Effects of Various Agents on Prednisolone-Induced Gastric Lesions in Rats

Youichi NOBUHARA, Koji TAKEUCHI and Susumu OKABE

Department of Applied Pharmacology, Kyoto College of Pharmacy,
Misasagi, Yamashina, Kyoto 607, Japan

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Abstract—We studied the effects of various agents on prednisolone-induced gastric lesions in rats. Gastric lesions were produced by subcutaneous administration of 50 mg/kg of prednisolone once daily for 4 days to non-fasted rats. Daily oral administration of anti-peptic, antisecretory agents and 16-dmPGE₂ significantly inhibited the lesions. Antacids and PGI₂ had little or no effect. These results suggest that the concomitant use of the above agents with steroid therapy to provide protection of the gastric mucosa warrants further attention.

Steroidal antiinflammatory agents, as well as nonsteroidal ones, often induce gastrointestinal lesions in man and animals (1, 2). Only a few studies, however, have been done to search for protective agents against steroid-induced lesions (3–6). We report herein the effects of various agents, which are categorized as antisecretory (including antacids and anti-peptic agents) or cytoprotective ones, on prednisolone-induced gastric lesions in rats.

Male Sprague-Dawley rats (Charles-River Japan), weighing 250–260 g, were used. Prednisolone-induced gastric lesions were produced by a modification of the method described by Robert and Nezamis (7). While they gave 25 mg/kg of prednisolone to fasted rats for 4 days, we gave 50 mg/kg of the agent to normally fed rats for 4 days. The dose and period of administration of prednisolone used in our study were selected by the following experiments: Prednisolone (Sigma), suspended in Tween 80-saline solution, was given subcutaneously at 12.5, 25 or 50 mg/kg in a volume of 1 ml/kg once daily (10:00 AM) for 2, 4 or 6 days. In some studies, prednisolone at 50 mg/kg was given once daily for 4 days to rats deprived of food but not of water for 24 hr before and during experiments. These animals were killed 24 hr after the final administration of prednisolone. The stomach was removed, inflated by

injecting 12 ml of 2% formalin, and then immersed into 2% formalin for 10 min to fix the inner and outer layers. The stomach was incised along the greater curvature and examined for lesions in the glandular portion. The length (mm) of each lesion was measured under a dissecting microscope with a square grid (10 x) to the nearest millimeter, summed, and used as a lesion index. The person measuring the lesion had no knowledge of which treatment an animal had received. The stomach was put into 10% formalin for microscopy. The following agents were given to various groups of 10 rats each to determine the effects on prednisolone-induced gastric lesions: Synthetic hydrotalcite (Mg₆ Al₂ (OH)₁₆CO₃ · 4H₂O, Kyowa) and Maalox (Rorer) as antacids, sucralfate (Chugai) and pepstatin (Banyu) as anti-peptic agents, cimetidine (Sigma) as a histamine H₂-receptor antagonist, propantheline bromide (Kongo Kagaku) as a conventional anticholinergic agent, pirenzepine hydrochloride (Boehringer Ingelheim) as a selective anticholinergic agent, and 16,16-dimethyl PGE₂ (16-dmPGE₂, Ono) and PGI₂ (Ono) as antisecretory and cytoprotective agents. Each drug was either suspended in distilled water (synthetic hydrotalcite, Maalox, sucralfate, pepstatin, cimetidine) or dissolved in distilled water (propantheline bromide, pirenzepine hydrochloride, 16-dmPGE₂). These drugs

were given by oral gastric intubation in a volume of 0.5 ml/100 g of body weight twice (9:00 AM, 6:00 PM) or thrice (9:00 AM, 2:00, 7:00 PM) daily for 4 days during the prednisolone treatment. PGI₂, firstly dissolved in 1 M Tris buffer and stored in a deep freezer, was diluted with 1.25% NaHCO₃ to a desired concentration and given subcutaneously in a volume of 0.2 ml/100 g of body weight, 4 times (9:00 AM, 0:00, 3:00, 6:00 PM) daily. The control groups were given only the corresponding vehicle. Student's *t*-test was used to determine the statistical significance of the data, and $P < 0.05$ was regarded as significant.

The administration of 12.5 or 25 mg/kg of prednisolone for 2 days produced few or no lesions in the glandular stomachs of rats (Fig. 1). Even at 50 mg/kg, there were only a few small lesions in the corpus in 20% of the animals. When prednisolone was given for 4 days, lesions were produced dose-dependently. When 50 mg/kg were given, there were multiple lesions in the corpus in all rats. Histologically, the lesion was limited to within the mucosal layer, i.e., it consisted of erosion and not of ulcer. Treatment with prednisolone for 6 days produced much more severe lesions in the corpus, but the lesions still remained within the mucosal layer. Since a 4 day treatment with 50 mg/kg of prednisolone produced consistent lesions at an incidence of 100%, the method was used

