Teprenone, an Anti-ulcer Agent, Increases Gastric Mucosal Mucus Level via Nitric Oxide in Rats

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ABSTRACT—We examined whether the increasing action of teprenone (TP) on mucus synthesis and content in rat gastric mucosa is related to nitric oxide (NO) formation via NO synthase (NOS) in the tissue. TP (200 mg/kg)-induced increases in levels of gastric mucosal hexosamine and adherent mucus were inhibited with decreased gastric mucosal NOS activity and nitrite/nitrate concentration by co-administration of *N*^G-monomethyl L-arginine (100 mg/kg), a NOS inhibitor, but not its D-isomer. These results suggest that TP exerts an increasing action on gastric mucus synthesis and content possibly under the condition of maintained NO production via NOS in gastric mucosal tissues, although the precise mechanisms for the action of TP is still unclear.

Keywords: Teprenone, Gastric mucus, Nitric oxide synthase

Teprenone (TP: 6,10,14,18-tetramethyl-5,9,13,17-nonadecatetraen-2-one, geranylgeranylacetone) is an anti-ulcer agent developed in Japan (1). It is known that TP protects gastric mucosal tissues against injury in various experimental gastric ulcer models through preservation of gastric mucus synthesis or content (1–3). Furthermore, it has been demonstrated that TP enhances mucus synthesis and content in cultured rat gastric mucosal cells (4). One of the mechanisms by which prostaglandins (PGs) protect against gastric mucosal lesions has been thought to involve an increase in gastric mucus synthesis and secretion (5). However, Terano et al. failed to demonstrate a positive relationship between increased gastric mucus generation and gastric mucosal PGs synthesis in cultured rat gastric cells treated with TP (4). In addition, it has been reported that the protective and preventive effects of TP on ethanol-induced gastric mucosal lesions in rats are not affected by co-administration of indomethacin, an inhibitor of PGs synthesis, suggesting that the increasing action of TP on gastric mucus synthesis and content may not be mediated by endogenous PGs (6). Thus, the mechanisms of TP-induced increase in gastric mucus generation are unclear at present. It has been demonstrated that nitric oxide (NO) generators and cyclic 3',5'-guanosine monophosphate (cGMP) stimulate mucus release from rat gastric mucosal cells (7). Furthermore, Ichikawa et al. (8) have reported that an inhibitor of NO synthase (NOS) com-

pletely prevents gastrin-induced enhancement of mucus synthesis in the gastric mucosa of rats. These findings suggest the possibility that NO facilitates mucus release and synthesis in the gastric mucosa. In this study, we attempted to clarify whether the increasing effect of TP on gastric mucus synthesis and content is related to endogenous NO produced by NOS in the gastric mucosal tissue. Namely, we examined the effect of administration of *N*^G-monomethyl L-arginine (L-NMMA), a non-specific inhibitor of NOS, on the TP-induced increases in levels of gastric mucosal hexosamine, a marker of gastric mucin, and adherent mucus in rats.

Seven-week-old male Wistar rats weighing 200–210 g, purchased from Shizuoka Laboratory Animal Center Co. (Hamamatsu), were used. These animals were starved for 24 hr prior to the experiments, but were allowed free access to water. TP (50, 100 or 200 mg/kg), suspended in 5% arabic gum, was orally administered. Five percent arabic gum used as a vehicle was given to control rats in the same manner. In a separate experiment, L-NMMA (100 mg/kg) or *N*^G-monomethyl D-arginine monoacetate (D-NMMA) (100 mg/kg), dissolved in physiological saline, was subcutaneously administered to rats immediately after the administration of TP (200 mg/kg). The same volume of physiological saline used as a vehicle was given to control rats in the same manner. These rats were sacrificed under ether anesthesia at 6 hr after the administrations and then their stomachs were removed. The

