EFFECTS OF SOFALCONE ON NECROTIZING AGENTS-INDUCED GASTRIC LESIONS AND ON ENDOGENOUS PROSTAGLANDINS IN RATS STOMACHS

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Attempts were made to investigate the effect of 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy) chalcone (sofalcone) on necrotizing agents-induced gastric lesions and on prostaglandin E₂ (PGE₂)-like activity of the gastric tissue in rats.

1. Sofalcone, 100 mg/kg i.p. and 300 mg/kg p.o., markedly suppressed 0.6 N HCl- or 100% EtOH-induced gastric lesions. Sofalcone, 100 mg/kg i.p., also significantly suppressed 0.2 N NaOH-induced gastric lesions.

2. Sofalcone suppressed 0.6 N HCl-induced gastric lesions with both oral and intraperitoneal routes, and the effect was particularly marked at 60 min. A dose of 100 mg/kg i.p. showed suppression lasting for up to 300 min.

3. 0.6 N HCl-induced gastric lesions were significantly aggravated by indomethacin treatment (10 mg/kg s.c.). Oral sofalcone (300 mg/kg) significantly suppressed the aggravation of gastric lesions by indomethacin given before and after sofalcone, but the i.p. (100 mg/kg) did not show significant suppression in the case of pretreatment with indomethacin.

4. PGs-like activity in the gastric tissue was increased in both of the fundus and the antrum by the administration of sofalcone without any dose-dependency. The increase was continuous and lasted for 6 h in the fundus of the stomach.

Keywords — sofalcone; gastric necrosis; prostaglandin; cytoprotection

INTRODUCTION

We have reported that newly synthesized 2'-carboxymethoxy-4,4'-bis (3-methyl-2-butenyloxy) chalcone (sofalcone) prevents the formation of various experimental ulcers and augmented ulcer healing.¹ We have also made it clear that sofalcone increased the gastric blood flow and stimulated the synthesis of mucusubstances of the gastric mucosa.¹⁻³ Various prostaglandins (PGs) have been known to play an important role in the regulation of the body functions in a wide range. The known effects on the stomach are suppression of gastric secretion,⁴ increase in blood flow⁵ and secretion of mucus.⁶ Robert et al.⁷ have reported that the gastric lesions induced by necrotizing agents such as 0.6N HCl and 100% EtOH were suppressed by pretreatment of PGs and they called the effect "cytoprotection". Similarity between above mentioned pharmacological effects of sofalcone and effects of PGs on the digestive tract suggests possible involvement of PGs in the anti-ulcer effect of sofalcone, and it has been a matter of concern whether or not sofalcone has cytoprotective action.

We report here the interesting findings obtained in a study of the effect of sofalcone on gastric lesions induced by necrotizing agents and on PGs-like activity of gastric tissue in rats.

MATERIALS AND METHODS

Male Wistar strain rats, weighing 180−220 g, were maintained with rat chow in a room air-conditioned at a temperature of 23 ±2°C with humidity of 55 ±5%. They were fasted for 24 h before use in experiments.
Test agents were sofalcone (Taisho), indomethacin (Sumitomo Chem.), atropine sulfate (Wako Junyaku), cyproheptadine (Banyu) and phenoxybenzamine (Tokyo Kasei). Fig. 1 shows the structure of sofalcone.

1. Lesion Induction — Animals were orally given 1 ml of either 0.6 N HCl, 100% EtOH or 0.2 N NaOH 30 min after an intraperitoneal administration or 60 min after an oral administration of the test agents and sacrificed 60 min later to examine gastric lesions by a dissecting microscopy. Measurements of length (mm) of gastric lesions were summed as a lesion index.

0.6 N HCl-induced gastric lesions were further studied in animals which had been given intraperitoneally or orally the test agents followed at a certain time (10, 30, 60, 90, 120, 180, 240, 300 and 360 min) by an oral dose of 1 ml/rat of 0.6 N HCl and killed 60 min later.

The effects of sofalcone in indomethacin treated rats were studied. Indomethacin was subcutaneously injected in a dose of 10 mg/kg 30 min before or after sofalcone, which was given in doses of 300 mg/kg orally or intraperitoneally, or 100 mg/kg intraperitoneally and its effect on the effect of sofalcone was studied. 1 ml/rat of 0.6N HCl was orally administered 60 min after sofalcone.

2. Effect on the PGs-Like Activity in the Gastric Tissue — Preparation of gastric samples and measurements of PGs-like activity were undertaken by using the method of Konturek et al. 8)

Isolated stomachs were immediately put in an ice-cold Krebs-Hensleit solution saturated with gaseous mixture of 95% O₂ + 5% CO₂ and separated into the fundus and the antrum. Each tissue was wiped off water with filter paper, weighed, cut into fine pieces, and washed twice with an ice-cold 0.05 M Tris buffer, pH 7.4. Each 300 mg tissue was mixed with 1 ml of the same buffer, shaken vigorously for 1 min, and centrifuged for 1 min at 3000 rpm to obtain the supernatant as a sample.

