Effect of 2'-Carboxymethoxy-4, 4'-Bis(3-Methyl-2-Butenyloxy) Chalcone (Sofalcone) on Chronic Gastric Ulcers in Rats

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Abstract—The anti-ulcer effect of sofalcone, an isoprenyl chalcone derivative, on acetic acid-induced gastric ulcers in rats was studied histologically and histochemically. After administrations of sofalcone at 50 and 200 mg/kg twice daily for 10 days, contraction of the ulcer, mucosal regeneration, accelerated development of the collagen fibers in the granulation tissue at the base of the ulcer, and increase of acid mucopolysaccharides, an alcian blue stain-positive substance covering the regenerated mucosa, were noted. The healing effect of sofalcone was balanced in mucosal regeneration and connective tissue proliferation (formation of the collagen fibers). Sofalcone of 50 mg/kg showed a greater healing effect than gefarnate at the same dose and had a similar healing effect as L-glutamine at 200 mg/kg.

2'-Carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy) chalcone (sofalcone) is a synthetic derivative of sophoradin (1), isolated from the root of a Chinese medical plant, *Sophora subprostrata* CHUN et T. CHEN. It was reported to have a preventive effect against various types of experimental gastric ulcers in rats and guinea-pigs (1, 2). From pathological examinations, it is well known that the repair process of acetic acid-induced gastric ulcers in rats closely resembles that of human peptic ulcers (3, 4). In this investigation, histological and histochemical evaluations of the anti-ulcer action of sofalcone were performed by using acetic acid-induced gastric ulcers in rats.

Materials and Methods

Animals

Male Wistar rats weighing 230–260 g were used in the present experiment. Each group contained 10 animals. They were housed in a room at constant temperature ($23\pm 2^{\circ}\text{C}$) and humidity ($55\pm 5\%$) and were maintained on commercial food (Oriental Yeast) and water ad libitum.

Acetic acid-induced ulcers

Laparotomies were performed on rats

under ether anesthesia, and then a gastric ulcer was induced in each by injecting 0.01 ml of 20% acetic acid solution into the gastric wall between the serosa and tunica muscularis on the border of the corpus and the antrum (3).

Drugs and methods of administration

Sofalcone was suspended in 0.4% (W/V) carboxymethyl cellulose (CMC) solution, and the suspension was orally administered at the volume of 5 ml/kg, twice (9:00 a.m. and 4:00 p.m.) daily for 10 days, starting on the 2nd post-operative day. The reference drugs were administered in a manner similar to that described above. In one experiment, animals received 50 mg/kg of either sofalcone or gefarnate and in the other, they received 200 mg/kg of either sofalcone or L-glutamine (Wako Pure Chemicals). In both of these experiments, the animals in the control group similarly received 0.4% CMC solution alone.

Gross observations

At the end of the administration, the animals were sacrificed, and the stomachs were removed, filled with 10 ml of 0.5% buffered formalin solution and immersed into 0.5% buffered formalin solution for 3 min to lightly fix the inner and other surfaces.

Then, the stomach was opened along the greater curvature in order to disclose the ulcer lesion. A measurement of the ulcerated area (mm^2) was made under a stereoscopic microscope (9x) with a square grid, and this was used as the size index.

Histology and histochemistry

The specimens were fixed in 10% buffered formalin solution, and sections perpendicular to the longer diameter of the ulcer were cut into step sections (3 sections per specimen) so that approximately 6 paraffin sections would include the center part of the lesion. The sections were stained with hematoxylin and eosin (H.E.), van Gieson (V.G.), and alcian blue (AB), and subjected to the following measurements and observations.

Histological measurement: In a manner similar to that described by Umehara et al. (5), the 8 variables were measured microscopically with an ocular micrometer on the vertical section of the ulcer stained with H.E. and recorded in mm after correction for magnitude. Using a section stained with V.G., the degree of development and the distribution of granulation tissue in the base of the ulcer were scored by the following criteria: Score 0: Though numerous fibroblasts and capillaries are observed, fibrosis of the granulation tissue rarely appears, and no collagen fibers appear on the basal portion of the ulcer. Score 20: Fibrosis of the granulation tissue arises, and a few collagen fibers are observed. Score 40: Fibrosis develops as collagen fibers are observed over one third of the microscopic field. Score 60: Fibrosis develops as the collagen fibers are observed over one half of the microscopic field. Score 80: Fibrosis of the granulation tissue progresses, and the basal portion of the ulcer is covered with reticular and collagen fibers. Score 100: Cicatrization is completed with a great deal of collagen fibers, after which neither capillaries nor fibroblasts are observed.