removed stomachs were cut open along the greater curvature and the gastric mucosa was removed on ice using a pair of small scissors. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of Fujita Health University. Gastric mucosal mucus extracted with Triton X-100 was hydrolyzed with hydrochloric acid. Hexosamine obtained from the hydrolyzed mucus was assayed by the method of Neuhaus and Letzring (9) using glucosamine as a standard. The determined gastric mucosal hexosamine was used as an indicator for gastric mucosal mucus synthesis. The removed stomach was rinsed with 10 ml of ice-cold 0.25 M sucrose, and 50 mm² (approx. 8 mm in diameter) of the glandular portion of the stomach was excised with a scalpel and weighed to determine its wet weight. Gastric mucosal adherent mucus was assayed by the method of Kitagawa et al. (10) using alcian blue staining. The determined gastric mucosal adherent mucus was used as an indicator for gastric mucus content. Total NOS activity in the 10% homogenate of gastric mucosa was measured spectrophotometrically using the oxidation of oxyhemoglobin to methemoglobin by NO as described previously (11). NO production in the gastric mucosa was quantitated indirectly as nitrite/nitrate by a commercial NO colorimetric assay kit (Boehringer Mannheim, GmbH, Germany) based on the Griess reaction-dependent method (12). Oxyhemoglobin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). L-NMMA, D-NMMA, alcian blue 8gx, and other chemicals were from Wako Pure Chemicals Ind., Ltd. (Osaka). TP (vitamin E free) was kindly provided from Eisai Co., Ltd. (Tokyo). Results obtained are expressed as means $\pm$ S.D. Results are analyzed by computerized statistical packages (SuperANOVA, Statview II; Abacus Concepts, Inc., Berkley, CA, USA) if necessary. Each mean value is compared by one-way analysis of variance and Fisher's protected least significant difference for multiple comparisons as the post hoc test. The level of significance was taken as $P < 0.05$.

The gastric mucosal hexosamine, used as an index for gastric mucus synthesis, and gastric mucosal adherent mucus, used as an indicator for gastric mucus content, were determined at 6 hr after administration of 50, 100 or 200 mg/kg of TP to rats. At 100 and 200 mg/kg, concentrations of gastric mucosal hexamine contents and gastric mucosal adherent mucus were each significantly increased, and both increases were dose-dependent (Table 1). These results are consistent with those reported by Seno et al. (3), which show that administration of TP (200 mg/kg) to rats increases hexosamine content and alcian blue-periodic acid Schiff stained mucus level in the gastric mucosa at 1 hr after the administration.

When rats were subcutaneously administered L-

Table 1. Dose-dependent effect of TP on gastric mucosal hexosamine and adherent mucus concentrations in rats

Drug	Hexosamine (mg/g tissue)	Adherent mucus (μ g alcian blue/g tissue)
Arabic gum	3.9 $\pm$ 0.6	152.4 $\pm$ 13.2
TP (mg/kg)		
50	4.2 $\pm$ 0.1	171.2 $\pm$ 17.0*
100	4.5 $\pm$ 0.4*	190.6 $\pm$ 17.1*
200	5.0 $\pm$ 0.7*	205.0 $\pm$ 20.5*

Rats received oral administration of TP (50, 100 and 200 mg/kg) or an equal volume of 5% arabic gum used as the vehicle. These rats were sacrificed 6 hr after the administrations. Each value represents the mean $\pm$ S.D. for 7 animals. * $P < 0.05$, compared with rats with vehicle treatment.

NMMA (100 mg/kg), but not D-NMMA (100 mg/kg), gastric mucosal hexosamine and adherent mucus concentrations significantly decreased, with decreased gastric mucosal NOS activity and nitrite/nitrate concentration at 6 hr after the administration (Table 2). Furthermore, the administration of TP (200 mg/kg) significantly increased gastric mucosal hexosamine and adherent mucus concentrations without any change in gastric mucosal NOS activity or nitrite/nitrate concentration at 6 hr after the administration (Table 2). In addition, the administration of TP (200 mg/kg) with L-NMMA (100 mg/kg), but not D-NMMA (100 mg/kg), completely inhibited TP-induced increases in gastric mucosal hexosamine and adherent mucus concentrations with decreased gastric mucosal NOS activity and nitrite/nitrate concentration at 6 hr after the co-administration (Table 2). Thus, both the basal gastric mucus content and the TP-induced enhancement of gastric mucus synthesis and content were reduced by pretreatment with L-NMMA. These results suggest that basal gastric mucus synthesis and content and the increasing effect of TP on gastric mucus synthesis and content could be closely related to NO formation via NOS in the gastric mucosal tissue. However, gastric mucosal NOS activity in rats pretreated with L-NMMA alone was lower than that in rats co-administered with TP and L-NMMA, suggesting that TP itself might interfere with the inhibitory effect of L-NMMA on gastric mucosal NOS activity. It has been demonstrated that NO acts in a paracrine fashion to stimulate soluble guanylate cyclase and to elevate cGMP level within the target cell (13). Brown et al. (7) have suggested that NO stimulates mucus secretion in rat gastric mucosal cells through the elevation of cGMP concentration within the cells. In this study, NOS activity did not change in the gastric mucosa of rats treated with TP (200 mg/kg). Furthermore, we have observed that TP in various concentrations up to 100 μ g/ml does not affect rat gastric mucosal NOS activity in the