The gastric strip for bioassay was obtained from Wistar strain male rats weighing 200-250 g. Two gastric strips were prepared; one end of each strip was fixed to the bottom of a Magnus tube and the other end was led to an isotonic transducer (Nihon Kohden, TD-112S).

The strips were superficially perfused by Krebs-Hensleit solution saturated with gaseous mixture of 95% O₂ + 5% CO₂ under room temperature with addition of the test agents. The perfusate contained atropine sulfate (10^-7 g/ml), cyproheptadine (10^-7 g/ml), phenoxybenzamine (10^-6 g/ml) and indomethacin (10^-6 g/ml) to suppress the contraction of the strip due to substances other than PGs.

The contraction of the strips by PGs was led to the displacement coupler by means of an isotonic transducer and recorded on the inkwriting recorder (Nihon Kohden WI-681G) through the amplifier (Nihon Kohden AA-600H) for the coupler.

A calibration curve was prepared with 0.5-15 ng of standard PGE₂ (Sigma) and the measurements were expressed as the amount corresponding to PGE₂ (ng/30 mg wet tissue).

For measurement of PGE₂-like activity in the fundus and the antrum, sofalcone was given orally in doses of 50, 100 or 200 mg/kg to animals, which were sacrificed 2 h later. Sofalcone was given orally also in a dose of 200 mg/kg and the animals were killed 0.5, 1.0, 2.0, 4.0 or 6.0 h later to measure PGE₂-like activity in the gastric tissue.

RESULTS

1. Lesion Induction

As shown in Fig. 2, 0.6 N HCl markedly induced gastric lesions. The lesions were aggravated significantly by pretreatment with indomethacin (10 mg/kg s.c.).

Fig. 3 showed the effect of sofalcone on the
gastric lesions induced by 0.6 N HCl, 100% EtOH or 0.2 N NaOH. Sofalcone, 100 mg/kg i.p. or 300 mg/kg p.o., markedly suppressed the gastric lesions induced by 0.6 N HCl or 100% EtOH. The dose of 100 mg/kg i.p. significantly suppressed 0.2 N NaOH-induced gastric lesions but the suppression was weaker than that in the case of 0.6 N HCl or 100% EtOH-induced lesions.

Time course changes in the effect of sofalcone

FIG. 2. Gastric Lesions Induced by Different Concentration of HCl

One ml of HCl was orally given and the animals were killed 1 h later. Indomethacin was injected subcutaneously in a dose of 10 mg/kg 30 min before HCl.

a) $p < 0.01$ compared with each concentration of HCl.

FIG. 3. Effect of Sofalcone on Gastric Lesions Induced by Three Different Necrotizing Agents

Drugs were intraperitoneally administered 30 min before necrotizing agents and orally 60 min before. Necrotizing agents were given orally in 1 ml and the animals were killed 1 h later.

a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$ compared with control.

FIG. 4. Time of Onset and Duration of Action of Cytoprotection by Sofalcone

Sofalcone was orally administered at various time intervals before 0.6 N HCl. ○ ○ 30, ○ ○ ○ 100, ○ ○ ○ ○ 300 mg/kg (p.o.). HCl was orally given and the animals were killed 1 h later.

a) $p < 0.05$, b) $p < 0.01$, compared with respective time control.

FIG. 5. Time of Onset and Duration of Action of Cytoprotection by Sofalcone

Sofalcone was intraperitoneally administered at various time intervals before 0.6 N HCl. ○ ○ 10, ○ ○ ○ 30, ○ ○ ○ ○ 100 mg/kg (i.p.).

a) $p < 0.05$, b) $p < 0.01$, c) $< 0.001$ compared with respective time control.
given orally and intraperitoneally were studied on 0.6 N HCl-induced gastric lesions and the results were shown in Figs. 4 and 5, respectively. The oral administration showed the maximum inhibitory effect at 60 min.

The intraperitoneal administration showed suppression at the dose level of 30 mg/kg or more and a significant suppression lasted for 30 min to 120 min after dosing at 30 mg/kg and for 10 min to as long as 300 min at 100 mg/kg.

The effect of indomethacin was studied on the effect of p.o. and i.p. sofalcone against 0.6 N HCl-induced gastric lesions and the results were shown in Figs. 6 and 7. As seen from Fig. 6, sofalcone, 300 mg/kg p.o., significantly suppressed the formation of gastric lesions, whereas indo-

![Image of a graph showing the effects of sofalcone and indomethacin on gastric lesions](image)

**FIG. 6.** *Effect of Combination of Sofalcone and Indomethacin on 0.6 N HCl-Induced Gastric Lesions*

Indomethacin was subcutaneously injected in a dose of 10 mg/kg 30 min before or after sofalcone which was orally administered in a dose of 300 mg/kg.

