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SU-840, A NOVEL SYNTHETIC FLAVONOID DERIVATIVE OF SOPHORADIN, WITH POTENT GASTROPROTECTIVE AND ULCER HEALING ACTIVITY

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Flavonoids derived from sophoradine are known to exhibit gastroprotective and ulcer healing properties but the mechanism of these actions are not fully explained. In this study we determined the effect of novel flavonoid derivative of sophoradin, SU-840, on gastric secretion, acute gastric lesions induced by acid-independent (100% ethanol) or acid-dependent ulcerogens (acidified aspirin (ASA) and stress) and on the healing of chronic gastric ulcers in rats. The number and area of gastric lesions was determined by planimetry, gastric blood flow (GBF) was measured using H_2-gas clearance technique and the mucosal samples were excised for the measurement of PGE_2 generation by radioimmunoassay. Exposure of rats to 100% ethanol or acidified ASA (100 mg/kg dissolved in 0.2 N HCl) or to water immersion and restraint stress (WRS) resulted in hemorrhagic gastric lesions accompanied by drastic fall in the GBF as compared to the values recorded in vehicle treated gastric mucosa. SU-840 (6.25—100 mg/kg i.g.) reduced dose-dependently gastric acid and pepsin secretion and gastric lesions induced by ethanol, acidified ASA and WRS, the dose inhibiting by 50% of these lesions (ID_{50}) being 28, 17 and 95 mg/kg, respectively. This protection required much lower doses as compared to original sofalcone or sucralfate and was obtained when this sofalcone-like drug was administered *via* parenteral route. The protective effect of SU-840 given i.g. or i. p. was accompanied by a marked rise in the GBF and mucosal generation of PGE_2. The protective activity of SU-840 showed longer duration of the action than that of sofalcone and occurred in the doses that failed to affect gastric secretion. Pretreatment with indomethacin to suppress endogenous PG reversed completely the protective and hyperemic effects of SU-840 against ethanol and stress induced damage whereas L-NNA, a potent inhibitor of NO-synthase, failed to affect protection but completely abolished the hyperemia evoked by this agent. NEM, an sulfhydryl alkylator, significantly attenuated the protective and hyperemic effects of SU-840 suggesting that endogenous sulfhydryls are involved in these effects. Seven day treatment with SU-840 accelerated significantly healing rate of chronic gastric ulcers and increased the GBF at the ulcer crater and ulcer margin. These effects were reversed by L-NNA and further restored by the addition to L-NNA of L-arginine, a substrate for NO-synthase. We conclude that SU-840 exhibits gastroprotective and hyperemic activity against acid-independent and acid-dependent irritants involving endogenous PG, sulfhydryls and hyperemia mediated by NO and 2) enhancement in gastric blood flow in the ulcer area mediated by NO appears to be essential for the acceleration of the ulcer healing by SU-840.

K e y   w o r d s: *sophoradin, gastroprotection, ulcer healing, prostaglandins, nitric oxide, gastric blood flow, sulfhydryls.*

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INTRODUCTION

Carbenoxolone-like agents such as synthetic isoprenyl flavonoid sofalcone derived from sophoradin, have been used successfully for a long time in the treatment of the gastric disorders including gastritis and peptic ulcer disease (1—4). This topically active antiulcer agent has been reported to prevent gastric mucosal lesions induced by various strong irritants presumably by enhancing of mucosal generation of prostaglandins (PG) suggesting the involvement of endogenous PG in its cytoprotective activity (2, 5—7). This was further supported by the demonstration that Sofalcone inhibits activity of 15-OH-PG-dyhydrogenase, an enzyme responsible for degradation of endogenous PG, thus leading to higher availability of protective PG (5, 6). Recent studies demonstrated that the enhancement of protective glycoproteins and mucus secretion (8—13), antioxidant (14) and antibiotic (15) properties of this agent significantly contribute to its protective and ulcer healing action on the gastric mucosa.

SU-840 is a novel agent originating from Sofalcone type of agents but due to complexing with beta-cyclodextrin this compound is expected to exhibit enhanced bioavailability and to act beneficially on the gastric mucosa at much lower doses than Sofalcone, therefore reducing the possibility of any side effects. Furthermore, it is unknown, whether SU-840 is effective after parenteral administration and whether its gastroprotective and ulcer healing actions of this Sofalcone derivative involve protective factors such as endogenous PG, nitric oxide (NO) and sulfhydryls (16—18)

This study was designed to determine 1) the gastroprotective activity of SU-840 in acid-independent (100% ethanol) and acid-dependent (acidified aspirin, stress) models of acute gastric damage; 2) to evaluate the possible mechanisms of protective effects of SU-840, particulary the role of gastric secretion, gastric blood flow, endogenous PG, NO and sulfhydryls; 3) to examine the effect of this agent on ulcer healing and gastric blood flow at the ulcer margin.

MATERIAL AND METHODS

Wistar rats of either sex, weighing 180-230 g were used both for secretory studies and for the production of gastric lesions or chronic ulcerations.

Gastric secretory studies

Gastric secretion was studied in 60 male rats prepared with a small metal canula to form gastric fistulas about 1 month before the secretory test as described previously (19). The rats were fasted for 18 h before the study but had free access to water 2 h before the experiment, when they were placed in individual Bollman cages with a wide-mesh bottom to prevent coprophagy. The

gastric fistula was opened, the stomach was rinsed with about 5 ml of tap water and the collection of gastric juice was started. After 1 h of basal secretion, SU-840 (a generous gift of dr Muramatsu, Taisho Pharmaceutical, Tokyo, Japan) was given intragastrically (i.g.) or intraperitoneally (i. p.) in various doses ranging from 6.25—100 mg/kg, each dose being administered as a single bolus given on a separate test day. In case of oral administration the drug was kept in the stomach by closing gastric fistula for 30 min and then reopening the fistula; the collection of gastric secretion was continued for the next 1 h. In control experiments, saline was administered i.g. or i.p. and the collection of gastric juice was carried out in the same way as in tests with SU-840 or Sofalcone. In all tested samples of gastric juice, the volume was measured and the concentration and output of acid and pepsin were determined as described before (19).

Production of gastric lesions

In all tests with experimental gastric lesions the rats were fasted for 24 h before the study but had free access to water. They were placed in individual Bollman cages to prevent coprophagy. Acute gastric lesions were induced by 100% ethanol, acidified aspirin (ASA) or 3.5 h of exposure to water immersion and restraint stress (WRS). Briefly, 100% ethanol in a volume of 1.5 ml was administered i.g. using a metal orogastric tube. One hour after ethanol administration, animals were sacrificed and the stomach was removed to determine the area of gastric lesions by planimetery (Morphomat, Carl Zeiss, Berlin, Germany) as described in our previous studies (21, 23).

Acidified ASA was administered i.g. in a dose of 150 mg/kg dissolved in 0.2 N HCl using a metal orogastric tube. After 1 h the animals were killed and the area of gastric lesions was determined planimetrically as described above. Stress lesions were provoked by placing the animals in special restraint cages and immersion into a water bath at 23°C for 3.5 h to the xyphoid level as described previously (20). The animals were then killed, the stomach removed and examined grossly for the number of gastric lesions.

