

## THE EFFECT OF CIMETIDINE AND HYPOXIA ON THE GASTRIC MACROMOLECULAR GLYCOPROTEIN IN RAT

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### ABSTRACT

In an experimental study in rats, cimetidine, a potent gastric-acid inhibitor (50 mg/kg, p.o.) reduced the macromolecular glycoprotein level of glandular stomach. The administration of cimetidine at a dose of 50 mg/kg, which inhibited the gastric lesions induced by hypoxia (13% O<sub>2</sub> for 6 hours), reduced the glandular stomach level of high molecular glycoproteins in animals receiving a low oxygen load as well as in those receiving no load. It was assumed that the reduction of macromolecular glycoprotein accelerated the gastric lesion induced by hypoxia load in rats and that cimetidine inhibited the lesions through a process other than that of recovering the glycoprotein level in the glandular stomach.

Key words: Glycoprotein, Rat, Hypoxia, Gastric mucosal lesion, Cimetidine

### INTRODUCTION

Although the mechanism of gastric mucosal protection is an important factor from the standpoint of the etiology and treatment of peptic ulcer<sup>1)</sup>, its properties complicate sample handling and there is no established view of its quantitative determination. Recently, Allen<sup>2)</sup> proposed a macromolecular glycoprotein, with a molecular weight  $2 \times 10^6$ , as a structure model for mucus, and Azuumi<sup>3)</sup> determined macromolecular glycoproteins by gel filtration. These works devoted to the development of study on the defensive mechanism of gastric mucosa. The earlier report from our laboratory<sup>4)</sup> demonstrated that the macromolecular glycoproteins are localized in the mucosal layer by using a modification of the original method of Azuumi *et al.* In the present report the authors tried to determine the effect of cimetidine, a Histamine H<sub>2</sub>-receptor antagonist which has an inhibitory effect on gastric acid secretion, on macromolecular and also to determine the variation in glycoprotein level during an acute low oxygen load<sup>5,6)</sup> which induced acute gastric mucosal lesions.

### MATERIALS AND METHODS

#### 1. Study Animals

The animals used were male Wistar rats weighing approximately 200 g. For 24 hours before the onset of the study they were offered nothing but water. Each animal was given 1 g of urethan per kg of body weight and all rats were treated under anesthesia.

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## 2. Study Design

The rats were divided into four groups. Animals from group A, the control group, received 1 ml of physiological saline through gastric intubation and then were kept in normal room air for 7 hours. Animals from group B were given cimetidine at a dose of 50 mg/kg and were similarly kept in normal room air for 7 hours. The remaining two groups were administered a low oxygen load: for group C, 1 ml physiological saline was administered into the stomach and for 6 hours after dose administration the animals were kept in a 60-liter incubator in low oxygen atmosphere consisting of 13% oxygen and 87% nitrogen, as described in a previous report<sup>5)</sup>. For group D, cimetidine was administered into the stomach at a dose of 50 mg/kg and the animals were kept in the same manner as those of group C.

After the experimental animal was decapitated and its stomach removed, an incision was made in the greater curvature of the excised stomach and it was examined grossly for mucosal lesions. According to the method of Takagi *et al.*,<sup>6)</sup> our findings were classified as "lesion-free" (–), "one echymosis or more or slight erosive lesion" (+), "moderate erosive lesion" (++) or "remarkable or numberless moderate erosive lesions" (+++). Then the forestomach was removed and only the glandular stomach, including the mucus layer, was subject to the study. Samples were immediately lyophilized and pulverized. As previously reported<sup>4)</sup>, 2 ml of a chloroform-methanol (2:1) mixture was added to 100 mg dry weight of the powdered sample and the solution was shaken for 24 hours and centrifuged. The supernatant was discarded to eliminate the effect of the lipid component. According to the method of Azuumi *et al.*,<sup>3)</sup> extraction was carried out twice with a pH 7.2 solution of 2% Triton x-100 and 50 mM Tris HCl. Using a Bio-Gel A 1.5 m column (diam., 1.1 cm; length, 80 cm), 1.5 ml of the combined extracts was eluted. The content of hexose in each fraction and the total hexose content were determined by the sulfuric acid method, using galactose as the standard<sup>7)</sup>.

The data obtained in this study were compared for samples of 100 mg dry weight and assessed by Student's t-test for significant differences ( $p < 0.05$ ).

## RESULTS

### 1. Gastric Mucosal Lesions Due to Experimental Hypoxemia

As shown in the Table, animals in group A (n=8) had few or no mucosal lesions, whereas all animals in group C (n=7) had lesions classified as (++) or (+++). Animals from group D (n=8) showed a tendency toward inhibition of the occurrence of mucosal lesions. The gastric mucosal lesions were divided into two groups, (1) (–) or (+) and (2) (++) or (+++), and were assessed by Fisher's exact probability test. There was a statistically significant difference between groups A and C at 1% level and between groups C and D at 5% level.

