Effects of Nicorandil on Experimentally Induced Gastric Ulcers in Rats: A Possible Role of K_{ATP} Channels

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ABSTRACT—The anti-ulcer effects of nicorandil [*N*-(2-hydroxyethyl)nicotinamide nitrate ester] were examined on water-immersion plus restraint stress-induced and aspirin-induced gastric ulcers in rats, compared with those of cimetidine. Nicorandil (3 and 10 mg/kg) given orally to rats dose-dependently inhibited the development of acid-related damage (water-immersion- and aspirin-induced gastric lesions) in the models. Cimetidine (50 mg/kg, p.o.) also had anti-ulcer effects in the same models. However, in the presence of glibenclamide (20 mg/kg, i.v.), an antagonist of K_{ATP} channels, nicorandil did not inhibit the formation of gastric lesions. Nicorandil (10 mg/kg) given intraduodenally (i.d.), like cimetidine (50 mg/kg), significantly reduced the volume of the gastric content, total acidity and total acid output in the pylorus ligation model. Glibenclamide reversed the changes caused by i.d. nicorandil. I.v. infusion of nicorandil (20 μ g/kg per min) significantly increased gastric mucosal blood flow, without affecting blood pressure and heart rate, but the increase in the blood flow was not observed after i.v. treatment with glibenclamide (20 mg/kg). These results indicate that nicorandil administered orally to rats produces the anti-ulcer effect by reducing the aggressive factors and by enhancing the defensive process in the mucosa through its K_{ATP} -channel-opening property.

Keywords: Nicorandil, Anti-ulcer effect, Gastric acid secretion, Gastric mucosal blood flow, Opening of K_{ATP} channels

It has been widely accepted that nicorandil, *N*-(2-hydroxyethyl)nicotinamide nitrate ester, an orally efficacious anti-anginal drug (1–3), possesses dual mechanisms of action (4), combining a K_{ATP} -channel-activating property and a nitrate-like nature. The finding of this property of nicorandil, as the first K_{ATP} -channel opener (5), has attracted the special interest of many researchers and facilitated research work in this field. Recently, Goswami et al. (6) reported that cromakalim, one of the K_{ATP} -channel openers (7), induces a potent anti-ulcer activity in different experimental models of rats and guinea pigs. On the basis of their finding (6), we thought that nicorandil also may possess anti-ulcer effects like those of cromakalim. The aim of the present experiments was to investigate in water-immersion plus restraint stress-induced and aspirin-induced gastric ulcer models of rats, whether nicorandil would have anti-ulcer activity, and if so, what mechanism is responsible for the action.

MATERIALS AND METHODS

Chemicals

Nicorandil was synthesized in the Chugai Organic Chemistry Laboratory. Cimetidine, aspirin, glibenclamide (all from Sigma Chemical Co., St. Louis, MO, USA) and carboxymethylcellulose (CMC) (Wako Junyaku, Osaka) were purchased. Glibenclamide was dissolved in 1 ml of 0.1 N NaOH, followed by slow addition of 4 ml of 5% glucose solution under sonication to reach a final concentration of 5 mg/ml (8). Other drugs were freshly suspended in 0.5% CMC or dissolved in and diluted with 0.9% saline solution, just before the experiments were started. The appropriate concentrations for administration volumes of 1 and 2 ml/kg for oral and i.d. administrations, respectively, were prepared. I.v. infusion of drugs was conducted at a rate of 0.1 ml/kg per min using a Terumo syringe pump (STC-525, Tokyo).

Animals

All experiments were carried out according to the

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Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Male Sprague-Dawley rats (Charles River Japan, Hino) weighing 180–230 g were used. The animals were deprived of food 24 hr prior to experiments, but allowed free access to water.

Experimental protocols for acute gastric ulcers

The methods described by Kinoshita et al. (9) were followed. The animals were killed by an overdose of ether after the completion of the experiments. The stomachs were excised, fixed with 1% formalin, opened along the greater curvature and then photographed. The area of lesions was measured on an Apple Macintosh computer (Quadra 800) using an NIH image program and converted to mm². Two observers unaware of the experimental protocols assessed the lesions.