Several groups of rats, each consisting of 8-12 animals, were used in studies with dose-dependency of the protective effect of SU-840 against all three ulcerogens and with duration of protection induced by SU-840: 1) vehicle followed 30 min later by 100% ethanol, acidified ASA (150 mg/kg) or WRS; 2) SU-840 (6.25-100 mg/kg i.g.) followed 30 min later by 100% ethanol; 3) SU-840 (6.25-100 mg/kg i.g.) followed 30 min later by acidified ASA or WRS and; 4) standard dose Sofalcone (100 mg/kg i.g.) or SU-840 (50 mg/kg i.g.) followed 30-360 min later by 100% ethanol.

Determination of the role of endogenous PG, NO and sulfhydryls in gastroprotection induced by SU-840.

The role of endogenous PG in the protection induced by SU-840 was examined by using 1) the pretreatment with indomethacin to inhibit endogenous PG in an attempt to reverse the gastroprotective activity of SU-840 against ethanol and stress lesions and 2) by direct measuring of the generation of PGE_2 by radioimmunoassay in tests with indomethacin. The following groups of rats were used: 1) vehicle (saline) followed 90 min later by 100% ethanol or stress; 2) vehicle (saline) followed 60 min later by SU-840 (50 mg/kg i.g.) and then 30 min later by 100% ethanol or stress; 3) indomethacin (5 mg/kg i. p.) followed 60 min later by vehicle (saline i.g.) and finally 30 min later by 100% ethanol or stress; 4) indomethacin (5 mg/kg i.p.) followed 60 min later by SU-840 (50 mg/kg i.g.) and finally 30 min later by 100% ethanol or stress.

To assess the possible role of NO in gastroprotection induced by SU-840 against ethanol damage, rats were pretreated intravenously (i.v.) with N^G-nitro-L-arginine (L-NNA) (21), a non-specific inhibitor of NO-synthase (purchased from Sigma Chemical Co, Ltd) and 15 min later SU-840 was applied i.g. followed by the oral administration of ethanol. In some tests, L-arginine, the substrate of NO-synthase or D-arginine, which does not serve as the substrate for NO-synthase (21) was added i.v. 10 min prior to L-NNA. The following groups of rats were used; 1) vehicle (saline 1 ml i.v.) followed 60 min later by 100% ethanol; 2) vehicle (saline i.v.) followed 30

min later by SU-840 and then 30 min later by 100% ethanol; 3) L-NNA (40 mg/kg i.v.) followed 30 min later by vehicle (saline i.v.) and then 30 min later by 100% ethanol; 4) L-NNA (40 mg/kg i.v.) followed 15 min later by SU-840 and then 30 min later by 100% ethanol; 5) L-arginine or D-arginine (300 mg/kg i.v.) followed 15 min later by L-NNA (40 mg/kg i. v.) and then 15 min later by SU-840 (50 mg/kg i.g.) and finally 30 min later by 100% ethanol.

The involvement of endogenous sulfhydryls in protection induced by SU-840 against ethanol damage was examined in rats pretreated with N-ethylmaleimide (NEM), a potent SH-depletor (22). These experiments included the following groups of animals; 1) vehicle (saline) followed 30 min later by 100% ethanol; 2) NEM (20 mg/kg s. c.) followed 30 min later by 100% ethanol; 3) SU-840 (50 mg/kg i.g.) followed 30 min later by 100% ethanol; 4) NEM (20 mg/kg s. c.) followed 30 min later by SU-840 (50 mg/kg i.g.) and then 30 min by 100% ethanol.

Measurement of gastric blood flow

In some rats treated with 100% ethanol, acidified ASA or WRS with or without pretreatment with SU-840, the GBF was measured using H_2-gas clearance technique as described previously (7). The rats were anesthetized with ether, the abdomen was opened, and the gastric contents were gently evacuated to the exterior through the cut in the forestomach. For GBF measurement, a double electrodes of electrolytic regional blood flowmeter (Biotechnical Science, Model RBF-2, Osaka, Japan) were inserted into the mucosa. One for these electrodes was used for the local generation of gaseous H_2 and another for the measurement of tissue H_2. With this method the H_2 generated locally is carried out by flow of blood while the polarographic current detector reads out decreasing tissue H_2. The clearance curve of tissue H_2 was used to calculate absolute flow rate (ml/min/100 g) in the oxyntic gland areas as described previously (7). The measurements were made in three areas of the oxyntic gland area in rats with or without ethanol administration and the mean values of these measurements were calculated and expressed as percentage of the flow rate recorded in the vehicle control gastric mucosa. All measurements were performed by the person unaware of the treatment given.

Determination of mucosal generation of PGE_2

In some tests, the mucosal samples of the oxyntic gland area were taken by biopsy (about 50 mg) immediately after the animals were killed, to determine the mucosal generation of PGE_2 by specific radioimmunoassay (RIA) as described previously (23). The mucosal sample was placed in preweighed Eppendorf vials, and 1 ml of Tris buffer (50 mM, pH 3.5) was added to each vial. The samples were finally minced (about 15 sec) with scissors, washed, and centrifuged for 10 sec, the pellet being resuspended again in 1 ml of Tris. Then each sample was incubated on a Vortex mixer for 1 min and centrifuged for 15 sec. The pellet was weighed and the supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mM) and kept at $-20°C$ until RIA. PGE_2 was measured in duplicate using RIA kits (New England Nuclear, Munich, Germany). The capability of the mucosa to generate PGE_2 was expressed in nanograms of wet tissue weight.

Production of chronic gastric ulcers

Gastric ulcers were produced using our modification (24) of acetic acid method originally proposed by Okabe et al (25). Under ether anesthesia the stomach was exposed and a plastic mold of 6 mm diameter was applied tightly to the serosal surface of the anterior wall of the stomach just proximal to the antral gland area. About 70 μl of 100% acetic acid was poured through the mold onto the surface of the stomach for 25 s. This produced an immediate necrosis of entire mucosa and submucosa within the area where the acetic acid was applied i.e. about 28 mm². Excess of acetic

acid was then removed and the serosa was gently washed with tap saline. Histologically, these ulcers became chronic within 2—3 days and healed within few weeks without perforation or penetration to the surrounding organs. After the application of acetic acid, the animals were allowed to recover from anesthesia and received only water on the day of operation (day O). Then, they were divided into various groups and fed normal chow and water *at libitum* for the next 7 days.

The GBF was measured after 7 days in rats with chronic gastric ulcers treated with vehicle, SU-840 and classic antiulcer agent, sucralfate applied i.g. without or with L-NNA using H_2-gas clearance method in similar manner as described above. At the end of 7 day treatment, the rats were lightly anesthetized with ether, the abdomen was opened and the stomach was exposed to assess the GBF at ulcer margin, ulcer crater and the adjacent intact mucosa on the opposite wall of the stomach. The GBF was expressed as the percentage of the basal flow recorded in the intact mucosa. All measurements were performed by the person unaware to which group animals belonged.

