INFLUENCE OF A NEW ANTIULCER AGENT, AMMONIUM 7-OXOBICYCLO (2, 2, 1) HEPT-5-ENE-3-CARBAMOYL-2-CARBOXYLATE (KF-392) ON GASTRIC LESIONS AND GASTRIC MUCOSAL BARRIER IN RATS

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Abstract—Antiulcer effects of KF-392 were studied in several experimental gastric ulcer models in rats. It was found that KF-392 given orally at 1.0 to 5.0 mg/kg had a marked suppression on the developments of Shay ulcer as well as the aspirin-, stress-, and reserpine-induced gastric lesions. The influence of KF-392 on gastric mucosal barrier was also studied. A back diffusion of H⁺ into the gastric mucosa and a fall of transmucosal potential difference were induced with KF-392 given orally at the above mentioned doses. KF-392 given s.c. at 5.0 mg/kg showed no inhibition on Shay ulcer and no induction of back diffusion of H⁺ into the gastric mucosa.

During the course of blind screening of various compounds in our laboratory, we found that a new compound, KF-392, produced a marked suppression on stress-induced gastric lesions in rats.

In the present study, attempts were made to determine the effects of KF-392 on several types of experimental gastric lesions and on the gastric mucosal barrier.

MATERIALS AND METHODS

The compound, KF-392, is a white powder and is soluble in water. The pH of the solution in water is approx. 7.0. The chemical formula of KF-392 is shown in Fig. 1. The compound was dissolved in deionized water prior to dosing.

Gastric lesions
Shay rat ulcers: Male Wistar strain rats, weighing 220 to 240 g, were housed in an individual cage with the wide mesh wire bottom to avoid coprophagy and were fasted for 48 hr. Under ether anesthesia, the pylorus of the animal was ligated. KF-392 was given either p.o. or s.c. immediately after the operation. Eighteen hr after the dosing, the totally fasting animal was sacrificed with an overdose of ether. The stomach was removed and the perforation ratio was determined. The content in the stomach without perforation was removed and the pylorus and cardia of each were ligated. The stomach was slightly inflated...
by an injection of saline and immersed in 1% formalin solution for 10 min for fixation of
the gastric wall. Subsequently the stomach was incised along the greater curvature and the
gastric lesions in the forestomach were observed. The lesion index was divided into the
following 6 grades: 0- Normal, 1- Hemorrhage, 2- One to 5 small lesions (less than 3 mm
diameter), 3- A large lesion (equal to or more than 3 mm diameter), 4- More than 2 large
lesions or a large lesion plus more than 6 small lesions and 5- Perforation.

Aspirin-induced gastric lesions: Male Donryu strain rats, weighing 200 to 240 g were
fasted for 48 hr and given orally either aspirin (100 mg/kg) suspended in 1% carboxy me-
thylethylcellulose (CMC) alone or aspirin plus KF-392 (1.0 to 5.0 mg/kg) immediately after the
pylorus of each was ligated. The animals were sacrificed 4 hr after dosing during which time
food and water were withheld. Ten min before death, the animals were injected with 1.0 ml
of a 5% solution of Pontamine sky blue 6-BX (pH 7.0). The length (mm) of the lesions in
the glandular stomach was summed to give a lesion index for each animal (1).

Stress-induced gastric lesions (water-immersion stress): Male Wistar strain rats, weigh-
ing 240 to 260 g were fasted for 18 hr. Immediately after an oral administration of KF-392,
the animal was immobilized in the stress cage devised by Takagi et al (2). The cage was then
immersed vertically in a water bath at 23±1°C for 7 hr to the height of xiphoid process.
After this procedure, the stomach of each was removed. The area of the lesion in the glandu-
lar stomach was summed to give a lesion index (mm²).

Reserpine-induced gastric lesions: Following the methodology in a previous report
(3), male Wistar strain rats, weighing 140 to 160 g, were fasted for 48 hr and then given
reserpine at 8 mg/kg i.p. followed immediately by an oral dosing of KF-392. Eighteen hr
later, the animals were sacrificed. The determination of lesion index was similar to that
described in stress-induced gastric lesion.

