Effect of H2-Receptor Antagonists on Acetaminophen-Induced Hepatic Injury

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Abstract—The effects of H2-antagonists on acetaminophen-induced hepatic injury were examined. Rats were administered acetaminophen at the dose of 800 mg/kg body, 60 hr after injection of 3-methylcholanthrene. As an H2-antagonist, cimetidine (200 mg/kg), ranitidine (50 mg or 100 mg/kg), or famotidine (20 mg or 40 mg/kg) was administered before and after acetaminophen injection. The group administered only acetaminophen had been severely damaged as evaluated by changes in serum transaminase, P-450 content, aminopyrine demethylation, glutathione content and histological study, but administration of 200 mg cimetidine together with acetaminophen significantly reduced the hepatic injury to nearly the control level. Ranitidine had no protective effect against hepatic injury at the dose of 50 mg, which appears to have the same antacid effect as 200 mg cimetidine, whereas it had a slight but significant protective effect as evaluated by the transaminase level, glutathione content and histological study at the dose of 100 mg. Famotidine had no effect against acetaminophen induced hepatic injury. Because famotidine had no effect, the protection by H2-antagonist against acetaminophen-induced hepatic injury cannot be explained by the decrease in hepatic blood flow alone. Therefore, inhibition of P-450 activity seems to be more important for reducing the generation of the reactive metabolites of acetaminophen than hepatic blood flow decrease.

The appearance of cimetidine revolutionized the medical care of peptic ulcers, but this drug also has strong inhibitory effects on the metabolism of other drugs. Cimetidine was reported to decrease plasma clearance of a number of drugs in man and animals, for example, warfarin (1), benzodiazepine (2), lidocain (3) and propranolol (4). The decrease in P-450 activity (5) and the reduction of liver blood perfusion (4, 6) are thought to be side effects of cimetidine, these two combined effects resulting in inhibition of drug metabolism. In this inhibition of drug metabolism by cimetidine, the contribution of each of these two mechanisms to the observed inhibition seemed to be different with each interacted drug. In 1981, Mitchell et al. reported that cimetidine had a protective effect on acetaminophen-induced liver damage (7). They explained that the inhibitory effect of cimetidine on P-450 activity reduced the generation of highly reactive metabolites and reduced the hepatotoxicity of acetaminophen, but they did not discuss whether the reduction of P-450 activity or hepatic blood flow decrease was more essential.

New H2-receptor antagonists such as ranitidine and famotidine have recently appeared and may soon be used routinely in clinics. Some studies have been performed on ranitidine (8–11), but no established opinion about inhibition of drug metabolism exists, and there have been no reports about famotidine.

Therefore, we examined the hepatoprotective effect of H2-antagonists (cimetidine, ranitidine, famotidine) on acetaminophen-
induced hepatic injury and attempted to reveal the mechanism of this protective effect on the liver.

Materials and Methods

Animals and pretreatment of 3-methylcholanthrene: Male Fisher rats each weighing 180 g (Charles River Co., Tokyo) were used. They were fed a standard laboratory chow (Oriental Yeast, Tokyo) and drinking water ad libitum. Since rats are not very sensitive to acetaminophen, they were given a single intraperitoneal injection of 3-methylcholanthrene (25 mg/kg body weight, Sigma Chemical Co., St. Louis, MO) to increase the susceptibility to acetaminophen according to the method of Mitchell et al. (7), and this induced state was established as a control state in the 3-methylcholanthrene pretreated group.

Treatment of acetaminophen and H2-receptor antagonists: Rats were administered intraperitoneally 800 mg/kg body weight of acetaminophen (Sigma Chemical Co.) after 3-methylcholanthrene injection. Cimetidine (200 mg/kg body weight, Sigma Chemical Co.), ranitidine (50, 100 mg/kg; generously provided by Sankyo Co., Osaka), famotidine (20, 40 mg/kg; generously provided by Yamanouchi Pharmaceutical Co., Tokyo) was administered twice intraperitoneally, 1 hr before and 1 hr after acetaminophen injection. These doses were used because they gave an equal effect on antacid action. Blood samples were taken from the jugular vein, and the rats were then killed by cervical dislocation. The liver was taken from the body. Part of the liver was used for histological study, and a part was used for microsomal preparation and glutathione measurement.

Assays of serum transaminases: The activities of serum glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) were determined by the procedure of Reitman and Frankel (12) and expressed as Karmen units.

