ELSEVIER

European Journal of Pharmacology 424 (2001) 69–74

ejp

www.elsevier.nl/locate/ejphar

The role of nitric oxide in the gastric acid secretion induced by ischemia–reperfusion in the pylorus-ligated rat

Junko Tanaka [a,*], Yasukatu Yuda [a], Shigeharu Inouye [a,1], Toshio Yamakawa [b]

[a] *Pharmacology Department Drug Research Laboratory, Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd., Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan*

[b] *Department of Biochemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan*

Received 17 January 2001; received in revised form 29 May 2001; accepted 1 June 2001

Abstract

In a rat model of the ischemia–reperfusion with pylorus ligation, gastric ulcer was formed, although gastric acid secretion was reduced. When the polymorphonuclear leukocytes were inactivated in advance, gastric ulcer was not formed, but acid secretion was increased, indicating that gastric acid is not a cause of the ulcer formation in this model. The mechanism of gastric acid suppression accompanied by ischemia–reperfusion was examined in relation to the role of oxygen-free radicals in this rat model. Prior administration of superoxide dismutase did not modulate acid secretion, but *N*-nitro-L-arginine methyl ester (L-NAME) increased acid secretion. The action of L-NAME was antagonized specifically by L-arginine, but not by D-arginine. *S*-nitroso-*N*-acetylpenicillamine did not inhibit basal acid secretion but antagonized the action of L-NAME. Aminoguanidine increased significantly the gastric acid output that was suppressed by ischemia–reperfusion. When polymorphonuclear leukocytes were inactivated by treatment with their antibody, the gastric acid output recovered to the level in the pylorus-ligated rat without ischemia–reperfusion. These results suggested that nitric oxide (NO) produced by the infiltrated polymorphonuclear leukocytes plays an important role in the suppression of acid secretion induced by ischemia–reperfusion.

Keywords: Nitric oxide (NO); Ischemia–reperfusion; Gastric acid; Polymorphonuclear leukocyte

1. Introduction

The mechanism of ulcer formation has been studied using various ulcer models of rats by a number of researchers. We established an ulcer model of ischemia–reperfusion in pylorus-ligated rats as a model of stress ulcer in humans. We reported previously that oxygen-free radicals and lipid peroxidation play a significant role in the pathogenesis of gastric mucosal injury induced by ischemia–reperfusion in pylorus-ligated rat (Tanaka and Yuda, 1993). A significant contribution of activated oxygen-free radicals in ulcer formation was also reported by Perry et al. (1986), Ito and Guth (1985) and Grisham et al. (1986). In order to elucidate further the mechanism of ulcer formation in this model, we now examined gastric acid secretion since it is well known that excessive acid output is a major factor of ulcer formation (Sun, 1974). The result obtained, however, was that gastric acid output was not increased but decreased. We have already reported that infiltrated polymorphonuclear leukocytes play a large part in the gastric mucosal injury induced by ischemia–reperfusion in the pylorus-ligated rat (Tanaka and Yuda, 1993). We therefore examined the effect of polymorphonuclear leukocytes and its product, nitric oxide (NO) on gastric acid secretion, using various chemical substances. These results are described in this paper.

2. Materials and methods

2.1. Animals

Male Donryu rats (SPF), 8-weeks old and weighing 200–230 g were used. The animals were obtained from Charles River, Tokyo.

* Corresponding author. Tel.: +81-45-541-1408; fax: +81-45-545-3194.

E-mail address: junko_tanaka@meiji.co.jp (J. Tanaka).

[1] Present address: Teikyo University Institute of Medical Mycology, Tokyo 192, Japan.

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01119-0

2.2. Materials

S-nitroso-*N*-acetylpenicillamine was purchased from RBI. *N*-nitro-L-arginine methyl ester (L-NAME), L-arginine and D-arginine were purchased from Calbiochem-NOVAbiochem. Superoxide dismutase and aminoguanidine were obtained from Sigma. The other chemicals were of reagent grade and were used without purification.

