EFFECTS OF THIOCYANATE AND ACETYLSALICYLIC ACID ON THE GASTRIC MUCOSAL BARRIER IN RATS

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The proposed presence of the gastric mucosal barrier system, which interferes with the back diffusion of hydrogen ion secreted in gastric juice (1), has been postulated to protect the possible occurrence of mucosal damage due to hydrochloric acid (2). Though the precise nature of the barrier remains biochemically unknown, mucous substances covering the mucous layer or mucosal epithelial cells are indicated to be related with barrier function (3).

Occurrence of the back diffusion of hydrogen ion in association with the equivalent increase in the intraluminal efflux of sodium ion in the surgically or pharmacologically vagotomized stomach of the rat or the dog indicated the equilibrated exchanges of hydrogen and sodium ions across the mucous epithels (4, 5).

Increase in the back diffusion of hydrogen ion by thiocyanate (2) and acetazolamide (6) in the vagotomized dog stomach suggests an essential role of a carbonic anhydrase in the maintenance of the gastric mucosal barrier, as the enzyme is present in the gastric mucosa and activity is inhibited by both agents.

Though thiocyanate as one of non-anticholinergic antacids has been an available pharmacological tool for elucidation of the mechanism of gastric acid secretion, especially in the gastric mucosa of frog in vitro, many discussions have been presented on the mode of its action (7-9).

Marked inhibition of the gastric acid secretion without affecting the volume of gastric juice by thiocyanate in the oral route prompted the study of the correlation between the inhibitory effect and increase in the back diffusion of hydrogen ion by thiocyanate.

METHODS
1. Sampling the gastric juice in rats

Male SD rats, 7 to 8 weeks of age and fasted previously for approx. 18 hr, were anesthetized with ether. The animals were divided into groups of 5. Exposition of the pyloric region of the stomach through abdominal incision along the middle line was followed by the ligation of the pylorus. Immediately thereafter, 2 ml of physiological saline was injected s.c. in order to prevent dehydration. Five hr after surgical procedures, the animals were sacrificed under deep ether anesthesia in order to extirpate the whole stomach. The volume (ml), hydrogen ion concentration and pepsin activity in the...
gastric juice were measured. Hydrogen ion (µEq/ml) was measured by titration with 0.01 N NaOH using phenolphthalein as an indicator. The activity of pepsin (µg/ml) was evaluated by the procedure of Kunitz (10), in which casein was used as a substrate.

Thiocyanate was administered orally 1 hr before surgery or s.c. immediately after the same procedure.

2. Back diffusion of hydrogen ion in the gastric mucosa of rats

Male SD rats previously fasted for 18 hr were used. These were divided into groups of Five. Following the procedure devised by Overholt et al. (5), surgicallaparotomy in the rat under ether anesthesia was followed by exposure of the stomach and ligation of the cardiac and pyloric rings with simultaneous bilateral section of the cardiac branch of the vagus nerve. The gastric luminal surface was washed with deionized water warmed at approx. 30°C until the return was clear. Thereafter, the infusion of 5 ml of the test solution (100 mM HCl containing 60 mM NaSCN, NaCl or Na₂SO₄) into the gastric lumen was followed by suture of the incised wound at the forestomach and closure of the abdominal cavity. Acetylsalicylic acid (ASA) was administered orally 3 hr prior to surgery. Two hr later, the animals were sacrificed under deep ether anesthesia for recovery of the gastric contents. Hydrogen ion concentration in the gastric content at the amount of 1/50 N NaOH consumed for neutralization to pH 7.0 by use of an autotitrator (Kyoto Denshi AT 05), sodium and potassium concentrations by use of a flame photometer (Coleman model 21) and concentration of chloride by use of a chloridmeter (Buchler-Cotlove) were estimated. The total amount of each ion initially instilled and later recovered from the stomach after 2 hr was calculated from the initial and recovered test solution. From these values net ion movement and volume change were determined using the equation given below. Net ion movement (Δ Concentration) = net ion recovered − net ion instilled. Net volume change (Δ Volume) = volume recovered − volume instilled. Positive values indicated a net gain of ion or volume. Negative values indicated a loss of volume or ion from the lumen.

