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Effect of Plaunotol on Gastric Injury Induced by Ischaemia-Reperfusion in Rats

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Abstract

Plaunatol, an anti-ulcer drug, increases prostaglandin content in gastric tissue but its effect on radical-mediated gastric damage or activity against reactive oxygen species is unknown. We examined the effects of oral administration of plaunotol (Kelnac) on the acute gastric mucosal lesion and its progression to ulcer lesion induced by ischaemia-reperfusion in rats.

Plaunotol (30 and 100 mg kg^{-1}, 15 min before ischaemia) significantly reduced the total erosion area observed immediately after ischaemia-reperfusion. When plaunotol (30 and 100 mg kg^{-1}, once a day) was administrated orally 60 min after reperfusion, it prevented the progression from erosion to ulcer. At 72 h after ischaemia-reperfusion, the total area of ulcers lesions was significantly reduced compared with that without plaunotol administration. Furthermore, treatment with plaunotol (100 mg kg^{-1}) significantly increased prostaglandin E_2 content in gastric tissues of both acute gastric mucosal lesion and gastric ulcer lesion. In in-vitro experiments, plaunotol (1–3 mg mL^{-1}) reduced the superoxide radicals generated by leucocytes, but not by xanthine oxidase.

These results indicate that plaunotol has protective effects on both the onset of acute gastric mucosal injury and its progression to ulcer lesion induced by ischaemia-reperfusion.

Both effects of plaunotol on increase in prostaglandin content in gastric tissues and inhibition of superoxide radical from leucocytes may play important roles on the protection against gastric mucosal injury.

Many reports have demonstrated that most injury of gastric mucosa is considered to occur during reperfusion rather than during ischaemia (Perry & Wadhwa 1988; Andrews et al 1992) and that the injury can be reduced by pretreatment with scavengers or inhibitors of reactive oxygen species (Perry et al 1986; Yoshikawa et al 1989; Wada et al 1995). Recently, it was indicated that some anti-peptic ulcer drugs, such as H_2-receptor antagonists, zinc compounds and sucralfate, have antioxidant properties on acute gastric mucosal lesions induced by ischaemia-reperfusion (Yoshikawa et al 1991; Naito et al 1995; Wada et al 1997). Thus, the antioxidant property of these drugs may play an important role on the protective effect against the acute gastric mucosal injury induced by ischaemia-reperfusion.

Plaunotol (Kelnac) has been used widely in the treatment of peptic ulcer in Japan. Plaunotol was shown to have protective effects on various ulcer models in rats (Kohda et al 1991). The main action of plaunotol was considered to be an increase of prostaglandin content in gastric tissue (Ushiyama et al 1987; Oda et al 1988). However, the effect of plaunotol on radical-mediated gastric damage, such as ischaemia-reperfusion injury, has not been investigated. Neither has the direct action of plaunotol against reactive oxygen species, such as the superoxide radical been examined.

Therefore, in this study, we investigated the protective effect of plaunotol against acute gastric mucosal injury caused by ischaemia-reperfusion in rats. In this model, temporal clamping of the coeliac artery can produce ischaemia-reperfusion state on gastric mucosa and the generated radicals cause lipid peroxidation in the gastric mucosa (Yoshikawa et al 1989; Wada et al 1995). Furthermore, we reported a new gastric ulcer model by development of the ischaemia-reperfusion model without any mechanical procedures to produce a gastric ulcer lesion in a time-dependent manner (Wada et al 1996a). Using the ulcer model with the damaged muscularis mucosae, we also examined the effect of plaunotol on aggravation of gastric mucosal lesions. In addition, the in-vitro effect of plaunotol against superoxide radicals derived from xanthine oxidase or human leucocyte was also investigated.

