EFFECTS OF L-GLUTAMINE ON ACETYLSALICYLIC ACID-INDUCED GASTRIC LESIONS IN NORMAL AND CIRRHOTIC RATS

Susumu OKABE, Koji TAKEUCHI, Tetsuro URUSHIDANI, Toshihiko NAGANUMA and Keijiro TAKAGI

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo, Tokyo 113, Japan

Accepted September 1, 1975

Abstract—Cirrhosis of the liver in rats was induced by the administration of carbon-tetrachloride (0.1 ml/100 g of body weight, s.c.) biweekly for 13 weeks. In the pylorus ligation preparation, acetylsalicylic acid (ASA) 100 mg/kg p.o. induced much more serious gastric damage in CCl₄-induced cirrhotic rats as compared with rats with a normal liver. L-glutamine 750 mg/kg p.o. prevented the ASA-induced gastric lesions in both normal and cirrhotic rats, even though the degree of the inhibition was weaker in cirrhotic rats. Gastric analysis indicated that L-glutamine 750 mg/kg p.o. markedly inhibited the gastric ionic changes (acid back diffusion) in response to ASA in both cirrhotic and normal rats.

Acetylsalicylic acid (ASA) is generally considered to be a hazardous agent on the stomach in both man and experimental animals (1, 2). Thus, the compound should not be given to cirrhotic patients who are known to bleed from the gastrointestinal tract (3-5). A series of studies from this laboratory indicated that L-glutamine markedly suppresses the ASA-induced gastric lesions in rats, possibly by preventing acid back diffusion caused by ASA (6-9). It was, therefore, of interest to examine whether or not, 1) ASA induces more serious gastric lesions in cirrhotic rats, and 2) L-glutamine concomitantly given with ASA prevents the increased gastric damages in cirrhotic rats, as observed in normal rats.

In this communication, we report the marked protection of gastric lesions and gastric ionic changes in response to ASA in both normal and cirrhotic rats by a concomitant administration of L-glutamine.

MATERIALS AND METHODS

Male Donryu strain rats, 180-220 g at the beginning of the experiments, were used.

Development of cirrhosis of the liver: In order to produce a cirrhosis of the liver, carbon-tetrachloride (CCl₄) in a dose of 0.1 ml/100 g of body weight was given to the rats s.c. biweekly for 13 weeks (10). Non-treated rats with a corresponding body weight were used as a control.

ASA-induced gastric lesions: Rats with normal or cirrhotic liver were deprived of food for 24 hr after which the pylorus was ligated under ether anesthesia. ASA 100 mg/kg suspended in 1% carboxymethylcellulose (CMC) solution was given orally to the animals.
10 min after pylorus ligation (6). Five hr after ASA dosing, the animals were sacrificed. The stomach of each was removed, treated with 1% formalin solution (11), and then examined for lesions. The sum of the length (mm) of all lesions, except the hardly visible ones, was used as a lesion index. L-glutamine 750 mg/kg suspended in 1% CMC solution or 1% CMC solution was given p.o. immediately after pylorus ligation.

Analysis of gastric contents: In order to examine the gastric secretory conditions in cirrhotic and normal rats, pylorus ligation preparation was also employed by applying the same time schedule (5 hr ligation after 24 hr fasting). ASA, ASA-L-glutamine, L-glutamine alone suspended in 1% CMC solution was given p.o. 10 min after the ligation. Five hr after dosing of these agents, the animals were sacrificed and gastric juices were collected. After centrifugation, the samples were analyzed for volume and titrated with 0.1 N NaOH to pH 7.0 on the pH meter for titratable acidity, which was expressed as meq/1. The concentrations of Na⁺ and K⁺ ions were measured by flamephotometry. The pepsin activity was determined by Anson's method (12) and was expressed as mg tyrosine per ml.

The level of significance was calculated using Student's t-test.