According to the method of Tabayashi (6), an index to represent the healing effect was calculated on the basis of these values. The healing index (A) is expressed in terms of the diameter of the mucosal defect as a percentage of the defect of the tunica muscularis, and the mucosal regeneration index (B) is

the regenerated mucosa as a percentage of the impaired mucosa. The collagen fiber proliferation index (C) represents the degree of development and distribution of the collagen fibers in the ulcer base. The arithmetic mean of these three indices was calculated to obtain the total healing index (D). Each of the three indices, A, B and C, was plotted on the assigned axis out of the three X, Y and Z axes on the same level, and the three points were connected to each other with lines to form a triangle (7). The effects of the drugs were evaluated by the area and pattern of the triangle.

Criteria for AB staining response: On the basis of the degree of AB staining, the acid mucopolysaccharide concentration in the regenerated mucosa was classified into the following 5 groups: -, no response; $\pm$, weak response; +, mild response; ++, moderate response; and +++, intense response. The results in the experimental groups were compared with those of the control groups.

Statistical analysis

Values are expressed as the mean $\pm$ S.E. of the mean. Statistical significance was evaluated with Student's *t*-test.

Results

Gross observations

When the ulcer lesions were disclosed by opening along the greater curvature, a round solitary ulcer was found in every stomach. The peripheral margin protruded a little and was roundish in shape. The base with fur was subsided or merely planar, and the surface was covered with a mucus substance. In most of the cases, part of the serosal surface was attached to the liver. The size indices are listed in Table 1. In the animals receiving 50 and 200 mg/kg of sofalcone, the healing rate for the ulcer was accelerated. The healing rates were 32.7% at a dose of 50 mg/kg and 35.6% at 200 mg/kg, and the acceleration was significant in comparison with controls. The healing rates were 27.2% and 23.7% in animals receiving 50 mg/kg of gefarnate and 200 mg/kg of L-glutamine, respectively.

Histological measurement

All the ulcers induced by the injection of acetic acid revealed a mucosal defect, and the part lacking mucosa extended deeply

Table 1. Effects of sofalcone, gefarnate and L-glutamine on the healing process of acetic acid ulcers in rats

Treatment	Dose (mg/kg)	Number of animals	Size index (mm ²) mean±S.E.	Healing rate (%)
Control	—	10	5.5±0.8	
Sofalcone	50	10	3.7±0.6*	32.7
Gefarnate	50	10	4.0±0.7	27.2
Control	—	10	5.9±0.8	
Sofalcone	200	10	3.8±0.5*	35.6
L-glutamine	200	10	4.5±0.5	23.7

Healing rate (%) = $\frac{C-T}{C} \times 100$ (C: Control, T: Test drug). Significantly different from the controls (*P<0.05).

into the gastric wall through the tunica muscularis, the class being UL-IV (8). The histological findings are shown in Table 2. The area of apparent mucosal defect decreased significantly after the administration of 200 mg/kg of either sofalcone (Figs. 1 and 2) or L-glutamine. The mucosal defect at the base was also significantly reduced after the administration of 200 mg/kg of sofalcone and tended to decrease after 50 mg/kg of sofalcone and 200 mg/kg of L-glutamine. Mucosal regeneration tended to be accelerated in each animal group receiving treatment. Healing index (A), mucosal regeneration index (B), collagen fiber proliferation index (C), and total healing index (D) are shown in Table 3.

Healing index (A): The values in animals receiving 50 mg/kg of sofalcone and gefarnate were higher than that in the control, and the values were 59.2 and 56.6, respectively, or calculated as 1.13 and 1.08, respectively, taking the value in the control as 1. Both of these values tended to increase after such treatments. The values after treatment with 200 mg/kg of sofalcone and L-glutamine were 62.9 and 59.9, respectively, increasing significantly to 1.23- and 1.17-fold over that in the control, respectively.

Mucosal regeneration index (B): The values in animals receiving 50 mg/kg of sofalcone and gefarnate were 60.0 and 55.4 respectively, or an increase of 1.14- and 1.05-fold over that in the control, respectively, suggesting a tendency to increase. In animals receiving 200 mg/kg of sofalcone and L-glutamine, the values were 64.9 and 60.6,

Fig. 1. A large and deep ulcer can be observed, and there is slight epithelial regeneration at the margin of the ulcer (A control rat, hematoxylin-eosin stain, ×40).

Fig. 2. The epithelization is well advanced in comparison with that of Fig. 1 (A sofalcone-treated rat, hematoxylin-eosin stain, ×40).

respectively, or 1.25 and 1.15 times as high as that in the control, respectively. Both increases were significant.