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Materials and Methods

Reagents

Plaunotol [(2E,6Z,10E)-7-hydroxymethyl-3,11,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol] was a gift from Sankyo Co. Ltd (Tokyo, Japan). The luminescence reagent, 2-methyl-6-[*p*-methoxyphenyl]-3,7-dihydroimidazo-[1,2-α]-pyrazin-3-one hydrochloride (MCLA) was obtained from Tokyo Kasei Co. Ltd (Tokyo, Japan). MCLA was prepared as previously described (Saniabadi & Nakano 1993) to detect superoxide radicals. Phorbol 12-myristate 13-acetate was obtained from Sigma (St. Louis, MO). Xanthine oxidase from buttermilk was obtained from Wako Chemicals (Tokyo, Japan). All chemicals were of reagent grade.

Preparation of acute gastric mucosal injury induced by ischaemia-reperfusion and drug treatment

All animal experiments were performed in accordance with the guideline for animal experimentation of the Faculty of Medicine, Tottori University. Male Wistar rats weighing 280–300 g

from SLC (Shizuoka, Japan) were fasted for 18 h before the experiments, but were allowed free access to water. Acute gastric mucosal injury was performed according to the method described previously (Wada et al 1995). Briefly, under pentobarbital anaesthesia (50 mg kg^{-1}), the coeliac artery was clamped with a small clamp (Sugita standard aneurysm clip, holding force 145 g; Mizuho Ikakogyo Co. Ltd, Tokyo, Japan) for 30 min and reperfused by removal of the clamp to obtain the ischaemia-reperfusion state. Sixty-minutes after the reperfusion, the rats were killed by exsanguination via the abdominal aorta, and the stomach was removed. Macroscopic gastric erosional damage, expressed as total area (mm^2), was measured by computer imaging analysis. Obtained gastric mucosal lesions were revealed by histological examination (Wada et al 1995). Plaunotol was dissolved in distilled water with a Tween 80 (final concentration 1%), as a solubilizing agent, and administered orally (30 and 100 mg kg^{-1}) 15 min before the ischaemia. A control group was given the vehicle.

Preparation of gastric ulcer induced by ischaemia-reperfusion and drug treatment

Gastric ulcers were produced according to the method described previously (Wada et al 1996a). Briefly, after the preparation of acute gastric mucosal injury (erosion), the surgical wound was sutured. Then, rats were awoken and allowed free access to water and food until they were killed. Seventy-two hours after the onset of erosions (30-min ischaemia and 60-min reperfusion), the rats were killed, and the stomachs were removed. Gastric damage (erosions and ulcers) was measured using an automated imaging analysis system and expressed as the total area (mm^2) described previously (Wada et al 1996a). Plaunotol was administered (30 and 100 mg kg^{-1}) orally just after gastric mucosal injury (onset of erosion), followed by the administration of an equal dose 24 and 48 h after the ischaemia-reperfusion.

Preparation of histological specimens

Preparation of histological specimens was performed according to the method previously described (Wada et al 1996a). Briefly, just after and 72 h after ischaemia-reperfusion (onset of erosion), the stomachs were immediately removed and fixed with 3·7% formaldehyde-saline. After the fixation, the stomach was horizontally sectioned at 3-mm intervals running across the glandular mucosa from 3 mm distal below to the limiting ridge of forestomach. Segments were stained with hematoxylin-eosin and processed for light microscopic observations.

Measurement of superoxide radicals generated from leucocytes or the xanthine-xanthine oxidase system in-vitro

Leucocyte suspensions were prepared from healthy volunteers by the modified method (Saniabadi & Nakano 1993). Human leucocyte-generated superoxide radicals were measured by the modified method described previously (Wada et al 1996b). Briefly, the leucocyte suspension (10^6 cells mL^{-1}, 1 mL) was incubated in the reaction vial containing plaunotol (0·1–3 mg mL^{-1} final concentration) for 1 min to equilibrate to 37°C and 1 μmol L^{-1} of MCLA was then added. Leucocytes were stimulated by addition of phorbol myristate acetate (final 0·3 μmol L^{-1}). Chemiluminescence intensity (superoxide radical generation) was measured with Aloka Luminescence Reader, BLR 301 (Aloka, Tokyo, Japan) for 10 min and expressed as kilo-counts per minute (kc min^{-1}).