RESULTS

Effect of SU-840 on gastric secretion

The effect of graded doses of SU-840 given i.g. or i.p. on gastric acid and pepsin secretion in rats with chronic gastric fistulae is shown in *Table 1*. In vehicle-treated control animals, basal acid output averaged 133 ± 19 µmol/30 min, while pepsin output was 0.89 ± 0.08 mg/30 min. Intragastric application of SU-840 (6.25-100 mg/kg) failed to affect significantly gastric acid or pepsin outputs at any dose tested. When SU-840 was applied i.g. or i.p. in larger dose of 200 mg/kg, it significantly inhibited gastric acid and pepsin secretion as compared to the respective values obtained in control rats treated with vehicle.

Table 1. Effect of SU-840 given orally in graded doses (6,25-200 mg/kg) on gastric acid and pepsin secretion in chronic gastric fistula rats. Mean ± SEM of 8-10 rats. Asterisk indicates a significant decrease below the value obtained in vehicle-control rats.

	Acid output (µmol/30 min)	Pepsin output (mg/30 min)
Vehicle (saline)	133 ± 19	$0,89 \pm 0,08$
Su-840 (mg/kg i.g.)		
6,25	135 ± 14	$0,90 \pm 0,09$
12,5	121 ± 12	$0,88 \pm 0,08$
25	123 ± 13	$0,85 \pm 0,06$
50	118 ± 9	$0,82 \pm 0,05$
100	112 ± 8	$0,75 \pm 0,06$
200	$87 \pm 5*$	$0,62 \pm 0,04*$
SU-840 (mg/kg i.p.)		
200	$94 \pm 8*$	$0,58 \pm 0,05*$

Effect of SU-840 on acute gastric lesions and gastric blood flow

Fig. 1 shows the effect of SU-840 given orally or injected parenterally (i.p.) on the area of gastric lesions and changes in the GBF induced by 100% ethanol. In vehicle-pretreated control rats, the i.g. application of 100% ethanol resulted in widespread gastric mucosal lesions and the area of these lesions averaged 76 ± 9 mm^2. The GBF, which in intact gastric mucosa averaged 51 ± 6 ml/min-100 g, was reduced by about 60% after ethanol administration. The i.g. pretreatment with SU-840 in graded doses ranging from 6.25—100 mg/kg reduced dose-dependently the area of ethanol-induced damage, the dose inhibiting by 50% ethanol lesions (ID$_{50}$) being about 25 mg/kg. This reduction in ethanol lesions was accompanied by a significant rise in GBF showing at 100 mg/kg of SU-840 about 75% increase above the value recorded in vehicle-treated rats. The pretreatment i.p. with SU-840 in graded doses also reduced the area of ethanol-lesions and this was accompanied by a significant increase in the GBF. However, the significant effect of i.p. SU-840 on the area of gastric lesions and gastric hyperemia was achieved at the dose (25 mg/kg i.p.) about twice higher than that obtained with i.g. application of this agent.
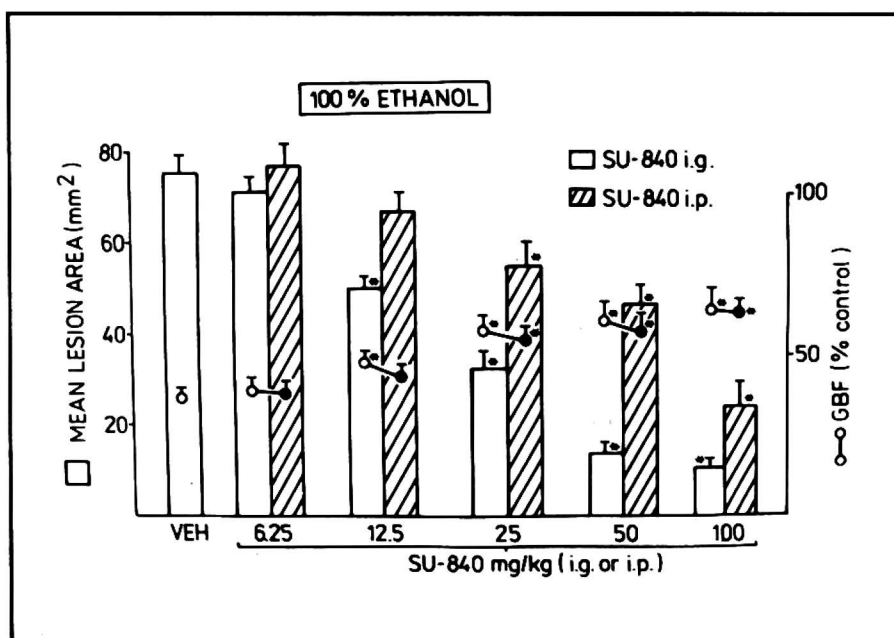

Fig 1. The effect of intragastric (i.g.) or intraperitoneal (i.p.) pretreatment with SU-840 given in graded doses ranging from 6.25 to 100 mg/kg on the mean lesion area and accompanying changes in the gastric blood flow (GBF). Means $\pm$ SEM or 6—8 rats. Asterisk indicates significant change as compared to the value obtained in rats pretreated with vehicle.

Fig. 2 shows the comparison of the duration of the protective effect of the same dose (50 mg/kg) of SU-840 and Sofalcone given i.g. against ethanol damage. Pretreatment with SU-840 caused significantly greater reduction in the area of ethanol-lesions than sofalcone. The protective effect of sofalcone was most pronounced during the first hour after the application but then disappeared after 2 h, while the protection induced by SU-840 was still observed at 6 h after application of this agent.