2.3. Experimental ischemia–reperfusion

The experimental plan and design were approved, in advance, to inspection by the Ethical Committee of Animal Experiments in our laboratory. Rats previously fasted for 19 h were anesthetized with pentobarbital sodium, and anesthesia was maintained with ether. Method 1: The pylorus of the rat was ligated and the celiac artery was immediately clamped for 30 min and then reperfused for 90, 150 or 270 min. The rats were killed, gastric juice was collected, and the ulcer index was determined. Method 2: 5 min after drug treatment, the pylorus was ligated and ischemia–reperfusion followed by collection of gastric juice was carried out as described above. All drugs were dissolved in saline immediately before use and administered in a volume of 5 ml/kg. A combination of two drugs was originally designed to be administered simultaneously, but due to technical difficulty, the second drug was given 5 min later. The administration schedules were as follows:

Group (1) Saline i.v. followed by L-NAME 5, 10 mg/kg i.v.
Group (2) Saline i.v. followed by *S*-nitroso-*N*-acetylpenicillamine 1 mg/kg i.v.
Group (3) Saline i.v. followed by superoxide dismutase 15 000 U/kg i.v.
Group (4) Saline i.v. followed by aminoguanidine 6 mg/kg i.v.
Group (5) L-NAME 5 mg/kg i.v. followed by superoxide dismutase 15 000 U/kg i.v.
Group (6) L-NAME 5 mg/kg i.v. followed by *S*-nitroso-*N*-acetylpenicillamine 1 mg/kg i.v.
Group (7) L-arginine 200 mg/kg i.v. followed by L-NAME 5 mg/kg i.v.
Group (8) D-arginine 200 mg/kg i.v. followed by L-NAME 5 mg/kg i.v.

The dosages in groups 2–8 were determined by referring to the literatures of (Tuchiya, 1987; Wright et al., 1992; Martinez-Cuesta et al., 1992; Hasan et al., 1993). Method 3: To obtain polymorphonuclear leukocyte-depleted rats, anti-rat polymorphonuclear leukocyte antibody was intraperitoneally injected at a dose of 10 ml/kg 15 h before ischemia production. The same amount of normal domestic rabbit serum as that of anti-polymorphonuclear leukocyte antibody solution was administered to the control group. The anti-rat polymorphonuclear leukocyte antibody was prepared following the technique of Ward and Cochrance (1965).

2.4. Acid secretion

The gastric juice stored was collected, and volume and acidity were measured. The acid was titrated with 0.1 N NaOH using an autoburette (TOA Electronics, Tokyo). Acid output was calculated by multiplying the volume of gastric juice by the acid concentration.

2.5. Ulcer index

Ulcer index was expressed as the size of ulcers measured with an image analyzer (IMCI) in the whole stomach.

2.6. Acid secretion in lumen-perfused rats

Male 8-week-old Donryu rats were fasted for 19 h. Gastric acid secretion was measured continuously according to the method of Ghosh and Schild (1958). A perfusion cannula made of polyethylene for collecting perfusate was inserted into the stomach under urethane anesthesia from a small incision in the duodenum, and fixed at the pyloric portion. The esophagus was ligated and the saline perfusate at 37 °C was circulated through the stomach at a flow rate of 10 ml/10 min. One hour after cannulation, histamine at a dose of 2.5 mg/kg was administered s.c. and 30 min after, the celiac artery was clamped for 30 min and then reperfused for 60 min. The gastric perfusate was collected at 6-min intervals. Its acidity was titrated with an autoburette.

2.7. Statistical analysis

All data are expressed as means ± S.E. Differences between groups were evaluated by Student's *t*-test for unpaired data. A probability of $P < 0.05$ was considered statistically significant.