Superoxide radicals were also generated from xanthine-xanthine oxidase system according to the method described previously (Wada et al 1996b). Briefly, 1·0 mL of reaction medium (Tris-HCl buffer, pH 7·4) containing 10 mU mL^{-1} of xanthine oxidase was incubated in the reaction vial containing plaunotol (0·1–3 mg mL^{-1} final concentration) for 1 min, and 1 μmol L^{-1} of MCLA was added. Xanthine (30 μmol L^{-1}) was added to generate superoxide radicals. Chemiluminescence intensity was measured for 5 min and expressed kilo-counts per minute (kc min^{-1}).

Measurement of prostaglandin content in gastric tissues

Preparation for the assay of gastric prostaglandin E_2 levels were performed according to the method described by Kobayashi et al (1985). Briefly, after the measurements of damaged area, gastric tissues of both acute gastric mucosal lesion for the erosional group and ulcer lesion for the ulcerative group were dissected. Whole layers of gastric wall (100 mg) were homogenized in 2 mL of 99·5% ethanol with 25 μmol L^{-1} of indomethacin and centrifuged at 10 000 *g* for 15 min. The supernatant was directly assayed by the prostaglandin E_2 EIA system (Amersham Japan Co., Tokyo, Japan). Prostaglandin E_2 levels in the gastric tissue were expressed as ng (mg protein)$^{-1}$.

Statistics

All results are expressed as means ± s.e.m. Statistical comparisons were with Student's *t*-test, Tukey or Scheffe's multiple comparison test after analysis of variance. The results were considered significantly different when $P < 0.05$.

Results

Effects of plaunotol on acute gastric erosion and gastric ulcer induced by ischaemia-reperfusion

The total area of erosions, a morphological index of acute gastric injury induced by ischaemia-reperfusion, was significantly decreased by the treatment with plaunotol (30 and 100 mg kg^{-1}, Table 1). In the microscopic observations, haemorrhage, erosion of mucosa and efflux of fibrin in the damaged area in control rats were observed (Fig. 1A). Pretreatment with plaunotol (30 and 100 mg kg^{-1}) improved these histopathological changes (Fig. 1B). No microscopic damage was observed after treatment with plaunotol (30 and 100 mg kg^{-1}) without the procedure of ischaemia-reperfusion (data not presented).

Table 1. Effect of plaunotol on the acute gastric mucosal injury induced by ischaemia-reperfusion in rats.

Treatment	n	Area of erosion (mm^2)
Control	7	158·4 ± 23·8
Plaunotol		
30 mg kg^{-1}	6	89·4 ± 17·1*
100 mg kg^{-1}	8	24·9 ± 8·4**

Gastric mucosal injury was produced by ischaemia-reperfusion resulting from clamping of the coeliac artery. Plaunotol was administered orally 15 min before the ischaemia. After 60 min of reperfusion, damaged areas were measured and expressed as area of erosion (mm^2). Values are mean ± s.e.m. *$P < 0.05$, **$P < 0.01$ compared with control (without plaunotol).

