order to investigate the effect of

Fig. 7. Effect of cimetidine on PD (impaired GMB by taurocholate).

Fig. 8. Comparison of PD in 4 groups. *C-D: $p<0.05$.

cimetidine on the PD during perfusion with 1/10 N HCl, comparison was made between groups A and B. The mean PD for groups A and B as expressed on the basis of percentage to basal phase level were 101.2 and 102.6% for period V and 98.1 and 102.3% for period VI, respectively. The value tended to be higher, with the lapse of time, in group B than in group A (Fig. 8), although the differences were statistically insignificant ($p > 0.05$).

(6) *Comparison of groups C and D.* The mean PD for groups C and D expressed as percentage to basal phase level were 79.7 and 88.1% for period III, 82.7 and 84.8% for period IV, 82.4 and 89.0% for period V and 84.7 and 90.4% for period VI, respectively. It became thus obvious that the reduction of the PD due to taurocholate-induced damage of the GMB was lessened in group D as compared with group C (Fig. 8). The differences in PD were significant ($p < 0.05$) for periods III, V and VI.

Fig. 9. Effect of cimetidine on volume of gastric juice and acid output stimulated with continuous intravenous injection of tetragastrin. Mean $\pm$ s.e. $n=3$.

Experiment 4

Cimetidine administered intravenously reduced volume of gastric juice and acid output significantly ($p < 0.05$) for a period from 75 to 135 min after initiation of the experiment. The maximum percent inhibition was attained at 90 min after initiation of the experiment both for volume of gastric juice (75.3%) and acid output (73.5%) (Fig. 9).

DISCUSSION

Cimetidine, known as histamine H_2 -receptor antagonist, has been demonstrated to have a potent inhibitory action on gastric acid secretion. (Burland et al. 1975; McCloy et al. 1979). Little is known, however, about the influence of cimetidine upon the GMB, a concept of the defense mechanism of gastric mucosa originating in the back diffusion theory advanced by Teorell (1939) and proposed later by Davenport et al. (1964). Very few reports (Gurll et al. 1977; Rees et al. 1977) are available on the effect of the drug on the GMB impaired by bile acids. The degree of impairment of the GMB has conventionally been estimated from the net flux of Na^+ and H^+ as indicators (Davenport 1968; Ivey et al. 1970). Unfortunately,

however, this method of evaluating GMB is so intricate that its application for clinical use is fraught with difficulties. The present experimental study was carried out in an effort to overcome such difficulties. The experiment 1, which pertained to the relationships of Na^+ and H^+ with PD, demonstrated a linear correlation between these parameters. The PD was thus considered a good indicator for the degree of impairment of the GMB, since it can be determined easily and has high reproducibility. This led us to use the PD as an index for the effect of cimetidine on the GMB.

Concerning the effect of cimetidine on the PD, Ivey and associates (1975) measured the PD in 5 healthy volunteers following an oral 300 mg dose of cimetidine using electrodes swallowed into the stomach under fluoroscopic control. According to these authors, the basal level of PD was -48 ± 1.0 mV. Beyond 30 min after administration of cimetidine, there was a gradual but significant increase of PD to -64 ± 2.4 mV. Kleist and his colleagues (1979) have also reported an increase of PD following intravenous administration of cimetidine in 38 healthy individuals. Our present experiment 3 also demonstrated a slight, though not significant, upward tendency of the PD following intravenous cimetidine when the GMB was kept intact.

The experiment 3 is made about the effect of cimetidine on the GMB impaired by bile acid. Davenport (1968) and Ivey et al. (1970), through estimating ionic net flux, and Black et al. (1971) and Ritchie (1975), through measurement of the PD, recognized that bile acids damage the GMB. In consistence with these studies, the present experiment showed that the addition of taurocholate to test solution results in an abrupt reduction of the PD. No reports are available, however, to date concerning the effect of H_2 -receptor antagonist on bile acid-induced damage of the GMB, except for the experiment with metiamide conducted by Gurll et al. (1977). According to the same authors, no significant changes in PD were noted following intravenous administration of metiamide at $10 \mu\text{M/kg/hr}$ during perfusion of the Heidenhain pouch with HCl plus 20 mM taurocholate in dogs. In contrast, our present experiments with taurocholate showed that the decrease of the PD was apparently smaller in the group pretreated with cimetidine than in not pretreated group. This discrepancy can be explained by the different bile acid concentration rather than the different H_2 -receptor antagonists employed in these two studies. In fact, the experiment 2 demonstrated that maximal response of the PD was obtained at a taurocholate concentration of 5 mM or above. It is likely, therefore, that taurocholate at a concentration of 20 mM used by Gurll et al. (1977), which elicits maximal response of the PD, causes irreversible changes in the GMB so that the PD could no longer respond to the H_2 -receptor antagonist. In the present experiment 2 taurocholate at a concentration of 2.8 mM, which elicits submaximal response of the PD, was used. Baskin et al. (1976), in their experiments on aspirin-induced damage of the GMB, found cimetidine to protect against such a deleterious effect of aspirin on the gastric mucosa. From these results it seems warrantable to reason that cimetidine protect the GMB impaired by bile acids as well as aspirin.

