Effects of Indomethacin and Prednisolone on the Different Stages of Healing of Acetic Acid-Induced Gastric Ulcers in the Rat

Yoshiyasu Ogihara and Susumu Okabe*

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607, Japan

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ABSTRACT—Repeated administration of indomethacin or prednisolone for 2 weeks apparently delayed the healing of initial gastric ulcers induced by acetic acid injection in rats. While indomethacin significantly interfered with the healing of partially healed 2-week-old ulcers, prednisolone did not. Indomethacin had no effect on the healing of “unhealed” 4-week-old ulcers. However, prednisolone significantly enhanced the healing of such “unhealed” ulcers, and it decreased the weight of the connective tissue at the ulcer base and the collagen content. These results suggest that prednisolone enhances the healing of “unhealed” gastric ulcers by degrading the fibroplasia that had amply developed in the connective tissue.

Keywords: Prednisolone, Fibroplasia, Ulcer (acetic acid-induced)

Various non-steroidal or steroidal anti-inflammatory agents delay the healing of gastric ulcers induced in experimental animals (1-4). Decreases in the endogenous prostaglandin level (3, 4) and the gastric mucosal blood flow around ulcers (5) and the inhibition of angiogenesis in the granulation tissue (6) have been postulated to be involved in the underlying mechanism. In addition, we proposed that the amply developed fibroplasia at the ulcer base on indomethacin treatment could interfere with the contraction of the ulcer, thereby resulting in clearly demarcated ulcers (designated as “unhealed” ulcers) (7). If so, it is conceivable that the degradation of this fibroplasia might accelerate the healing of unhealed ulcers. Steroidal anti-inflammatory agents decrease the collagen content and/or the weight of pre-existing granulation tissue such as carrageenin granuloma tissue (8). Therefore, we examined the effect of prednisolone as well as that of indomethacin on the spontaneous healing of 2-week-old ulcers and 4-week-old “unhealed” ulcers.

Male Donryu rats (Nihon SLC), weighing 240-260 g, were used. The rats were fasted for 5 hr prior to ulcer production to facilitate the injection of the acetic acid solution into the gastric wall. Gastric ulcers were induced by submucosal injection of 20% acetic acid (0.03 ml) into the border between the antrum and corpus on the anterior wall of the stomach (9). After closure of the abdomen, the rats were maintained in the usual manner. Since well-defined, deep ulcers were observed 5 days after the acid injection, we defined the 5th day as the day of ulceration. After ulceration, the rats were randomly divided into the following 3 groups. a) Indomethacin (Sigma) at 1 mg/kg or prednisolone (Sigma) at 5 mg/kg was administered subcutaneously once daily (9:00 AM) for 4 or 2 weeks after ulceration, respectively. Control animals received the vehicle alone. b) Indomethacin at 1 mg/kg or prednisolone at 5 mg/kg was administered subcutaneously once daily for 2 weeks, starting from 2 weeks after ulceration. Control animals received the vehicle alone. c) Indomethacin at 1 mg/kg was administered subcutaneously once daily for 4 weeks after ulceration to induce “unhealed” ulcers. After the treatment, the animals were subdivided into two groups; one group was further administered indomethacin at 1 mg/kg, subcutaneously or the vehicle alone once daily for 2 weeks, and the other was given prednisolone at 5 mg/kg, subcutaneously or the vehicle alone for 2 weeks. After the final administration, the animals were fasted for 24 hr (water was given freely) and then killed with an overdose of ether. Their stomachs were removed, inflated with 8 ml of 2% formalin and then immersed in 2% formalin.
for 10 min to lightly fix the gastric wall. Subsequently, the stomachs were incised along the greater curvature and the ulcerated area (mm²) was determined under a dissecting microscope (Olympus, × 10) with a square grid. The person (S.O.) measuring the ulcers did not know the treatment given to the animals.