Table: The inhibitory effect of cimetidine on gastric mucosal lesion induced by hypoxia.

Groups	Grade of Lesions			
	(–)	(+)	(++)	(+++)
(A) Control (n=8)	7	1	0	0
(C) Hypoxia (n=7)	0	0	4	3
(D) Hypoxia with Cimetidine (n=8)	1	6	1	6

2. Variations in Glycoproteins, Especially in the Macromolecular Fraction

The elution pattern by gel filtration of the sample was obtained from the control group as shown in Figure 1. Each sample separated into three fractions designated as peak-1, peak-2, and peak-3, beginning with the fraction of the greatest molecular weight. Figure 2 shows the total hexose content and peak-1 hexose content, which represents macromolecular glycoproteins, for groups A and B. The total hexose content was  $149 \pm 52 \mu\text{g}/100 \text{ mg}$  dry weight (mean  $\pm$  SEM) for group A (n=8) and  $1309 \pm 66 \mu\text{g}$  for group B (n=6) which tended to show lower hexose content than group A, but the difference was not statistically significant. On the other hand, the peak-1 hexose content was  $519 \pm 57 \mu\text{g}$  and  $330 \pm 34 \mu\text{g}$  for groups A and B, respectively, the

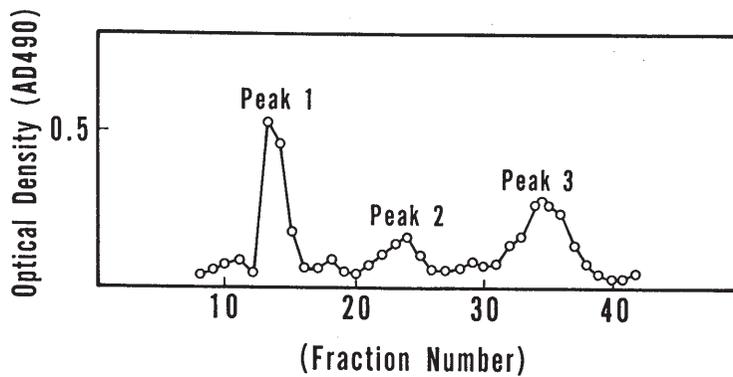


Fig. 1 Elution pattern in Bio-Gel A 1.5 m column chromatography of the extract from rat glandular stomach.

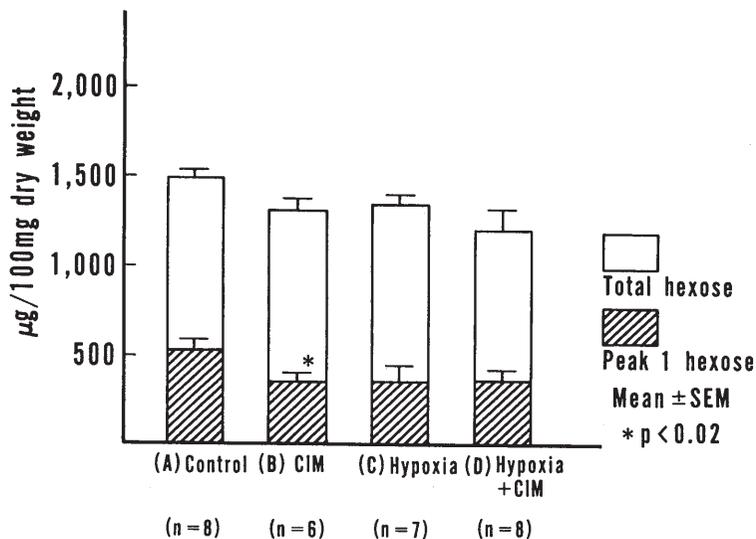


Fig. 2 The quantitative change of glycoprotein by cimetidine (CIM) and hypoxia. The peak-1 hexose of group B shows a statistically significant difference compared with that of group A ( $p < 0.02$ ).

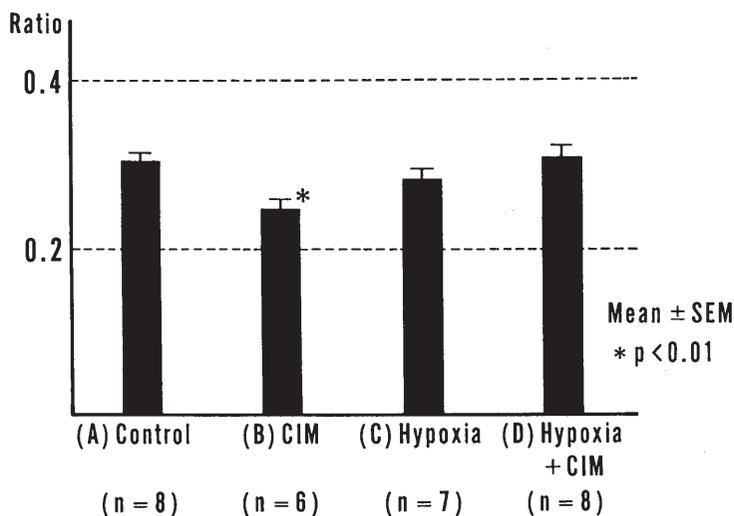


Fig. 3 The ratio of peak-1 hexose to total hexose. The ratio of group B shows a statistically significant difference compared with that of group A ( $p < 0.01$ ).