One ml of HCl was orally given 60 min after sofalcone and the animals were killed 1 h later.

- a) \( p < 0.01 \)
- b) \( p < 0.001 \) compared with control.
- c) \( p < 0.001 \) compared with indomethacin alone.

![Image of a graph showing the effects of sofalcone on PGE₂-like activity](image)

**FIG. 7.** *Effect of Combination of Sofalcone and Indomethacin on 0.6 N HCl-Induced Gastric Lesions*

Indomethacin was subcutaneously injected in a dose of 10 mg/kg 30 min before or after sofalcone which was intra-peritoneally administered in a dose of 100 mg/kg. One ml of HCl was orally given 60 min after sofalcone and the animals were killed 1 h later.

- a) \( p < 0.05 \)
- b) \( p < 0.01 \)
- c) \( p < 0.001 \) compared with control.
- d) \( p < 0.05 \) compared with indomethacin alone.

![Image of a graph showing PGE₂-like activity](image)

**FIG. 8.** *Effect of Sofalcone on the PGE₂-Like Activity in the Rat Gastric Tissue*

Sofalcone was orally administered and the animals were killed 2 h later.

- a) \( p < 0.05 \)
- b) \( p < 0.01 \) compared with control.

[fundus, antrum]
methacin alone aggravated gastric lesions significantly regardless of the time of administration. In the combined administration groups, the aggravation was significantly suppressed both in the groups of sofalcone before or after indomethacin.

As shown in Fig. 7, sofalcone, 100 mg/kg i.p., significantly suppressed gastric lesions, whereas indomethacin alone aggravated gastric lesions significantly. Combined administration of sofalcone and indomethacin significantly suppressed the aggravation of gastric lesions due to indomethacin when indomethacin was given after sofalcone. Pretreatment with indomethacin did not suppress the aggravation of the gastric lesions.

2. Effect on the PGs-Like Activity in Gastric Tissue

PGE$_2$ showed a concentration-dependent contraction of the rat fundus strip with the application range of 0.5–15 ng. In another sample from animals treated with indomethacin, 0.1–10.0 mg/kg s.c. 2 h before sacrifice, the contraction of the gastric strip was suppressed dose-dependently by the dose of 1 mg/kg or more. These results were in accordance with the previous study by Konturek et al.$^8$)

Significant increases in PGE$_2$-like activity were noted at the dose of 50 mg/kg or more in the antrum and at 100 mg/kg or more both in the fundus and the antrum as compared to that in the control, but there was no dose-dependency (Fig. 8). Fig. 9 shows time course changes of

**FIG. 9. Time Course Changes of PGE$_2$-Like Activity in the Rat Gastric Tissue**

Sofalcone was orally administered at a dose of 200 mg/kg and the animals were killed 0.5, 1, 2, 4 and 6 h later respectively.

a) $p < 0.05$, b) $p < 0.01$ compared with control.

**FIG. 10. Effect of Combination of Sofalcone and Indomethacin on the PGE$_2$-Like Activity in the Rat Gastric Tissue**

Sofalcone was orally administered at a dose of 300 mg/kg and indomethacin was subcutaneously injected at a dose of 20 mg/kg and the animals were killed 2 h later.

a) $p < 0.05$, b) $p < 0.01$ compared with control.

□ fundus, ■ antrum.
PGE$_2$-like activity in the gastric tissue following sofalcone administration. Sofalcone 200 mg/kg p.o. significantly increased the PGE$_2$-like activity compared with nontreated control groups at 0.5, 2.0 and 4.0 h in the antrum and 2.0, 4.0 and 6 h in the fundus, respectively.

The effect of combination of indomethacin with sofalcone was studied by measuring PGE$_2$-like activity 2 h after concomitant administration of sofalcone, 300 mg/kg p.o. and indomethacin 20 mg/kg s.c.

As shown in Fig. 10, indomethacin-alone group gave a marked reduction in PGE$_2$-like activity compared to the control group. The combination group showed an increasing tendency of the activity compared to indomethacin-alone group, but there was no significant difference.

**DISCUSSION**

These studies show that the pretreatment of sofalcone greatly suppressed gastric lesions induced by necrotizing agents, indicating that sofalcone has PGs-like cytoprotection.