Gastric juice

Pylorus-ligated rats: Male Wistar strain rats, weighing 210 to 230 g, fasted for 48 hr
were given KF-392 either p.o. or s.c. after the pylorus of each was ligated. Six hr after the
dosing during which time food and water had been withheld, the animals were sacrificed and
the gastric juice was centrifuged and titrated for acidity to pH 7.4 with 0.05 N NaOH on
the pH meter. The concentrations of Na⁺ and K⁺ were measured by flame photometry.
The peptic activity was measured by Anson’s method (4) and represented as L-tyrosine
content (mg).

Gastric secretion by aspirin (pylorus-ligated rat): Male Donryu strain rats, weighing
200 to 240 g, fasted for 48 hr were given Aspirin and/or KF-392 orally at the doses described
in the experiment of aspirin-induced gastric lesions. The animals were sacrificed and the
gastric juice was collected 4 hr after the pylorus of each had been ligated. The analysis of
gastric juice was conducted by the same procedure as mentioned above.

Buffer action and peptic activity in in vitro experiments: The mixed solution of 10 mEq/
1 HCl plus several concentrations of KF-392 were titrated to pH 7.0 with 10 mEq/1 NaOH
on the pH meter. The peptic activity was studied using 10 times diluted gastric juice collected
in pylorus-ligated rats as the enzyme solution. Two ml of the enzyme solution and 10 ml
of several concentrations of KF-392 solution were incubated for 10 min at 37°C. Each experiment was done in duplicate.

Transmucosal potential difference (P.D.)

Two male Wistar strain rats, weighing 480 to 520 g, were allotted to each experiment. Under urethane anesthesia, a small incision was made on the forestomach of each and the content was rinsed away gently with saline. One specimen was used for the intragastric pH measurement with the pH electrode inserted into the gastric lumen, and the other for the P.D. measurement. P.D. was measured according to the method of Black et al (5). The agent was given by gastric intubation.

Transport of charcoal meal

Male dd strain mice, weighing 19 to 21 g, fasted for 24 hr were given orally KF-392 and 5% charcoal suspension (10% CMC) concomitantly. Twenty min later the entire intestinal tract was removed. The total length of the intestine and the position of the head of the charcoal transport in the intestine were measured. The transport ratio was obtained by the following formula:

\[
\text{Transport ratio} \quad \%
\text{Distance from pylorus to charcoal head} \over \text{Total length of intestinal tract} \times 100
\]

RESULTS

Gastric lesions

Shay rat ulcers (Table 1): KF-392 given orally at more than 2.0 mg/kg showed a complete preventive effect on the development of gastric lesions in the forestomach. A significant inhibition was found at 0.5 mg/kg (34.8%, \( p < 0.01 \)). A slight hemorrhage in the glandular stomach was observed with KF-392 at 5.0 mg/kg. The perforation ratio was 70%, in the control and 40% in case of KF-392 at 0.2 mg/kg. There was no perforation in the animals given KF-392 at more than 0.5 mg/kg p.o. KF-392 given s.c. 5.0 mg/kg had no influence on either the lesion index or the perforation ratio.

Aspirin-induced gastric lesions (Table 2): KF-392 suppressed the gastric lesion, dose-
dependently. The inhibitory effect of KF-392 (1.0 mg/kg) on the gastric lesion was significant (41.7%, \( p<0.001 \)).

**Stress-induced gastric lesions (water-immersion stress)** (Table 3): The inhibition in the lesion index was 96.9, 86.7 or 85.7% at doses of 5.0, 2.0 or 1.0 mg/kg of KF-392 respectively, as compared with the control. All the values were highly significant (\( p<0.001 \)). Neither gastric lesion nor gastrointestinal hemorrhage was noted with 5.0 mg/kg of KF-392.

**Reserpine-induced gastric lesions** (Table 4): The preventive effect of KF-392 at 1.0 mg/kg on reserpine-induced gastric lesions was significant as compared with the control (54.9%, \( p<0.01 \)). The inhibition was dependent upon the dose of KF-392.