Fig. 2. Comparison of the duration of the protective effect of SU-840 and Sofalcone applied i.g. at various time intervals before administration of 100% ethanol. Means ± SEM of 5—7 rats. Asterisk indicates significant change as compared to the value obtained with 100% ethanol alone.
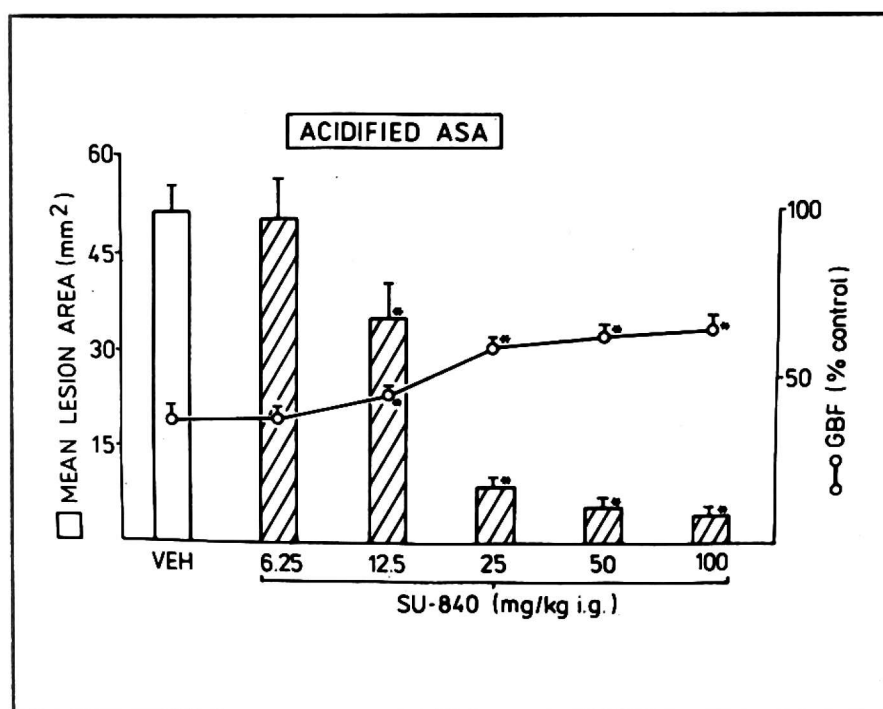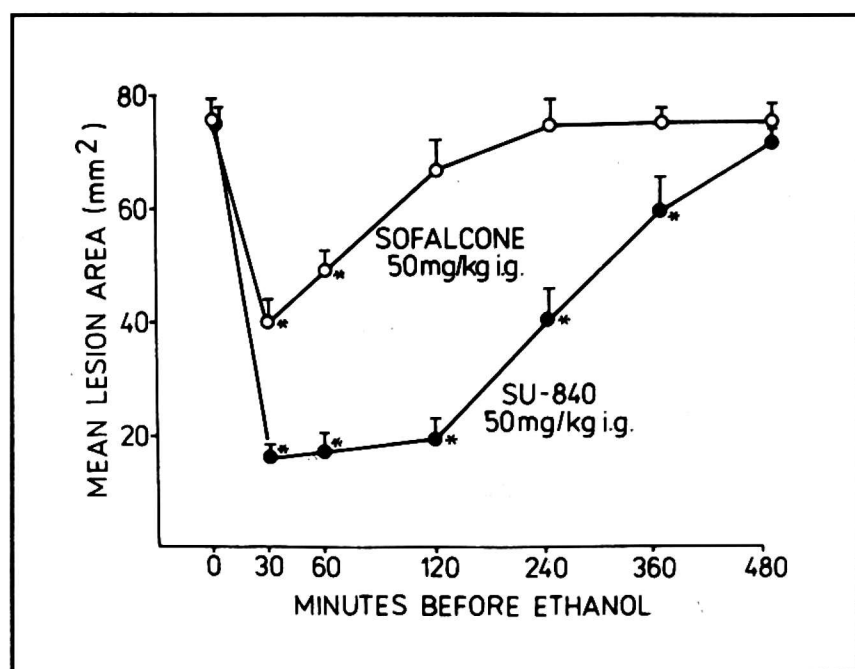

Fig. 3. The effect of pretreatment with SU-840 applied i.g. in graded doses ranging from 6.25 to 100 mg/kg on the area of gastric lesions induced by acidified aspirin (ASA) and accompanying changes in the GBF. Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in gastric mucosa pretreated with vehicle (control).

Figs. 3 and 4 show the effect of i.g. pretreatment with acidified ASA or water immersion and restraint stress (WRS). In control experiments, the oral application of acidified ASA resulted in gastric hemorrhagic lesions with the mean area of $52 \pm 7 mm^2$, while the number of gastric lesions provoked by 3.5 h exposure of gastric mucosa to WRS averaged 18 ± 4. The pretreatment with SU-840 caused a dose-dependent reduction in ASA- and WRS-lesions, the ID_{50} for these lesions being 17 mg/kg and 94 mg/kg respectively. This reduction by SU-840 in ASA- or WRS-induced gastric lesions was accompanied by the gradual increase in the GBF, reaching the maximum at the dose of 50 mg/kg. At the highest dose tested (100 mg/kg i.g.) SU-840 prevented the formation of ASA- and WRS-lesions to a similar extent as SU-840 given in a dose of 50 mg/kg.
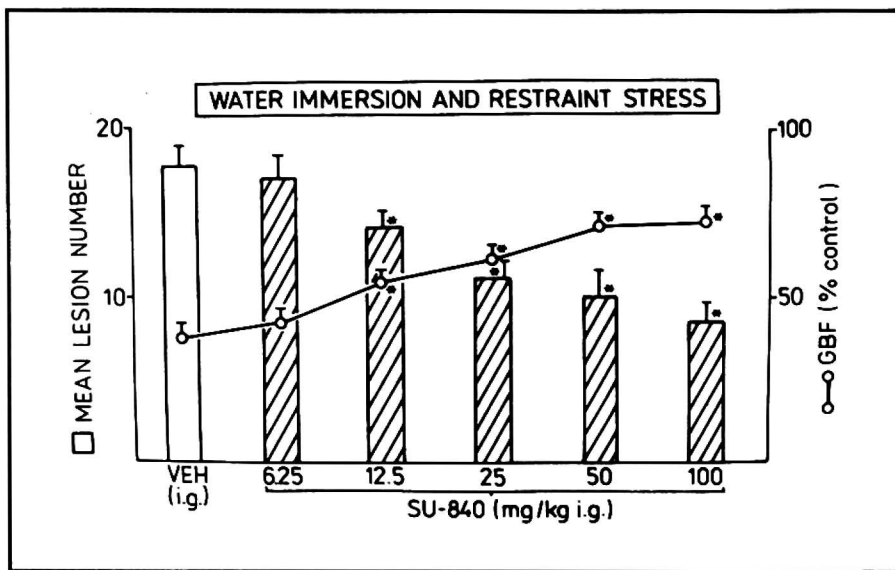
Fig. 4. The number of gastric lesions induced by water immersion and restraint stress and accompanying changes in the GBF in rats pretreated with vehicle (saline) or SU-840 applied i.g. in graded doses ranging from 6.25 to 100 mg/kg. Means ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in the gastric mucosa pretreated with vehicle.

Effect of indomethacin L-NNA and NEM on gastroprotection and changes in the gastric blood flow induced by SU-840

The effects of topical administration of standard dose (50 mg/kg) SU-840 without or with the pretreatment with indomethacin on gastric lesions induced by ethanol or WRS and accompanying changes in the GBF are presented in *Figs. 5 and 6.* The pretreatment with i.g. SU-840 caused a significant reduction in the area of ethanol- and WRS-induced lesions by about 73% and 68%, respectively, and enhanced the GBF over the value recorded in vehicle-control rats exposed to 100% ethanol or to 3.5 h of WRS. Pretreatment with indomethacin, which by itself produced a small but significant increase in ethanol- and WRS-damage, failed to affect significantly the fall in the GBF induced by these ulcerogens. Such pretreatment with indomethacin, completely reversed the reduction in the area of gastric lesions induced by SU-840 against 100% ethanol or WRS-lesions and accompanying rise in the GBF caused by SU-840.
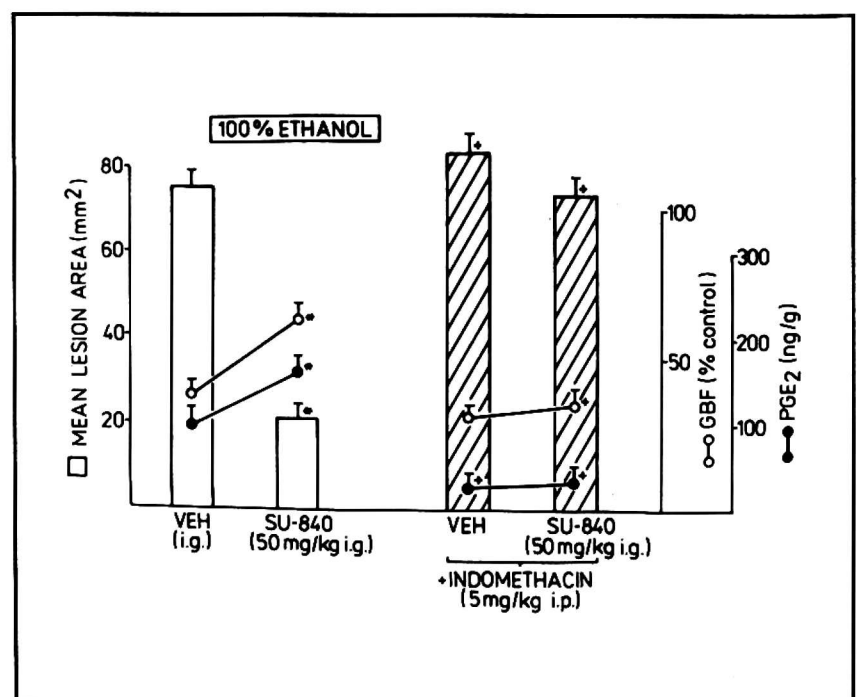
Fig. 5. The effect of vehicle or SU-840 (50 mg/kg i.g.) without or with pretreatment with indomethacin on the area of ethanol lesions, mucosal generation of PGE_2 and changes in the GBF. Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle (control) rats. Cross indicates significant change as compared to the values in rats without indomethacin pretreatment.