3. Anti-ulcer evaluation in rats

Phenylbutazone (PBZ) and ASA were used as ulcerogenic stimuli. 200 mg/kg of PBZ or 260 mg/kg ASA suspended in 5% gumi arabicum was orally administered to the male SD rats fasted previously for approx. 18 hr. Six hr later, the animals were sacrificed under deep ether anesthesia. Gastric ulcers and hemorrhages in the glandular mucosa were scored by the following grading; 0: intact mucosa, 1: mucous or submucous hemorrhages associated with mucous erosions, 2: mucosal ulcers O to 1 mm in size and less than 10 in number, 3: mucosal ulcers 1 to 3 mm in size and less than 10 in number or those O to 1 mm in size and more than 10 in number, 4: those 3 to 5 mm and less than 10 or those 1 to 3 mm and more than 10, and 5: mucosal ulcers more than 5 mm in size, as well as O: no hemorrhage, 1: hemorrhages at the partial surface of the mucosa, 2: those covering more than 50 % of the gastric mucosa, and 3: diffuse hemorrhages on the whole surface of the gastric mucosa. Thioyanate was administered 1 hr before application of the ulcerogenic stimuli.

Thioyanate concentration in gastric juice or serum was determined by the spectrophoto-meteric method (11).
4. Estimation of carbonic anhydrase (CAase) activity

Following the method of Philpot (12), the activity of the enzyme was estimated. The bubbling of 0.3 M Na₂SO₃ solution containing 0.2 M NaHCO₃ with CO₂ gas converted the previous pH 9.5–10.0 to 7.0 in 60 to 75 seconds due to formation of HCO₃⁻. The presence of CAase prompted this reaction in 10 to 15 sec. The enzyme activity was measured, by estimating the length of time required to complete this reaction.

RESULTS

1. Effect of thiocyanate on the gastric juice secretion

As shown in Table 1, s.c. doses of 100 and 200 mg/kg of thiocyanate in the pylorus-ligated rats insignificantly decreased gastric secretion in volume and acidity. Marked decrease of both parameters was obtained by the dose of 500 mg/kg. However, one out of the 5 rats treated with 500 mg/kg died from toxicity. Oral administration of 200 mg/kg of thiocyanate resulted in a marked decrease of the gastric secretion but only regarding acidity.

2. Effect of thiocyanate on the back diffusion of hydrogen ion across the gastric mucosa

The infusion of HCl solution into the pylorus-ligated stomach with vagal innervation in rats produced the slightly increased concentration of hydrogen ion corresponding with an increase in concentration of chloride ion. The same procedure with HCl solution in the bilaterally vagotomized rats however produced a decreased level of hydrogen ion concentration in the test solution indicating occurrence of a back diffusion of hydrogen ion across the gastric mucosa and almost equivalent elevation (approx. 20 mEq/l) of sodium ion level in the test solution.

<table>
<thead>
<tr>
<th>Table 1. Effect of thiocyanate on the gastric secretion in 5-hr-phlorus-ligated rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper panel</strong>: Thiocyanate in doses described were injected s.c. immediately after surgical ligation of the pylorus.</td>
</tr>
<tr>
<td><strong>Lower panel</strong>: Thiocyanate in a dose of 200 mg/kg was orally administered one hr before ligation of the pylorus.</td>
</tr>
<tr>
<td>Each number indicates mean of 5 rats and standard error of the mean.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Volume (ml)</th>
<th>Acid concentration (μEq/ml)</th>
<th>Pepsin concentration (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Free acid</td>
<td>Total acid</td>
</tr>
<tr>
<td>Saline</td>
<td>5.9 ± 2.0</td>
<td>108 ± 12</td>
<td>111 ± 9</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>100 s.c.</td>
<td>4.7 ± 0.8</td>
<td>78 ± 8</td>
</tr>
<tr>
<td></td>
<td>200 s.c.</td>
<td>3.0 ± 0.8</td>
<td>64 ± 6</td>
</tr>
<tr>
<td></td>
<td>500 s.c.</td>
<td>0.6 ± 0.1*</td>
<td>3 ± 2 ***</td>
</tr>
<tr>
<td>Saline</td>
<td>5.3 ± 1.6</td>
<td>104 ± 18</td>
<td>135 ± 9</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>200 p.o.</td>
<td>6.0 ± 1.0</td>
<td>13 ± 6**</td>
</tr>
</tbody>
</table>

* p < 0.05
** p < 0.01 Significant difference in each saline group.
*** p < 0.001

Mean ± S.E.
Changes in Volume and Electrolytes Following Intragastric Instillation of a 100 mM HCl Solution

Oral administration of acetylsalicylic acid (ASA) in doses described 3 hr before pyloric ligation in vagotomized rats resulted in dose-dependent increases of both the back diffusion of hydrogen ion and the intraluminal efflux of sodium ion in the gastric juice. The control group consists of 5 non-vagotomized rats.