3. Result

Table 1 summarizes the time course of gastric acid secretion with ischemia–reperfusion in the pylorus-ligated rat. The gastric juice volume at 90 min was not different from that of the control without ischemia–reperfusion but was significantly increased at 150 and 270 min over that of the control. The pH in the gastric juice of ischemia–reperfusion rats was raised as compared with that of the control without ischemia–reperfusion at 90, 150 and 270 min. Acidity and acid output were significantly decreased by

Table 1
Time course of gastric acid secretion with ischemia–reperfusion in the pylorus-ligated rat

		After reperfusion (min)		
		90	150	270
Gastric volume (ml)	N	2.40 ± 0.30	3.07 ± 0.38	5.48 ± 0.40
	I	2.38 ± 0.14	4.54 ± 0.50[a]	7.58 ± 0.58[b]
pH	N	2.29 ± 0.09	2.30 ± 0.05	2.43 ± 0.07
	I	4.64 ± 0.35[b]	3.23 ± 0.24[b]	3.72 ± 0.48[b]
Acidity (meq/l)	N	86.34 ± 7.66	68.00 ± 5.82	76.31 ± 7.02
	I	15.49 ± 2.87[b]	34.15 ± 4.28[b]	32.03 ± 5.59[b]
Acid output (μeq/h)	N	108.08 ± 20.80	85.91 ± 13.77	84.72 ± 11.40
	I	19.38 ± 4.48[b]	54.88 ± 11.64[a]	51.24 ± 12.50[a]
Ulcer index (mm)	I	519.05 ± 22.64	438.50 ± 42.07	469.50 ± 42.48

Gastric acid secretion was measured 90, 150 and 270 min after reperfusion. Each value is mean ± S.E. ($n = 5–6$).

N, pylorus ligation alone; I, ischemia–reperfusion during pylorus ligation.

[a] $P < 0.05$.

[b] $P < 0.01$ significantly different from pylorus ligation alone at each point.

ischemia–reperfusion, compared to those of the control rats for every measurement. Ulcer indices at 90, 150, 270 min remained unchanged though the acid output increased time dependently. The time course of gastric acid secretion induced by ischemia–reperfusion in the lumen-perfused rat was similar to that with pylorus ligation as shown in Fig. 1. Namely, gastric acid output was reduced more by ischemia than that of the control, and remained reduced after reperfusion. The reduction in gastric acid output of the ischemia–reperfusion group was significantly greater than that of the control group up to 60 min after reperfusion.

Figs. 2–5 indicate the effect of various chemical substances on the gastric acid output in the ischemia–reperfusion model. Since the gastric juice output remained constant at 90 min, the gastric acid output was determined at 90 min. Intravenous injection of the O_2^- inhibitor, superoxide dismutase at 15 000 U/kg to the rats caused no change (Fig. 2), but an NO synthase inhibitor, L-NAME, induced an increase in acid secretion, and the acid output returned to a level comparable to that of the saline control when administered intravenously at 5 mg/kg in combination with superoxide dismutase (Fig. 2) or alone at a dosage of 5 mg/kg (Fig. 3). Essentially, the same result was obtained using the 5 mg/kg dose of L-NAME. Therefore, we used the result from the lower dosage as the effect of L-NAME. This effect of L-NAME was significantly inhibited by i.v. pretreatment with L-arginine at 200 mg/kg; pretreatment with D-arginine, however, had no effect (Fig. 3). Pretreatment i.v. with *S*-nitroso-*N*-acetylpenicillamine, an exogeneous NO donor, at 1 mg/kg, gastric acid secretion was slightly increased compared to that of the saline control. (Fig. 4). Moreover, *S*-nitroso-*N*-acetylpenicillamine cancelled the effect of L-NAME. With intravenous pretreatment with aminoguanidine, an inducible NO synthase inhibitor, at 6 mg/kg, the increase in gastric acid secretion was not marked but was significant, as that with