as the standardized one for the assay of various agents. Daily weight loss was about 5 g on the average during the prednisolone treatment. When prednisolone was given in a dose of 50 mg/kg for 4 days to fasted rats, gastric lesions developed in all rats, and the lesion index was 43.1 ± 7.3 ($N=10$). The value was almost the same as that observed in non-fasted rats, i.e., 45.6 ± 8.3 ($N=10$). At that time, daily weight loss was about 11 g on the average. Pepstatin, cimetidine, propantheline bromide, and pirenzepine hydrochloride dose-dependently inhibited the prednisolone-induced gastric lesions (Fig. 2). It should be noted that 20 mg/kg/day of pepstatin inhibited the lesion formation by about 90% as compared with the controls. Both sucralfate at 600 mg/kg/day and 16-dmPGE₂ at 0.06 mg/kg/day significantly inhibited the prednisolone-induced lesions, but increase in the dose did not increase the inhibition. Synthetic hydrotalcite, given at 3000 or 6000 mg/kg/day, tended to inhibit the formation of gastric lesions in response to prednisolone. Maalox given at 3000 or 6000 mg/kg/day had no effect on lesion formation. The administration of PGI₂ at 0.04 or 0.12 mg/kg/day tended to inhibit the prednisolone-induced gastric lesions, but the inhibition was not significant.

These studies indicated that repeated administration of prednisolone to non-fasted rats, at least at the dose of 50 mg/kg for 4 days, induced visible gastric lesions at a high incidence. Other investigators (3-9) produced gastric lesions by giving various steroids, either alone or in combination, for 4 to 6 days to fasted rats. In this study, we found that the incidence and severity were not different between fasted and non-fasted animals. However, one difference between these two groups was the extent of weight loss which occurred in the fasted rats. Therefore, we used non-fasted rats in this experiment.

Dodi et al. (6) reported that concomitant administration of cimetidine, 15 mg/kg intravenously plus 200 mg/kg subcutaneously, was effective in preventing gastric lesions induced with 6-methyl prednisolone plus cortisone acetate in rats. They suggested that cimetidine may be useful for patients under

Fig. 1. Development of gastric lesions in rats by repeated subcutaneous administration of prednisolone at various doses for 2, 4 and 6 days. The number in the parenthesis is the incidence (%) of lesion formation. Data represent mean $\pm$ one S.E.

Fig. 2. Effects of various agents on the development of gastric lesions induced by prednisolone (50 mg/kg given subcutaneously once daily for 4 days) in rats. Data represent mean $\pm$ S.E.

long term steroid treatment. We also found that cimetidine, at the dose which significantly inhibits gastric secretion (10), potentially inhibited prednisolone-induced gastric lesions. Other antisecretory agents, propantheline bromide and pirenzepine hydrochloride also markedly inhibited the gastric lesions induced by prednisolone. These results are consistent with those by Robert and Nezamis (3) who showed a marked inhibition of cortisol-induced lesions in rats with methscopolamine bromide. Of interest was the finding that sucralfate and pepstatin, both anti-peptic agents (11–14), markedly inhibited these lesions. These results taken together suggest that gastric secretory factors, particularly pepsin, play an important role in mechanisms involved in prednisolone-induced gastric lesions.

Robert et al. (4) already reported that PGE₁, given by subcutaneous infusion for 4 days, markedly inhibited prednisolone-induced gastric lesions in rats. Lancaster and

Robert (15) found that gastric lesions (including perforated ones) as well as intestinal ones were produced by repeated administration of prednisolone for 8 days to non-fasted rats. They found that while 16-dmPGE₂ given orally at 0.1 mg/kg twice daily potentially inhibited the intestinal lesions, it had no effect on gastric lesions. In contrast, we found that 16-dmPGE₂, given twice daily in both antisecretory (0.1 mg/kg) and non-antisecretory doses (0.03 mg/kg) (16), significantly inhibited the gastric lesions in response to prednisolone. One consideration is that the gastric lesions induced by Lancaster and Robert (15) were too severe to be prevented by 16-dmPGE₂. Glucocorticoids, such as prednisolone, prevent the liberation of arachidonic acids from membrane phospholipids, presumably by blocking phospholipase A₂ and by so doing inhibit the biosynthesis of endogenous prostaglandins (17). Lancaster and Robert (15) implicated that the preventive activity of prostaglandin

was due to replacement therapy in an intestine rendered deficient in endogenous prostaglandin(s). Our findings and those of others (4) strongly suggest that a deficiency of endogenous prostaglandin in the stomach is also involved in the etiology of steroid-induced lesions.

The insignificant effect of two antacids on the gastric lesions caused by prednisolone appears to be induced by their short term neutralization of gastric juice, despite administration of 3 times daily. The same explanation may be applicable even in the case of PGI_2 . We gave PGI_2 in four divided doses daily and found only a tendency toward inhibition of prednisolone-induced gastric lesions. This may be due to a rapid destruction of this PG in the body. Continuous intravenous or subcutaneous infusion may result in a significant inhibition of these lesions.

The data suggest that the concomitant use of antipeptic, antisecretory, or cytoprotective agents with steroid therapy to provide protection of the gastric mucosa warrants further attention.

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