EFFECT OF N-ACETYLL-L-GLUTAMINE ALUMINUM COMPLEX (KW-110), AN ANTIULCER AGENT, ON THE NON-STEROIDAL ANTI-INFLAMMATORY DRUG-INDUCED EXACERBATION OF GASTRIC ULCER IN RATS

Hiroshi TANAKA, Katsuichi SHUTO and Hirofuto MARUMO
Pharmaceuticals Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.,
1188 Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan

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Abstract—Gastric ulcer induced by the injection of acetic acid (0.025 ml of 20%) into the gastric wall of rats was healed considerably 5 days after the injection of acetic acid. Non-steroidal anti-inflammatory drugs (NSAID) such as aspirin, indomethacin, and phenylbutazone were given consecutively for 5 days, and they exacerbated the ulcer and enlarged the ulcer area. Aspirin caused exacerbation when it was given for the initial 5 days of the ulcer healing process. Phenylbutazone caused exacerbation by the administration for 5 days at the middle stage of the ulcer healing process. In contrast, indomethacin caused exacerbation not only when it was given for the initial 5 days but also when it was given for the middle 5 days. The effect of the antiulcer agent N-acetyl-L-glutamine aluminum complex (KW-110) on the exacerbation was studied. KW-110 at an oral dose of 500 mg/kg inhibited remarkably the exacerbation induced by all of the NSAID used. The development of gastric lesions induced by these NSAID was also prevented by KW-110. Further study was carried out with regard to the influences of KW-110 on the pharmacological properties of NSAID. The results showed no influences of KW-110 on the antiedematous and antipyretic actions of the NSAID.

As far as we know, there is no report about an agent to inhibit the relapse or the exacerbation of ulcer. In order to study the effect of N-acetyl-L-glutamine aluminum complex (KW-110) (1–6), an antiulcer agent, on the exacerbation of ulcer, we attempted to make an experimental model for exacerbation. Ulcers were exacerbated when non-steroidal anti-inflammatory drugs (NSAID) that are known to produce gastric damages and aggravate ulcers in humans (7–9) were given during the healing processes of acetic acid induced ulcers in rats. Acetic acid ulcer has been often used as the chronic ulcer model (10, 11).

The present work was concerned with the effects of combining KW-110 with NSAID on this exacerbation model as well as the influence of combined KW-110 and NSAID on the antipyretic and antiedematous actions of NSAID.

MATERIALS AND METHODS

Healing process of acetic acid ulcer: Male Donryu strain rats weighing 190 to 210 g were used in this experiment. Under ether anesthesia, 0.025 ml of acetic acid (20%) was injected into the submucosal layer of the
glandular stomach according to the method of Takagi et al. (10). The rats were killed by CO₂ inhalation on day 3, 5, 7, 10 or 15 of ulcer formation. Each stomach was cut open along the greater curvature, and the ulcer was observed. The longitudinal and abscissal lengths of the ulcer area were measured under a magnifying glass, and the multiplication product of these two quantities designated as the ulcer index (mm²).

Exacerbation of ulcer induced by NSAID: The method of the ulcer formation was the same as described above. The 1st experiment (EXP I) was conducted as follows: NSAID were given once a day for 5 consecutive days from the day after ulcer formation. NSAID used in this study were aspirin (ASP, Sanko Seiyaku), indomethacin (IND, Sigma) and phenylbutazone (PHE, Sigma). NSAID were given orally 30 min before the oral dose of KW-110 (Kyowa Hakko). In the 2nd experiment (EXP II), NSAID were given consecutively for 5 days from day 6 after ulcer formation. In both experiments, rats were killed on day 15 of ulcer formation.

Effect of KW-110 on NSAID-induced gastric lesions: Male Donryu strain rats weighing 190 to 210 g were used. They were deprived of food, but water was allowed ad libitum for 48 hr. KW-110 was given orally 30 min after the oral dose of NSAID. Seventeen hr later, the rats were killed by CO₂ inhalation. The gastric lesions were observed, and the total length (mm) was measured as the lesion index.

