Gastric-Acidity-Controlled Rabbits: Control of Gastric pH of Rabbits by the Use of Antacids

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A method for controlling the gastric pH of rabbits to low acidity (pH > 4) by using different antacids was investigated, and the physiological state of rabbits during gastric-acidity control and repeated bioavailability tests was also examined in terms of hemocytological and clinico-biochemical parameters. Neither dried aluminum hydroxide gel (DAH) nor synthetic aluminum silicate (SAS) could elevate the gastric pH to more than 4. In the case of sodium bicarbonate (SBB), the gastric pH increased transiently to around 6 immediately after ingestion of the diet containing SBB and then decreased rapidly to the region of high acidity (pH < 3). On the other hand, magnesium aluminosilicate (MAS) could maintain the gastric pH in the range from 4 to 6 over a period of 3 h after ingestion of the diet containing MAS. Thus, we conclude that MAS is the preferred antacid for maintaining the gastric pH at low acidity over a long period. Analysis of hemocytological and clinico-biochemical data suggested that the physiological state of rabbits is hardly affected by gastric-acidity control and repeated bioavailability tests.

Keywords—gastric pH; antacid; low acidity; gastric-acidity-controlled rabbit; physiological state

Introduction

Since the gastric pH or the gastric acidity in humans varies between subjects,1,2 the bioavailability of drug preparations whose dissolution and stability will be influenced by the gastric pH commonly varies between subjects.3-6 Therefore, the gastric acidity of humans and experimental animals should be taken into consideration when bioavailability tests and bioequivalent tests with regard to such preparations are carried out. The gastric pH in fasting humans varies between subjects and can be divided into two groups: above and below pH1,2). Ogata et al.3) divided the gastric fluid acidity of humans into two groups before bioavailability studies by using Gastrotest tablets (Chugai Pharmaceuticals, Tokyo), which contain a protein-bound dye7) that is released in the stomach by acid at pH 3 or less. The groups evaluated as hypo- or anacidic and as normal or hyperacidic by means of the Gastrotest8) were designated as low and high acidity subjects, respectively. Thus, we designated rabbits having gastric pHs of less than 3 and more than 4 as high and low acidity rabbits, respectively. In our previous paper,9) a method for controlling the gastric pH of rabbits to pH > 4 (low acidity) by using magnesium aluminosilicate (MAS) as an antacid and to pH < 3 (high acidity) by using hydrochloric acid was partly presented. In addition, the usefulness of rabbits having gastric pHs of low and high acidities (gastric-acidity-controlled rabbits: GAC-rabbits) has been demonstrated in bioavailability studies.9-11) However, when the gastric contents of GAC-rabbits were observed after bioavailability tests, the gastric contents of some rabbits sometimes contained hair, which may retard the gastric emptying of food or controlled-release preparations that do not disintegrate in the stomach. Thus, we considered that gastric lavage should be incorporated in the method for gastric-acidity control to remove hair.
Materials—Commercial solid diet (CR-1, Nihon Clea Co.) and special solid diet (CR-S, Nihon Clea Co.), which is prepared by removing alfalfa from CR-1, were employed for pre-feeding and controlling the gastric emptying, respectively. Magnesium aluminosilicate (Neusilin, Fuji Chemical Industry Co.; MAS), sodium bicarbonate (JPS grade: SBB), dried aluminum hydroxide gel (Kyowa Chemical Industry Co.; DAH) and synthetic aluminum silicate (Kyowa Chemical Industry Co.; SAS) were employed as antacids.

Preparation of Special Soft Diet—Special soft diet (soft CR-S) was prepared by adding 60 parts of water to 40 parts of CR-S according to the method reported by Maeda et al.12) This mixture was allowed to stand overnight in a cool place to swell and was divided into prescribed amounts and made into balls. Special soft diet with antacids (soft CR-A) was prepared by adding 30 g of water to a mixture consisting of 20 g of pulverized CR-S and 1, 2 or 3 g of antacid.