**Gastric juice**

**Pylorus-ligated rats** (Table 5): The influence of KF-392 given either p.o. or s.c. on the gastric secretion was studied. It was found that KF-392 given orally at 0.5 mg/kg showed a significant decrease of \( H^+ \) output (\( p<0.001 \)) and a significant increase of \( Na^+ \) output (\( p<0.05 \)), though there was no change in the volume of juice. An increased \( K^+ \) was evident

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**Table 2. Effect of KF-392 on aspirin-induced gastric lesions in pylorus-ligated rats**

| Treatment                  | No. of animals | Lesion index (mm) (Mean±S.E.) | Inhibition ratio (%) |
|----------------------------|----------------|-------------------------------|---------------------|
| Control deionized water    | 12             | 45.8±3.3                      |                     |
| 0.5 ml/100 g p.o.          | 12             | 18.9±6.0***                   | 58.7                |
| KF-392 5.0 mg/kg p.o.      | 12             | 20.5±3.9***                   | 55.2                |
| 2.0                       | 12             | 26.7±4.2***                   | 41.7                |
| 1.0                       |                |                               |                     |

*** \( p<0.001 \) vs. Control.

**Table 3. Effect of KF-392 on stress-induced gastric lesions in rats**

| Treatment                  | No. of animals | Lesion index (mm²) (Mean±S.E.) | Inhibition ratio (%) |
|----------------------------|----------------|--------------------------------|---------------------|
| Control deionized water    | 10             | 9.8±0.7                        |                     |
| 0.5 ml/100 g p.o.          | 10             | 0.3±0.1***                     | 96.9                |
| KF-392 5.0 mg/kg p.o.      | 10             | 1.3±0.5***                     | 86.7                |
| 2.0                       | 10             | 1.4±0.4***                     | 85.7                |
| 1.0                       |                |                               |                     |

*** \( p<0.001 \) vs. Control.

**Table 4. Effect of KF-392 on reserpine-induced gastric lesions in rats**

| Treatment                  | No. of animals | Lesion index (mm²) (Mean±S.E.) | Inhibition ratio (%) |
|----------------------------|----------------|--------------------------------|---------------------|
| Control deionized water    | 10             | 5.1±0.6                        |                     |
| 0.5 ml/100 g p.o.          | 10             | 0.5±0.3***                     | 90.2                |
| KF-392 5.0 mg/kg p.o.      | 10             | 1.5±1.0**                      | 70.6                |
| 2.0                       | 10             | 2.3±0.7**                      | 54.9                |
| 1.0                       |                |                               |                     |

*** \( p<0.001 \) vs. Control. ** \( p<0.01 \) vs. Control.
with more than 1.0 mg/kg. The increase of peptic activity was significant at 0.5 mg/kg (p<0.05). KF-392 given orally at more than 2.0 mg/kg resulted in an increased total output of H⁺, Na⁺ and K⁺. On the other hand, KF-392 given s.c. had no influence on the ionic outputs.

*Gastric secretion by aspirin (pylorus-ligated rat) (Table 6): A decrease of H⁺ output and an increase of Na⁺ output induced by aspirin were enhanced by KF-392 and such was dose dependent.

*Buffer action and peptic activity in in vitro experiment (Tables 7, 8): KF-392 at 1.0 to 4.0 mg/ml had no influence on either antacid or buffer action. KF-392 at 0.1 to 2.5 mg/ml had no effect on the peptic activity.

### Table 5. Effect of KF-392 on gastric juice in pylorus-ligated rats (6 hr ligation after 48 hr fasting)

| Treatment                  | No. of animals | Volume (ml) | Analysis of gastric juice ion output (µEq/hr) | Peptic activity (mg as L-tyrosine) |
|----------------------------|----------------|-------------|-----------------------------------------------|-----------------------------------|
| Control deionized water    | 10             | 8.8 ± 0.4   | 54.6 ± 4.2                                    | 35.8 ± 7.8                       |
| 0.5 ml/100 g p.o.          |                |             |                                               | 5.2 ± 1.9                        |
| KF-392 5.0 mg/kg p.o.      | 10             | 9.7 ± 0.3   | 10.0 ± 3.1                                    | 150.8 ± 8.8***                   |
| 2.0                        |                |             |                                               | 8.9 ± 1.5                        |
| 1.0                        | 10             | 9.3 ± 0.6   | 16.8 ± 1.2                                    | 89.2 ± 15.6***                   |
| 0.5                        |                |             |                                               | 7.0 ± 2.4                        |
| 5.0 mg/kg s.c.             | 10             | 8.8 ± 0.5   | 33.1 ± 1.4                                    | 68.3 ± 9.0*                      |
|                            |                |             |                                               | 5.3 ± 1.0                        |