Oral administration of 125 and 250 mg/kg of ASA 3 hr before pyloric ligation in vagotomized rats resulted in dose-dependent increases of both the back diffusion of hydrogen ion and the intraluminal efflux of sodium ion in the test solution (Fig. 1). Both increases were again almost equivalent. The increased back diffusion of hydrogen ion by ASA in the vagotomized stomach has already been demonstrated by Davenport (13) and Overholt (14).

As shown in Fig. 2, the intragastric infusion of 100 mM HCl solution containing 15, 30 or 60 mM thiocyanate dose-dependent produced increases in the back diffusion of hydrogen ion and the intraluminal efflux of sodium ion from/to the test solution, however, the increase in the efflux of sodium ion was markedly less than that in the back diffusion of hydrogen ion. Though the intragastric presence of thiocyanate in the vagotomized rats did not affect the gastric juice secretion regarding volume and concentration of potassium, the back diffusion of chloride ion was slightly increased.

The intragastric infusion of NaCl, NaI or Na₂SO₄ produced little change in either
Fig. 2. Dose-response relationship of back diffusion of hydrogen ion and intraluminal efflux of sodium ion from/to the gastric juice of thiocyanate. Ordinate indicates net flux of sodium and hydrogen ion concentration (mEq/L). Abscissa indicates the concentration of thiocyanate (mM).

Fig. 3. Effects of intragastrically administered NaCl, NaSCN, NaI and Na₂SO₄ on the gastric mucosal barrier. Changes in volume and electrolytes following intragastric instillation of a 100 mM HCl solution containing 60 mM inorganic Na salts into vagotomized stomach of rats.
parameter as compared with that of thicyanate (NaSCN) (Fig. 3). Accordingly, such effects of sodium thiocyanate cannot be ascribed to the sodium ion but rather to the thiocyanate anion.

The loss of hydrogen ion (mEq/1) from the test solution in the presence of thiocyanate

![Graph showing the loss of hydrogen ion (mEq/l) from the gastric juice in the presence of thiocyanate and ASA plotted against the gains of sodium ion (mEq/1) to the gastric juice in bilaterally vagotomized rats.]

**Fig. 4.** The losses of hydrogen ion (mEq/l) from the gastric juice in the presence of thiocyanate and ASA were plotted against the gains of sodium ion (mEq/l) to the gastric juice in bilaterally vagotomized rats.

![Graph showing changes in volume and electrolytes following intragastric instillation of a 100 mM HCl solution containing 60 mM NaCl into vagotomized stomach of rats.]

**Fig. 5.** Effects of thiocyanate on the gastric mucosal barrier in s.c. doses of 200 and 500 mg/kg.

Changes in volume and electrolytes following intragastric instillation of a 100 mM HCl solution containing 60 mM NaCl into vagotomized stomach of rats.
and ASA were plotted against the gains of sodium ion (mEq/l) to the test solution in the bilaterally vagotomized rats (Fig. 4). Linear correlation between both parameters indicates the exchange of hydrogen and sodium ion at the same ratio as in the case of ASA and at the ratio of 2 to 1 in the case of thiocyanate. Difference in the slope of the curves between thiocyanate and ASA appeared, to be partly the contribution of the sodium ion form of thiocyanate, since the same ratio in mucosal exchange of hydrogen and sodium ions was also demonstrated by intragastric presence of NaCl or NaI. In addition, potassium thiocyanate (KSCN) resulted in the same event at the ratio of 1 to 1.

On the other hand, thiocyanate in a s.c. dose of 200 and 500 mg/kg did not increase the back diffusion of hydrogen ion and the efflux of sodium ion (Fig. 5).

Table 2 shows thiocyanate concentration in gastric juice and blood 5 and 8 hr after s.c. injection of thiocyanate (300 mg/kg) to pylorus ligated rats. At these times, the concentration of 5.0 mM in the gastric juice was not attained, though this dose of thiocyanate decreased the gastric secretion in volume and acidity in pylorus ligated rats. The blood levels of thiocyanate in these experiments were 16.5±9.0 and 34.0±8.5, respectively.

It was confirmed herein that direct contact with the gastric mucosa of thiocyanate above 10 mM is a determining factor for activating the back diffusion of hydrogen ion and the efflux of sodium ion.

### Table 2. Thiocyanate concentration (mM) in the gastric juice and plasma 5 and 8 hr after s.c. injection of thiocyanate in pylorus-ligated rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Volume (ml)</th>
<th>Total acid (μEq/ml)</th>
<th>Thiocyanate concentration</th>
<th>Gastric juice (mM)</th>
<th>Plasma (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4.0±0.7</td>
<td>105±2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiocyanate 300</td>
<td>1.2±0.2**</td>
<td>66±13*</td>
<td>3.0±0.8</td>
<td>16.5±9.0</td>
<td></td>
</tr>
<tr>
<td>8 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>6.3±0.7</td>
<td>119±7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiocyanate 300</td>
<td>2.0±0.5**</td>
<td>65±13**</td>
<td>4.8±0.5</td>
<td>34.0±8.5</td>
<td></td>
</tr>
</tbody>
</table>

* P<0.05
** P<0.01  Significant difference from each saline group.

