EFFECKS OF AN ANTIULCER AGENT, N-ACETYL-L-GLUTAMINE ALUMINUM COMPLEX (KW-110) ON THE DUODENAL AND GASTRIC ULCER MODELS IN THE RAT

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Abstract—Antiulcer effects of N-acetyl-L-glutamine aluminum complex (KW-110) were studied in experimental gastric and duodenal ulcer models in the rat, comparing its effect to that of atropine sulfate. It was found that KW-110, given orally 3 times daily for 8 days, had an accelerating activity on the healing process of duodenal ulcer whereas atropine sulfate, given s.c. twice daily for 8 days at a glutamine dose to sufficiently suppress gastric secretion, had a significant effect. In addition, KW-110 showed remarkable effects on prevention of acutely induced experimental gastric ulcers. In regard to the Shay ulcer or aspirin-induced gastric lesions, KW-110 showed enhanced effects which exceeded the apparently maximum effects of atropine sulfate, even though the dosage of the former was much higher than that of the latter. While atropine sulfate completely inhibited the stress-induced gastric lesions, KW-110 also revealed a strong inhibition on the lesions.

Tanaka et al. have reported excellent effects of a new antiulcer agent, N-acetyl-L-glutammine aluminum complex (KW-110), on several kinds of experimental gastric ulcers in rats (1). In particular, the agent was found to have an accelerating activity on healing of chronic gastric ulcer. Thus, the present study was designed to determine whether or not the agent has a curative effect on duodenal ulcer in rats while comparing the effects with those of atropine sulfate. In addition, several preventive tests for gastric ulcers induced in rats, i.e., stress- or aspirin-induced gastric lesions and Shay ulceration, were also performed and the inhibitory effect of the agent on those acute ulcers was investigated.

MATERIALS AND METHODS

Duodenal ulceration in rats for healing test

Male Donryu rats (200-230 g) were used in this study. As in the methods of Okabe et al. (2), the abdomen of the rat was incised under ether anesthesia and a metal mold (6 mm in diameter) was tightly placed upon the serosal surface of the duodenum wall, about 5 mm distal to the pylorus. Acetic acid (100%, 0.06 ml) was poured into the mold and allowed to remain for 30 sec. After removal of the acetic acid, the abdomen was closed and the animals were provided a usual diet. One day following this surgery the animals
were given KW-110 dissolved in saline 3 times daily \( \times 8 \) per os. On the 10th post-op day, these animals were sacrificed utilizing an overdose of ether. Atropine sulfate in saline was given one day following the surgery s.c. for 8 days twice daily. The duodenum was removed, filled with 1% formalin and immersed in 1% formalin for 10 min to fix the inner and outer surface of duodenum wall for easy observation of the lesions (3). The duodenum was then opened and the ulcerated area was examined and measured under the dissecting microscope with a square grid (10x). The depth of the ulceration was grossly estimated. The ulcer index represents the damaged area \((\text{mm}^2)\) and in the deeply penetrated cases (confined perforation) the area was multiplied by 1.5 times to adjust the severity of ulceration. Healing rate was calculated as follows:

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\text{Healing rate (\%)} = \frac{\text{Ulcer Index (Control)} - \text{Ulcer Index (Drug)}}{\text{Ulcer Index (Control)}} \times 100
\]

**Stress-induced gastric lesions for preventive test**

As previously described by Takagi and Okabe (4), rats (230-255 g) were placed in a stress cage and immersed to the level of xiphoid process in a water bath \((23°C)\) for 7 hr. The animals were then sacrificed by a blow on the head and examined for the gastric lesions under the dissecting microscope. The stress ulcer index was estimated as the sum of the length of each lesion in the glandular portion of the stomach. Drugs were given orally or s.c. thirty min prior to the stress treatment.