Fig. 6. The effect of vehicle or SU-840 (50 mg/kg i.g.) without or with pretreatment with indomethacin on the area of stress induced gastric lesions and accompanying changes in the GBF. Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle (control) rats. Cross indicates significant change as compared to the values in rats without indomethacin pretreatment.
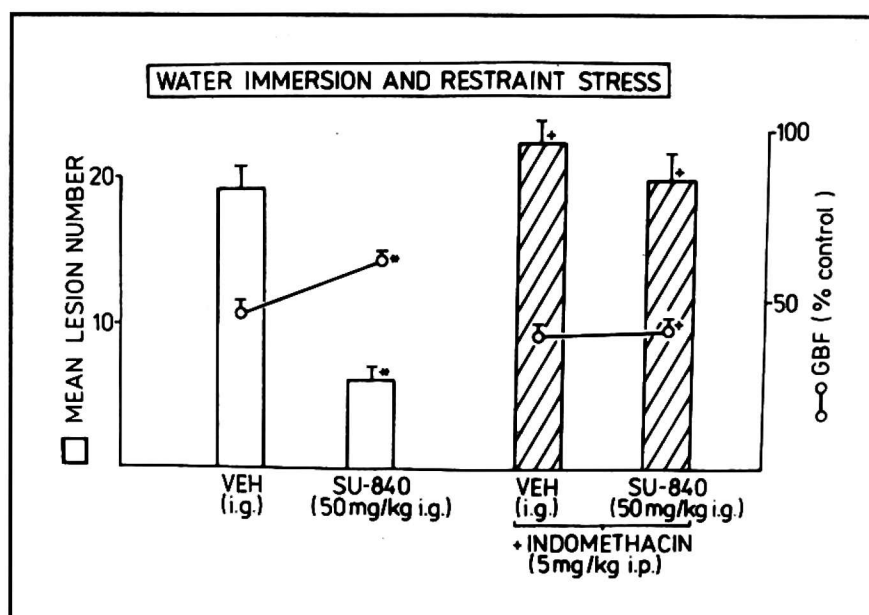
The generation of PGE_2 in the gastric mucosa of intact rats averaged about 120 ng/g of wet tissue weight, and the administration of 100% ethanol did not cause any significant alteration in mucosal content of PGE_2 but the pretreatment with indomethacin resulted in significant reduction in mucosal generation of PGE_2 by about 85% (Fig. 5). Pretreatment with SU-840 produced a significant increase in the generation of mucosal PGE_2 and this effect was completely abolished in rats pretreated with indomethacin (Fig. 5).
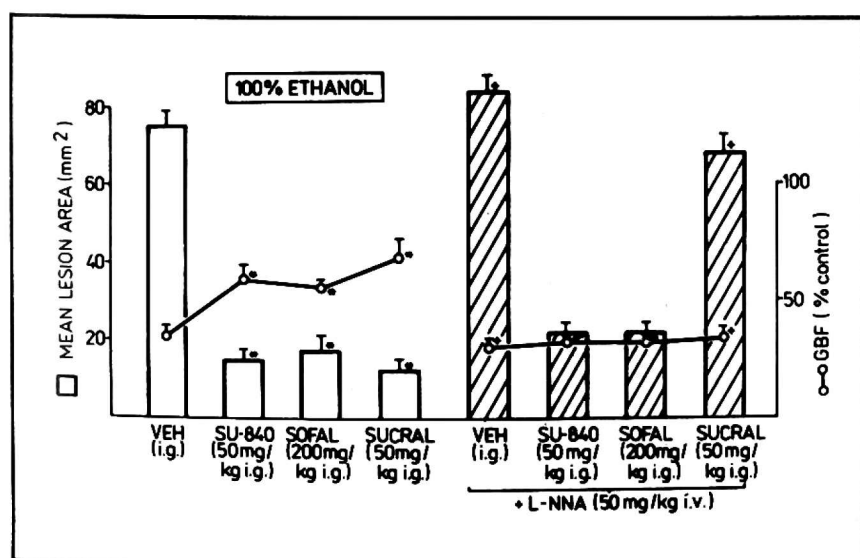
Fig. 7. The effect of topical administration of SU-840 (20 mg/kg) Sofalcone (200 mg/kg) or Sucralfate (50 mg/kg) without or with L-NNA pretreatment on the area of gastric lesions and changes in the GBF. Mean ± SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in vehicle control gastric mucosa. Cross indicates significant change as compared to the value obtained in rats without L-NNA pretreatment.

As shown in Fig. 7 the i.v. pretreatment with L-NNA caused a significant increase in the area of gastric lesions induced by ethanol and this was accompanied by a significant decrease in the GBF as compared to the value recorded in ethanol-treated rats. When the pretreatment with SU-840 (50 mg/kg) or Sofalcone (200 mg/kg) was applied i.g., typical reduction in ethanol lesions accompanied by the rise in the GBF was observed. Pretreatment with L-NNA produced only a small and unsignificant decrease in