Water-immersion plus restraint stress-induced gastric ulcer

The animals were divided into 7 groups (n=10 for each), which were given p.o. vehicle (0.5% CMC, 1 ml/kg), nicorandil (1, 3 and 10 mg/kg) and cimetidine (50 mg/kg), respectively, and either vehicle or nicorandil (10 mg/kg) given p.o., 30 min after i.v. injection of a single dose of glibenclamide (20 mg/kg over 5 min). Thirty minutes later, the rats were placed in a stainless-mesh cage and immersed vertically to the level of the xiphisternum process in a water bath maintained at 23±1°C. Five hours later, they were taken out and killed by an overdose of ether.

Aspirin-induced gastric mucosal lesions

The rats were divided into 5 groups (n=10 for each), which were given p.o. vehicle (0.5% CMC), nicorandil (1, 3 and 10 mg/kg) and cimetidine (50 mg/kg), respectively. One hour after the dosing of the test drugs, aspirin (200 mg/kg) was administered p.o. About 4 hr later, the rats were killed by an overdose of ether, and then the stomach was excised.

Determination of basal gastric acid secretion

The rats were divided into 5 groups (n=5 for each). After laparotomy under light ether anesthesia, the pylorus was ligated and the abdomen was closed by suturing. Three of the groups received i.d. vehicle (0.5% CMC, 1 ml/kg), nicorandil (10 mg/kg) or cimetidine (50 mg/kg) just after the pylorus was ligated. The remaining 2 groups were given i.d. CMC or nicorandil, as described above, 10 min after ending i.v. administration of glibenclamide (20 mg/kg over 5 min). The animals were sacrificed 3 hr later by an overdose of ether, and the stomachs were removed. The gastric contents were collected and centrifuged at

3,000 rpm for 10 min. The volume of supernatant was measured, and the acid concentration was determined by automatic titration (TTA81; Radiometer, Copenhagen, Denmark) to pH 7.0 with 0.01 mol/l NaOH. The total acid output during the 3-hr period was calculated.

Measurement of gastric mucosal blood flow

The rats were randomly divided into 4 groups (n=5 for each). Under pentobarbital (55 mg/kg, i.p.) anesthesia, the rats were placed on a table warmed to 37°C. The stomach was exposed by laparotomy, and an incision was made into the forestomach. A laser probe (ϕ 0.25×2, EG-type; Omegawave Inc., Tokyo) was placed to make gentle contact with the mucosal surface of the corpus using a balancer, and gastric mucosal blood flow was measured by a laser Doppler flowmeter (Model FLO-XII; Omegawave, Inc.) (10). The abdominal incision was covered with a wrap film to prevent tissue dehydration. Polyethylene tubes (PE 10) were inserted into the right and left femoral veins for i.v. injection or infusion of the drug. Arterial blood pressure was measured from the right femoral artery with a Nihon Kohden pressure transducer (DX-360, Tokyo). Heart rate was determined by means of a heart rate counter (Nihon Kohden, AT-601G). All recordings were made on a chart by using a Graphtec Linearcorder (WR-3101, Tokyo). Following surgery, a period of at least 30 min was allowed for stabilization of preparations.

After the measured hemodynamic parameters were stabilized, i.v. infusion of either 0.9% saline or nicorandil (20 µg/kg per min) solution was started by means of a Terumo syringe pump (STC-525, Tokyo) at a rate of 0.1 ml/kg per min, and changes in the parameters were observed over 1 hr. The dose of nicorandil was chosen according to the previous reports (11, 12), so that it was pharmacologically effective and had little effects on basal blood pressure and heart rate. In 2 among 4 groups, the effect of glibenclamide (20 mg/kg, 4 ml/kg, i.v.), an antagonist of K_{ATP} channels (13), which almost completely blocked the 40 mmHg decrease in mean arterial blood pressure caused by cromakalim (30 µg/kg, i.v.) in rats (14), was examined. Ten minutes after the dose of glibenclamide, which had virtually no effects on basal blood pressure and heart rate, was given i.v. over 5 min, 0.9% saline or nicorandil (20 µg/kg per min) solution was infused i.v. over 1 hr. In a single preparation, only a single infusion of each agent was made.