**Shay ulceration for preventive test**

Rats (195-215 g) were deprived of food but allowed free access to water for 36-48 hr prior to the experiments. Under ether anesthesia, the pylorus was ligated as described by Shay et al. (5). Sixteen hr later, the animals were sacrificed by an overdose of ether, the stomach was removed and examined for lesions. The damaged areas were measured under the dissecting microscope. The measured area was graded from 1 to 5 according to the size of the area; i.e., 1–10 mm\(^2\) as 1, 11–20 mm\(^2\) as 2, 21–30 mm\(^2\) as 3, 31–40 mm\(^2\) as 4 or perforated cases as 5. At the time of autopsy, gastric juice was collected, centrifuged and the gastric contents analyzed by a routine procedure. The acidity was determined by titrating with 0.1 N NaOH to pH 7.4 on the Hitachi pH meter. The pepsin concentration was determined by using Anson's hemoglobin method (6).

**Aspirin-induced gastric lesions for preventive test**

The rats (195-215 g) were fasted for 24 hr but allowed free access to water after which the pylorus was ligated as described previously. Immediately after this ligation, 100 mg/kg of aspirin suspended in 1% CMC solution was given orally (7). Seven hr later, the animals were sacrificed by an overdose of ether, but 10 min before death, these animals were subjected to a 5% solution of pontamine sky blue 6 BX (dissolved in saline and adjusted the pH to 7.2 with 0.5 N HCl) (8). After the formalin treatment, the length of each lesion produced in the glandular stomach was measured under the dissecting microscope (10 X) and the sum of the length per rat was referred to as the ulcer index (mm). The level of significance was calculated by using Student's t-test.
RESULTS

Effects of KW-110 and atropine sulfate on the healing of duodenal ulcers

It was found that KW-110 had a significant accelerating activity on the healing of duodenal ulcer at 1000 mg/kg per day (38.0%; P<0.05) or 3000 mg/kg per day (40.0%; P<0.001) (Table 1). Even at 300 mg/kg, the agent showed a tendency to accelerate the healing, though not significantly different from the control (P>0.05). Atropine sulfate at 20 mg/kg per day also showed a tendency to promote healing but not significantly (24%; P>0.05).

| Treatment                  | Dose & Route (mg/kg per day) | No. of rats | Body weight (g) | Ulcer index M±S.E. | Healing ratio (%) | P value |
|----------------------------|------------------------------|-------------|-----------------|-------------------|------------------|---------|
| Control (saline)           | P.O.                         | 23          | 203             | 25.0±3.0          |                  |         |
| KW-110                     | 300  P.O.                    | 14          | 202             | 17.6±1.9          | 29.6             | NS      |
|                            | 1000 P.O.                    | 16          | 206             | 15.5±2.7          | 38.0             | <0.05   |
|                            | 3000 P.O.                    | 15          | 203             | 15.0±1.2          | 40.0             | <0.001  |
| Control (saline)           | S.C.                         | 13          | 223             | 22.0±4.1          |                  |         |
| Atropine sulfate           | 20  S.C.                     | 13          | 235             | 16.7±4.0          | 24.0             | NS      |

Drugs were given 2 or 3 times a day for 8 consecutive days beginning one day post op

Effects of KW-110 and atropine sulfate on the stress-induced gastric ulcer

It was demonstrated that KW-110 had a strong inhibitory activity on the stress ulcer as the dose was increased (Table 2). The inhibition was 66.0% (P<0.001) or 81.7% (P<0.001) at the dose of 500 or 1000 mg/kg of the agent respectively. As expected, atropine sulfate at 10 mg/kg completely inhibited the stress ulcer formation.