*** P<0.001 vs. Control. ** P<0.01 vs. Control. * P<0.05 vs. Control. All values represent mean ± S.E.

### Table 6. Effect of KF-392 and aspirin on gastric juice in pylorus-ligated rats (4 hr ligation after 48 hr fasting)

| Treatment                  | No. of animals | Volume (ml) | Analysis of gastric juice ion output (µEq/hr) |
|----------------------------|----------------|-------------|-----------------------------------------------|
| Control (1% CMC 0.5 ml/100 g + deionized water 0.5 ml/100 g p.o.) | 12             | 9.4 ± 0.3   | 130.6 ± 5.2                                    | 61.7 ± 4.5                       |
| Aspirin 100 mg/kg p.o.     |                |             |                                               | 10.0 ± 0.8                       |
| Aspirin 100 mg/kg + deionized water 0.5 ml/100 g p.o.             |                |             |                                               |                                  |
| Aspirin 100 mg/kg + KF-392 5 mg/kg p.o.                           | 12             | 9.9 ± 0.2   | 15.8 ± 2.4                                    | 178.6 ± 17.5***                  |
| Aspirin 100 mg/kg + KF-392 2 mg/kg p.o.                           | 12             | 9.4 ± 0.3   | 32.2 ± 2.5                                    | 153.2 ± 18.1*                    |
| Aspirin 100 mg/kg + KF-392 1 mg/kg p.o.                           | 12             | 8.6 ± 0.2   | 26.0 ± 8.8*                                   | 137.4 ± 9.3*                     |

*** P<0.001 vs. Control. ** P<0.01 vs. Aspirin alone. * P<0.05 vs. Aspirin alone. All values represent mean ± S.E.
TABLE 7. Effect of KF-392 on buffer action in in vitro experiment

| Concentration of KF-392 (mg/ml) | Acidity titrated (μEq/ml) | pH of mixed solution |
|--------------------------------|---------------------------|----------------------|
| 0.0                            | 5.0                       | 2.2                  |
| 1.0                            | 4.9                       | 2.3                  |
| 2.0                            | 5.0                       | 2.3                  |
| 3.0                            | 5.0                       | 2.3                  |
| 4.0                            | 4.8                       | 2.3                  |

KF-392 (0.5 ml) was added to 0.5 ml of 0.01 N HCl. The mixture solution was titrated by 0.01 N NaOH. The titration was done in duplicate.

TABLE 8. Effect of KF-392 on peptic activity of rat gastric juice in vitro

| Concentration of KF-392 mg/ml* | Percentage of antipeptic activity (%) |
|-------------------------------|---------------------------------------|
| 0.10                          | 0.0                                   |
| 0.25                          | -1.2                                  |
| 0.50                          | -4.8                                  |
| 1.00                          | -6.0                                  |
| 2.50                          | -3.6                                  |

* Concentration of enzyme reaction mixture. KF-392 and enzyme were pre-incubated. Gastric juice was diluted ten times. Peptic activity was assayed in duplicate.

TABLE 9. Effect of KF-392 on charcoal transport in mice

| Treatment                        | No. of animals | Transport ratio (%) Mean±S.E. | Inhibition ratio (%) |
|----------------------------------|----------------|--------------------------------|----------------------|
| Control deionized water 0.5 ml/100 g p.o. | 10             | 65.9±5.1                      | —                    |
| KF-392 100 mg/kg p.o. 10          | 10             | 86.7±2.6**                    | -31.6                |
| KF-392 100 mg/kg p.o. 1           | 10             | 69.8±4.8                      | -5.9                 |
| KF-392 100 mg/kg p.o. 1           | 10             | 62.2±4.0                      | 5.6                  |

** P<0.01 vs. Control.

Transmucosal potential difference (P.D.) (Figs. 2, 3)

In the resting state, the intragastric pH was approx. 2.0 and the stomach of rat generated a P.D. of approx. -30 mV. A fall in P.D. and a rise of pH were observed with KF-392 at 5.0 mg/kg. On the other hand, with aspirin 100 mg/kg, a more rapid fall in P.D. was seen and recovery to the original level was observed 60 min after dosing, and with no influence on the pH. The combination of KF-392 (5.0 mg/kg) with aspirin (100 mg/kg) resulted in a marked fall in P.D. to -6 mV and marked rise of pH to approx. 7.0 at 15 min after dosing and these effects lasted for 60 min.