3. Anti-ulcerogenic effect of thiocyanate

The hemorrhagic ulcers of the gastric mucosa caused by oral administration of ASA were hypothetically proposed to be induced by the increased back diffusion of hydrogen ion present in the gastric juice due to the destruction of mucosal barrier system (13).

As shown in Fig. 6, the intragastric administration of both 30 mM thiocyanate and 30 mM ASA more strongly activated the back diffusion of hydrogen ion in vagotomized rats than did individual administration however, an oral dose of 250 mg/kg of thiocyanate
FIG. 6. Intragastric administration of both 30 mM thiocyanate and ASA more strongly activated the back diffusion of hydrogen ion and the efflux of sodium ion than did individual administration.

TABLE 3. Effect of thiocyanate on the phenylbutazone (260 mg/kg, p.o.) and acetylsalicylic acid (200 mg/kg, p.o.) ulcer.

Phenylbutazone ulcer

<table>
<thead>
<tr>
<th>Treatment (mg/kg) p.o.</th>
<th>Intragastric pH</th>
<th>Score of hemorrhage index</th>
<th>Score of ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2.5 ± 0.5</td>
<td>1.8 ± 0.5</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Thiocyanate 200</td>
<td>2.2 ± 0.4</td>
<td>1.4 ± 0.5</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>Thiocyanate 500</td>
<td>4.5 ± 0.4**</td>
<td>0.4 ± 0.3*</td>
<td>0.8 ± 0.2*</td>
</tr>
</tbody>
</table>

Acetylsalicylic acid ulcer.

| Saline                | 3.0 ± 0.4       | 0.8 ± 0.2                 | 2.4 ± 0.4            |
| Thiocyanate 250       | 2.1 ± 0.1       | 0.5 ± 0.2                 | 1.0 ± 0.4*           |

* p < 0.05
** p < 0.01 Significant difference in each saline group.

did not aggravate the ulcer formation by ASA in vagally intact rats. Reversely, this dose of thiocyanate inhibited the formation of the gastric ulcer caused by ASA. In addition, oral doses of 200 and 500 mg/kg of thiocyanate also inhibited the development of phenylbutazone ulcer dose-dependently.
4. Effect of thiocyanate and ASA on the activity of carbonic anhydrase (CAase) in the rat's gastric mucosa

The oral administration of 200 mg/kg of ASA or thiocyanate produced increases in both the back diffusion of hydrogen ion and the efflux of sodium ion to/from the gastric juice. As shown in Table 4, each treatment with ASA and thiocyanate produced a marked decrease of CAase activity of the homogenate and the supernatant fraction of the gastric mucosa. In addition, treatment with thiocyanate also produced a decrease of CAase activity of the mitochondrial fraction, while no increase was observed with ASA.

<table>
<thead>
<tr>
<th>Treatment (mg/kg) p.o.</th>
<th>Activity of CAase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mitochondria</td>
</tr>
<tr>
<td>Control</td>
<td>10.1±2.8</td>
</tr>
<tr>
<td>ASA</td>
<td>8.0±3.0</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>0.8±0.1***</td>
</tr>
</tbody>
</table>

# Mitochondria fractionated by centrifugation at 6,000 g for 10 min.
## Supernatant fractionated by centrifugation at 57,000 g for 60 min.
* p<0.05
*** p<0.001. Significant difference from control group.

DISCUSSION

In the present experiments, oral administration of thiocyanate similar to that of ASA increased the back diffusion of hydrogen ion and the intraluminal efflux of sodium ion from/to the gastric juice in vagotomized rats. Similar results have been reported by Moody and Davis (2) and Lawrence et al. (6). These are the activation of the mucosal exchange of hydrogen and sodium ions by the intravenous injection of thiocyanate (2) as well as that of acetazolamide (6) in the dog's stomach containing the isotonic solution of HCl. Since the oral dose of thiocyanate prevented the formation of the gastric ulcer caused by ASA in vagally intact rats, both agents seemed to differ considerably in the mode of action on the mucosal barrier. At the same time, this evidence throws some doubt on the hypothesis (13) that ASA produces hemorrhagic ulcers of the gastric mucosa in virtue of destruction of the mucosal barrier followed by the increased back diffusion of hydrogen ion.