Statistical analyses

Data are expressed as means±S.E.M. Significant differences were determined by means of the unpaired Student's *t*-test. Analysis of variance (ANOVA) was used for the statistical analysis of multiple comparisons of

data. When multiple comparisons were made with a single control, Dunnett's test was used to determine the level of statistical significance. A P value of less than 0.05 was considered to be statistically significant.

RESULTS

Effects of nicorandil and cimetidine on acute gastric lesion formation

Water-immersion plus restraint stress-induced gastric ulcer: Rats subjected to water immersion at 23°C for 5 hr elicited pronounced gastric damage mainly in the glandular segment, accompanied by intraluminal bleeding in

these animals. Nicorandil (3 and 10 mg/kg, p.o.) dose-dependently decreased the ulcerated area. Cimetidine (50 mg/kg, p.o.) also caused a significant reduction in the total lesion area. The combined administration of glibenclamide (20 mg/kg, i.v.) and 0.5% CMC tended to increase the ulcerated area, whereas that of glibenclamide (20 mg/kg) and nicorandil (10 mg/kg) tended to recover the formation of gastric lesions induced by glibenclamide, even though their effects were not significant. The activity of 50 mg/kg cimetidine, preventing the formation of the lesions, corresponded to that of 10 mg/kg nicorandil. Summarized data are shown in Fig. 1.

Aspirin-induced gastric lesions: Aspirin (200 mg/kg,

Fig. 1. Effects of nicorandil (NCR) and cimetidine given orally on water-immersion plus restraint stress-induced gastric ulcer in the absence and presence of glibenclamide (Glib; 20 mg/kg, i.v.). Each column represents the mean $\pm$ S.E.M. from 10 rats. **P < 0.01, compared with the corresponding values from the vehicle (control)-treated group.

Fig. 2. Effects of nicorandil (NCR) and cimetidine given orally on aspirin-induced gastric lesions. Each column represents the mean $\pm$ S.E.M. from 10 rats. *P < 0.05, **P < 0.01, ***P < 0.001, compared with the corresponding values from the vehicle (control)-treated group.

p.o.) induced marked gastric lesions mainly in the glandular segments of the stomach, and the stomach wall was frequently fragile and thin. Nicorandil (3 and 10 mg/kg, p.o.) prevented the formation of aspirin-induced lesions in a dose-dependent manner, as depicted in Fig. 2. Cimetidine (50 mg/kg, p.o.) also significantly inhibited the formation of the lesions.

Effects of nicorandil and cimetidine on basal gastric acid secretion in the absence and presence of glibenclamide

The effects of nicorandil (10 mg/kg) and cimetidine (50 mg/kg) given intraduodenally on the basal acid secretion were examined in pylorus-ligated rats. As demonstrated

in Fig. 3, significant reductions in the volume of the gastric content, in total acidity and increase in pH were observed after dosing of nicorandil or cimetidine. Glibenclamide (20 mg/kg, i.v.) not only significantly increased basal gastric content and total acidity, but also reversed the changes caused by intraduodenal nicorandil.

Effects of nicorandil on gastric mucosal blood flow in the absence and presence of glibenclamide

The basal values of mean arterial blood pressure, heart rate and gastric mucosal blood flow, just before the administrations of 0.9% saline and drugs, are shown in Table 1. After the preparations were stabilized, i.v. infusion

Fig. 3. Effects of intraduodenal administration of nicorandil (NCR) and cimetidine on basal gastric acid secretion in the absence and presence of glibenclamide (Glib; 20 mg/kg, i.v.). Each column represents the mean $\pm$ S.E.M. from 5 rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the corresponding values from the vehicle (control)-treated group.