Robert *et al.*[^10] have described that suppression of gastric lesions induced by necrotizing agents with PGs is not due to the inhibition of gastric secretions and disappeared by pretreatment with indomethacin. As the inhibitory effect of sofalcone on gastric secretions was notably weak,[^11] the cytoprotection by sofalcone was not thought to be due to the inhibition of gastric secretions. The effect of indomethacin on sofalcone-produced cytoprotection was investigated in the 0.6 N HCl-induced gastric lesion model. Indomethacin alone markedly aggravated 0.6 N HCl-induced gastric lesions, but in the combined use of sofalcone and indomethacin the aggravation of the lesion due to indomethacin was significantly suppressed in either case of indomethacin before or after sofalcone when sofalcone was given orally. On the other hand, when sofalcone was given intraperitoneally, suppression was significant in case of post-treatment with indomethacin but was not significant in the case of pretreatment.

When sofalcone was either orally or intraperitoneally given before indomethacin, indomethacin’s aggravating effect was suppressed, but intraperitoneal administration of sofalcone after indomethacin failed to show suppression. This would indicate a possibility that sofalcone when orally administered has direct effect on the stomach in relation to its suppression of lesion aggravation due to indomethacin.

Based on the above finding that sofalcone had PGs-like cytoprotection and on the similarity between the various pharmacological activities of sofalcone and the effect of PGs on the digestive tract, an attempt was made to investigate sofalcone’s effect on the PGs-like activity in gastric tissue.

Our results concerning the contents of PGs in gastric tissue by bioassay were almost in agreement with those of Konturek *et al.*[^8] The PGE$_2$-like activity (including PGE$_1$ and PGF$_2$) measured by bioassay in nontreated normal control animals was larger in the antrum than in the fundus.

Sofalcone (100 and 200 mg/kg, p.o.) increased PGE$_2$-like activity in the fundus and the antrum about twice and one and a half, respectively, 2 h later. An increasing effect of the gastric PGE$_2$-like activity reached maximum in the fundus and in the antrum by 100 and 50 mg/kg of sofalcone, respectively.

Sofalcone, 200 mg/kg p.o., markedly increased gastric PGE$_2$-like activity at 2 and 4 h and the effect lasted even for 6 h in the fundus. The similar results were obtained in the antrum.

There was, however, difference in the duration of effects between the inhibitory effects of sofalcone on necrotizing agents-induced gastric lesions and increasing effects on PGE$_2$-like activity in gastric tissues.

Oral sofalcone, in doses of 100 and 200 mg/kg p.o., showed inhibition of gastric lesions even at 10 min after administration (Fig. 4). On the other hand, sofalcone, 200 mg/kg p.o., showed increases in PGE$_2$-like activity at more than 2.0 h (Fig. 9).

As the rapid onset of inhibitory effects on gastric lesions have been reported to be peculiar to
PGs,\textsuperscript{7} the inhibitory effects of sofalcone on gastric lesions would be in part due to PGs.

In a series of studies of sofalcone, we have already made it clear its preventive effect on various experimental gastric ulcers,\textsuperscript{1} the increasing effects of the gastric blood flow\textsuperscript{2} and the gastric tissue mucosubstances.\textsuperscript{3} These effects are in agreement with the reported effects of PGs or PGs-derivatives.\textsuperscript{4–6} The pharmacological effect of sofalcone on gastric ulcers would partly be explained to be due to increased tissue PGs contents. Sofalcone increases PGE\textsubscript{2}-like activity in gastric cells, exerting protective effect on the stomach which might have resulted in the prevention of experimental ulcers.

PGs have been well known to protect the alimentary tract against various necrotizing agents\textsuperscript{7} and indomethacin induces ulcers by suppressing PGs biosynthesis.\textsuperscript{10} In the present study, indomethacin aggravated HCl-induced gastric lesions. In addition, indomethacin (1–10 mg/kg, s.c.) markedly decreased PGE\textsubscript{2}-like activity in the gastric tissue. These facts suggest the important role of PGs in the cytoprotection and anti-ulcer effect on the gastric mucosa.

The mechanism by which sofalcone increases PGs activity in the gastric tissue might involve stimulation of PGs biosynthesis or suppression of its decomposition. Our earlier study showed sofalcone had no effect on cyclooxygenase activity but suppressed PG-15-OH dehydrogenase.\textsuperscript{11} The reason that pre-administered sofalcone suppressed aggravation of HCl-induced gastric lesions may be understood as the result of PGs retention in the gastric tissue resulting from dehydrogenase suppression by sofalcone. However, in the case of pretreatment with indomethacin, oral sofalcone suppressed aggravating effect on its gastric lesions. This could not be explained by the increase in PGs contents, because PGs biosynthesis was suppressed. Other pharmacological effects such as increasing effects of gastric blood flow,\textsuperscript{3} therefore, may contribute to its suppression of aggravating effect.

Based on the above findings, the anti-ulcer effect of sofalcone would be in part due to the increasing effect on gastric tissue PGs contents.

REFERENCES