In Vitro Tests of Antacid Potency—The use of 25 ml of 0.1 N hydrochloric acid was selected as an initial condition because it seemed likely to simulate the condition in the empty stomach of rabbits, as shown in Fig. 1. First, 50 g of soft CR-S or CR-A and 20 ml of water were added to 25 ml of 0.1 N hydrochloric acid. This mixture was then vigorously shaken for 10 min at 37°C and was allowed to stand for 5 min to sediment the solid materials in the mixture. The pH of the upper liquid layer was measured with a pH-meter. This pH was taken as that immediately after ingestion of the soft diet. Subsequently, 20 ml of 0.1 N hydrochloric acid was added. The mixture was shaken for 20 min and then the pH was measured as described above. These procedures were repeated after every addition of hydrochloric acid.

Procedures for Gastric-Acidity Control—Gastric acidity of rabbits was controlled by a slight modification of the previous method.9) White male rabbits fed with CR-1, weighing about 3 kg, were used for this experiment. After fasting for 24 h (stage I), the rabbit was lightly anesthetized by intravenous injection of pentobarbital sodium (15 mg/kg), and the stomach was washed with about 200 ml of warm saline (37°C) using a rubber stomach tube. After gastric lavage (stage II), the rabbit was given 100 g of CR-S per day for 5 d. After fasting again for 24 h (stage III), the rabbit was given 100 g of soft CR-S per day for 2 d. On the next day (stage IV), the rabbit was given 50 g of soft CR-A. During gastric-acidity control, the rabbit was allowed water freely, and a cangue was fixed to the neck to prevent coprophagy.

Measurement of pH and Amount of Gastric Contents—After the rabbits at stage IV had been fed soft CR-S or soft CR-A with antacids other than MAS, 50 ml of water was orally administered through a rubber stomach tube to aid in collecting the gastric contents. About 5 ml of the gastric contents was withdrawn by suction with a syringe at predetermined times and centrifuged, and the pH of the supernatant was measured. On the other hand, in the cases of the rabbits fed soft CR-A with MAS and the rabbits at stages II—IV, a rabbit was sacrificed at predetermined times and the stomach was immediately isolated. The wet weight of the gastric contents was measured, the contents were centrifuged to obtain a solid layer and a liquid layer, and the pH of the liquid layer and the dry weight of the solid layer were measured. The difference between wet weight and dry weight was taken as representing the amount of the gastric juice.

Measurement of Physiological Parameters—Hemocytological and clinico-biochemical parameters were measured at stages I—IV of gastric-acidity control and at each stage of a sham bioavailability test. The sham bioavailability test was carried out as follows. About 2 ml of arterial blood of a rabbit in which the gastric pH was controlled by MAS was drawn by cardiac puncture six times at intervals of 2 h after oral administration of one capsule with no drug. After washout for 2 weeks, the gastric acidity of the rabbit was again controlled and the sham bioavailability test was repeated three times. The physiological parameters were measured at the following stages: immediately after the 1st administration (stage A), 12 d after the 1st administration (stage B) and 12 d after the 2nd administration (stage C).

Arterial blood (5 ml) was collected by cardiac puncture when the physiological parameters were measured. With regard to the hemocytological and clinico-biochemical parameters, the range of assay values obtained with 12 untreated rabbits was taken as the normal clinical range. It was estimated by comparing the range for treated rabbits with the normal range whether rabbits are normal or not.

Hemocytological Analysis—The blood specimen was immediately mixed with ethylenediaminetetraacetic acid tripotassium salt (EDTA-3K) and hemocytological parameters were analyzed by using a Microcellcounter CC-150 (Sysmex, Toa Medical Electronics Co.). Parameters measured were packed cell volume (PCV), hemoglobin.
concentration, white blood cell count (WBC) and red blood cell count (RBC).