Transport of charcoal meal (Table 9)

The transport ratio was lowered by 31.6% with KF-392 at 100 mg/kg. However, KF-392 at less than 10 mg/kg had no effect on the charcoal transport.
The influence of a new compound, KF-392, on several types of induced gastric lesions and the gastric mucosal barrier were investigated. KF-392 given orally had marked inhibitory effects on the gastric lesions developed in a variety of experimental ulceration models. Gastric lesions developed in both stress- and Shay rats were strongly inhibited by approx. 80% with KF-392 (1.0 mg/kg). On both aspirin- and reserpine-induced gastric lesions, KF-392 at 1.0 mg/kg showed an inhibition ratio of more than 40%.

It is generally accepted that gastric lesions in Shay rats are induced by an accumulated gastric juice in the gastric lumen. When KF-392 was given orally at 2.0 mg/kg no ulcers developed and a significant inhibitory effect was found at 0.5 mg/kg. The high frequency of perforation in the control animal made collection of gastric juice impossible. The animal was sacrificed to collect the gastric juice 6 hr after the pylorus-ligation, and during this time, gastric lesions did not develop. Thus a decrease H⁺ in the gastric lumen was found when KF-392 was given orally, yet KF-392 has no antacid or buffer actions. On the other hand, a marked increase in Na⁺ was shown in the KF-392 treated animals. In addition a tendency toward increase of K⁺ output was found and the leaks of blood and protein were detected by the clinical test paper when KF-392 was given orally.

It was concluded that KF-392 given orally may evoke a back-diffusion and disruption of the gastric mucosal barrier.

Peptic activity in the pylorus-ligated rat was investigated and a rise in peptic activity was observed when KF-392 was given orally. However, KF-392 had no effect on peptic activity in the in vitro experiment. Such increase may be due to increased pepsin secretion or the disruption of the gastric mucosal barrier followed by the leak of pepsinogen from the gastric mucosa.
These responses were not observed when KF-392 was given s.c. at 5.0 mg/kg.

KF-392 given orally had a significant inhibitory effect on the aspirin-induced gastric lesion. A variety of factors are accepted as the mechanisms by which aspirin induces the gastric lesion (6, 7). It has been described that one of the mechanisms was the back diffusion of $H^+$ into the gastric mucosa (8). In our study, it was observed that both decreased $H^+$ and increased $Na^+$ in the gastric lumen were induced with aspirin in rats. In addition, increased $K^+$ and the considerable leaks of blood and protein were found in aspirin treated animal and were enhanced with KF-392. Thus it is unlikely that the suppression of back diffusion of $H^+$ is a mechanism by which KF-392 inhibits the aspirin-induced gastric lesion.

It had been reported that in rats an augmented gastric motility was caused under conditions of stress and an augmentation was one of the mechanisms by which the gastric lesion developed in stressed rats (9). In the present work, KF-392 given orally at 10 mg/kg had no effect on charcoal transport. It might be that the inhibitory effect of KF-392 on the stress-induced gastric lesion was not due to the suppression of the augmented gastric motility.

The occurrence of both sedative effect and diarrhea induced by reserpine was not inhibited with KF-392 at 5.0 mg/kg.

Many compounds e.g., aspirin, bile acid and alcohol are known to disrupt the gastric mucosal barrier and to cause a fall in P.D. (5, 10, 11). In the present study we measured the change of P.D. to confirm the effect of KF-392 on the gastric mucosal barrier. A fall in P.D. slightly prior to the rise of pH was observed when KF-392 was given orally. The effect lasted for 60 min and was dose dependent. On the other hand, aspirin caused a more rapid fall in P.D. which recovered to the original level 60 min after dosing as well as a slight change in pH. KF-392 enhanced both a fall in P.D. to approx. 0 InV and a rise of pH to approx. 7.0 caused by aspirin. It was thus confirmed that KF-392 evoked a disruption of gastric mucosal barrier and had marked antiulcer effects on several types of induced gastric lesions.

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