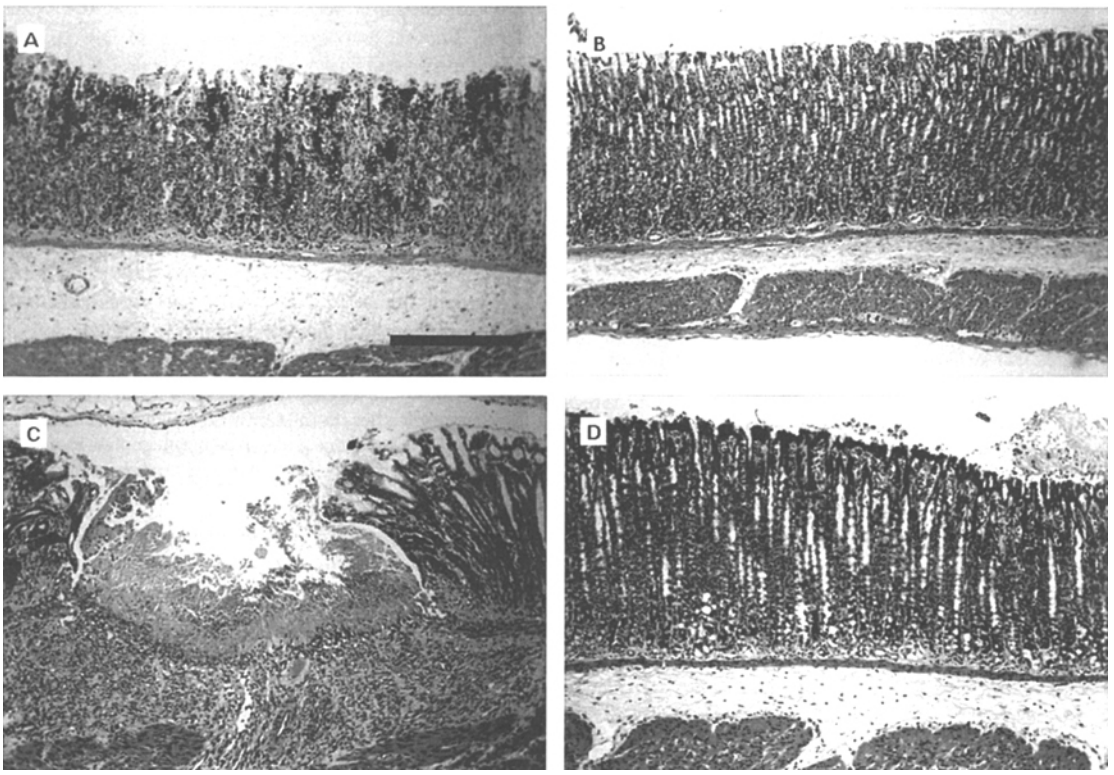

FIG. 1. Light microscopic photographs of gastric tissue obtained from the rats just after (A, B) and 72 h after (C, D) ischaemia (30 min) and reperfusion (60 min). Plaunotol (100 mg kg^{-1}) was administered orally 15 min before the ischaemia (B), or immediately, 24 and 48 h after ischaemia-reperfusion (D). The group undergoing pretreatment with plaunotol (B) showed improvement of haemorrhagic erosion of gastric mucosa compared with control (A). In the group administered plaunotol after ischaemia-reperfusion (D) was observed the maintenance of muscularis mucosae and the regeneration of gastric mucosa compared with the ulcer-control (C). Segments were stained with hematoxylin-eosin. Calibration bar = 300 μm.

Seventy-two hours after the ischaemia-reperfusion, gastric ulcers with damage to muscularis mucosae were observed in the areas of the gastric glands (Fig. 1C). Administration of plaunotol (30 and 100 mg kg^{-1}, once a day, p.o.) significantly decreased the total area of ulcers 72 h after the onset of erosions, compared with controls (Table 2). In the histological observations, no damages of muscularis mucosae were observed, although the regeneration of gastric mucosa in the damaged area were observed (Fig. 1D).

Plaunotol showed not only a protective effect on acute gastric mucosal injury, but also an inhibitory effect against progression to gastric ulcer from erosion.

Table 2. Effect of plaunotol on the progression to gastric ulcer from erosion in rats.

Treatment	n	Area of ulcer (mm^2)
Control	6	185·2 ± 15·4
Plaunotol		
30 mg kg^{-1}	6	116·3 ± 21·8*
100 mg kg^{-1}	8	46·3 ± 11·1**

After the preparation of acute gastric mucosal injury, the surgical wound was sutured. Plaunotol was administered orally immediately, 24 and 48 h after ischaemia-reperfusion injury (onset of erosions). Seventy-two hours after ischaemia-reperfusion, damaged areas were measured and expressed as total area of ulcers. Values are mean ± s.e.m. *$P < 0.05$, **$P < 0.01$ compared with control (without plaunotol).