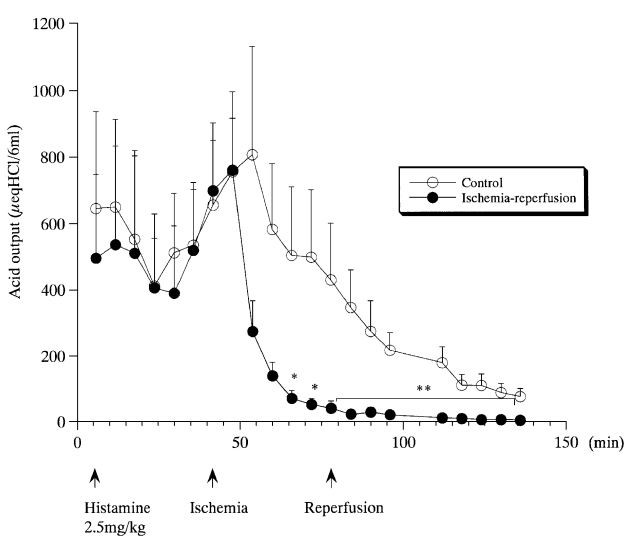

Fig. 1. Time course of gastric acid secretion in the lumen-perfused rats. Acid secretion in the gastric perfusate was measured at 6-min intervals. Thirty minutes after stimulation with 2.5 mg/kg of histamine, a celiac artery was clamped for 30 min and then reperfused for 60 min. Each value is mean ± S.E. ($n = 7–12$). ○, histamine stimulation; ●, ischemia–reperfusion with histamine stimulation. $^{**}P < 0.01$, $^{*}P < 0.05$ significantly different from histamine stimulation at each point.

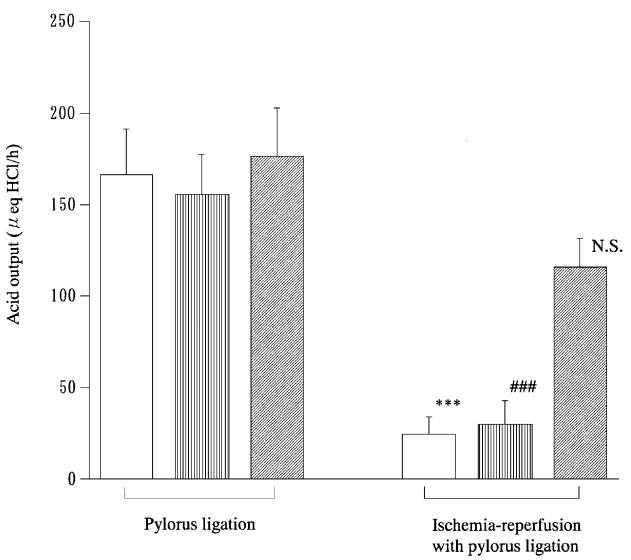

Fig. 2. Effect of SOD on acid output in gastric juice induced by ischemia–reperfusion in the pylorus-ligated rat. Gastric acid secretion was measured 90 min after reperfusion. □, saline 1 ml/kg; ▥, SOD 15 000 U/kg i.v.; ▨, L-NAME 5 mg/kg + SOD 15 000 U/kg i.v. All drugs were administered at least 5 min before ischemia. Each value is mean ± S.E. ($n = 5–6$). $^{***}P < 0.001$ significantly different from pylorus ligation alone. $###P < 0.001$ significantly different from ischemia–reperfusion control.