Influence of KW-110 on antipyretic action of NSAID: To male Donryu strain rats weighing 140 to 160 g, yeast (brewer yeast, Nutritional Biochemical Co.) suspended in physiological saline at a concentration of 250 mg/ml was injected s.c. in an amount of 2.5 ml/rat, and the animals were deprived of food. Seventeen hr after the injection of yeast, rats whose rectal temp. had risen to about 38°C or more were selected and used in this test. NSAID were given orally 30 min before the administration of KW-110. Rectal temp. was measured before and at 1, 2 and 3 hr after NSAID medication.

Influence of KW-110 on antiedematous action of NSAID: Male Wistar strain rats weighing 140 to 160 g were used. They were given NSAID, KW-110 or NSAID plus KW-110 by the oral route. One hr after the administration of NSAID, a phlogistic agent (1% carrageenin solution) was injected s.c. into the hind paw. The swelling was measured 3 hr after the injection.

Statistical analysis: Values are expressed as the mean±S.E. of the mean. Differences were determined by the Student’s t-test, and P-values of less than 0.05 were considered significant.

RESULTS

Healing process of acetic acid ulcer: The ulcer healing process was represented in Fig. 1. The healing started rapidly between day 3 and day 5 after ulcer formation. The ulcer index of day 10 or day 15 was one-eighth of that of day 3.

Exacerbation of ulcer induced by NSAID: In EXP I, both ASP and IND inhibited the healing of acetic acid ulcer (Fig. 2). The ulcer indices of groups treated with these two drugs were almost double those of the control, and the increment of the index by IND was greater than that by ASP. The augmentations of the ulcer index induced by both IND and ASP were also significantly prevented by KW-110 at the dose of 500 mg/kg. Figure 3 shows that both IND and PHE remarkably inhibited the decrease of the ulcer index in EXP II, but ASP did not decrease the index. The inhibition by IND was greater than that by PHE. The inhibition was also significantly prevented by combining KW-110 with these NSAID.

Effect of KW-110 on NSAID-induced
Fig. 1. Healing process of acetic acid ulcer. Each value is the mean, and the horizontal bars represent the S.E. of the mean.

gastric lesion: In control rats receiving only NSAID, gastric lesions were observed. The ASP-induced lesion was almost linear and appeared almost in the corpus of the stomach. The IND-induced lesion developed in the corpus and/or the antrum. The shapes of these lesions were linear and circular, respectively. The PHE-induced lesion was like the ASP induced one, though the former was a little finer than the latter. The development of gastric lesions by all of the NSAID used in this experiment was significantly prevented by KW-110 at doses of more than 1 g/kg. KW-110 significantly inhibited the formation of the lesion by both ASP and IND at 500 mg/kg (Figs. 4–6).

Influence of KW-110 on antipyretic action of NSAID: The rectal temp. was measured for 3 hr after the administration of NSAID.
The yeast-induced fever in rats was reduced by the oral dosing of NSAID. ASP at 200 mg/kg reduced significantly the rectal temp. and the peak fall appeared 2 hr after the medication. The fall was not affected by the combination of KW-110 with ASP.

**Fig. 4.** The preventive effect of KW-110 on ASP-induced gastric lesions in rats. Control: Aspirin, 200 mg/kg p.o. Each value is the mean, and the horizontal bars represent the S.E. of the mean. Statistical significance of difference from the control: ***P<0.001.

**Fig. 5.** The preventive effect of KW-110 on IND-induced gastric lesions in rats. Control: Indomethacin, 20 mg/kg p.o. Each value is the mean, and the horizontal bars represent the S.E. of the mean. Statistical significance of difference from the control: ***P<0.001.

**Fig. 6.** The preventive effect of KW-110 on PHE-induced gastric lesions in rats. Control: Phenylbutazone, 200 mg/kg p.o. Each value is the mean, and the horizontal bars represent the S.E. of the mean. Statistical significance of difference from the control: **P<0.01, ***P<0.001.
until 3 hr after the dosing with ASP. Both IND at 20 mg/kg and PHE at 200 mg/kg also induced significant falls of rectal temp. KW-110 combined with these NSAID showed no influences on the fall induced by these drugs (Figs. 7–9). KW-110 itself had almost no antipyretic action.