RESULTS

Cirrhosis of the liver

Severe cirrhosis of the liver was observed macroscopically in all of the rats treated with carbon-tetrachloride for 13 weeks (Fig. 1). Coarse granules ranging 1 to 3 mm in diameter were observed on the surface of the liver. The ascites found in the abdominal cavity in most of the rats was quite apparent. About 30% of the cirrhotic rats used in this experiment had a few erosions and numerous hardly visible lesions in the glandular stomach while others appeared to have a normal mucosa.

L-glutamine on ASA-induced gastric lesions

As shown in Table 1, L-glutamine 750 mg/kg p.o. produced a 80.7% inhibition

| Table 1. Effects of L-glutamine on acetylsalicylic acid (ASA)-induced gastric lesions in normal or cirrhotic rats (24 hr fasting, 5 hr ligation) |
|---|---|---|---|---|
| Group | Rats | Treatment | Dose (mg/kg) | No. of rats | Lesion Index (Mean ± S.E.) | % inhibition |
| 1 | Normal | Control | | 10 | 57.4 ± 7.4 |
| 2 | Normal | L-glutamine | 750 | 10 | 11.1 ± 2.8 | 80.7 |
| 3 | Normal | Control | | 10 | 83.5 ± 9.7 |
| 4 | Cirrhotic | Control | | 10 | 31.0 ± 6.8 | 62.7 |
| 5 | Cirrhotic | L-glutamine | 750 | 10 | 4.2 ± 1.2 |

*P < 0.001*

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ASA-induced gastric lesions in cirrhotic rats

It was revealed that ASA dosing in cirrhotic rats produced much more serious gastric damage (P<0.05) as compared with that seen in the control rats with a normal liver. L-glutamine 750 mg/kg also significantly (P<0.001) prevented the ASA-induced gastric lesions in cirrhotic rats (Fig. 2), even though the degree of inhibition (62.7%) was weaker than that seen in normal rats.

Gastric secretion in normal or cirrhotic rats

Details of the data as to the gastric secretion in rats are shown in Table 2. It was found that ASA 100 mg/kg p.o. produced a significant (P<0.001) decrease of gastric acid concentration and increment of Na⁺ ion concentration in both normal and cirrhotic rats to almost the same degree. It should be noted that L-glutamine 750 mg/kg p.o. given

| Group | Rats    | Treatment          | Dose (mg/kg) | No. of rats | Volume (ml) | Gastric juices | Pepsin (mg/ml) |
|-------|---------|--------------------|--------------|-------------|-------------|----------------|----------------|
|       |         |                    |              |             | Acid        | Na⁺ (meq/l)   | K⁺            |
| 1     | Control | ASA 100            | 10           | 12.4 ± 0.2  | 108.2 ± 2.8 | 38.7 ± 2.2    | 7.1 ± 0.4      | 21.4 ± 1.1 |
| 2     | Normal  |                    | 10           | 11.8 ± 0.6  | 74.8 ± 4.0  | 69.8 ± 4.9    | 6.4 ± 0.6      | 22.1 ± 0.9 |
| 3     | ASA+    | glutamine 750      | 10           | 11.7 ± 0.5  | 100.5 ± 2.4 | 51.0 ± 2.6    | 8.0 ± 1.1      | 19.1 ± 0.5 |
| 4     | glutamine|                | 750          | 11.7 ± 0.5  | 123.8 ± 2.4 | 38.5 ± 1.4    | 4.9 ± 0.3      | 18.8 ± 0.6 |
| 5     | Control | ASA 100            | 10           | 13.3 ± 1.2  | 106.4 ± 4.3 | 42.7 ± 2.9    | 7.6 ± 0.8      | 17.9 ± 0.5 |
| 6     | Cirrhotic|                | 10           | 13.7 ± 0.8  | 70.3 ± 4.1  | 71.6 ± 6.1    | 8.2 ± 0.8      | 16.6 ± 1.4 |
| 7     | ASA+    | glutamine 750      | 10           | 14.4 ± 1.2  | 115.7 ± 8.8 | 44.9 ± 3.2    | 8.2 ± 1.2      | 16.3 ± 0.4 |
| 8     | glutamine|                | 750          | 15.3 ± 0.4  | 128.6 ± 3.8 | 31.2 ± 2.3    | 6.5 ± 1.1      | 14.7 ± 0.5 |