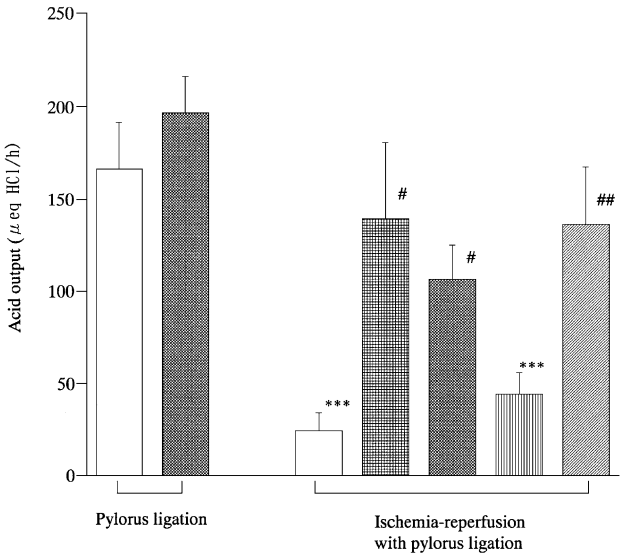

Fig. 3. Effect of L-NAME on acid output in gastric juice induced by ischemia–reperfusion in the pylorus-ligated rat. Gastric acid secretion was measured 90 min after reperfusion. □, saline 1 ml/kg; ⊞, L-NAME 10 mg/kg i.v.; ▩, L-NAME 5 mg/kg i.v.; ▥, L-arginine 200 mg/kg + L-NAME 5 mg/kg i.v. ▨, D-arginine 200 mg/kg + L-NAME 5 mg/kg i.v. All drugs were administered at least 5 min before ischemia. Each value is mean ± S.E. ($n = 5$–6). $^{***}P < 0.001$ significantly different from only pylorus ligation. $\#P < 0.05$, $\#\#P < 0.01$ significantly different from ischemia–reperfusion control.

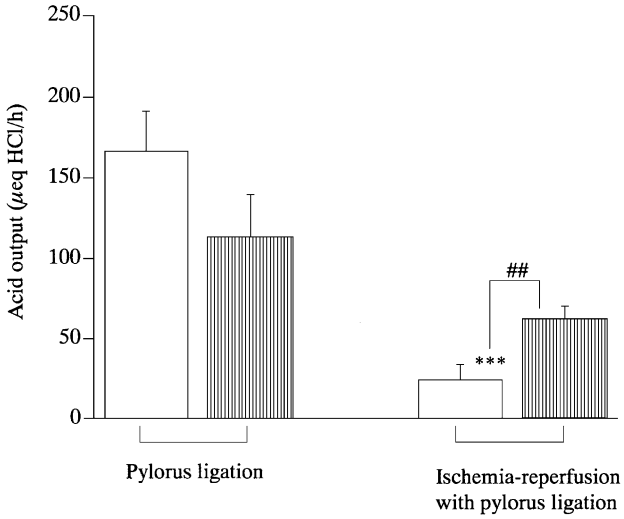

Fig. 5. Effect of aminoguanidine on acid output in gastric juice induced by ischemia–reperfusion in the pylorus-ligated rat. Gastric acid secretion was measured 90 min after reperfusion. □, saline 1 ml/kg; ▥, aminoguanidine 6 mg/kg. Aminoguanidine was administered 5 min before ischemia. Each value is mean ± S.E. ($n = 5$–7). $^{***}P < 0.001$ significantly different from only pylorus ligation. $\#\#P < 0.01$ significantly different from ischemia–reperfusion control.

saline (Fig. 5). Fig. 6 shows the effect of polymorphonuclear leukocyte on gastric acid secretion. When polymorphonuclear leukocytes were inactivated by treating with the antibody, the acid output was increased significantly over that of the saline control and returned to the level of the ligated rats without ischemia–reperfusion. No damage to the gastric mucosa was observed when polymorphonuclear leukocytes were depleted before ischemia–reperfusion.