**Clinico-biochemical Analysis** — The blood specimen was centrifuged and the plasma was subjected to clinico-biochemical analysis using a Technicon SMA 12/60 and a Technicon AA BASIC (Technicon Inst. Co.). Parameters measured were as follows: sodium, potassium, calcium, inorganic phosphorus, chloride, creatinine, bilirubin, total protein, albumin, glucose, urea nitrogen, total cholesterol, alkaline phosphatase, glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase.

**Results and Discussion**

**pH and Amount of Gastric Contents during Gastric-Acidity Control**

Figure 1 shows the pH and amount of the gastric contents at each stage of gastric-acidity control. On the morning after gastric lavage (stage II), no injury to the stomach wall was observed and no solid material was detected in the gastric contents. This indicates that gastric lavage was effectively performed. The amount of the gastric contents, consisting of only the gastric juice, was 29.3 ± 6.4 g and the pH was 1.15 ± 0.02 (mean ± S.E., n = 3). Thus, it was found that the rabbit has a basal secretion of acid. When rabbits were fasted for 24 h after feeding with CR-S for 5 d (stage III), the amount of the gastric contents was 44.3 ± 2.3 g, more than 90% of which was gastric juice with the pH of 2.06 ± 0.08 (n = 3). On the morning after rabbits had been given 100 g of soft CR-S (stage IV), the amount of the gastric contents was 24.9 ± 2.3 g, about 95% of which was gastric juice with the pH of 1.08 ± 0.02 (n = 3). This gastric condition was approximately the same as that after gastric lavage: the amount of solid materials remained negligibly small. Although gastric lavage transiently produced loss of appetite, the rabbit’s appetite was restored by stage IV. Thus, we could make the stomach of rabbits almost empty by these procedures.

After rabbits at stage IV had freely ingested CR-S for 1 h, the amount of the gastric contents increased remarkably and in particular, the amount of the gastric juice (123.3 ± 6.6 g; n = 3) increased about four times as compared with stage IV. This appears to be due to secretion of acid, promoted by ingestion of the diet. The amount of acid secretion by 1 h after diet ingestion was roughly estimated at 100 ml or more. However, the pH of the gastric contents (3.81 ± 0.12, n = 3) did not become as low as expected. This is considered to be due to the buffering capacity of the diet used in this experiment.

![Fig. 1. Amount and pH of Gastric Contents during Gastric-Acidity Control](image-url)
Gastric pH after Ingestion of Soft CR-S

Each line in Fig. 2 shows the individual gastric pH–time profile after a rabbit at stage IV ingested 50 g of soft CR-S. Although the gastric pH was about 1 before ingestion of soft CR-S, the gastric pH in each rabbit, except for one (solid line), increased and was maintained in the range of 3–4 during the first 1 h after ingestion because of the buffering capacity of soft CR-S. Thereafter, one of 4 rabbits (dotted line) showed a slow decrease of the gastric pH while the other three showed relatively rapid decreases of the gastric pH, and the pH at 3 h after ingestion became 3 or less. On the other hand, the gastric pH of the rabbit which is represented by the solid line increased initially, then decreased rapidly from 0.5 h and again increased gradually from 1.5 h. The reason for this is not clear. Thus, the changes of gastric pH after ingestion of soft CR-S showed moderate inter-subject variation. This seems to be ascribable to inter-subject variation in the extent of acid secretion and in the rate of gastric emptying of soft CR-S having the buffering capacity. The gastric pH values at all times in this experiment were about one unit or more lower than that in our previous paper. This may be ascribable to the differences between the groups in acid secretion and gastric emptying.

Thus, the pH change of the gastric contents after ingestion of soft CR-S showed inter-subject and inter-group variations, and the gastric pH changed from near low acidity (pH > 4) to high acidity (pH < 3). Therefore, although the gastric emptying can be controlled by CR-S, the rabbit in which the gastric pH is not controlled is not a suitable animal to test the bioavailability of drug preparations that show gastric pH-dependent bioavailability in humans. Thus, we attempted to maintain the gastric pH at low acidity over a long period by using different antacids.