the area of ethanol lesions but almost completely reversed the rise in GBF as compared to the values recorded in gastric mucosa of vehicle-pretreated rats given 100% ethanol. In contrast, the reduction in both the area of gastric lesions and the gastric accompanying hyperemia induced by sucralfate were significantly attenuated by the pretreatment with L-NNA.
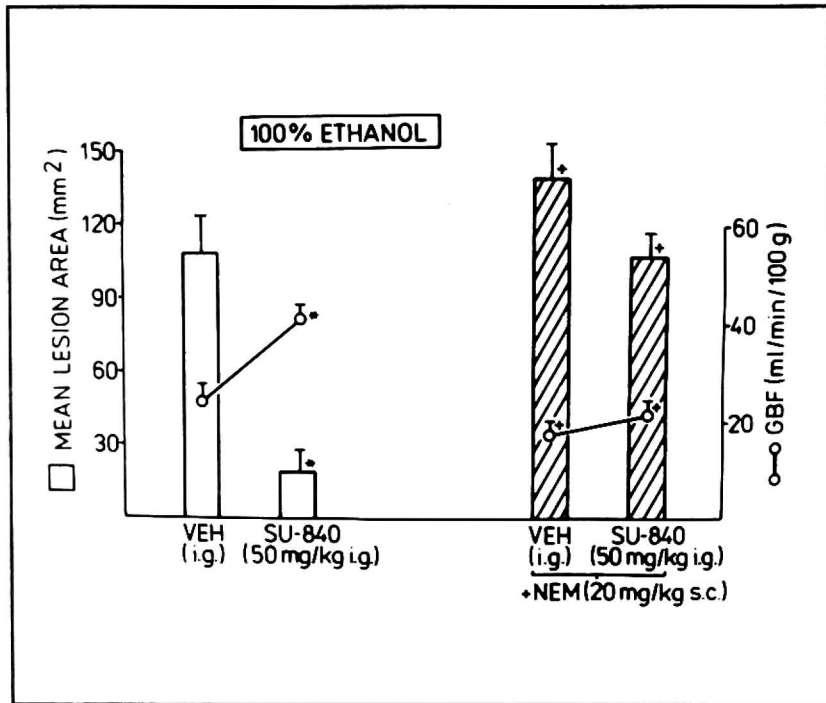

Fig. 8. Mean area of gastric lesions and GBF in rats treated with SU-840 (50 mg/kg i.g.) or vehicle (saline) without or with pretreatment with NEM (20 mg/kg s.c.). Mean ± SEM of 6—8 animals. Asterisk indicates significant change as compared to the value obtained in vehicle control rats. Cross indicates significant change as compared to the value obtained in rats without NEM pretreatment.

The pretreatment with NEM (20 mg/kg s.c.), to block nonprotein sulfhydryls, significantly increased the area of ethanol induced gastric lesions and almost completely reversed the gastroprotective and hyperemic effects of SU-840 in these animals (*Fig. 8*).

Effect of vehicle, SU-840 and sucralfate without or with L-NNA on the ulcer healing and gastric blood flow in ulcer area

Fig. 9 shows the effects of seven day treatment with vehicle, SU-840 (100 mg/kg i.g.) or sucralfate (400 mg/kg i.g.) or their combination without or with administration of L-NNA (40 mg/kg i.g.) on the area of chronic gastric ulcers and changes in the GBF at the ulcer margin. Following 7 days after ulcer induction the area of ulcers in vehicle-treated controls was reduced by about 58% and the GBF at the ulcer margin was significantly higher than that recorded at ulcer crater but reached lower value as compared to that in intact non-ulcerated gastric mucosa (*Fig. 9, Table 2*). Oral treatment with SU-840 or sucralfate resulted in a significant decrease by about 62% or 54% of the ulcer area and a significant increase by about 21% or 19% of the GBF at the ulcer margin, respectively. In contrast rats treated daily with L-NNA throughout 7 day period showed a significant increase in the area of gastric ulcers and this was accompanied by a significant decrease in the GBF at both the crater and

margin of ulcer (*Fig. 9, Table 2*). When SU-840 or sucralfate was combined with L-NNA, a significant increase in the area of gastric ulcers and the fall in the GBF at ulcer margin were observed as compared to those obtained in rats treated with SU-840 or sucralfate without L-NNA administration.
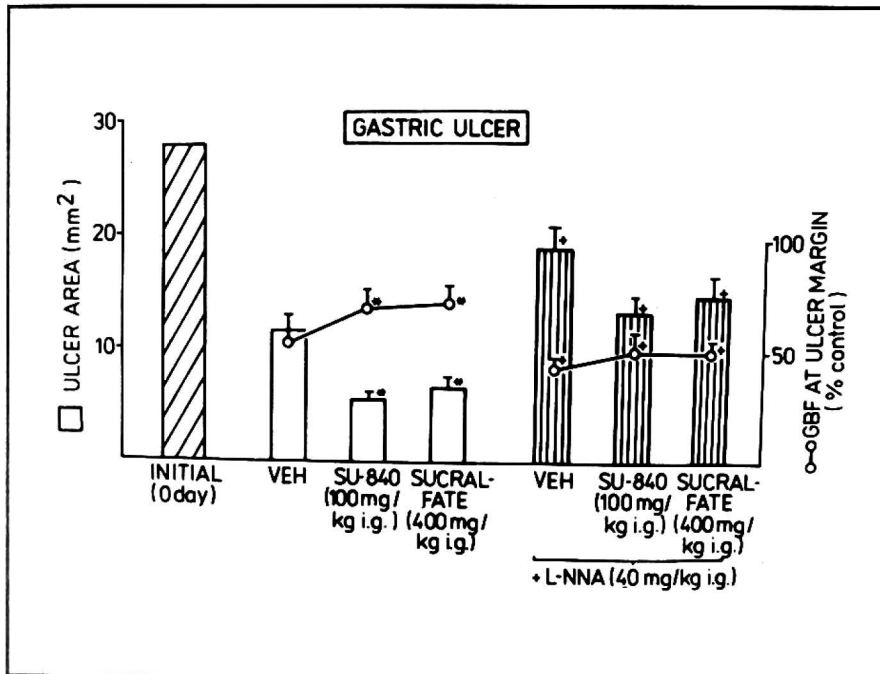

Fig. 9. Area of chronic gastric ulcers and GBF at ulcer margin in rats recorded after 7 day treatment with vehicle SU-840 or sucralfate without or with addition of L-NNA (40 mg/kg per day i.g.). Means±SEM of 6—8 rats. Asterisk indicates significant change as compared to the value obtained in animals treated with vehicle (saline). Cross indicates significant change as compared to the value recorded in rats without addition of L-NNA.

Table 2. Effect of 7 day administration of vehicle (saline), SU-840 (100 mg/kg i.g.) or sucralfate (400 mg/kg i.g.) without or with the combination with L-NNA (40 mg/kg i.g.) on the changes in GBF at ulcer crater, ulcer margin and intact gastric mucosa. Mean ± SEM of 6-8 rats per group. Asterisk indicates significant change as compared to the values obtained in rats treated with vehicle. Cross indicates a significant change as compared to blood flow in the gastric mucosa without L-NNA administration.