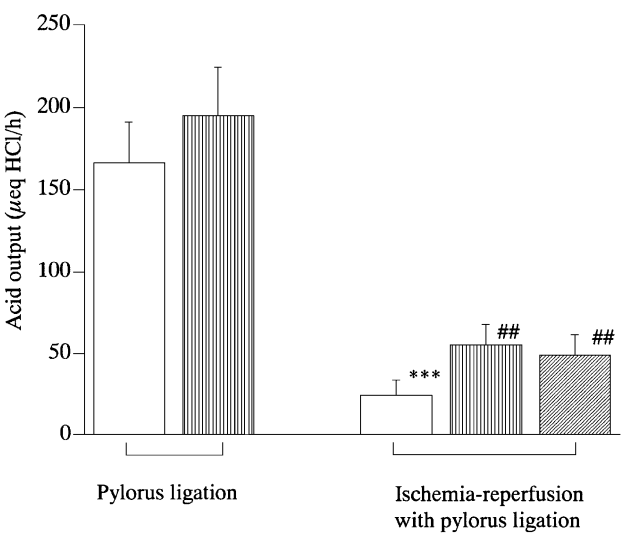

Fig. 4. Effect of SNAP on acid output in gastric juice induced by ischemia–reperfusion in the pylorus-ligated rat. Gastric acid secretion was measured at 90 min after reperfusion. □, saline 1 ml/kg; ▥, SNAP 1 mg/kg; ▨, L-NAME 5 mg/kg + SNAP 1 mg/kg. All drugs were administered at least 5 min before ischemia. Each value is mean ± S.E. ($n = 5$–6). $^{***}P < 0.001$ significantly different from pylorus ligation alone. $\#\#P < 0.01$ significantly different from ischemia–reperfusion control.

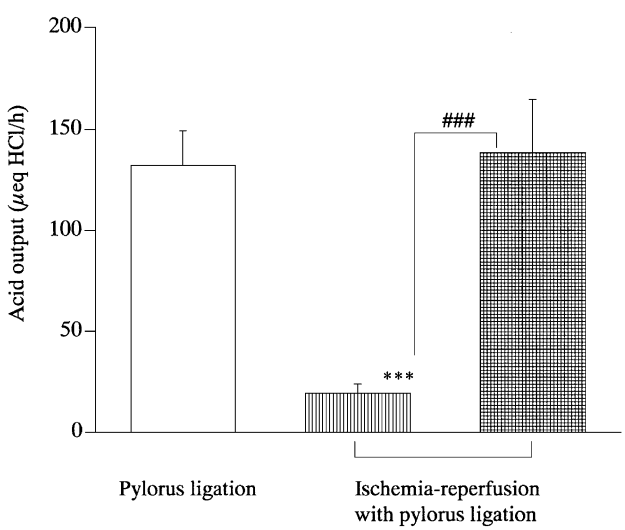

Fig. 6. Effect of PMN-depression on acid output in gastric juice induced by ischemia–reperfusion in the pylorus-ligated rat. □, normal rabbit serum 10 ml/kg i.p.; ▥, normal rabbit serum with ischemia–reperfusion; ⊞, anti-PMN serum 10 ml/kg i.p. 15 h before ischemia. Each value is mean ± S.E. ($n = 5$–7). $^{***}P < 0.001$ significantly different from only pylorus ligation. $\#\#\#P < 0.001$ significantly different from ischemia–reperfusion control.

4. Discussion

The present study showed that gastric acid secretion was suppressed by ischemia–reperfusion in the pylorus-ligated rat. A similar reduction in acid secretion was observed with ischemia–reperfusion in the lumen-perfused rat. It was thought originally that the mucosal injury in this ulcer model might be caused by the excessive secretion of gastric acid. Contrary to our expectation, it was found that acid secretion was not increased but was decreased up to 270 min, indicating that acid secretion is not a major factor in this ulcer model. This was supported by the fact that, though acid secretion was increased by inactivation of polymorphonuclear leukocyte, no ulcer was formed. We already reported that the gastric mucosal injury caused by ischemia–reperfusion was induced by oxygen-free radicals (Tanaka and Yuda, 1993). We therefore supposed that oxygen-free radicals might suppress the gastric acid secretion accompanying ischemia–reperfusion. Using a variety of chemical substances, we examined what kind of oxygen-free radical took part in this suppression of acid secretion. Since pretreatment with superoxide dismutase did not alter gastric acid secretion, O_2^- might not be important for the suppression. Pretreatment with L-NAME, an inhibitor of NO synthase, increased gastric acid output, the effect of which was antagonized with L-arginine, but not D-arginine, indicating the structure-specific antagonism of NO synthase. *S*-nitroso-*N*-acetylpenicillamine, which supplies NO directly without affecting NO synthase, did not increase the acid output but canceled the action of L-NAME that increased the acid output during ischemia–reperfusion.