Effects of KW-110 and atropine sulfate on Shay ulceration

It was confirmed that KW-110 had a significant inhibitory effect on Shay ulcer at 500 and 1000 mg/kg, in particular 1000 mg/kg of KW-110 completely abolished the ulceration (Table 3). The gastric juice volume was not at all affected by either 500 or 1000 mg/kg of the agent. Acidity was not determined because KW-110 in the gastric contents inter-
fered with the accurate titration with NaOH by producing aluminum complex. In con
trast to the pH value of control (1.25±0.04), the pH values were significantly raised by
KW-110 at 500 and 1000mg/kg, being 3.43, 10.14 (P<0.001) and 3.97, 10.12 (P<0.001)
respectively. The pepsin activities were significantly decreased by KW-110 in the amount
of 500 or 1000 mg/kg, being 36.0; (P<0.001) or 57.9% (P<0.001) respectively. Atro
pine sulfate at 10 nag/ kg also reduced the ulceration (74.4/ ; P<0.01) in comparison with
the control in which 58.3% of the number of rats had a perforation.

**Effects of KW-110 and atropine sulfate on aspirin-induced gastric lesions**

KW-110 had a significant inhibitory effect on aspirin lesions; the inhibition was 32.8%
and 69.1% at 500 and 1000 mg/kg respectively (Table 4). Atropine sulfate showed a con-
siderable effect as compared with the control (33.8%; P<0.05).

| Treatment                        | Dose & Route (mg/kg) | No. of rats | Body weight (g) | Lesion Index (mm) | % Inhibition | P value |
|----------------------------------|----------------------|-------------|-----------------|-------------------|--------------|---------|
| Control (saline)                 | P.O. 500 P.O.       | 10          | 199             | 53.6±3.4          | 32.8         | <0.05   |
| KW-110                           | 1000 P.O.           | 10          | 193             | 35.6±5.1          | 69.1         | <0.01   |
| Control (saline)                 | S.C. 10             | 14          | 188             | 59.7±3.6          | 33.8         | <0.05   |
| Atropine sulfate                 | S.C. 10             | 14          | 184             | 39.8±6.8          | 33.8         | <0.05   |

**DISCUSSION**

In the present study, KW-110 was found to have an accelerating effect on the healing
of experimentally induced duodenal ulcers. The effect of KW-110 was greater than that
of atropine sulfate, even though the dose of former was much higher than that of the latter. The dose of atropine sulfate used in this study (10 mg/kg) is a near maximal dose to adequately suppress gastric secretion in rats (9). Therefore, gastric secretion does not play an important role in the healing process while KW-110 does play a role in the recovery of the damaged mucosa itself. In every preventive test, KW-110 showed excellent effects on the acutely produced gastric lesions. Both stress and Shay ulcerations were in particular considerably suppressed by KW-110, which effects were almost comparable with or even more excellent than those of atropine sulfate, even though the dose level was different. The mechanisms of the effect of KW-110 and atropine sulfate appear to be quite different since Tanaka et al. (1) have reported that KW-110 had no anticholinergic effect in the pharmacologic properties. In addition, in a previous study by the authors it was indicated that either L-glutamine or aluminum hydroxide had a considerably less effect on the prevention of stress ulcer (unpublished data) so that the effect of KW-110 on stress ulceration would appear to be quite unique. Although KW-110 strongly prevented the development of Shay ulceration, the prevention appeared to be caused by the raised pH value and reduced pepsin activity. The effect of KW-110 on gastric acid and pepsin appears to be due to the solubilized aluminum hydroxide involved in the structure of the drug. It is significant that KW-110 had a remarkable effect on the suppression of aspirin-induced gastric lesions dose dependently whereas atropine sulfate had a weak effect in such a case. In a preliminary study, the authors found that L-glutamine inhibited gastric lesions induced by aspirin and assumed that the prevention may be caused by the suppression of back diffusion of acid into the gastric mucosa the same of which has been proposed by Davenport (10). Therefore, the effect of KW-110 on aspirin-induced gastric lesions may be partly due to this preventive activity of the back diffusion of acid.

From the above data, KW-110 was confirmed to be a promising antiulcer agent in experimental gastrointestinal ulcer models, regardless of the related mechanisms.

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