Effects of KB-5492, 1-(3,4,5-Trimethoxybenzyl)-4-((4-Methoxyphenyl)oxycarbonylmethyl)piperazine Monofumarate Monohydrate, on Gastric Lesions and Gastric Secretion in Rats

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Abstract—Effects of a newly synthesized compound, KB-5492, on gastric lesions and gastric secretion were studied in rats. Oral KB-5492 inhibited the lesions induced by HCl-ethanol, HCl-aspirin, water-immersion stress, indomethacin, histamine and prednisolone at 30-300 mg/kg. The ED50 values varied from about 35 to 98 mg/kg. KB-5492 had no effect on gastric acid secretion even at 300 mg/kg. KB-5492 appeared to have a much more potent protective effect than a known anti-ulcer drug, sofalcone, against acute gastric lesions.

It is generally accepted that peptic ulcers result from an imbalance between aggressive and defensive factors (1). Therefore, antiulcer drugs have been used to suppress aggressive factors or strengthen defensive factors. After random screening of many compounds at the Pharmaceuticals Research Center of Kanebo, Ltd., it was found that KB-5492, 1-(3,4,5-trimethoxybenzyl)-4-((4-methoxyphenyl)oxycarbonylmethyl)piperazine monofumarate monohydrate, had a preventive activity on a few experimental ulcer models. We herein describe the effects of KB-5492 on gastric mucosal lesions and gastric secretion in rats.

Male Sprague-Dawley rats (220-280 g, Nippon Charles-River Co.) were deprived of food (but not water) for 24 hr before the experiments, unless otherwise noted. Gastric mucosal lesions were produced by the following methods. Necrotizing agent-induced lesions were produced by p.o. administration of either 60% ethanol (v/v) or 150 mg/kg of aspirin (Sigma) in 150 mM HCl (HCl-ethanol or HCl-aspirin) (2, 3). The animals were killed 1 hr later. The stomachs were removed, inflated by injecting 8 ml of 2% formalin, and immersed for 10 min in 2% formalin to fix the gastric wall. The formalin treatment was performed for all the following experiments. The stomachs were then incised along the greater curvature and examined for lesions. The length (mm) of each lesion was measured under a dissecting microscope (×10) with a square grid and summed per stomach. Test drug or the vehicle alone was given p.o. 30 min before administration of HCl-ethanol or HCl-aspirin. Stress- or indomethacin-induced lesions were produced by either immersing animals in a water bath (23°C) using the restraint box (Toudai-Yakusaku type) (4) or giving indomethacin (Merck, suspended in Tween-saline) at 25 mg/kg, s.c. Eight or seven hours later, the animals were killed, and the stomachs were examined for lesions (mm). Test drug or the vehicle alone was given p.o. 30 min before stress or administration of indomethacin. Histamine-induced lesions: Under ether anesthesia, the abdomen of rats was incised, and the pylorus and the gastric artery were ligated. Histamine•2HCl (Nacalai Tesque, dissolved in saline) was given at 40 mg/kg, s.c. (5). The animals were killed 3 hr later, and the stomachs were examined for lesions (mm²). Test drug or the vehicle alone was given p.o. 30 min before ligation. Prednisolone-induced lesions: Rats, without fasting prior to and during the experiments, were administered prednisolone (Sigma, suspended in Tween-saline) at 50 mg/kg, s.c., once daily for 4 days (6). Twenty-four hours after the final administration of prednisolone, the animals were killed, and the...
stomachs were examined for lesions (mm²). Test drug or the vehicle alone was given p.o. twice daily (30 min before and 9 hr after prednisolone treatment) for 4 days.