Brodie and Chase (15) found that vagotomy markedly decreased the incidence of ASA-induced mucosal ulceration in the rat, however, when acid (100 mM HCl solution) was infused into the stomach, mucosal ulceration again occurred. Our results show that when 100 mM HCl solution was infused into the surgically vagotomized rat's stomach, significant back diffusion of hydrogen ion and mucosal ulceration occurred in the case of ASA, but the former occurred and the latter did not in the case of thiocyanate. From these results, it is reasonable to speculate that the increased back diffusion of hydrogen ion dose not always
produce mucosal ulcer and that HCl solution is the essential factor in the production of ASA ulcer, not the vagus.

Increase in the back diffusion of hydrogen ion by thiocyanate or ASA in vagotomized rats suggests an essential role of carbonic anhydrase (CAase) in the maintenance of the gastric mucosal barrier, because the enzyme is present in the supernatant fraction of the gastric mucosa and its activity is inhibited by thiocyanate or ASA. Treatment with thiocyanate in vagotomized rats also inhibited the activity of CAase in the mitochondrial fraction, but that with ASA did not. Accordingly, both agents differed in the mode of action on the CAase activity of the mitochondrial fraction.

Davenport (16) has suggested that when the barrier is broken, hydrogen ion rapidly penetrates the underlying cells and stimulates them to release histamine, which in turn stimulates the secretion of more acid and caused the blood vessels in the mucosa to dilate and become more permeable. He concluded that this is the cause of the drastic hemorrhagic ulcers with ASA.

It has already been confirmed that histamine, tetragastrin or carbachol stimulated the activity of the CAase of mitochondrial fraction in the rat's gastric mucosa (17), consequently, this enzyme is presumed to be related with acid secretion.

From these facts, it is reasonable to speculate that thiocyanate inhibits CAase activity of the mitochondrial fraction and this effect causes an inhibit ion of the acid secretion and not a breakdown in capillary walls. Accordingly, thiocyanate reversely inhibited the formation of the gastric ulcer.

Thiocyanate behaved differently on the gastric juice secretion depending on the oral or s.c. route. Logothetopoulos, and Myant (18) have demonstrated the active transport of i.m. injected thiocyanate and iodine through the gastric mucosa and resultant increase in the concentration of these ions in the gastric mucosa and urine in the hamster, rat, mouse and guinea-pig. In view of this a considerable amount of thiocyanate was secreted in the gastric juice, even if an s.c. administrat ion was given. As cited above, the therapeutic dose of thiocyanate in the oral route but not in the s.c. route profoundly decreased the gastric juice secretion in acidity but not in volume. In addition, the s.c. administration of thiocyanate did not produce increases in the back diffusion of hydrogen ion and the efflux of sodium ion.

From these results, it seems reasonable to postulate that the oral route and the s.c. route of thiocyanate behaved differently regarding the mode of action in the decrease of acid secretion. Decrease through the former route is proposed to be derived from the increased back diffusion of hydrogen ion. That of the latter route awaits further investigation.

SUMMARY

1. S.C. dose of 200 mg/kg of thiocyanate (NaSCN) in pylonus ligated rats slightly decreased the gastric secretion in volume and acidity. The oral administration of the same dose of thiocyanate markedly decreased the gastric secretion but only in acidity.
2. Intragastric infusion of 100 mM HCl solution containing 15, 30 or 60 mM thiocyanate activated the back diffusion of hydrogen ion and the intraluminal efflux of sodium to/from the gastric content dose-dependently, whereas that of 60 mM NaCl, NaI or Na2SO4 did not.

3. Oral doses of 200 or 250 mg/kg thiocyanate did not aggravate the ulcer formation by acetylsalicylic acid (ASA) or phenylbutazone (PBZ). Reversely, these doses of thiocyanate inhibited the formation of the gastric ulcers caused by ASA or PBZ.

4. Oral administration of 200 mg/kg of thiocyanate or ASA produced marked inhibition of carbonic anhydrase (CAase) activity of the homogenate and supernatant fraction in the gastric mucosa. The same treatment of thiocyanate also inhibited CAase activity of the mitochondrial fraction while that of ASA did not.

From these results, it is concluded that thiocyanate is qualitatively different from ASA in the mode of action on the mucosal barrier system, since the oral dose of thiocyanate prevented the formation of the gastric ulcers caused by ASA, despite the fact that oral administration of thiocyanate similar to that of ASA produced increases in the back diffusion of hydrogen ion and the intraluminal efflux of sodium ion from/to the gastric content in vagotomized rats.

These points have been discussed in connection with CAase activity of the gastric mucosa.

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