These results indicated that NO was associated with the reduction of the acid output. This was further confirmed by the use of aminoguanidine, an inducible NO synthase inhibitor (Misko et al., 1993), though the release of suppression of the gastric acid secretion was not markedly prevented. Although there was a potential increase of the histamine level with aminoguanidine, it was reported that the histamine level was decreased by ischemia (Fujisaki et al., 1993), and therefore, the contribution from histamine might not be significant. Furthermore, it was reported that NO did not affect the histamine release from mast cells (Lau and Chow, 1999) and stabilized the rat mast cell membrane (Vural et al., 2000). Since *S*-nitroso-*N*-acetylpenicillamine is a supplier of NO, *S*-nitroso-*N*-acetylpenicillamine, which increased acid secretion in a similar way to aminoguanidine, may not increase the histamine level.

Since NO was suggested to be a suppressing factor of acid secretion in this investigation, we examined the effect on acid secretion of polymorphonuclear leukocytes infiltrated into gastric mucus immediately after reperfusion because NO appeared to be released from the activated polymorphonuclear leukocytes influx in the gastric mucosa (Hortelano et al., 1993). We have already shown that polymorphonuclear leukocytes were activated by ischemia–reperfusion (Tanaka and Yuda, 1993), and the present study indicated that the suppression of acid secretion was completely eliminated by inactivating polymorphonuclear leukocytes. Summarizing these results, NO arising from the activated polymorphonuclear leukocytes appeared to be a dominant factor in the suppression of gastric acid secretion caused by ischemia–reperfusion.

There are many reports describing the relation of NO and pentagastrin-stimulated gastric acid secretion. Gastric acid secretion stimulated with pentagastrin in the rat was suppressed by endotoxin, which produced NO (Martinez-Cuesta et al., 1992) or by an NO donor (Kato et al., 1998). Similarly, Brown et al. (1993) and Barrachina et al. (1994) showed that NO donors inhibit acid secretion in vitro and in vivo. On the contrary, Bilski et al. (1994) reported that the pentagastrin-induced gastric acid secretion in dogs was suppressed with L-nitro-L-arginine, an NO synthase inhibitor. Walder et al. (1990) reported also that the pentagastrin-stimulated acid secretion in rats was accelerated by endogenous NO. Pique et al. (1992) reported that the NO synthase inhibitor did not modulate the acid secretion stimulated with pentagastrin. Our data were in agreement with the results of Martinez-Cuesta et al. (1992), Kato et al. (1998), Brown et al. (1993) and Barrachina et al. (1994), although the ulcer models are different. The discrepancy between our data and those of Bilski et al. (1994), Walder et al. (1990) and Pique et al. (1992) remained unresolved. It was concluded that NO released by infiltrated polymorphonuclear leukocytes takes part in the suppression of gastric acid secretion on ischemia–reperfusion in the pylorus-ligated rat. As far as we know, this is the first report that neutrophils play a significant role for acid secretion, as well as ulcer formation.

References

Barrachina, D., Calatayud, S., Esplugued, J., Whittle, B.J.R., Moncada, S., Esplugues, J.V., 1994. Nitric oxide donors preferentially inhibit neuronally medicated rat gastric acid secretion. Eur. J. Pharmacol. 262, 181–183.

Bilski, J., Konturek, S.J., Cieszkowski, M., Pawlik, W.W., 1994. Endogenous nitric oxide in the regulation of gastric acid secretion, gastrin release and blood flow. Biomed. Res. 15 (Suppl. 2), 63–67.

Brown, J.F., Hanson, P.J., Whittle, B.J.R., 1993. The nitric oxide donor, *S*-nitroso-*N*-acetyl-penicillamine, inhibits secretory activity in rat isolated parietal cells. Biochem. Biophys. Res. Commun. 195, 1354–1359.