Basal gastric secretion: Under ether anesthesia, the abdomen was incised and the pylorus ligated (7). Four hours later, the animals were killed, and the gastric contents were collected and analyzed for volume and acidity. Acidity was determined by titrating the gastric juice against 0.1 N NaOH to pH 7.0 using an automatic titrater (Hiranuma). Titratable acid output was expressed as μEq/hr. Test drug or the vehicle alone was given p.o. 30 min before the ligation. Histamine-stimulated gastric secretion: Under ether anesthesia, the abdomen was incised and the pylorus ligated. An acute fistula, with an esophageal cannula for drainage of saliva and a duodenal cannula for drug administration, was provided in the forestomach (8). Gastric samples which were collected through the fistula during the first 2 hr were discarded. The following 2 hr sample was considered to be the basal secretion. Histamine was administered at 20 mg/kg, s.c., twice, once immediately after collection of the basal secretion and again 2 hr later. Gastric samples were analyzed for volume and acidity. Test drug or the vehicle alone was given intraduodenally via the duodenal cannula after collection of the basal secretion. Drugs used were KB-5492 (Kanebo, Ltd.) and sofalcone (extracted from a commercial product, Solon®, Taisho Ltd.). Both KB-5492 and sofalcone were suspended in 0.5% carboxymethylcellulose (CMC) and given in a volume of 1 ml/200 g body wt. Statistical significance was examined by one-way analysis of variance followed by Dunnett's test. The ED50 value, the dose required for 50% inhibition of lesions, was determined by the method of least squares.

HCl-ethanol, HCl-aspirin, water-immersion stress, indomethacin, histamine and prednisolone produced multiple and extensive mucosal lesions in the glandular stomach. KB-5492, given at 30–300 mg/kg, significantly inhibited the development of these lesions (Table 1). The ED50 values varied from about 35 to 98 mg/kg. Sofalcone, given at 30–300 mg/kg, also significantly inhibited the lesions induced by HCl-ethanol, HCl-aspirin, water-immersion stress and indomethacin, but had little or no effect on histamine- and prednisolone-induced lesions (Table 1). KB-5492 and sofalcone given at 30–300 mg/kg did not significantly affect either basal or histamine-stimulated gastric acid secretion (Table 2).