After determination of the ulcerated area, the connective tissue at the ulcer base was isolated from the surrounding mucosa under a dissecting microscope (× 20), as previously described (7). The isolated tissues were individually put into test tubes and boiled in ethanol (99%, v/v), defatted with ether-acetone (1:1, v/v) for 48 hr and then dried using a Freeze-Dryer 80 (Eyela) under reduced pressure. The dried tissues were then weighed (mg), hydrolyzed in 6 N HCl for 3 hr and neutralized with 6 N NaOH. The hydrolyzed samples were used for determination of hydroxyproline as collagen (10). The collagen content and concentration were expressed as μg of hydroxyproline and μg of hydroxyproline/mg dry tissue weight, respectively. The drugs used in the present study were dissolved in a trace of Tween-80 and then suspended in saline. The drugs were prepared immediately before use and administered in a volume of 1 ml per 200 g body weight. All data represent the mean±S.E.M. The statistical significance of the data was determined by Student’s t-test or Dunnett’s multiple comparison test, at the level of P<0.05.

On the day of ulceration, the ulcerated area was about 40 mm². These ulcers spontaneously decreased in size to 4.2±0.5 or 2.7±0.7 mm² at 2 or 4 weeks, respectively. Treatment with indomethacin for 4 weeks or prednisolone for 2 weeks after ulceration significantly delayed the healing of ulcers (Fig. 1A). In the group treated with indomethacin, the delay in healing was much greater than that in the animals treated with prednisolone. Indomethacin administered for 2 weeks starting from 2 weeks after ulceration also significantly delayed the healing of the partially healed ulcers (Fig. 1B). In contrast, prednisolone administered for 2 weeks had little or no effect on the healing of partially healed ulcers. After a 4-week treatment with indomethacin, the ulcerated area was 12.4±2.9 mm², and there was no further healing after a subsequent 2-week treatment with saline (Fig. 1C). A subsequent 2-week treatment with indomethacin also had no effect on the healing of "unhealed" ulcers. On the other hand, a subsequent 2-week treatment with prednisolone significantly accelerated the healing of "unhealed" ulcers, the healing rate being 42.9%.

Fig. 1. Effects of indomethacin and prednisolone on the healing of acetic acid-induced gastric ulcers in rats. A: Indomethacin or prednisolone was administered subcutaneously for 4 or 2 weeks starting from the day of ulceration, respectively. B: Agents were administered subcutaneously for 2 weeks starting from 2 weeks after ulceration. C: Agents were administered subcutaneously for 2 weeks starting from 4 weeks after ulceration, during which time 1 mg/kg of indomethacin was administered once daily to produce "unhealed ulcers". Control animals received the vehicle alone for 2 weeks. Data are values of the mean±S.E.M. * Statistically significant as compared with the control values at P<0.05.
Fig. 2. Effects of indomethacin (1 mg/kg/day) and prednisolone (5 mg/kg/day) on the connective tissue at the base of acetic acid-induced gastric ulcers in rats. Agents were administered for 2 weeks to rats with "unhealed" 4-week-old ulcers (caused by indomethacin, 1 mg/kg/day). Data are values of the mean ± S.E.M. (N=12). *, * Statistically significant as compared with the corresponding control values at 4 or 6 weeks at P <0.05. ○: Control, ●: Indomethacin, ▲: Prednisolone.

After a 4-week treatment with indomethacin, the weight of the isolated connective tissue, collagen content and concentration at the ulcer base were 15.5 ± 2.1 mg, 593.0 ± 71.4 µg and 39.6 ± 2.2 µg/mg dry tissue weight, respectively. These values were significantly larger than those in the control group, i.e., 8.4 ± 1.5 mg, 217.8 ± 37.3 µg and 25.8 ± 0.8 µg/mg dry tissue weight, respectively (Fig. 2). After a subsequent 2-week treatment with indomethacin, the weight and collagen content tended to be decreased, but the collagen concentration was slightly increased. However, a subsequent 2-week treatment with prednisolone significantly decreased the weight of the connective tissue and the collagen content of the unhealed ulcers, the values being 7.2 ± 0.9 mg and 365.7 ± 35.5 µg vs. 15.5 ± 1.7 mg and 616.8 ± 42.0 µg in the control group, respectively. The agent had little effect on the collagen concentration.