All figures represent mean ± S.E. The values in the parentheses represent the net difference from control.
together with ASA produced a marked inhibition of acid and Na⁺ ion changes in response to ASA in normal and cirrhotic rats. L-glutamine itself significantly (P<0.001) increased the acid concentration with a significant reduction of Na⁺ (cirrhotic rats) or without reduction of Na⁺ (normal rats) as compared with corresponding control values. L-glutamine consistently reduced the pepsin activity in comparison with the control. It was noted at autopsy that L-glutamine had had no effect on the few erosions developed in the cirrhotic rats.

**DISCUSSION**

An advanced stage of cirrhosis of the liver was found by treatment of CCl₄ in all rats, such as has already been described by others (10, 13, 14). Although histological examinations were not carried out, coarse granules on the surface of the liver and severe ascites provided firm evidence for the development of advanced cirrhosis. Using such cirrhotic rats, we confirmed that ASA induces much more serious mucosal damages in the stomach than in normal rats. This finding appears to be consistent with the clinical data in which ASA is quite toxic when prescribed for cirrhotic patients. Even without ASA treatment, it is well documented that cirrhotic patients often have bleeding in the upper gastrointestinal tract (4,5, 15, 16). Gheorghiu et al (17) have demonstrated the occurrence of severe gastric mucosal hemorrhages and erosions in the glandular stomach of cirrhotic rats (CCl₄ given more than 4 months). They suggested that diminution of mucosubstances might play an important role in the pathogenesis of gastric damages in CCl₄-induced cirrhotic rats. The present authors also observed the formation of a few erosions in the glandular part of the stomach in rats with advanced cirrhosis. These results led to the speculation that gastric mucosal permeability may be increased in cirrhotic rats and as a result serious gastric ionic changes are induced as compared with those in normal rats. In the present study, however, the degree of ionic changes in response to ASA, most likely the occurrence of acid back diffusion, was found to be almost the same in both cirrhotic and normal rats, suggesting the normal integrity of the stomach in cirrhotic rats.

Of great interest was the evidence that L-glutamine, which prevents the ASA-induced gastric lesions in normal rats, also prevented to a great extent the gastric lesions developed in cirrhotic rats. However, the degree of glutamine protection in cirrhotic rats was weaker than that in normal rats. The dose of L-glutamine used was the same as that used in previous work (6, 7) in which a marked inhibition of ASA-induced gastric lesions in normal rats was induced by that dose level. Gastric analysis indicated that L-glutamine prevents the reduction of acid and a corresponding increment of Na⁺ in response to ASA to the same degree in both normal and cirrhotic rats. These findings suggest that the pathogenic mechanisms of the aggravated development of ASA-induced gastric lesions in cirrhotic rats involve gastric ionic changes (acid back diffusion) leading to mucosal damages and reduced activity of ASA metabolizing enzyme. Menguy et al. (16), including one of the present authors (S.O.), found that ASA-acyl-hydrolase, which metabolizes ASA to salicylate and acetic acid, is significantly lower in serum of cirrhotic patients than in normal subjects. They speculated that the lowered enzyme activity in cirrhotic patients allows ASA to exist in the
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blood for a longer period so as to exert to a greater extent a noxious effect on the gastric mucosa. In a preliminary study, we also determined the serum ASA-acyl-hydrolase activity in cirrhotic rats and found about 50% reduction of the activity in comparison with that of normal rats. Therefore, it is likely that the delayed degradation of ASA in blood in cirrhotic rats may partly evoke acid back diffusion and partly other changes in the body, such as an inhibition of platelet aggregation, which is not affected by L-glutamine. Regardless of the mechanism, ASA, when given together with L-glutamine, may be prescribed even for cirrhotic patients with little risk of damaging the gastric mucosa.

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