In Vitro Antacid Potency

Figure 3 shows the relationship between the amount of 0.1 N hydrochloric acid added to soft CR-S and the in vitro pH, together with the in vivo results described above. The in vitro pH change with every 20 ml addition after 40 ml of 0.1 N hydrochloric acid was initially added corresponded better with the gastric pH change every 0.5 h from 0.5 h after ingestion of soft CR-S. This indicates that this in vitro method may permit the prediction of antacid activity in vivo after ingestion of soft diet containing antacid (soft CR-A). The antacid activity of each antacid was evaluated under in vitro conditions corresponding to the half life (about 2 h) of gastric emptying with regard to soft CR-S. Figure 4 shows the in vitro pH when 100 ml of 0.1 N hydrochloric acid was added to soft CR-S (50 g) containing 1, 2 or 3 g of each antacid. When no antacid was added, the pH was 2.5. In the cases of DAH and SAS, the pH did not exceed 2.5 even when 3 g of each antacid was added. In the cases of MAS and SBB, the pH increased with increasing added amount of antacid and when 1 g of antacid was added, the pHs with MAS and SBB were 4.3 and 5.8, respectively. SBB had the strongest antacid
potency, although it reacts with hydrochloric acid to produce carbonic acid gas. When soft CR-A with 1 g or more of MAS or SBB is ingested, it is predicted that the gastric pH will be maintained in the region of low acidity (pH > 4) for 2 h or more.

Thus, MAS and SBB were considered to be promising candidates as antacids for controlling the gastric pH to low acidity. In this experiment, 2 g of antacid was used for control of the gastric pH to make sure that the gastric pH is maintained at low acidity.

Gastric-Acidity Control by the Use of Different Antacids

Figure 5 shows the individual time courses of gastric pH after rabbits at stage IV ingested 50 g of soft CR-A with 2 g of antacid. With regard to MAS, each point represents the gastric pH of an individual rabbit because the gastric pH was measured by using the isolated gastric contents at each time. Neither DAH nor SAS showed antacid activity as effectively as had been expected from the in vitro results. In the case of SBB, the gastric pH increased rapidly to low acidity and then decreased rapidly to high acidity within 3 h after ingestion. The reasons why SBB could not maintain the gastric pH at low acidity (pH > 4) over a long period are considered to be as follows. SBB was rapidly emptied from the stomach because it is readily water-soluble, and/or physicochemical stimulating actions on the stomach wall, such as pressure of carbonic acid gas or acute pH elevation, might promote acid secretion. Another serious disadvantage of SBB is that most of the rabbits refused to ingest soft CR-A containing SBB, possible because the rabbits dislike the taste of the diet. Consequently, we did not adopt SBB as an antacid for controlling the gastric pH to low acidity.

On the other hand, although the gastric pH at each time varied widely between subjects, MAS could maintain the gastric pH in the region of low acidity (pH 4—6) over a period of 3 h after ingestion. MAS, which is poorly soluble in water, reacts with the acid to dissolve in the gastric juice. In generally, the liquid layer in the gastric contents is emptied from the stomach faster than the solid layer. Therefore, most of the MAS is presumably in the solid state in the stomach because enough MAS to neutralize the acid is added to soft CR-A. Accordingly, MAS seems to remain for a long period in the stomach as compared with water-soluble SBB.
and to exert a prolonged antacid action. However, the gastric pH at each time varied in the range of 4—6, although it was within the low-acidity region, and one of 7 rabbits at 0.5 h and 3 of 12 rabbits at 3 h after ingestion showed gastric pH below the low-acidity region (pH < 4). As a good correlation between the amount and the pH of the remaining contents in the stomach at 3 h after ingestion of soft CR-A with MAS was observed, as shown in Fig. 6, the variation and noncontrol of the gastric pH seem to be ascribable to inter-subject variation in the gastric emptying rate, besides the acid secretion.