This study demonstrated that KB-5492 potently protects the gastric mucosa of rats from development of various acute lesions without inhibiting gastric secretion. Among the lesion models used, KB-5492 markedly inhibited HCl-ethanol- and HCl-aspirin-induced gastric lesions. In this study, ethanol or aspirin was administered into the stomach together with a sufficient amount of exogenous acid (150 mM HCl). In these models, the protective effect of a drug against these lesions may not be associated with its antisecretory or acid-neutralizing action. Robert et al. (9) reported that exogenous prostaglandins (PGs, at non-antisecretory dose) markedly inhibited the gastric mucosal lesions induced by aspirin, indomethacin, absolute ethanol or boiling water and described these actions as cytoprotection. In the present study, KB-5492 had no effect on gastric secretion, suggesting that the drug has a mucosal protective activity. KB-5492 also inhibited gastric mucosal lesions induced by stress or indomethacin. The fact that H2-antagonists or antacids inhibit stress- or indomethacin-induced lesions clearly shows that the mechanism of formation of these lesions is related to gastric secretion (4, 10). Moreover, Yano et al. (11) and Takeuchi et al. (12) proposed that enhanced gastric contraction in response to stress or indomethacin is responsible for the development of lesions. In another series of studies on KB-5492, we observed that the agent inhibited spontaneous and indomethacin-stimulated gastric motility in rats (K. Shimohara et al., in preparation). Since KB-5492 had no antisecretory effect, the inhibition of stress- or indomethacin-induced lesions by KB-5492 may be due in part to inhibition of gastric motility. KB-5492 also significantly inhibited the development of histamine- and prednisolone-induced lesions. The pathogenesis of histamine-induced lesions remains un-
| Lesion models          | Treatment (mg/kg, p.o.) | No. of rats | Length (mm) or area (mm²) of lesions Mean±S.E. | Inhibition (%) | ED50 (mg/kg) | Treatment (mg/kg, p.o.) | No. of rats | Length (mm) or area (mm²) of lesions Mean±S.E. | Inhibition (%) | ED50 (mg/kg) |
|-----------------------|-------------------------|-------------|-----------------------------------------------|----------------|--------------|-------------------------|-------------|-----------------------------------------------|----------------|--------------|
|                       | KB-5492                 |             |                                               |                |              | Sofalcone              |             |                                               |                |              |
| Control               | 8                       | 109.5± 7.2 (mm) | 15.9                                          | 108.3±10.3     | 7.7          | Control               | 8                       | 117.3± 5.6 (mm) | —              |              |
| HCl-ethanol           | 30                      | 62.9± 8.8*  | 42.6                                          | 30             | 76.1± 8.2*  | 57.5                | 30                      | 76.1± 8.2*  | 35.1          |
|                       | 60                      | 38.5± 6.6*  | 64.8                                          | 100            | 29.8± 5.1*  | 74.6                | 100                     | 29.8± 5.1*  | 74.6          |
|                       | 150                     | 26.1± 5.4*  | 76.1                                          | 300            | 25.4± 7.2*  | 78.3                | 300                     | 25.4± 7.2*  | 78.3          |
| Control               | 8                       | 88.9± 7.1   | 49.6                                          | 10             | 91.8± 8.9   | —                   | 8                       | 91.8± 8.9   | 22.0          |
| HCl-aspirin           | 30                      | 44.8± 7.8*  | 60.1                                          | 30             | 69.3± 5.1*  | 24.5                | 30                      | 69.3± 5.1*  | 24.5          |
|                       | 60                      | 35.5± 7.5*  | 63.6                                          | 100            | 69.9± 7.8   | 23.9                | 100                     | 69.9± 7.8   | 23.9          |
|                       | 100                     | 32.4± 7.8*  | 88.7                                          | 200            | 46.5±10.4*  | 49.3                | 200                     | 46.5±10.4*  | 49.3          |
| Water-immersion stress| 8                       | 16.5± 1.9   | 31.1                                          | 30             | 14.1± 2.5   | 28.8                | 30                      | 14.1± 2.5   | 28.8          |
|                       | 200                     | 8           | 8.5± 0.8*                                     | 100            | 16.3± 1.7   | 17.7                | 100                     | 16.3± 1.7   | 17.7          |
|                       | 300                     | 8           | 2.9± 1.0*                                     | 300            | 6.8± 1.7*   | 65.7                | 300                     | 6.8± 1.7*   | 65.7          |
| Control               | 16                      | 10.3± 2.2   | 42.7                                          | 16             | 12.5± 2.6   | —                   | 8                       | 12.5± 2.6   | —             |
| Indomethacin          | 30                      | 5.9± 1.2    | 46.1                                          | 30             | 9.3± 2.8    | 25.6                | 30                      | 9.3± 2.8    | 25.6          |
|                       | 150                     | 3.6± 0.9*   | 65.0                                          | 100            | 5.4± 1.1    | 56.8                | 100                     | 5.4± 1.1    | 56.8          |
|                       | 200                     | 0.8± 0.6*   | 92.2                                          | 200            | 5.0± 1.4*   | 60.0                | 300                     | 5.0± 1.4*   | 60.0          |
| Control               | 6                       | 188.2±24.6 (mm²) | 42.7                        | 30             | 129.7±23.0  | 31.1                | 30                      | 148.4±36.3  | 3.2           |
| Histamine             | 6                       | 102.2±35.2  | 59.9                                          | 100            | 163.7±44.7  | -6.8                | 100                     | 163.7±44.7  | -6.8          |
|                       | 100                     | 68.7±22.0*  | 100                                           | 150            | 83.3         | 118.1±36.0          | 150                     | 83.3         | 118.1±36.0   |
|                       | 150                     | 31.5±10.2*  | 48.9± 4.6*                                   | 8              | 47.4± 6.0   | —                   | 8                       | 47.4± 6.0   | —             |
| Prednisolone          | 30                      | 27.7± 4.8*  | 56.6                                          | 30             | 40.3± 5.0   | 15.0                | 30                      | 40.3± 5.0   | 15.0          |
|                       | 60                      | 21.3± 4.6*  | 54.2                                          | 100            | 44.9± 6.9   | 5.3                 | 100                     | 44.9± 6.9   | 5.3           |
|                       | 100                     | 23.6± 4.4*  | 51.7                                          | 200            | 46.4± 8.9   | 2.1                 | 200                     | 46.4± 8.9   | 2.1           |
|                       | 200                     | 20.0± 6.1*  | 59.1                                          |                |              |                      |                          |