latter showing a significantly decreased value ( $p < 0.02$ ). As shown in Figure 3, the ratio of peak-1 hexose content to total hexose content was  $0.304 \pm 0.010$  for group A and  $0.248 \pm 0.012$  for group B, the difference being significant ( $p < 0.01$ ).

In animals from group C, which were administered a low oxygen load for 6 hours, the total hexose content of the gastric mucosa was  $1322 \pm 57 \mu\text{g}$  and peak-1 hexose content was  $370 \pm 78 \mu\text{g}$ . Both levels showed a tendency to decrease in group C as compared with group A. For group D ( $n=8$ ), the total hexose and peak-1 hexose levels were  $1191 \pm 122 \mu\text{g}$  and  $355 \pm 50 \mu\text{g}$ , respectively, both exhibiting a tendency toward a slight decrease.

The ratio of peak-1 hexose content to total hexose content was depressed at  $0.280 \pm 0.017$  for group C, while it was elevated at  $0.308 \pm 0.012$  for group D.

## DISCUSSION

The theory that mucus in the digestive tract protects the mucosa against chemical stimuli and enzyme-induced lesions such as gastric acid and pepsin has been supported by a variety of experiments. It has been found that mucus which covers the mucosal surface in jelly form not only withstands mechanical stimuli due to food and other factors but also exerts antipeptic action on proteolytic enzymes such as pepsin and acid<sup>8)</sup> and acts on  $\text{H}^+$  as a buffer<sup>9)</sup>. In spite of the important role played by mucus in mucosal protection, its mechanisms of biosynthesis and secretion still remain to be elucidated. Attempts have been made to clarify its mechanisms with slit-lamp<sup>10)</sup> and electron microscopic observation<sup>11)</sup>, and with histochemical methods, such as PAS staining<sup>12)</sup>, Alcain blue staining<sup>13)</sup>, and their combination, as well as with paradoxical concanavalin A staining. Various biochemical approaches have also been made to find a solution to the problem. A major glycoprotein complex present in the gastric mucosa has been found to be mucous glycoproteins. Variations in gastric mucosal glycoprotein content have been investigated through determination of hexose, hexosamine, sialic acid, and other related substances,

using the sugar portion of glycoprotein as a parameter. In the present study we determined the effect of cimetidine on gastric mucosal glycoproteins and the change during a low oxygen load, using hexose as an index and using a delipidizing technique, by which the level of macromolecular components could be determined more accurately, as described in a previous report<sup>4</sup>). In rats with experimentally induced hypoxemia, a 6-hour 13%-oxygen gas load resulted in a significant decrease of PaO<sub>2</sub> to 57.4 + 3.6 Torr, and under such conditions an ulcer occurred centering around the corpus. Both the peak-1 hexose level and the ratio of the peak-1 hexose to total hexose were decreased after 6 hours of oxygen load (O<sub>2</sub> 13%). This finding suggests that macromolecular glycoproteins are associated with mucosal lesions. It has been reported that the mucosal blood flow in this model is decreased, especially, in the corpus, as compared to the pyloric portion<sup>14</sup>). Although the mechanism of mucosal lesion development is under study in relation to the findings of decreased mucosal blood flow, the following assumption may be made. Briefly, low oxygenation state, which is caused at the cellular level in the mucosa by hypoxia and a disturbance of circulation, can also occur in mucous cells. As a result, macromolecular glycoproteins are decreased, the gastric mucoprotective mechanism breaks down, and an ulcer ultimately develops.

The present study showed that peak-1 hexose level was significantly decreased after administration of cimetidine. This may be explained as follows. Earlier studies<sup>15,16</sup>) have shown that histamine causes the covering cell cyclic AMP level to increase and also exerts a direct action on the covering cells. The other authors reported that histamine H<sub>2</sub>-receptor agonists promoted mucous secretion via adenylate cyclase<sup>17</sup>). Histamine H<sub>2</sub>-receptor antagonist was assumed to inhibit the occurrence of gastric mucosal lesions, exerting an intense inhibitory action on gastric juice secretion<sup>18,19</sup>).

Based on these facts, it is conceivable that cimetidine causes a decrease in consumption of macromolecular glycoproteins, but that through inhibition of biosynthesis it lowers the glycoprotein level of gastric mucosa. We are therefore inclined to think that if gastric acid, which plays an especially important role in gastric mucosal lesion development, is controlled, the magnitude of decrease in macromolecular glycoproteins is reduced as a secondary event.

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