Thus, although it is difficult to control the gastric pH of all rabbits in the low-acidity region because some rabbits always have high acid secretion and/or rapid gastric emptying, we could control the gastric pH of most rabbits in the low-acidity region by using MAS as an

Fig. 5. Individual Gastric pH–Time Profiles after Ingestion of Soft Diet with Different Antacids

In the case of D, the solid line is constructed with the mean value at each time. Antacid: A, DAH; B, SAS; C, SBB; D, MAS.

Fig. 6. Correlation between Remaining Amount of Gastric Contents and Gastric pH

Remaining amount is dry weight. The solid line is the regression line; *r* = 0.844, *p* < 0.01.
Physiological State of Rabbits during Gastric-Acidity Control

The influence of each procedure of gastric-acidity control on the physiological state of rabbits was examined in terms of blood parameters. The range of each parameter at each stage is shown in Tables I and II. All parameters in these tablets were approximately within the normal range. Accordingly, it was found that the physiological state of rabbits was not affected by the gastric-acidity control procedures, including ingestion of special diet, fasting and gastric lavage, although the body weight of the rabbits was reduced by about 6% (200 g) by fasting and gastric lavage.

Physiological State in Bioavailability Tests

In considering the practical use of GAC-rabbits, it is very important to know whether the rabbit can endure repeated bioavailability test (crossover test), including repeated blood sampling and repeated gastric-acidity control. In order to examine this, sham bioavailability tests were carried out in GAC-rabbits. Tables III and IV show the ranges of hemocytological

### Table I. Hemocytological Parameters in Rabbits during Gastric-Acidity Control

<table>
<thead>
<tr>
<th>Stage&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>PCV (%)</th>
<th>WBC (10&lt;sup&gt;9&lt;/sup&gt;/mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Hemoglobin (g/dl)</th>
<th>RBC (10&lt;sup&gt;12&lt;/sup&gt;/mm&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>36—42</td>
<td>63—114</td>
<td>11.3—13.5</td>
<td>533—676</td>
</tr>
<tr>
<td>I</td>
<td>40—44</td>
<td>63—96</td>
<td>12.3—14.0</td>
<td>564—658</td>
</tr>
<tr>
<td>II</td>
<td>32—43</td>
<td>76—117</td>
<td>9.7—13.7</td>
<td>424—626</td>
</tr>
<tr>
<td>III</td>
<td>34—41</td>
<td>62—118</td>
<td>10.4—12.8</td>
<td>540—592</td>
</tr>
<tr>
<td>IV</td>
<td>37—46</td>
<td>96—130</td>
<td>11.5—14.4</td>
<td>546—683</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each stage is described in Experimental. Data represent the ranges for three (stages I—IV) or twelve (untreated) animals.

### Table II. Clinico-biochemical Parameters in the Plasma of Rabbits during Gastric-Acidity Control

<table>
<thead>
<tr>
<th>Stage&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>Na&lt;sup&gt;+&lt;/sup&gt; (meq/l)</th>
<th>K&lt;sup&gt;+&lt;/sup&gt; (meq/l)</th>
<th>Cl&lt;sup&gt;-&lt;/sup&gt; (meq/l)</th>
<th>Ca&lt;sup&gt;2+&lt;/sup&gt; (mg/dl)</th>
<th>P&lt;sup&gt;+&lt;/sup&gt; (mg/dl)</th>
<th>Glu&lt;sup&gt;+&lt;/sup&gt; (mg/dl)</th>
<th>UN&lt;sup&gt;+&lt;/sup&gt; (mg/dl)</th>
<th>Cr&lt;sup&gt;+&lt;/sup&gt; (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>135—143</td>
<td>2.7—6.1</td>
<td>98—109</td>
<td>10.0—13.3</td>
<td>3.4—6.7</td>
<td>138—200</td>
<td>13—25</td>
<td>0.6—1.2</td>
</tr>
<tr>
<td>I</td>
<td>135—139</td>
<td>3.6—3.8</td>
<td>102—106</td>
<td>11.4—11.7</td>
<td>3.4—4.8</td>
<td>151—160</td>
<td>20—39</td>
<td>1.0—1.2</td>
</tr>
<tr>
<td>II</td>
<td>136—139</td>
<td>5.8—5.9</td>
<td>95—103</td>
<td>12.6—13.7</td>
<td>5.4—6.0</td>
<td>162—173</td>
<td>15—22</td>
<td>1.1</td>
</tr>
<tr>
<td>III</td>
<td>142—146</td>
<td>4.2—4.8</td>
<td>102—103</td>
<td>12.2—13.7</td>
<td>4.9—5.7</td>
<td>151—165</td>
<td>12—19</td>
<td>1.0—1.5</td>
</tr>
<tr>
<td>IV</td>
<td>135—139</td>
<td>7.7—8.0</td>
<td>99—102</td>
<td>11.0—12.5</td>
<td>8.1—9.7</td>
<td>69—175</td>
<td>13—19</td>
<td>1.0—1.1</td>
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### Table III. Clinico-biochemical Parameters in the Plasma of Rabbits during Gastric-Acidity Control