In-vitro effect of plaunotol on the superoxide radicals generated from leucocytes or the xanthine-xanthine oxidase system

Plaunotol, at more than 1 mg mL^{-1}, caused a significant reduction in superoxide generation from leucocytes in-vitro (Table 3). On the other hand, in the xanthine-xanthine oxidase system, plaunotol showed only a tendency to decrease the generation of superoxide radicals (735·4 ± 40·6 kc min^{-1},

Table 3. Effect of plaunotol on superoxide radicals generated from human leucocytes in-vitro.

Concn of plaunotol (mg mL^{-1})	Chemiluminescence intensity (kc min^{-1})
0·0	1699·1 ± 224·2
0·1	1564·6 ± 103·9
0·3	1448·3 ± 280·9
0·5	1277·3 ± 145·2
1·0	1146·7 ± 106·2*
2·0	280·3 ± 132·8*
3·0	175·7 ± 91·1*

The leucocyte suspension (10^6 cells mL^{-1}) was incubated in the reaction vial containing plaunotol (0–3 mg mL^{-1} final concn) and 1 μmol L^{-1} of MCLA was then added. Stimulation was initiated by addition of phorbol myristate acetate (0·3 μmol L^{-1} final concn). Chemiluminescence intensity (superoxide level) was measured and expressed as kilo-count per minute (kc min^{-1}). Values are mean ± s.e.m. (n = 6) *P, 0·05, **P, 0·01 compared with control (without plaunotol).

Table 4. Effect of plaunotol on the prostaglandin E_2 content of gastric tissue in rats.

	PGE_2 content (ng (mg protein)$^{-1}$)	
	Erosions	Ulcers
Normal rats	5·72 ± 0·33	–
Control rats	5·61 ± 0·44	13·24 ± 1·59
Plaunotol (100 mg kg^{-1}) -treated rats	14·06 ± 1·57**	30·60 ± 6·07*

After measurement of damaged areas, gastric tissues of both acute gastric mucosal and ulcer lesion were homogenized and centrifuged. The supernatant was assayed by the prostaglandin E_2 EIA system and was expressed as ng (mg protein)$^{-1}$. *$P < 0·05$, **$P < 0·01$ vs control (without plaunotol). Each value represents the mean ± s.e.m. from 6–8 observations.

n = 4), compared with controls (840·0 ± 28·5 kc min^{-1}, n = 4).

Effect of plaunotol on prostaglandin content in gastric tissues

Since the main action of plaunotol has been considered to be an increase in prostaglandin content in gastric tissue, we measured the prostaglandin content in gastric tissues treated with ischaemia-reperfusion. Pre-treatment with plaunotol significantly increased the prostaglandin E_2 content in the gastric tissues, compared with that of erosion-control (Table 4). Plaunotol also significantly increased the prostaglandin E_2 content compared with that of ulcer-control (Table 4).

Discussion

In the present study, plaunotol inhibited the increase in area of gastric mucosal lesions induced by ischaemia-reperfusion in rats. These protective effects were observed at oral doses of 30 and 100 mg kg^{-1} administered 15 min before ischaemia. We also investigated the effects of plaunotol (30 and 100 mg kg^{-1}, p.o.) on the development of erosive lesions to ulcer. Administration of plaunotol significantly decreased the total area of ulcers 72 h after the ischaemia-reperfusion (onset of erosion). In the histological observations, not only the maintenance of the muscularis mucosae but also the regeneration of the gastric mucosa in the damaged regions was observed. These results indicate that plaunotol has protective effects on both the onset of acute gastric mucosal injury and its progression to ulcer lesion induced by ischaemia-reperfusion, suggesting that plaunotol may inhibit the progression to gastric ulcer derived from erosion or may promote the healing of erosive damage.