In this study, we reconfirmed our earlier findings that both indomethacin and prednisolone could delay the healing of initial ulcers when administered repeatedly for up to 4 weeks. In addition, we found that indomethacin significantly delayed the healing of 2-week-old partially healed ulcers. Satoh et al. (11) reported that acetic acid-induced gastric ulcers (4 weeks after acid injection) significantly relapsed when indomethacin (0.3–3 mg/kg) was administered for the following 2 weeks. These data suggest that indomethacin always exerts an unfavorable effect on ulcer healing, regardless of whether the ulcers are at the initial, partially healed or healed stage. However, the mechanism underlying the aggravating action of indomethacin seems to differ between initial, partially healed and healed ulcers. It is possible to partly explain the delayed healing of initial ulcers as due to interference with the contraction of the ulcer base. However, the aggravation of healed ulcers would involve a mechanism other than the inhibition of the contractile response of the ulcer base because the amount of fibroplasia is too little to interfere with ulcer contraction.

In contrast, the effect of prednisolone changed according to the stage of ulcer healing. The agent apparently aggravated the initial ulcers, had no appreciable effect on partially healed ulcers, and rather accelerated the healing of unhealed ulcers. Steroidal anti-inflammatory agents are well known to aggravate the initial stage of experimental gastric ulcers since Williams (1) reported the noxious effect of cortisone. Steroidal anti-inflammatory agents decrease the collagen content and the weight of pre-existing granulation tissue such as carrageenin granuloma tissue (8). The mechanism by which steroidal agents interfere with ulcer healing seems to involve a decrease in the granulation tissue formed at the ulcer base (7), which is the first step of wound healing.

In addition, there have been several reports that such agents can significantly or tend to cause reulceration of partially healed or healed gastric ulcers (12–14). Okabe et al. (12) reported that cortisone, administered at 25 or 75 mg/kg for 10 to 20 days, starting from the 50th day after ulceration, tended to aggravate partially healed acetic acid-induced ulcers. Kahn et al. (13) and Ito et al. (14) reported that cortisone (25 mg/kg) or hydrocortisone (20
mg/kg) administered for 10 to 20 days starting from the 50th or 40th day after ulceration induced by thermocautery or acetic acid injection in rats apparently caused the ulcers to recur. The reason why prednisolone failed to aggravate the partially healed ulcers remains to be elucidated.

It should be noted that prednisolone enhanced the healing of “unhealed” ulcers when administered for 2 weeks. The dose of the agent used is that which extensively delayed the healing of the initial ulcers. As provided in the present study, the weight of connective tissue and the collagen content at the base of “unhealed” ulcers were significantly higher than those of healed ulcers at 4 weeks. The subsequent treatment with prednisolone caused a significant decrease of these parameters. These results strongly suggest that the mechanism by which prednisolone enhances the healing of “unhealed” ulcers involves degragation of the fibroplasia at the ulcer base, which interfered with the ulcer healing. In addition, these data support our hypothesis that the mechanism underlying the delay in ulcer healing caused by indomethacin partly involves interference with the contraction of irregularly and amply developed fibroplasia. The reason why indomethacin had no effect on “unhealed” ulcers might be that it had no effect on preliminary developed granulation tissues (8). Recently, it was reported that some steroidal agents potentially have angiogenic activity (15). As shown by Tarnawski et al. (6), angiogenesis of the ulcer base plays an important role during the healing of gastric ulcers. Therefore, there is a possibility that prednisolone might promote the angiogenesis in the connective tissue at the ulcer base, leading to the enhanced healing of “unhealed” ulcers. In any event, the favorable effect of prednisolone on “unhealed” gastric ulcers would afford a new approach for the treatment of chronic, unhealed or intractable ulcers with various drugs.

We conclude that in contrast to indomethacin, prednisolone exerts either an unfavorable or beneficial effect on experimental gastric ulcers, depending on the stage of healing, i.e., aggravation of initial ulcers and acceleration of the healing of “unhealed” gastric ulcers.

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