Table 2. Effects of sofalcone, gefarnate and L-glutamine on the histologic measurement of 8 variables during the healing process of acetic acid ulcers in rats

Treatment	Dose (mg/kg)	Measurement (mm)							
		Apparent defect of the mucosa	True defect of the mucosa (the base of the ulcer)	The regener- ation of the mucosal layer	Distance of the ruptured mucosa	Distance of the ruptured muscularis proplica	Thickness of the base of the ulcer	Thickness of the margin of the ulcer	Thickness of the necrosis
Control	—	2.2±0.2	2.2±0.2	2.3±0.2	4.6±0.2	4.1±0.3	1.2±0.3	1.6±0.1	0.18±0.02
Sofalcone	50	2.0±0.2	1.9±0.2	2.6±0.1	4.5±0.2	4.7±0.2	1.2±0.1	1.6±0.1	0.18±0.02
Gefarnate	50	2.2±0.3	2.1±0.3	2.6±0.1	4.8±0.4	5.2**±0.1	1.3±0.1	1.6±0.1	0.23±0.03
Control	—	2.3±0.2	2.1±0.2	2.2±0.2	4.2±0.3	3.9±0.2	1.3±0.1	1.5±0.1	0.29±0.03
Sofalcone	200	1.4**±0.2	1.5*±0.2	2.6±0.2	3.9±0.3	4.3±0.3	1.1±0.03	1.3±0.1	0.26±0.03
L-glutamine	200	1.6*±0.2	1.7±0.1	2.5±0.1	4.1±0.2	4.3±0.2	1.2±0.03	1.4±0.1	0.27±0.03

All values represent the mean±S.E. obtained with tissues from 10 animals. Significantly different from the controls (*P<0.05, **P<0.01).

Table 3. Effects of sofalcone, gefarnate and L-glutamine on the healing process of acetic acid ulcers in rats

Treatment	Dose (mg/kg)	The healing index (A)	The mucosal regeneration index (B)	Collagen fiber proliferation index (C)	The total healing index (D)
Control	—	52.6 ±3.9 (1.00)	52.6 ±3.5 (1.00)	34.0 ±1.0 (1.00)	45.5 ±2.2 (1.00)
Sofalcone	50	59.2 ±2.9 (1.13)	60.0 ±2.7 (1.14)	42.0**±1.2 (1.24)	51.4 ±2.0 (1.13)
Gefarnate	50	56.6 ±2.8 (1.08)	55.4 ±3.2 (1.05)	33.3 ±1.0 (0.98)	48.2 ±1.3 (1.06)
Control	—	51.0 ±2.5 (1.00)	52.6 ±2.0 (1.00)	37.9 ±1.0 (1.00)	47.7 ±1.7 (1.00)
Sofalcone	200	62.9**±2.1 (1.23)	64.9**±2.3 (1.25)	43.3**±1.1 (1.14)	56.9*±2.5 (1.19)
L-glutamine	200	59.9* ±2.1 (1.17)	60.0**±1.4 (1.15)	41.1* ±1.0 (1.08)	53.1*±1.1 (1.11)

All net values represent the mean±S.E. obtained with tissues from 10 animals. Significantly different from the controls (*P<0.05, **P<0.01). The value in the parentheses is the ratio calculated by taking each index in the controls as 1.

Collagen fiber proliferation index (C): After treatment with 50 mg/kg of sofalcone,

the value was 42.0 and 1.24 times as high as that in the control, suggesting a significant

Fig. 3. Granulation tissue in the base of the ulcer. Collagen fibers are thinly distributed (A control rat, van Gieson stain, $\times 250$).

Fig. 4. Granulation tissue in the base of the ulcer. Adult collagen fibers are closely distributed compared with those of Fig. 3 (A sofalcone-treated rat, van Gieson stain $\times 250$).

Fig. 5. Effects of sofalcone, gefarnate and L-glutamine on the healing index (A), regenerated mucosa index (B) and collagen fiber proliferation index (C) of gastric ulcer induced by acetic acid in rats. A, B and C are expressed as the ratio calculated by taking each index in the controls as 1. S indicates the area of the triangle (Control: $S=1.30$).

acceleration of the development of the collagen fibers. In contrast, after treatment with 50 mg/kg of gefarnate, the value was 0.98-fold that in the control and remained at the level of the control. In animals treated with 200 mg/kg of sofalcone and L-glutamine, the values were 43.3 and 41.1, respectively, or 1.14 and 1.08 times as high as that of control, respectively, indicating that the development of the collagen fibers was enhanced significantly (Figs. 3 and 4).