Table 1. Basal values of mean arterial blood pressure (MAP), heart rate (HR) and gastric mucosal blood flow (GMBF), just before the administration of saline and drugs

	MAP (mmHg)	HR (beats/min)	GMBF (ml/min/100 g)
Saline	111.0±3.3	386.0±14.0	29.0±4.0
Nicorandil (20 µg/kg/min)	104.4±5.0	398.0±21.1	26.8±2.4
Glibenclamide (20 mg/kg)	108.3±4.0	386.7±16.3	32.7±3.9
Glibenclamide (20 mg/kg) + Nicorandil (20 µg/kg/min)	117.0±1.2	364.0±15.7	28.0±2.3

Basal values (explained above) are expressed as the mean±S.E.M. from 5 preparations in each group. The values from each group showed no significant differences relative to the corresponding values from the saline-treated group.

of either 0.9% saline (0.1 ml/kg per min) or nicorandil (20 µg/kg per min) was started in the absence of glibenclamide. Following i.v. infusion of nicorandil, the mucosal blood flow gradually increased, and 30 min later, it reached a steady-state level, as shown in Fig. 4. No significant changes in arterial blood pressure (AP) and heart rate were observed between the corresponding values from 0.9% saline- and nicorandil-treated groups: at 30 min after starting infusion, mean AP and heart rate: 105±5 mmHg and 354±10 beats/min, respectively, for the 0.9% saline-treated group; 100±7 mmHg and 388±19 beats/min, respectively, for the nicorandil-treated

ed group, n=5 for each. A single dose of glibenclamide (20 mg/kg) given i.v. over 5 min significantly reduced the mucosal blood flow, with a transient rise (about 5%) of mean arterial blood pressure and virtually little change in heart rate, although 30 min later, there were no significant changes in both parameters between groups treated and not treated with glibenclamide. When nicorandil was infused i.v. at a rate of 20 µg/kg per min, in the presence of glibenclamide (20 mg/kg), the decrease in the mucosal blood flow was recovered.

DISCUSSION

The present results revealed that nicorandil given orally to rats significantly reduced the formation of lesions in water-immersion plus restraint stress-induced and aspirin-induced gastric ulcers. Similarly, cimetidine given orally also elicited significant reduction of the lesions.

Recently, Goswami et al. (6) reported the dose-dependent anti-ulcer activity of cromakalim, a potent K_{ATP} channel opener (7), compared with cimetidine, a histamine H_2 -receptor antagonist (15), against experimentally induced gastric and duodenal ulcers in rats and guinea pigs, although they did not analyze possible mechanisms for the anti-ulcer effects of cromakalim. In the present study, nicorandil (10 mg/kg) given intraduodenally to rats, like cimetidine (50 mg/kg), had a potent inhibitory activity against gastric acid secretion in pylorus-ligated rats. Furthermore, it was noted that nicorandil infused i.v. at the rate of 20 µg/kg per min significantly increased gastric mucosal blood flow virtually without affecting blood pressure and heart rate. If nicorandil was assumed to be infused for 5 hr at this rate, it almost corresponds to the dose (total 6 mg/kg) that was effectively utilized in the present two acute gastric ulcer models. As has been widely accepted, successful peptic ulcer therapy depends on the restoration of the compromised integrity of the gastroduodenal mucosa by reducing the aggressive factors and/or enhancing the defensive process in the mucosa (16). Although it is possible that nicorandil develops anti-ulcer effects at least partly through inhibition of gastric acid secretion (aggressive factor) and/or increase in the mucosal blood flow (defensive factor), the question arises as to how these mechanisms contributed to the anti-ulcer effects of nicorandil on two ulcer models.