<table>
<thead>
<tr>
<th>Stage&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>TC&lt;sup&gt;f&lt;/sup&gt; (mg/dl)</th>
<th>TP&lt;sup&gt;g&lt;/sup&gt; (g/dl)</th>
<th>Alb&lt;sup&gt;h&lt;/sup&gt; (g/dl)</th>
<th>Bil&lt;sup&gt;i&lt;/sup&gt; (mg/dl)</th>
<th>Al-P&lt;sup&gt;j&lt;/sup&gt; (u/l)</th>
<th>GPT&lt;sup&gt;k&lt;/sup&gt; (u/l)</th>
<th>GOT&lt;sup&gt;m&lt;/sup&gt; (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>47—179</td>
<td>5.4—6.9</td>
<td>3.3—4.3</td>
<td>0.05—0.15</td>
<td>55—154</td>
<td>27—105</td>
<td>16—105</td>
</tr>
<tr>
<td>I</td>
<td>88—114</td>
<td>5.5—6.3</td>
<td>3.6—3.8</td>
<td>0.10</td>
<td>74—77</td>
<td>59—74</td>
<td>61—146</td>
</tr>
<tr>
<td>II</td>
<td>59—76</td>
<td>5.1—5.9</td>
<td>3.3—3.7</td>
<td>0.05—0.10</td>
<td>74—103</td>
<td>29—89</td>
<td>45—78</td>
</tr>
<tr>
<td>III</td>
<td>45—69</td>
<td>5.0—5.9</td>
<td>3.4—3.8</td>
<td>0.10</td>
<td>62—120</td>
<td>31—59</td>
<td>40—71</td>
</tr>
<tr>
<td>IV</td>
<td>76—185</td>
<td>5.4—7.5</td>
<td>3.1—4.6</td>
<td>0.10—0.20</td>
<td>75—96</td>
<td>36—59</td>
<td>21—54</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each stage is described in Experimental. b) Inorganic phosphorus. c) Glucose. d) Urea nitrogen. e) Creatinine. f) Total cholesterol. g) Total protein. h) Albumin. i) Bilirubin. j) Alkaline phosphatase. k) Glutamic-pyruvic transaminase. m) Glutamic-oxaloacetic transaminase. Data represent the ranges for three (stages I—IV) or twelve (untreated) animals.
and clinicobiochemical parameters at each stage, respectively. All parameters at each stage were approximately within the normal range, although urea nitrogen at stage A showed a somewhat high value. With regard to the reduction of the body weight caused by fasting and gastric lavage, the body weight recovered very smoothly during the period of washout and rabbits apparently recovered well before the next bioavailability test. Consequently, it was found that the physiological state of GAC-rabbits is hardly affected by repeated bioavailability tests.

From the results obtained in this study, we conclude that MAS is the preferred antacid for controlling the gastric pH of rabbits in the low-acidity region and that it is possible to use GAC-rabbits for crossover bioavailability studies from a physiological point of view.

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References