Tetraprenylacetone Promotes Healing Process of Ethanol-Induced Gastric Damage in the Rat

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Received July 26, 1990 Accepted November 5, 1990

ABSTRACT—Tetraprenylacetone (TPA: teprenon, geranylgeranylacetone) is a novel anti-ulcer agent developed in Japan. The aim of this study was to test whether TPA has the ability to promote the healing process of rat gastric mucosal injury induced by absolute ethanol (ET). Fasted rats received orally 5 ml/kg of absolute ET. Sixty minutes later, TPA (200 mg/kg) or saline (control) was administered intragastrically. Thereafter, the same dose of TPA or saline was given orally every 8 hours. To investigate the role of endogenous prostaglandins, indomethacin was given intraperitoneally every 8 hours. Twenty four or 48 hours after the first administration of TPA or saline, rats were sacrificed and the stomachs were removed. Administration of TPA significantly reduced lesion indices from $100 \pm 12.9\%$ (control) to $57.0 \pm 12.8\%$ (24 hours, $P < 0.05$) and from $100 \pm 15.3\%$ (control) to $17.6 \pm 3.4\%$ (48 hours, $P < 0.01$). Addition of indomethacin did not significantly affect this effect of TPA. Ultrastructural studies revealed that TPA stimulated regeneration of gastric mucosa damaged by ET after 24 and 48 hours. These results indicate that TPA has the ability to promote the healing process of gastric mucosal damage induced by absolute ET. It is, however, unlikely that endogenous prostaglandins are involved in this promotive effect of TPA on the healing process of gastric injury.

Tetraprenylacetone (TPA: teprenon, geranylgeranylacetone) is a novel anti-ulcer agent developed in Japan (1, 2). It is reported that this drug has the ability to protect gastric mucosa against damaging-agents such as ethanol (ET) without inhibition of gastric acid secretion (3). It, however, remains elusive what effect this drug has on the healing process of gastric mucosal damage induced by necrotizing-agents. The aim of the present study was to test whether or not TPA is able to promote the healing process of rat gastric mucosal injury induced by absolute ET in the rat.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Doken, Ibaragi, Japan) weighing 200–250 g were used. The animals were fasted in individual wire-bottom cages for 24 hours prior to the experiments. Water was also withheld 3 hours before starting the studies.

Studies

Fasted rats received orally 5 ml/kg of absolute ET. Sixty minutes later, TPA (200 mg/kg) or saline (control) was administered

intragastrically. Thereafter, the same dose of TPA or saline was given orally every 8 hours. The number of animals employed in this study was seven for each group. To investigate the role of endogenous prostaglandins, indomethacin (5 mg/kg) was given intraperitoneally every 8 hours. Sixty minutes and 24 or 48 hours after the first administration of TPA or saline, animals were sacrificed with ether. The stomachs were removed and then opened along the greater curvature.

As parameters of gastric damage, lesion indices and scanning electron microscopic studies were employed.

Macroscopic study

After rinsing the stomachs with tap water, they were fixed on the board and photographed. The lesions (erosion or ulcer) were evaluated by measuring the length of red streaks and linear erosions or ulcer by an observer who was unaware of the treatment. The results were expressed as a percentage of the control.

Scanning electron microscopy

The specimens obtained from the linear lesions, which were distinguishable from the remaining area, were fixed for 3 hours in 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.2). After they were immersed in 0.1 M cacodylate buffer with 3.4% sucrose (pH 7.2) for more than 24 hours, they were refixed in 0.1 M cacodylate buffered 1.0% osmic acid for 1.5 hours. The samples were dehydrated using a graded series of ET (50%, 70%, 80%, 90%, 95% and 100%) and then immersed in iso-amyl acetate solution. They were dried by the critical point method and coated with platinum palladium. They were observed by a scanning electron microscope (Hitachi S450 LB).

Statistical analysis

Statistical significance of lesion indices was evaluated by Student's *t*-test. The results were expressed as the mean $\pm$ S.E.M. of the percentage of lesion indices compared with the

control value.

RESULTS

Macroscopic study

Sixty minutes after intragastric administration of absolute ET, extensive red streaks (hemorrhagic necrosis) were produced in the gastric fundic area (lesion index, 8.5 ± 1.6 cm).

Twenty-four and 48 hours after instillation of absolute ET, the length of the red streaks in the gastric fundic area was 9.3 ± 1.2 cm and 9.8 ± 1.5 cm, respectively.

Administration of TPA (200 mg/kg), however, significantly reduced lesion indices from $100 \pm 12.9\%$ (control) to $57.0 \pm 12.8\%$ (24 hours, $P < 0.05$) and from $100 \pm 15.3\%$ (control) to $17.6 \pm 3.4\%$ (48 hours, $P < 0.01$), as depicted in Fig. 1.

Addition of indomethacin (5 mg/kg) did not significantly affect this effect of TPA, as shown in Fig. 2. In this study, the treatment

Fig. 1. Effect of tetraphenylacetone (TPA) (200 mg/kg) on the healing process of rat gastric damage induced by absolute ethanol (24 and 48 hours after administration of TPA or saline). The actual length of lesions of 24 and 48 hour treatment by TPA or saline was 9.3 ± 1.2 cm and 9.8 ± 1.5 cm, respectively. The results are expressed as the percentage of lesion index (length of red streaks or erosions) ($n = 7$). Values are the mean $\pm$ S.E. of the percentage of lesion indices compared with the control value (* $P < 0.05$; ** $P < 0.01$ vs. vehicle group).