Table 2. Effect of co-administration of TP and L-NMMA on gastric mucosal hexosamine, adherent mucus and nitrite/nitrate concentrations and NOS activity in rats

Drug	Hexosamine (mg/g tissue)	Adherent mucus (μ g alcian blue/g tissue)	Nitrite/nitrate (nmol/g tissue)	NOS (nmol/min/g tissue)
Arabic gum				
+ Saline	3.9 $\pm$ 0.3	152.4 $\pm$ 13.2	100.4 $\pm$ 9.9	2.8 $\pm$ 0.6
+ L-NMMA	3.2 $\pm$ 0.3*	108.7 $\pm$ 10.5*	58.6 $\pm$ 1.2*	0.7 $\pm$ 0.2*
+ D-NMMA	3.9 $\pm$ 0.4	146.9 $\pm$ 10.1	103.4 $\pm$ 15.6	2.6 $\pm$ 0.1
TP				
+ Saline	5.1 $\pm$ 0.6*	206.7 $\pm$ 36.0*	100.0 $\pm$ 11.2	2.7 $\pm$ 0.2
+ L-NMMA	3.9 $\pm$ 0.3 [†]	166.7 $\pm$ 25.7 [†]	69.7 $\pm$ 7.0* [†]	1.7 $\pm$ 0.2* [†]
+ D-NMMA	4.9 $\pm$ 0.4*	206.1 $\pm$ 10.8*	102.5 $\pm$ 6.2	2.7 $\pm$ 0.3

Rats received oral administration of TP (200 mg/kg) or an equal volume of 5% arabic gum used as the vehicle. L-NMMA (100 mg/kg) or D-NMMA (100 mg/kg) was subcutaneously administered to rats immediately after TP administration. The same volume of physiological saline used as a vehicle for L-NMMA and D-NMMA was given to control rats in the same manner. These rats were sacrificed 6 hr after the administrations. Each value represents the mean $\pm$ S.D. for 7 animals. * P <0.05, compared with rats with arabic gum treatment without TP treatment. [†] P <0.05, compared with rats with saline and TP treatment.

presence or absence of L-NMMA in vitro (unpublished data). These findings suggest that TP may facilitate the action of NO on cGMP production via guanylate cyclase in gastric mucosal tissues, leading to stimulation of gastric mucus synthesis and secretion without affecting NOS activity and NO production in the tissue. As above-mentioned, while basal gastric mucus synthesis and content are regulated by the amount of NO produced by NOS in the gastric mucosal tissue, the increasing effect of TP on gastric mucus synthesis and content might be due to not only the maintenance of NO production but also the changes in guanylate cyclase activity and cGMP production in the gastric mucosal tissues. However, further investigation, especially as to the change in gastric mucosal cGMP production, is required to clarify this proposed mechanism.

It has been demonstrated that PGs increase the cGMP level in isolated rat gastric mucosal cells (14). In addition, it has been thought that an increase in gastric mucus synthesis and secretion is one of the mechanisms through which PGs exert their protective effect against gastric mucosal lesions (5). Therefore, there is a possibility that the increasing effect of TP on gastric mucus content and/or synthesis is due to PGs-induced elevation of cGMP concentration in the gastric mucosal tissue. Hence, we examined the effect of co-administration of indomethacin at a dose of 5 mg/kg on TP-induced increases in gastric mucosal hexosamine and adherent mucus concentrations in normal rats because this dose of indomethacin is known to inhibit cyclooxygenase activity by 90%, leading to suppression of PGs production in gastric mucosal tissues with maintenance of gastric mucosal integrity (15). Indeed, the co-administration of TP (200 mg/kg) and

indomethacin (5 mg/kg) inhibited TP-induced increases in gastric mucosal hexosamine and adherent mucus concentrations; however, the co-administration of indomethacin to rats caused decreases in gastric mucosal NOS activity and nitrite/nitrate concentration at the same time (unpublished data). Thus, it is unclear in this study whether the increasing effect of TP on gastric mucus synthesis and content is mediated by endogenous PGs. However, it has been demonstrated that TP does not cause any significant increase in PGs (E_2 and I_2) production in rat gastric cultured cells (4). It has also been shown that administration of indomethacin with TP does not affect the stimulatory action of TP on gastric mucus synthesis and secretion in rats with ethanol-induced gastric mucosal lesions (6). Accordingly, it seems unlikely that the increasing effect of TP on the gastric mucus synthesis and content is mediated by endogenous PGs.

In conclusion, the results obtained in this study suggest that TP exerts an increasing action on gastric mucus synthesis and content possibly under the condition of maintained NO production via NOS in gastric mucosal tissues, although the precise mechanisms for the action of TP is still unclear.

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