Influence of KW-110 on antiedematous action of NSAID: All of NSAID used in this experiment significantly inhibited the development of edema as compared with the control (P<0.001). The antiedematous effect of NSAID was unaffected by KW-110 in combination with NSAID (Fig. 10). KW-110 itself had almost no effect on the development of edema.

**DISCUSSION**

In this paper, it was shown that acetic acid ulcer induced at the dose used in this experiment healed rapidly. The decrease in ulcer area started early on the day after ulcer formation. In order to make an exacerbation model, NSAID were applied to animals with acetic acid ulcers for a certain period since it is established that NSAID produced damages in the stomachs of humans as well as in experimental animals (7–9, 12). The obvious suppression of healing of the ulcer was obtained by administration of NSAID. Differences in the rate of decrease the ulcer area were observed both with different days of NSAID dosing and with the different types of NSAID used. Though there is not an obvious explanation for these differences, it is known that each of the NSAID has its own influences on the mucus content, the
blood supply in gastric mucosa, the mucosal regeneration, etc. (13-18). For example, only ASP among the NSAID used was reported to impair the gastric mucosal barrier as indicated by the increased back diffusion of hydrogen ions (14). The gastric hexosamine content was obviously lowered by ASP, slightly by PHE, but not by IND (14). Another report indicates that hypercontractility of the stomach was essential for the ulceration by PHE (18). The mucosal blood flow was decreased by IND and ASP (15). Mucus secretion was reported to be decreased by ASP and IND as well as by PHE (13, 19, 20).

The mucus content of the gastric mucosa in the ulcer healing process has been studied, and it was found that the hexosamine content in the gastric mucosa increased immediately after the ulcer formation (21, 22).

In this report, we tried to test the effect of the antiulcer agent KW-110 (1-6) against this model of ulcer exacerbation induced by NSAID. The agent showed a remarkable ability to prevent the exacerbation induced by all of the NSAID used in this experiment.

Further, the development of gastric lesions induced by NSAID was significantly prevented by KW-110. The mechanisms by which KW-110 acts to cure the ulcer are mucosal protection, acceleration of mucosal regeneration, and antacid action in addition to the increment of hexosamine in the gastric mucosa (2, 3, 23, 24). The prevention of exacerbation by KW-110 was based on these effects.

Further studies were carried out on the influences of KW-110 on the anti-inflammatory and antipyretic effects of the NSAID used. The results showed that there were no influences on these effects when KW-110 was used in combination with the NSAID.