Total healing index (D): The total healing index in animals receiving 50 mg/kg of sofalcone and gefarnate were improved 1.13- and 1.06-fold over the value in the control, respectively, indicating a tendency to increase. In animals treated with 200 mg/kg of sofalcone and L-glutamine, the values were 56.9 and 53.1, respectively, increasing significantly 1.19- and 1.11-fold over the value in the control, respectively.

These three indices, A, B and C, were

plotted on X, Y and Z axes of the same dimension, and the area of the triangle formed was calculated (Fig. 5). The areas thus obtained were 1.89 in the 200 mg/kg sofalcone group, 1.76 in the 50 mg/kg sofalcone group, 1.67 in the 200 mg/kg L-glutamine group, 1.39 in the 50 mg/kg gefarnate group, and 1.30 in the controls. The shape was approximately a regular triangle in all cases, suggesting that each of these four treatments was balanced in mucosal regeneration and collagen fiber development.

Histochemistry

Acid mucopolysaccharide concentration in the regenerated mucosal tissue were semi-quantitatively estimated by the AB staining technique, and the results are shown in Table 4. Compared with the controls, intensification of the staining was apparent for animals treated with sofalcone for both 50 and 200 mg/kg doses (Figs. 6 and 7).

Table 4. Effects of sofalcone, gefarnate and L-glutamine on AB staining reactivity of regenerated mucosa during the healing process of acetic acid ulcers in rats

Treatment	Dose (mg/kg)	Number of animals	—	±	+	++	+++
Control	—	10			6	4	
Sofalcone	50	10				7	3
Gefarnate	50	10			3	5	2
Control	—	10			4	6	
Sofalcone	200	10				7	3
L-glutamine	200	10			4	6	

+++ : very strong staining, ++ : moderate staining, + : slight staining, ± : weak staining, — : no staining

Fig. 6. Edge of the ulcer. Mild response of alcian blue staining reactivity is seen in the regenerative mucosa (A control rat, $\times 100$).

Fig. 7. Edge of the ulcer. Intensification of alcian blue staining is observed in the regenerative mucosa (A sofalcone-treated rat, $\times 100$).

Discussion

The effect of sofalcone on acetic acid-induced ulcers, an experimental model for chronic gastric ulcers, was estimated chiefly on the basis of histological measurements. Sofalcone administered in a 50 mg/kg dose tended to produce a contraction of the ulcer and acceleration of regeneration of mucosal tissue and formation of granulation tissue. At a dose of 200 mg/kg, a significant healing effect was demonstrated in all of the responses of these tissues.

Histopathologically, the mechanism for the healing of peptic ulcers includes tissue responses such as reepithelization, formation of granulation tissue, and contraction of the ulcer. An independent operation of any one of these factors makes the ulcer intractable. A balanced operation of all the repair mechanisms is essential (9). The healing effect of sofalcone appeared to be the result of a proportionate operation of all these factors.

It was previously reported (10) that sofalcone accelerated the biosynthesis of acid mucopolysaccharides in the gastric wall in an experiment using an acetic acid-induced ulcer. This is consistent with our present results which showed that the acid mucopolysaccharide layer covering the regenerated mucosal tissue was intensified after the administration of sofalcone. Fukawa et al. (11) described increase of the acid mucopolysaccharides covering the regenerated epithelium as one of factors in inducing indirect acceleration of healing as well as enhancing the defensive ability against ulcers. Ishimori et al. (12) also suggested the participation of mucosal acid mucopolysaccharides in the acceleration of healing of ulcer lesions. Consequently, the increase of acid mucopolysaccharides by sofalcone seems to account for the tissue repairing action of sofalcone.

Of the two reference drugs, gefarnate did not accelerate the development of the collagen fibers at the base of the ulcer at a dose of 50 mg/kg. On the other hand, L-glutamine at a dose of 200 mg/kg improved all of the ulcer indices calculated in the present experiments, suggesting its accelerating effect on the healing of an ulcer.

Thus, sofalcone showed a better healing effect than gefarnate at the same dose of 50 mg/kg and showed a similar healing effect as L-glutamine at 200 mg/kg.

According to Matsuo et al. (13), sofalcone locally increased gastric blood flow when determined by the inhalation hydrogen gas clearance technique. Weakening of the gastric mucosal tissue resulting from a decreased local blood flow due to excitation of the adrenergic nerves was indicated as an important factor in the etiology of peptic ulcers, and it is essential in healing to increase the local blood flow and to maintain this higher level. Thus, the increase in the local blood flow in the stomach noted following the administration of sofalcone may contribute to the acceleration of the healing of peptic ulcers to a considerable degree.

Consequently, sofalcone increases the defensive ability of the gastric mucosa and acts favorably in restoring the tissue, accelerating the healing of ulcers.

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