Fig. 2. Effect of tetraphenylacetone (TPA) (200 mg/kg) and/or indomethacin (IDM) on the healing process of rat gastric damage induced by ethanol (ET) (48 hours after administration of TPA or saline). The results are expressed as lesion indices (the length of red streaks or erosions is presented as a percentage of that in the vehicle group; $n = 7$). Values are the mean $\pm$ S.E. of the percentage of lesion indices compared with the control group (* $P < 0.05$ vs. vehicle group, NS: not statistically significant).

by indomethacin did not induce macroscopic gastric lesions.

Ultrastructural study

Scanning electron microscopy demonstrated the extensive exfoliation of the surface epithelial cells and the denuded lamina propria observed after absolute ET treatment (Fig. 3A).

Twenty-four hours later, the damage of the gastric surface epithelium was not improved (Fig. 3B). The surface of the fundic area was somewhat covered with immature cells after 48 hours of ET instillation, as presented in Fig. 3C.

The administration of TPA, however, remarkably restored the gastric epithelium from the extensive exfoliation induced by absolute ET after 24 and 48 hours (Fig. 3D).

DISCUSSION

A great number of papers have been published regarding preventive effect of many agents against gastric damage, such as prostaglandins and sucralfate, since Robert reported the concept of cytoprotection of prostaglandins in 1979 (4–6). TPA is one of the drugs that have cytoprotective action against gastric injury induced by necrotizing agents (1–3). We reported previously that pretreatment with TPA can prevent gastric damage induced by absolute ET, and this action of TPA may be due to the stimulation of gastric mucus production (7, 8). Arakawa et al. demonstrated the protective effect of TPA against gastric injury caused by ET in human subjects (9). Further, it has been reported that this effect of TPA may involve the metabolism of mucosal lipids (10–12).

In general, however, the studies concerning the effect of various drugs on the healing process of gastric injury induced by damaging agents like ET have been very few compared with those of the preventive effect of drugs. For example, a large number of papers regarding the "cytoprotective" (preventive) effect of prostaglandins have been reported to date, whereas very few papers concerning the effect of PGs on lesion-healing have been found.

Therefore, we decided to test whether or not TPA has a healing effect on ET-induced gastric injury in rats. The reason why we selected ET as a gastric damaging agent is that ET has been experimentally employed as an injurious agent to the gastric mucosa in the majority of studies on drug-induced gastric damage. Of importance is that ET may be clinically relevant to human gastric damages like acute gastric lesion. In addition, a surgical procedure is avoided in ET-induced damage, while the model of acetic acid-induced ulcer requires such a procedure.

The current study has revealed that TPA has the ability to promote the healing process of rat gastric injury induced by absolute ET. An ultrastructural study using scanning elec-

Fig. 3. Scanning electron micrographs of rat gastric mucosa 24 or 48 hours after tetraprenylacetone (TPA) or saline administration. A) ethanol (ET) plus vehicle: 24 hour after administration of TPA or saline. B) ET plus vehicle: 48 hour after administration of TPA or saline. C) ET plus TPA (200 mg/kg): 24 hour after administration of TPA or saline. D) ET plus TPA (200 mg/kg): 48 hour after administration of TPA or saline.

tron microscope further confirmed these results. This suggests that TPA is a beneficial agent for treatment of acute gastric damage as well as prevention of drug-induced gastric damage.

To disclose the mechanism of this effect of TPA, we attempted to investigate the effect of indomethacin on this healing effect of TPA. The result obtained in this study indicates that indomethacin did not affect the healing effect of TPA: i.e., endogenous PGs did not play a leading role in this action of TPA. Endogenous PGs have been reported to play a crucial role in the action of many gastric protective agents like sucralfate as well as in so-called adaptive cytoprotection (13). Recently, however, Hawky et al. claimed that endogenous PGs do not play an important role in adaptive cytoprotection (14). They insist that a gastric "mucoid cap" produced by mild irritants protects gastric mucosa against injury induced by strong damaging agents like absolute ET. Furthermore, this mucoid cap may act as a scavenger of free radicals produced by necrotizing agents. As we previously reported, TPA has the ability to stimulate gastric mucus in a cell culture system (8). In addition, TPA acts as a scavenging agent of free radicals like hydroxyl radicals in gastric cultured cells (15). Bilsky et al. demonstrated that the gastric mucosal protective action of TPA may involve the metabolism of mucosal phospholipids, but not the stimulation of endogenous PGs (16).

Taken together, it seems that the promoting effect of the healing process of TPA is not mediated by stimulation of endogenous PGs, but mediated by other factors such as augmentation of mucus or phospholipid production by gastric mucosal cells as well as scavenging action of free radicals.

In summary, the results obtained from the current study indicate that TPA has the ability to promote the healing process of gastric mucosal damage induced by absolute ET. It is, however, unlikely that endogenous prostaglandins are involved in this promotive effect of TPA on the healing process of gastric injury.

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