In our previous studies, we have demonstrated that superoxide radicals derived from xanthine oxidase or leucocytes after ischaemia-reperfusion play important roles on both the onset of acute gastric mucosal injury and its progression to gastric ulcer (Wada et al 1995; Wada et al 1996a). Therefore, we investigated the effect of plaunotol on superoxide radicals generated from human leucocytes and from xanthine oxidase in-vitro. Plaunotol (1–3 mg mL^{-1}) significantly inhibited superoxide radical generation from the stimulated leucocytes. Plaunotol may have an inhibitory effect on the leucocyte function. In addition, in an ex-vivo study, it was reported that oral administration of plaunotol (480 mg per day) for seven days significantly decreased production of superoxide radicals from human leucocytes (Okabe et al 1995). One of the mechanisms by which leucocytes damage the gastroduodenal mucosa is considered to be the release of reactive oxygen species, such as superoxide radicals or hydrogen peroxide (Wallace et al 1990). Those reports support our present data that one of the protective mechanisms of plaunotol against gastric injury induced by ischaemia-reperfusion may be due to the inhibition of superoxide radical generation from leucocytes. On the other hand, in the xanthine-xanthine oxidase system, plaunotol did not show any significant inhibition of superoxide radical generation (only a tendency to inhibit was observed). These data suggest that main source of superoxide radical in the gastric tissue after ischaemia-reperfusion may be leucocytes rather than xanthine oxidase, although the other mechanisms may be concerned with the protective effects of plaunotol on the gastric mucosal injury and gastric ulcer induced by ischaemia-reperfusion.

Prostaglandins are well known to possess a cytoprotective effect against various gastric injury models (Main & Whittle 1973; Henagan et al 1989; Konturek 1990; Wallace 1992; Nishizaki et al 1994). In the present experiment, treatment with plaunotol (100 mg kg^{-1}) significantly increased prostaglandin E_2 content in gastric tissues of both acute gastric mucosal and ulcer lesion. It has been reported that the increase in the prostaglandin content of gastric tissue is due to the inhibition of the 15-hydroxyprostaglandin dehydrogenase, the first-step enzyme in the biological inactivation of prostaglandins (Oda et al 1988). Furthermore, in the histological observations, no microscopic damage was noted during pretreatment with plaunotol, with or without the procedure of ischaemia-reperfusion, although the prostaglandin content increased in the gastric tissue. This suggests that the increase in the prostaglandin content of gastric tissue treated with plaunotol is mainly due to the inhibition of the prostaglandin-inactivation enzyme.

It was not clarified in this study whether or not prostaglandins play a main role in the protection of gastric damage in the ischaemia-reperfusion model. Many mechanisms have been reported for gastroprotection of prostaglandins, such as increase in mucus secretion, reduction in gastric contractility, increase in blood flow and increase in bicarbonate secretion (Henagan et al 1989; Konturek 1990; Wallace 1992). Recently, it has been reported that leucocytes play an important role in gastric mucosal damage induced by nonsteroidal anti-inflammatory drugs, such as indomethacin and aspirin (Wallace 1992; Doble & Bahl 1994; Konturek et al 1994; McCafferty et al 1995; Santucci et al 1995; Anthony et al 1996). Therefore, plaunotol-induced increase in prostaglandin content in gastric tissue may be one of the important mechanisms to protect against leucocyte-mediated gastric damage caused by ischaemia-reperfusion. However, more detailed studies are necessary to clarify the mechanisms of plaunotol on the protections against acute gastric mucosal and ulcer lesions induced by ischaemia-reperfusion.

In conclusion, plaunotol protected against gastric damage induced by ischaemia-reperfusion in rats. This protection was evident against both the development of acute gastric mucosal lesion and its progression to ulcer lesion, probably due to the increase in prostaglandin content in gastric tissue or due to the inhibition of the superoxide generation from leucocytes.

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