It should be noted that most of the previous studies on mucosal protection centered on prostaglandins. However, recently, it has been suggested that both calcitonin gene-related peptide (CGRP) (17) and nitric oxide (NO) (18, 19) play an important role in gastric mucosal protection. More recently, Rossowski et al. (20) also reported that adrenomedullin, amylin, CGRP and their fragments powerfully inhibit gastric acid secretion in

Fig. 4. Effects of 0.9% saline (0.1 ml/kg per min) and nicorandil (20 µg/kg per min) infused i.v. on gastric mucosal blood flow (GMBF) in the absence (○ for 0.9% saline; △ for nicorandil) and presence (● for 0.9% saline; ▲ for nicorandil) of glibenclamide (20 mg/kg, i.v.). Vertical bars represent the mean±S.E.M. from 5 rats. *P<0.05, **P<0.01, ***P<0.001, compared with the corresponding values from the 0.9% saline-treated group.

rats. CGRP and adrenomedullin are potent endogenous vasodilators (21), acting partly through opening of K_{ATP} channels (14, 22–25). It appears that CGRP is present in the afferent, capsaicin-sensitive nerve fibers innervating the rat gastric mucosa (26), and released from isolated rat stomach by capsaicin, as measured with a chemiluminescent enzyme immunoassay (27). On the other hand, adrenomedullin-containing cells were also found in the gastric mucosa (28, 29). Thus, recent studies have suggested that both peptides are involved in the pathophysiological regulation of gastric functions such as acid secretion (20) and mucosal blood flow (30, 31), and they contribute to the mucosal protective mechanism against noxious stimuli. The present finding that glibenclamide, an antagonist of K_{ATP} channels (13), significantly decreased basal gastric mucosal blood flow and increased basal gastric acid secretion may support this view.

According to our recent studies, nicorandil synergistically interacts with several naturally occurring vasodilators involving CGRP (12) and adrenomedullin (24, 25) on adenosine-induced vasodepression in rats, partly through K_{ATP} -channel activation (32). Therefore, it is possible to consider that nicorandil elicited inhibition of gastric acid secretion and increase in gastric mucosal blood flow by its own activity or by cooperating with CGRP and other endogenous vasodilators, partly through opening of K_{ATP} channels. This view is strongly supported by a recent report by Doi et al. (31) that Y-26763, a K_{ATP} -channel opener, and CGRP caused an increase in the mucosal blood flow in rats, which was attenuated by glibenclamide. Further support is given by the results from the present study: 1) nicorandil, infused i.v. to rats, significantly increased the mucosal blood flow, which was attenuated by glibenclamide; 2) nicorandil given intraduodenally to pylorus-ligated rats significantly reduced gastric acid secretion, which was reversed by glibenclamide; 3) anti-ulcer effects of nicorandil on stress-induced gastric ulcer were not observed in the group treated with glibenclamide; and 4) i.v. treatment with glibenclamide alone tended to exacerbate stress-induced gastric ulcer, even though the effect was not significant.

In summary, the present experiments demonstrate: 1) nicorandil (3 and 10 mg/kg, p.o.), like cimetidine (50 mg/kg, p.o.), protected against the acute gastric damage in water-immersion plus restraint stress- and aspirin-induced gastric ulcer models of rats, but after i.v. pretreatment with glibenclamide, the ulcer lesions were increased; 2) like cimetidine, nicorandil (10 mg/kg, i.d.) significantly inhibited gastric acid secretion, but the observed reduction caused by nicorandil did not occur after i.v. treatment with glibenclamide; and 3) nicorandil (20 μ g/kg per min) infused i.v. significantly increased gastric mucosal blood flow. Glibenclamide significantly de-

creased the blood flow, whereas the combination with nicorandil significantly recovered the decrease in the blood flow induced by glibenclamide alone. Even though a possible involvement of NO in the anti-ulcer effect of nicorandil, having a hybrid property between a nitrate and a K_{ATP} -channel activator (4), remains to be investigated, it is presumed that the contribution of NO to the anti-ulcer effect is minor, on the basis of the fact that the effect of nicorandil was almost completely blocked by glibenclamide. Taken together, these findings indicate that nicorandil produces the anti-ulcer effect by enhancing the defensive capacity as well as by reducing the aggressive factors in the mucosa, mainly in a close link with K_{ATP} channels.

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