Preparation of microsomes: Rat liver microsomes were prepared according to our previous method (13, 14). In brief, excised livers were thoroughly perfused with cold 0.15 M KCl and homogenized in 4 volumes of 0.15 M KCl solution containing 10 mM EDTA using a Tefron pestle in a Potter-type glass homogenizer. The homogenate was centrifuged at 10,000×g for 15 min in a Hitachi 20PR refrigerated centrifuge. The supernatant was then centrifuged at 105,000×g for 60 min in a Hitachi 65P preparative ultracentrifuge. The pellet was homogenized in the homogenizing solution again and centrifuged again as described above. The resulting pellet was suspended in 20 mM potassium-phosphate buffer, pH 7.4, containing 15% glycerol and used for several assays described in the next section. All procedures were done at 0–4°C.

Assays of microsomal protein, P-450 content, aminopyrine demethylation activity and total glutathione content: Microsomal protein was measured by the method of Lowry et al. (15), and the cytochrome P-450 was assayed by the method of Omura and Sato (16). Aminopyrine demethylation activity was assayed according to the method of Nash (17). Total glutathione content of the liver was estimated as described by Tietze (18).

Statistical analysis: Statistical analysis was performed with Student's t-test.

Results

The changes in serum GOT, GPT and serum albumin

Nothing-pretreated group: No significant change was seen in any of the results.

3-Methylcholanthrene-pretreated group: Maximal increases in GOT and GPT values were observed in the rats given only acetaminophen. The transaminase levels of the rats given cimetidine along with acetaminophen remained at nearly the control levels and were significantly different from those in the rat given only acetaminophen. On the other hand, the levels of the transaminases in the rats administered 50 mg ranitidine and acetaminophen were high values similar to those of the rats given only acetaminophen, but those given 100 mg ranitidine had slightly but significant lower values than those of the rats given only acetaminophen. The rats given famotidine had transaminase values similar to those given only acetaminophen without regard to the doses. The
serum albumin concentration of the rats given cimetidine along with acetaminophen remained at the normal level, whereas those of the rats given acetaminophen alone or along with the other H₂-receptor antagonist were severely reduced (Table 1). The change in microsomal protein, P-450 content and aminopyrine demethylation activity

**Nothing-pretreated group:** No significant change was seen in any of the results.

**3-Methylcholanthrene-pretreated group:** Though the content of P-450 in the nothing-pretreated rats was 11.4 ng/g liver, the values of control animals in the 3-methylcholanthrene-pretreated group increased resulting from induction. In the induced state by 3-methylcholanthrene, acetaminophen administration reduced the content of P-450. In the rats given cimetidine along with acetaminophen, the content of P-450 in the liver remained at nearly the control level, clearly differing from that in the rats given only acetaminophen. Neither the dose of ranitidine nor that of famotidine had a clear protective effect. The content of microsomal protein per gram liver also decreased in the rats given only acetaminophen, but remained at nearly the control level in the rats given cimetidine along with acetaminophen. No protective effect was seen in the other groups. aminopyrine demethylation activity also showed a change similar to that of P-450 content (Table 2).

The change in total glutathione content

**Nothing-pretreated group:** No significant change was seen in any of the results.

**3-Methylcholanthrene-pretreated group:** Glutathione content in the rats given acetaminophen alone decreased markedly. Cimetidine and 100 mg ranitidine had protective effects, but 50 mg ranitidine and famotidine gave no protection against the decrease in glutathione content induced by

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Table 1. Effects of H₂-receptor antagonists on serum trans-aminases and albumin content in rats administered acetaminophen

|                      | s-GOT (KU/ml) | s-GPT (KU/ml) | s-Albumin (g/dl) |
|----------------------|---------------|---------------|-----------------|
| **Nothing-pretreated group** |               |               |                 |
| Control (n=11)       | 184±34        | 28±10         | 3.58±0.40       |
| AA alone (n=10)      | 192±40        | 31±9          | 3.64±0.39       |
| C200 mg alone (n=5)  | 210±52        | 33±15         | 3.62±0.54       |
| R50 mg alone (n=5)   | 195±46        | 32±14         | 3.50±0.50       |
| R100 mg alone (n=5)  | 200±48        | 34±13         | 3.62±0.55       |
| F20 mg alone (n=5)   | 192±52        | 29±12         | 3.74±0.58       |
| F40 mg alone (n=5)   | 196±47        | 30±14         | 3.70±0.48       |
| **3-Methylcholanthrene-pretreated group** |               |               |                 |
| Control (n=9)        | 190±30        | 30±12         | 3.75±0.42       |
| AA alone (n=13)      | 12000±4730    | 3880±1560     | 3.01±0.21       |
| C200 mg alone (n=5)  | 182±48        | 35±13         | 3.66±0.42       |
| C200 mg+AA (n=9)     | 357±261       | 47±42         | 3.44±0.17       |
| R50 mg alone (n=5)   | 194±50        | 30±14         | 3.70±0.40       |
| R50 mg+AA (n=9)      | 11100±9650    | 4100±3720     | 3.16±0.27       |
| R100 mg alone (n=5)  | 208±58        | 34±15         | 3.68±0.38       |
| R100 mg+AA (n=9)     | 3030±3010     | 1020±986      | 3.10±0.17       |
| F20 mg alone (n=5)   | 210±52        | 30±16         | 3.68±0.44       |
| F20 mg+AA (n=9)      | 12800±8450    | 4230±2150     | 3.00±0.27       |
| F40 mg alone (n=5)   | 204±54        | 31±14         | 3.66±0.48       |
| F40 mg+AA (n=7)      | 8268±5595     | 3340±2856     | 2.63±0.41       |