Fujisaki, J., Fujimoto, K., Oohara, A., Sakata, T., Hirano, M., Ohyama, T., Iwakiri, R., Yamaguchi, M., 1993. Role of histamine and diamine oxidase in mucosa of rat small intestine after ischemia–reperfusion. Dig. Dis. Sci. 38, 1195–1200.

Gosh, M.N., Schild, H.O., 1958. Continuous recording of acid gastric secretion in the rat. Br. J. Pharmacol. Chemother. 13, 54–61.

Grisham, M.B., Hernandez, L.A., Granger, D.N., 1986. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am. J. Physiol. 251, G567–G574.

Hasan, K., Heesen, B.-J., Corbett, J.A., McDaniel, M.L., Chang, K.,

Allison, W., Wolffenbuttel, B.H.R., Williamson, J.R., Tilton, R.G., 1993. Inhibition of nitric oxide formation by guanidines. Eur. J. Pharmacol. 249, 101–106.

Hortelano, S., Genaro, A.M., Bosca, L., 1993. Phorbol esters induce nitric oxide synthase and increase arginine influx in cultured peritoneal macrophage. FEBS Lett. 320, 135–139.

Ito, M., Guth, P.H., 1985. Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. Gastroenterology 88, 1162–1167.

Kato, S., Kitamura, M., Korol Kiewicz, R.P., Takeuchi, K., 1998. Role of nitric oxide in regulation of gastric acid secretion in rats: effect of NO donors and NO synthase inhibitor. Br. J. Pharmacol. 123, 839–846.

Lau, H.Y., Chow, S.M., 1999. Nitric oxide dose not affect histamine release from rat peritoneum mast cells. Inflammation Res., Suppl. 1, S27–S28.

Martinez-Cuesta, M.A., Barrachina, M.D., Pique, J.M., Whittle, B.J.R., Espluques, J.V., 1992. The role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. Eur. J. Pharmacol. 218, 351–354.

Misko, T.P., Moore, W.M., Kasten, T.P., Nickols, G.A., Corbett, J.A., Tilton, R.G., Mcdaniel, M.L., Williamson, J.R., Currie, M.G., 1993. Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. Eur. J. Pharmacol. 233, 119–125.

Perry, M.A., Wadhwa, S., Parks, D.A., Pickard, W., Granger, D.N., 1986. Role of radicals in ischemia-induced lesions in the cat stomach. Gastroenterology 90, 362–367.

Pique, J.M., Esplugues, J.V., Whittle, B.J.R., 1992. Endogenous nitric oxide as a mediator of gastric mucosal vasodilation during acid secretion. Gastroenterology 102, 168–174.

Sun, D.C.H., 1974. Etiology and pathology of peptic ulcer. In: Bockus, H.L. (Ed.), Gastroenterology, vol. 1, Saunders, Philadelphia, pp. 579–610.

Tanaka, J., Yuda, Y., 1993. Role of lipid peroxidation in gastric mucosal lesions induced by ischemia–reperfusion in the pylorus-ligated rat. Biol. Pharm. Bull. 16, 29–32.

Tuchiya, M. (Ed.), 1987. Gatric Mucosal Damages and Free Radicals. Nihon Igakukan, Tokyo, pp. 33–40.

Vural, K.M., Liao, H., Oz, M.C., Pinsky, D.J., 2000. Effects of mast cell membrane stabilizing agents in a rat lung ischemia–reperfusion model. Ann. Thorac. Surg. 69, 228–232.

Walder, C.E., Thiemermann, C., Vane, J.R., 1990. Endothelium derived relaxing factor participates in the increased blood flow in response to pentagastrin in the rat stomach mucosa. Proc. R. Soc. London, Ser. B 241, 195–200.

Ward, P.M., Cochrance, S.G., 1965. Bound complement and immunologic injury of blood vessels. J. Exp. Med. 121, 215–237.

Wright, C.E., Rees, D.D., Moncada, S., 1992. Protective and pathological roles of nitric oxide in endotoxin shock. Cardiovasc. Res. 26, 48–57.