The present study shows that the exacerbation induced by NSAID was prevented by KW-110. It is suggested that KW-110 can be therapeutic in the prevention of ulcer exacerbation in humans without decreasing the effects of the NSAID. The consecutive administration of NSAID for a certain period to animals with acetic acid ulcer was an interesting method to make an exacerbation model.
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REFERENCES
1) Yamagata, S., Ishimori, A. and Ogawa, N.: Phase III study of N-acetyl-L-glutamine aluminum complex (KW-110). J. Adult Disease 4, 894–906 (1974) (in Japanese)
2) Tanaka, H., Shuto, K., Ishi, S., Orima, H. and Takahira, H.: Antiulcerogenic actions and other pharmacological properties of N-acetyl-L-glutamine aluminum complex (KW-110). Folia pharmacol. japon. 68, 602–617 (1972) (Abs. in English)
3) Tanaka, H., Nagashima, Z. and Takahira, H.: Study of N-acetyl-L-glutamine aluminum complex (KW-110) on experimental chronic gastric ulcer. Pharmacometrics 7, 1035–1045 (1973) (Abs. in English)
4) Takagi, K., Takeuchi, K., Nakamura, K., Morita, A. and Okabe, S.: Effects of antiulcer agent, N-acetyl-L-glutamine aluminum complex (KW-110) on the duodenal ulcer models in the rat. Japan J. Pharmacol. 24, 357–361 (1974)
5) Harada, M. and Yano, S.: Inhibitory effect of N-acetyl-L-glutamine aluminum complex (KW-110) and related compounds on gastric erosion and mortality in stressed animals. Pharmacometrics 8, 1–6 (1974)
6) Tanaka, H., Shuto, K. and Marumo, H.: Effect of N-acetyl-L-glutamine aluminum complex (KW-110) on experimental duodenal ulcer in rats. Pharmacometrics 20, 185–193 (1980) (Abs. in English)
7) Miller, T.A. and Jacobson, E.D.: Gastrointestinal cytoprotection by prostaglandin. Gut 20, 75–87 (1979)
8) Daniel, E.E.: The effects of drugs on the gastrointestinal tract. In Gastroenterology, Edited by Bogoch, A., p. 119–120. McGraw-Hill Book Co., U.S. (1973)
9) Menguy, R.: Gastric mucosal injury from common drugs. Postgrad. med. J. 63, 82–86 (1978)
10) Takagi, K., Okabe, S. and Saziki, R.: A new method for the production of chronic gastric ulcer in rats and the effect of several drugs on its healing. Japan. J. Pharmacol. 19, 418–426 (1969)
11) Bates, R.F.L., Buckley, G.A. and Sterettle, R.J.: The effect of salmon calcitonin on acetic acid induced chronic gastric ulceration in rat. Brit. J. Pharmacol. 69, 339P–340P (1980)
12) Wilhelmi, G. and Menassé-Gdynia, R.: Gastric mucosal damage induced by non-steroid antinflammatory agents in rats of different ages. Pharmacology 8, 321–328 (1972)
13) Menguy, R. and Master, Y.S.: Effects of aspirin on gastric mucus secretion. Surgery, Gynecol. Costet. 120, 92–98 (1985)
14) Narumi, S. and Kanno, M.: Effects of non-steroidal antiphlogistics on the gastric mucosal barrier and hexosamine content in rats. Japan. J. Pharmacol. 22, 675–684 (1972)
15) Kauffman, G.L., Jr., Aures, D. and Grossman, M.I.: Intravenous indomethacin and aspirin reduced basal gastric mucosal blood flow in dogs. Am. J. Physiol. 238, G 131–134 (1980)
16) Max, M. and Menguy, R.: Influence of adrenocorticotropic, cortisone, aspirin and phenylbutazone on the rate of exfoliation and the rate of renewal of gastric mucosal cells. Gastroenterology 58, 329–336 (1970)
17) Murray, H.S., Strottman, M.P. and Cooke, A.R.: Effect of several drugs on gastric potential difference. Brit. med. J. 1, 19–21 (1974)
18) Mersereau, W.A. and Hinchee, E.J.: Synergism between hypercontractility and acid in the genesis of the phenylbutazone ulcer in the rat. Gastroenterology 78, 1221 (1980)
19) Menguy, R. and Desbaillets, L.: Influences of phenylbutazone on gastric secretion of mucus. Proc. Soc. exp. Biol. Med. 125, 1108–111 (1967)
20) Menguy, R. and Desbaillets, L.: Role of inhibition of gastric mucous secretion in the phenomenon of gastric mucosal injury by indomethacin. Am. J. Dig. Dis. 12, 862–866 (1967)
21) Tanaka, H., Kojima, T. and Marumo, H.: Effects of N-acetyl-L-glutamine aluminum complex (KW-110) on hexosamine content in gastric mucosa. Pharmacometrics 9, 519–522 (1975) (Abs. in English)
22) Suzuki, Y., Ito, M. and Sudo, Y.: Changes in connective tissue components in ulcer tissue during healing process of acetic acid ulcer in rat. Japan. J. Pharmacol. 29, 821–828 (1979)
23) Tanaka, H., Kojima, T. and Marumo, H.: Relation between the antulcer effect and the formation of the adhered complex to the mucosa by N-acetyl-L-glutamine aluminum complex (KW-110). Pharmacometrics 11, 71–76 (1976) (Abs. in English)
24) Shiraki, H., Mineura, K. and Takahira, H.: Biochemical studies on N-acetylglutamine aluminum complex (KW-110). II. The effects of KW-110 on gastric secretory compounds in rats. Yakugaku Zasshi 94, 559–565 (1974) (Abs. in English)