*mean±S.D. AA; acetaminophen. C; cimetidine. R; ranitidine. F; famotidine. *Significantly different at P<0.01.
Table 2. Effects of H2-receptor antagonists on P-450 content, microsomal protein content and aminopyrine demethylation activity in rats administered acetaminophen

|                                | P-450 (nmol/g liver) | Microsomal protein (mg/g liver) | Aminopyrine demethylation (nmol/g liver/min) |
|--------------------------------|----------------------|---------------------------------|---------------------------------------------|
| **Nothing-pretreated group**   |                      |                                 |                                             |
| Control (n=11)                 | 11.4±1.25            | 10.35±1.42                      | 142.3±20.1                                  |
| AA alone (n=10)                | 12.0±1.18            | 11.22±1.23                      | 135.4±18.8                                  |
| C200 mg alone (n=5)            | 10.8±2.23            | 9.88±2.52                       | 133.2±28.4                                  |
| R50 mg alone (n=5)             | 11.6±1.72            | 10.64±1.95                      | 138.2±28.4                                  |
| R100 mg alone (n=5)            | 12.0±1.85            | 11.68±2.01                      | 132.0±39.4                                  |
| F20 mg alone (n=5)             | 10.9±1.98            | 11.35±2.32                      | 140.8±30.5                                  |
| F40 mg alone (n=5)             | 11.8±2.12            | 11.42±1.94                      | 139.3±29.4                                  |
| **3-Methylcholanthrene-pretreated group** |          |                                 |                                             |
| Control (n=9)                  | 37.5±4.80            | 15.85±3.23                      | 299.5±68.8                                  |
| AA alone (n=13)                | 23.8±4.50            | 13.31±1.87                      | 186.0±48.7                                  |
| C200 mg +AA (n=5)              | 38.0±8.32 *          | 15.96±4.34                      | 288.5±89.8 *                                |
| C200 mg +AA (n=9)              | 42.1±6.11            | 16.82±1.87                      | 272.8±49.8                                 |
| R50 mg alone (n=5)             | 35.4±9.88            | 15.33±4.24                      | 278.0±95.0                                  |
| R50 mg +AA (n=9)               | 20.6±5.52            | 11.80±2.29                      | 207.3±65.0                                  |
| R100 mg alone (n=5)            | 37.0±8.94            | 14.84±3.88                      | 280.2±98.4                                  |
| R100 mg +AA (n=9)              | 26.5±10.4            | 13.99±2.77                      | 198.8±65.1                                  |
| F20 mg (n=5)                   | 39.7±9.45            | 16.00±7.01                      | 270.1±95.4                                  |
| F20 mg +AA (n=9)               | 21.2±6.47            | 11.13±2.63                      | 190.3±57.1                                  |
| F40 mg (n=5)                   | 40.5±8.33            | 15.43±5.84                      | 284.8±88.5                                  |
| F40 mg +AA (n=7)               | 23.3±5.50            | 11.50±2.10                      | 181.6±42.2                                  |

Mean±S.D. AA; acetaminophen, C; cimetidine, R; ranitidine, F; famotidine. *Significantly different at P<0.01.

Histological study
Severe necrosis and intrahepatic bleeding were observed in 3-methylcholanthrene pretreated rats given acetaminophen (Fig. 1). However, hardly any was detected in the rats given cimetidine together with acetaminophen (Fig. 2). In the specimens from animals treated with ranitidine (100 mg), necrosis was somewhat mild compared with that in the acetaminophen only administered group and intact parts are seen like islands (Fig. 3). However ranitidine (50 mg) and famotidine showed no protective effect (Figs. 3 and 4).