The mechanism whereby histamine H_2 -receptor antagonists increase the PD still remains obscure. Gurll et al. (1977), and Read and Levin (1975) suggested that the mechanism might be due to the inhibitory action of gastric secretion. The results of the experiment 4 indicate that cimetidine at the aforementioned dosage inhibited gastric secretion to a significant extent; however, since bile acids have been shown to damage the GMB not through inhibition of gastric secretion but a direct action, the mechanism seems to be not only by inhibition of gastric secretion, but also by cytoprotection as induced by prostaglandin (Odonkor et al. 1981).

References

- 1) Baskin, W.N., Ivey, K.J., Jeffrey, G.E. & Burks, M. (1976) Protective effect of cimetidine on aspirin-induced alteration of gastric potential difference in man. *Clin. Res.*, **24**, 44A.
- 2) Black, R.B., Hole, D. & Rhodes, J. (1971) Bile damage to the gastric mucosal barrier — The influence of pH and bile acid concentration. *Gastroenterology*, **61**, 178–184.
- 3) Burland, W.L., Duncan, W.A.M., Hesselbs, T., Mills, J.G. & Sharpe, P.C. (1975) Pharmacological evaluation of cimetidine, a new histamine H_2 -receptor antagonist, in healthy man. *Brit. J. clin. Pharmacol.*, **2**, 481–486.
- 4) Davenport, H.W. (1968) Destruction of the gastric mucosal barrier by detergents and urea. *Gastroenterology*, **54**, 175–181.
- 5) Davenport, H.W., Warner, H.A. & Code, C.F. (1964) Functional significance of gastric mucosal barrier to sodium. *Gastroenterology*, **47**, 142–152.
- 6) Gurll, N.J., Zinner, M.J. & Callahan, W. (1977) Effect of histamine H_2 -antagonism by metiamide on the response of the canine gastric mucosa to acid and bile salt. *Gastroenterology*, **73**, 255–259.
- 7) Ivey, K.J., Denbesten, L. & Clifton, J.A. (1970) Effect of bile salts on ionic movement across the human gastric mucosa. *Gastroenterology*, **59**, 683–690.
- 8) Ivey, K.J., Baskin, W. & Jeffrey, G.E. (1975) Effect of cimetidine on gastric potential difference in man. *Lancet*, **2**, 1072–1073.
- 9) Kleist, D., Stopik, D. & Hampel, K.E. (1979) Effect of cimetidine and ranitidine on mucosal potential difference. *Lancet*, **2**, 1071–1072.
- 10) McCloy, R.F., Parmenter, S.C. & Baron, J.H. (1979) Cimetidine and salbutamol combined inhibition of gastric acid secretion in the dog. *Digestion*, **19**, 340–345.
- 11) Momono, S., Sekine, T., Kameyama, J. & Sasaki, I. (1980) Experimental studies on the influence of jaundice on gastric mucosal barrier. *J. Jpn. Surg. Soc.*, **81**, 416–425.
- 12) Odonkor, P., Mowat, C. & Himel, H.S. (1981) Prevention of sepsis-induced gastric lesions in dogs by cimetidine via inhibition of gastric secretion and by prostaglandin via cytoprotection. *Gastroenterology*, **80**, 375–379.
- 13) Read, N.W. & Levin, R.J. (1975) Cimetidine and gastric potential difference. *Lancet*, **2**, 1314.
- 14) Rees, W.D.W., Rhodes, J., Wheeler, M.H., Meek, E.M. & Newcombe, R.G. (1977) The role of histamine receptors in the pathophysiology of gastric mucosal damage. *Gastroenterology*, **72**, 67–71.
- 15) Ritchie, W.P., Jr. (1975) Acute gastric mucosal damage induced by bile salts, acid and ischemia. *Gastroenterology*, **68**, 699–707.
- 16) Teorell, T. (1939) On the permeability of the stomach mucosa for acids and some other substances. *J. gen. Physiol.*, **23**, 263–274.