	GBF (% control)		
	ULCER CRATER	ULCER MARGIN	INTACT
VEHICLE (CONTROL)	41 ± 2	48 ± 3	100 ± 1
SUCRALFATE (100 mg/kg i.g.)	$52 \pm 5^*$	$74 \pm 6^*$	102 ± 2
SUCRALFATE (400 mg/kg i.g.)	$68 \pm 4^*$	$85 \pm 8^*$	101 ± 2
L-NNA (40 mg/kg i.g.)	$24 \pm 2^*$	$38 \pm 4^*$	95 ± 3
L-NNA + SU-840	$41 \pm 4^*$	$51 \pm 3^*$	96 ± 2
L-NNA + SUCRALFATE	$36 \pm 3^*$	$41 \pm 4^*$	97 ± 3

DISCUSSION

This study demonstrates that SU-840, a novel synthetic flavonoid derivative of sophoradin is highly protective against various noxious agents and that this action is accompanied by a marked rise in the gastric blood flow over that obtained with gastric irritant alone. The protection against ethanol lesions and gastric hyperemia were achieved when this sofalcone-like drug was

administered both by oral and parenteral route and required much lower doses of SU-840 as compared to original sofalcone or sucralfate. Moreover, this protection occurred in the doses which did not influence gastric secretion, suggesting that this drug is cytoprotective because it acts on gastric mucosa, similarly to exogenous PG but without affecting secretory activity of gastric mucosa. Endogenous PG, NO and sulfhydryls are involved in protective and hyperemic action of SU-840 because these effects were attenuated by indomethacin to inhibit PG-cyclooxygenase, L-NNA to suppress NO-synthase and pretreatment with NEM to deplete endogenous sulfhydryls. Chronic treatment with SU-840 accelerated significantly healing rate of gastric ulcers and this effect was reversed by L-NNA and further restored by the addition to L-NNA of L-arginine, a substrate for NO-synthase.

Sofalcone has been reported previously to prevent the damage induced by various topical ulcerogens including ethanol, taurocholate and indomethacin *in vivo* and *in vitro* (2, 3, 6, 7, 27, 30). This protection was attributed to the ability of this carbenoxolone-resembling analog to stimulate generation of endogenous PG (5—7), to enhance the physicochemical quality of protective mucus synthesis (11—13, 31) and to reverse lipolytic activity of *Helicobacter pylori* (15). In another studies, sofalcone was shown to enhance availability of endogenous PG by suppressing 15-OH-PG-dehydrogenase an enzyme involved in the degradation of these endogenous protective compounds (5, 6, 32). Recent studies revealed that sofalcone could be a relatively suitable candidate for combination with antibiotics such as amoxycilin or clarythromycin in eradication of *Helicobacter pylori* (33). However, in most of these studies, sofalcone was administered topically and it remains unknown whether any sofalcone-like drugs could be beneficial in protecting of injury without any contact with gastric mucosa.

SU-840 is a novel agent, which originates from the sofalcone-like drugs that due to complexing with betacyclodextrin showed higher bioavailability and reduced ethanol damage after intraduodenal administration (Dr Muramatsu, personal communication). In the present study, we demonstrated that SU-840 prevented dose-dependently the gastric lesions induced by acid-independent (100% ethanol) or acid-dependent (acidified ASA, stress) ulcerogens. The protective activity of SU-840 persisted over 6 h and showed longer duration than that of sofalcone. This protection was accompanied by a marked increase in gastric blood flow and appears to be unrelated to gastric acid secretion because occurred in nonantisecretory dose. SU-840 exhibit protective activity when given parenterally but this effect seems to be much weaker than that obtained after administration of this drug by topical route.

The protective action of SU-840 could be attributed to the endogenous PG because this agent enhanced generation of PGE_2 and pretreatment with

indomethacin in at the dose of 5 mg/kg i.p. that was shown previously (19) to suppress PG-cyclooxygenase activity by about 90%, significantly attenuated the SU-840 induced gastroprotection against lesions induced by ethanol and stress. On the other hand the finding that SU-840 was also effective against damage induced by acidified ASA, when the generation of mucosal PG was almost completely suppressed, militates against a major role for PG in this protection. This is the reason, why we studied, whether NO, a potent vasodilator is involved in the protective and hyperemic action of SU-840. We employed L-NNA, a selective inhibitor of NO-synthase, which was originally shown to eliminate of carbenoxolone-induced gastroprotection (34). Besides carbenoxolone, the activation of NO system was recently implicated in the protective action of other cytoprotective drugs such as aluminium-containing antacids and sucralfate (21).

In our present study, L-NNA failed to effect the gastroprotective activity of SU-840 but completely abolished the enhancement in gastric mucosal blood flow induced by this agent. This suggest that activation of NO by SU-840 contribute mostly to the increase in microcirculatory response and that gastroprotection induced by this agent is unaffected by blockade of NO-system. This does not exclude the possible interaction of NO and endogenous PG activated by SU-840 in the maintenance of microcirculatory response accompanying protection afforded by this agent and in ulcer healing activity of sofalcone-like drugs.

Another factors that may be implicated in gastroprotection induced by SU-840 are non-protein sulfhydryl compounds. Sulfhydryls protect the gastric mucosa against the damage induced by variety of noxious agents (18), and the pretreatment with sulfhydryl alkylator NEM, abolished the activity of protective agent such as PG, growth factors and gastrointestinal hormones (22, 35). In the present study, NEM almost completely reversed SU-840-induced protection suggesting, that this protection may also he mediated by nonprotein sulfhydryls and the sulfhydryl-induced increase in microvascular integrity. This could be corroborative with previous observations that strengthening of microvasculature by sulfhydryls renders the mucosa less vulnerable to the damaging effect of ethanol (18, 22).

It is of interest that SU-840 accelerated significantly healing of chronic gastric ulcers with the extent similar to that observed for sofalcone and sucralfate but applied in much higher daily doses. Administration of L-NNA delayed ulcer healing and markedly diminished microcirculatory response around the ulcer during healing. The addition of L-NNA to SU-840 completely reversed the acceleration of ulcer healing and an increase in gastric blood flow around the ulcer caused by SU-840. This suggests that NO does not seem

to play any major role in gastroprotective action of SU-840 but appears to be essential for the process of ulcer healing and hyperemia around ulcer induced by this agent.

REFERENCES

1. Kishimoto S, Okamoto K, Kambara A, Kajiyama G, Miyoshi A, Suwa T. Terapeutic effects of sofalcone on experimental gastritis. *Arzneimittelforschung* 1987; 37: 944—947.

2. Muramatsu M, Tanaka M, Murakami S, Aihara H. Effect of sofalcone on gastric mucosal prostaglandin metabolism in taurocholate induced gastritis in rats. *Res Commun Chem Pathol Pharmacol* 1986; 53: 289—300.

3. Isobe Y, Hirose H, Muramatsu M, Aihara H. Cytoprotective effect of sofalcone in the rat gastric mucosa. *Arzneimittelforschung* 1985; 35: 138—41.

4. Kimura M, Saziki R, Arai I, Tarumoto Y, Nakane S. Effect of 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy) chalcone (sofalcone) on chronic gastric ulcers in rats. *Jpn J Pharmacol* 1984; 35: 389—96.

5. Muramatsu M, Tanaka M, Murakami S, Aihara H. Inhibition of 15-hydroxy prostaglandin dehydrogenase and increase of prostaglandin E_2; effect of sofalcone on rat gastric mucosa. *Life Sci* 1987; 41: 315—22.

6. Konturek SJ, Radecki T, Brzozowski T *et al.* Antiulcer and gastroprotective effects of solon a synthetic flavonoid derivative of sophoradin. Role of endogenous prostaglandins. *Eur J Pharmacol* 1986; 125: 185—192.

7. Konturek SJ, Brzozowski T, Drozdowicz D, Pawlik W, Sendur R. Gastroprotective and ulcer healing effects of solon a synthetic flavonoid derivative of sophoradin. *Hepatogastroenterology* 1987; 34: 164—170.