Discussion
Cimetidine is now widely used as a specific drug for peptic ulcer disease. In 1979, Rendic et al. reported that cimetidine had an inhibitory effect on the monoxygenase system of liver microsomes, and they considered that it was caused by the imidazole base of cimetidine (5). On the other hand, Feely et al. reported that cimetidine reduced hepatic blood flow, and they speculated that this effect was caused by the H2-antagonistic effect itself (4). Therefore, the inhibition of drug metabolism by cimetidine has been considered to be due to both the reduction in P-450 activity and decrease in hepatic blood flow, but the contribution of each of these two mechanisms to the observed inhibition seems to be different with each interacted drug. Cimetidine interact with benzodiazepine or warfarin mainly by the reduction of P-450 activity (1, 2), and liver blood flow decrease is more important in the inhibition of lidocaine metabolism (3), and propranolol was inhibited by a mixture of the two mechanisms, both contributing equally (4).

Ranitidine and famotidine, new H2-antagonists that show more strong H2-antagonistic action, have structures similar to acetaminophen (Table 3).
Table 3. Effects of H₂-receptor antagonists on total glutathione in rats administered acetaminophen

|                              | Total glutathione content (mg/g liver) |
|------------------------------|---------------------------------------|
| Nothing-pretreated group     |                                       |
| Control                      | 1.51±0.75                             |
| AA alone                     | 1.55±0.65                             |
| C200 mg alone                | 1.58±0.68                             |
| R50 mg alone                 | 1.48±0.66                             |
| R100 mg alone                | 1.54±0.75                             |
| F20 mg alone                 | 1.42±0.78                             |
| F40 mg alone                 | 1.44±0.64                             |
| 3-Methylcholanthrene-pretreated group |                            |
| Control                      | 1.48±0.62                             |
| AA alone                     | 0.33±0.17                             |
| C200 mg alone                | 1.44±0.63 *                           |
| C200 mg+AA                   | 0.74±0.43                             |
| R50 mg alone                 | 1.42±0.52                             |
| R50 mg+AA                    | 0.41±0.17                             |
| R100 mg alone                | 1.60±0.66                             |
| R100 mg+AA                   | 0.71±0.33                             |
| F20 mg alone                 | 1.48±0.60                             |
| F20 mg+AA                    | 0.36±0.19                             |
| F40 mg alone                 | 1.55±0.48                             |
| F40 mg+AA                    | 0.41±0.21                             |

(mean±S.D.) AA: acetaminophen, C: cimetidine, R: ranitidine, F: famotidine. *:* Significantly different at P<0.01 or P<0.05, respectively.

that of cimetidine as a whole, but differ in that they have no imidazole base. In comparison with cimetidine, there is relatively limited information about the effect of these two drugs on drug metabolism. Heagarty et al. found that ranitidine failed to interact with propranolol (8), whereas Spahn et al. showed that ranitidine delayed the clearance of metoprolol, which is also a β-blocker (9). Knodell et al. reported that ranitidine had no influence on the microsomal monooxygenase because it lacked an imidazole base (10); but on the other hand, Hoensch et al. showed that ranitidine was bound to microsomal enzymes like cimetidine (11). Thus the discussion about the effect of ranitidine has not ceased. Furthermore, no reports have been made on the interaction between famotidine and other drugs.

Mitchell et al. (7) reported that cimetidine protected against acetaminophen-induced hepatic injury and considered that cimetidine blocked the activity of P-450, resulting in the reduction of highly reactive metabolites. Peterson et al. (19) and Miners et al. (20) further examined cimetidine protection against acetaminophen-induced hepatic injury. These reports indicated a protection of the liver itself, but was different to ours in detail; they could not explain this phenomenon clearly.

For cimetidine, we observed a prominent protective effect as reported previously (7, 19, 20), and ranitidine had a slight protective effect on the transaminase value, glutathione content and histological study only at large doses. Ranitidine seems to have a weak but significant protective effect. Famotidine had no protective action against acetaminophen-induced hepatic injury in our experiment. However, nobody knows whether famotidine has an inhibitive action on P-450 or not; the protection by H₂-antagonist cannot be explained by the decrease in blood flow alone because famotidine which has strong H₂-antagonistic effect showed no action on
acetaminophen-induced hepatic injury. Therefore, the hepatic blood flow decrease due to the H₂-antagonistic action itself seems to have no connection with the protection against acetaminophen-induced hepatic injury. Since ranitidine binds liver microsomal enzymes like a cimetidine (11) and since Mitchell et al. also showed in another report (21) that ranitidine dose-dependently inhibits the metabolism of...
acetaminophen in vitro, we presume that ranitidine binds microsomes and inhibits the activity of P-450 in spite of the lack of an imidazole base, but the degree of ranitidine action will be weak. If famotidine does not inhibit P-450 activity at all, our result can be clearly explained by only the mechanism of inhibition of P-450. At the present stage, we presume that the inhibition of P-450 is the main factor in the protection by H2-antagonist against acetaminophen-induced hepatic injury, and the decrease in the hepatic blood flow has little effect on this phenomenon.

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