* Statistically significant difference as compared to the controls. P<0.05 (Dunnett's test).
Table 2. Effects of KB-5492 and sofalcone on basal and histamine-stimulated gastric acid secretion in pylorus-ligated rats

| Drugs      | Dose (mg/kg, p.o.) | No. of rats | Basal secretion | Acid-output (μEq/hr) | Inhibition (%) |
|------------|-------------------|-------------|-----------------|----------------------|---------------|
|            |                   |             | Volume (ml/rat) | Inhibition (%)        |               |
| Control    | 8                 | 30          | 6.1±0.7         | 174.0±27.1           |               |
|            | 100               | 5.4±0.7     | 11.5            | 149.1±18.2           | 14.3          |
| KB-5492    | 150               | 5.3±0.8     | 24.6            | 117.0±9.5            | 32.8          |
|            | 300               | 5.5±1.0     | 13.1            | 138.8±24.1           | 20.2          |
| Control    | 8                 | 5.0±0.4     | 9.8             | 153.6±17.6           | 11.8          |
|            | 30                | 5.7±0.9     | 18.0            | 124.5±11.5           | 28.4          |
| Sofalcone  | 100               | 6.3±0.9     | -10.5           | 183.5±28.7           | -14.7         |
|            | 300               | 7.0±0.6     | -23.3           | 193.8±22.0           | -21.1         |
|            |                   | 6.1±0.6     | -7.0            | 159.3±14.7           | 0.4           |

| Drugs      | Dose (mg/kg, i.d.) | No. of rats | Basal secretion | Acid-output (μEq/hr) | Stimulated secretion |
|------------|-------------------|-------------|-----------------|----------------------|----------------------|
|            |                   |             | Volume (ml/hr)  |                      |                      |
| Control    | 7                 | 0.47±0.19   | 55.6±24.2       | 0.78±0.24            | 108.9±36.4          |
|            | 7                 | 0.40±0.13   | 23.1±9.5        | 1.13±0.11            | 150.3±17.2          |
| KB-5492    | 100               | 0.71±0.24   | 71.4±32.3       | 1.11±0.15            | 148.7±24.2          |
|            | 300               | 0.54±0.17   | 51.8±38.6       | 0.80±0.12            | 111.1±18.6          |
| Control    | 8                 | 0.36±0.12   | 21.5±11.3       | 0.91±0.20            | 116.3±27.0          |
|            | 8                 | 0.29±0.07   | 19.0±7.9        | 0.66±0.16            | 81.0±23.1           |
| Sofalcone  | 100               | 0.30±0.08   | 19.6±9.6        | 0.59±0.12            | 73.7±17.6           |
|            | 300               | 0.45±0.13   | 28.2±15.1       | 0.67±0.18            | 80.2±20.3           |

All values represent the mean±S.E. i.d.: intraduodenal administration.

known. In the model of histamine-induced lesions produced by histamine at 100 mg/kg, i.p., propantheline bromide and cimetidine were effective for inhibiting the lesions, thereby indicating the participation of gastric acid and pepsin (13). In another study, it was confirmed that histamine interfered with gastric mucosal circulation in guinea pigs (14). In the present study, the gastric artery was ligated with histamine treatment. Therefore, the mucosal circulation may play a significant role in the formation of histamine-induced lesions under these conditions. Since KB-5492 did not inhibit gastric secretion, its inhibitory effect on histamine-induced lesions may be due to improvement of the damaged mucosal circulation. The pathogenesis of prednisolone-induced lesions also remains unknown. However, the fact that antisecretory drugs, such as cimetidine or pirenzepine, inhibited this type of lesion might indicate that the development of these lesions is related to gastric secretion (6). On the other hand, exogenous PGs (at non-antisecretory dose) significantly inhibited prednisolone-induced lesions (6). Konturek et al. (15) reported that sofalcone inhibited gastric lesions produced by acidified aspirin and water-immersion stress, suggesting that the protective effects of this drug may be mediated in part by endogenous PGs. In our present study, sofalcone inhibited HCl-ethanol-, HCl-aspirin-, water-immersion stress- and indomethacin-induced gastric lesions without any antisecretory action, thereby confirming the mucosal protection afforded by this agent. However, sofalcone had no significant effect on histamine- and prednisolone-induced gastric lesions. Based on ED50 values, KB-5492 was about 2 times more potent than sofalcone in the cases of water-immersion stress- and indomethacin-
induced lesions. All these findings taken together indicate that KB-5492 has gastric mucosal protective effects on the development of acute lesions. KB-5492 was already demonstrated to have no important toxicity (the LD50 value of KB-5492 is >5 g/kg, p.o., in rats) (T. Unno et al., unpublished data). Accordingly, KB-5492 is expected to be an effective drug for the treatment of human acute gastric mucosal disease (acute gastritis or acute gastric ulcer).

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