8. Muramatsu M, Murakami S, Arai I *et al.* Effect of sofalcone on acute gastric mucosal lesions induced by aspirin and ethanol in reference to the biosynthesis of gastric mucosal glycoprotein. *Res Commun Chem Pathol Pharmacol* 1986; 54: 321—37.

9. Slomiany BL, Liau YH, Carter SR, Sarosiek I, Tsukada H, Slomiany A. Enzymatic sulfation of mucin in gastric mucosa effect of sofalcone sucralfate and aspirin. *Digestion* 1987; 38: 178-86.

10. Murakami S, Muramatsu M, Aihara H, Honda A, Mori Y. Enzymatic sulfation of gastric mucous glycoprotein in rat changes in glycoprotein sulfotransferase activity with stress and antiulcer agent sofalcone. *Res Commun Chem Pathol Pharmacol* 1987; 57: 77—98.

11. Murakami S, Yamada H, Muramatsu M *et al.* Effect of sofalcone on gastric mucous glycoprotein in experimental gastritis induced by sodium taurocholate. *Res Commun Chem Pathol Pharmacol* 1988; 62: 461—82.

12. Piotrowski J, Yamaki K, Tamura S, Slomiany A, Slomiany BL. Enhancement of the physicochemical qualities of gastric mucus by sofalcone. *J Physiol Pharmacol* 1991; 42: 293—304.

13. Slomiany BL, Tamura S, Piotrowski J, Slomiany A. Effects of sofalcone administration on the physicochemical properties of gastric mucus. *J Clin Gastroenterol* 1992; 14: Suppl 1: 88—93.

14. Yoshikawa T, Nakamura S, Takahashi S, Naito Y, Kondo M. Effect of sofalcone on gastric mucosal injury induced by ischemia reperfusion and its antioxidant properties. *J Clin Gastroenterol* 1993; 17: Suppl 1: 111—115.

15. Sunairi M, Watanabe K, Suzuki T, Tanaka N, Kuwayama H, Nakajima M. Effects of antiulcer agents on antibiotic activity against *Helicobacter pylori*. *Eur J Gastroenterol Hepatol* 1994; 6: Supp 1. 1: 121—124.

16. Konturek SJ. Mechanisms of gastroprotection. *Scand J Gastroenterol* 1990; 25: Suppl. 1: 74: 15—28

17. Brzozowski T, Drozdowicz D, Szlachcic A, Pytko-Polonczyk J, Majka J, Konturek SJ. Role of nitric oxide and prostaglandins in gastroprotection induced by capsaicin and papaverine. *Digestion* 1993; 54: 24—31

18. Konturek SJ, Brzozowski T, Piastucki I, Radecki T, Dupuy D, Szabo S. Gastric protection by agents altering gastric mucosal sulfhydryls. Role of prostaglandins. *Digestion* 1987; 37: 65—71.

19. Konturek SJ, Brzozowski T, Majka J, Dembinski A, Slomiany A. Transforming growth factor alpha and epidermal growth factor in protection and healing of gastric mucosal injury. *Scand J Gastroenterol* 1992; 27: 649—655.

20. Brzozowski T, Konturek SJ, Pytko-Polonczyk J, Warzecha Z. Gastric adaptation to stress: Role of sensory nerves salivary glands and adrenal glands. *Scand J Gastroenterol* 1995; 30: 6—16.

21. Konturek SJ, Brzozowski T, Majka J, Szlachcic A, Pytko-Polonczyk J. Implications of nitric oxide in the action of cytoprotective drugs on gastric mucosa. *J Clin Gastroenterol* 1993; 17: Suppl 1: 140—145.

22. Szabo S, Trier JS, Frankel PW. Sulfhydryl compounds may mediate gastric cytoprotection. *Science* 1981; 214: 200—202.

23. Konturek SJ, Brzozowski T, Stachura J, Dembinski A, Majka J. Role of gastric blood flow neutrophil infiltration and mucosal cell proliferation in gastric adaptation to aspirin in the rat. *Gut* 1994; 35: 1189—1196.

24. Konturek SJ, Stachura J, Radecki T, Drozdowicz D, Brzozowski T. Cytoprotective and ulcer healing properties of prostaglandin E_2, colloidal bismuth and sucralfate in rats. *Digestion* 1987; 38: 103—113.

25. Okabe S, Pfeifer CI, Roth IL. A method for experimental penetrating ulcers in rats. *Am J Dig Dis* 1971; 16: 277—284.

26. Brzozowski T, Konturek SJ, Drozdowicz D, Dembinski A, Stachura J. Healing of chronic gastric ulcerations by L-arginine. Role of nitric oxide prostaglandins, gastrin and polyamines. *Digestion* 1995; 56: 463—471.

27. Fujimoto N, Murakami S, Suwa T, Mori Y. The studies on the role of gastric glycoproteins with reference to cytoprotection: protective effect of prostaglandin E_2 and sofalcone on ethanol induced necrosis. *J Pharmacobiodyn* 1984; 7: 864—71.

28. Saziki R, Arai I, Isobe Y, Hirose H, Aihara H. Effects of sofalcone on necrotizing agents induced gastric lesions and on endogenous prostaglandins in rats stomachs. *J Pharmacobiodyn* 1984; 7: 791—797.

29. Arakawa T, Nakamura A, Yamada H *et al*. Protection of gastric surface epithelial cells of rats by 16,16-dimethyl prostaglandin E_2 and sofalcone a synthetic flavonoid derivative of sophoradin against ethanol. *Digestion* 1988; 41: 61—67.

30. Kobayashi K, Arakawa T, Nakamura A, Nakamura H. Protective effect of sofalcone and 16, 16-dimethyl-PGE_2 on isolated rat gastric cells. *J Clin Gastroenterol* 1990; 12: Suppl 1: 177—80.

31. Murakami S, Fujimoto N, Mori Y. Prostaglandin cytoprotection linked to the adhesion of mucous glycoprotein to the gastric epithelium. *J Pharmacobiodyn* 1986; 9: 359—67.

32. Kobayashi K, Higuchi K, Arakawa T, Matsumoto T, Nagura H. Effect of sofalcone on localization of 15-hydroxyprostaglandin dehydrogenase, an enzyme that metabolizes prostaglandin E_2 in rat gastric mucosa: an immunohistochemical study. *J Clin Gastroenterol* 1992; 14: Suppl 1: 39—42.

33. Kodama R, Fujioka T, Fujiyama K, Kawasaki H, Kubota T, Nasu M. Combination therapy with clarithromycin and sofalcone for eradication of *Helicobacter pylori*. *Eur J Gastroenterol Hepatol* 1994; 6: Suppl 1: 125—8.

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34. Dembinska-Kiec A, Pallapies D, Simmet T, Peskar BM, Peskar MA. Effect of carbonoxolone on the biological activity of nitric oxide: relation to gastroprotection. *Br J Pharmacol* 1991; 104: 811—816.
35. Konturek SJ, Brzozowski T, Pytko-Polonczyk J, Drozdowicz D. Exogenous and endogenous cholecystokinin protects gastric mucosa against the damage caused by ethanol in rats. *Eur J Pharmacol* 1995; 273: 57—62.

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