Dissolution of Antisecretory and Cytoprotective Action of PGE₂ in Rats

Jian Ying WANG, Koji TAKEUCHI and Susumu OKABE

Department of Applied Pharmacology, Kyoto Pharmaceutical University,
Misasagi, Yamashina, Kyoto 607, Japan

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Abstract—Oral administration of 60% ethanol in 150 mM HCl (HCl-ethanol) to rats produced gastric mucosal lesions within 1 hr. PGE₂ given s.c. at 0.1 to 3 mg/kg 0.5 hr before HCl-ethanol significantly prevented the lesion formation. The protection afforded with 3 mg/kg of PGE₂ persisted for 6 or 12 hr after administration. PGE₂ given s.c. at 3 mg/kg significantly inhibited gastric secretion in pylorus-ligated and intact rats, but the action disappeared 6 hr later.

Prostaglandins (PGs) have been shown to inhibit gastric acid secretion and prevent ulcer formation in rats (1). It was postulated at first that the antiulcer effect of PGs is causally related to its antisecretory activity. However, Robert et al. (2) reported that certain PGs, at non-antisecretory doses, protect the gastric mucosa of rats against lesions induced by various necrotizing agents such as absolute ethanol or 0.6 N HCl. This phenomenon was termed "gastric cytoprotection", but the mechanism remains unknown. No report has correlated the duration of cytoprotection (including the protection caused by the anti-secretory doses) and antisecretory activities. The present study was designed to determine the duration of cytoprotective activity on HCl-ethanol-induced gastric lesions and anti-secretory activities after administering PGE₂ to rats.

Male Sprague-Dawley rats, weighing 200–220 g, were used. The animals were fasted in a cage with a raised mesh bottom for 22 hr, but had free access to water. Water was withheld for only 2 hr before the experiments. Gastric lesions were produced by giving 60% ethanol in 150 mM HCl, p.o., in a volume of 1 ml/200 g body wt. (3). One hour later, the animals were killed and the stomachs were removed. After gastric contents were withdrawn through the duodenum by gentle pushing of the stomach, 8 ml of 2% formalin was injected into the stomach which was

then put into 2% formalin for 10 min. Subsequently, the stomach was incised along the greater curvature. The length of each lesion was measured under a dissecting microscope ($\times 10$) by an observer who was unaware of the treatment given. The degree of lesions was expressed by the sum of the length of the lesions. PGE₂ (Ono; dissolved first with absolute ethanol, 5 mg/ml, and then diluted with saline to the desired volume) or saline was given s.c. at 0.5, 6 or 12 hr before HCl-ethanol administration.

The effect of PGE₂ on gastric secretory function was tested using pylorus ligated and intact (unoperated) rats. The advantage of pylorus ligated rats is that it permits collection of all gastric juice that accumulates during a given time interval. The advantage of the intact, unoperated rats is that the secretion obtained is influenced only by the treatment given and is not altered by pylorus ligation, a procedure known to stimulate gastric acid secretion. Under ether anesthesia, the abdomen was incised, and the pylorus was ligated. Four hr later, the animals were killed, and the gastric contents were collected and centrifuged for 10 min at 3,000 rpm. The volume of each sample was measured, and acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Autoburette, Radiometer). Titratable acid output was expressed as microequivalents per hour. Prostaglandin E₂ was given s.c. 0.5 or

6 hr before the pylorus was ligated. The control group was given the vehicle alone. In the case of intact rats, animals were killed 0.5 or 6 hr after subcutaneous administration of PGE₂. At autopsy, the esophagus and the pylorus were clamped, and the gastric contents were collected into a test tube. Since there was too little secretion for direct titration, the volume was first measured, and then 1 ml of water was added to the test tube. The acidity of the diluted samples was determined by titrating 0.5 ml with 0.1 N NaOH. The amount of acid in the stomach was calculated as described by Robert et al. (2).

Oral administration of 60% ethanol in 150 mM HCl consistently produced gastric mucosal necrosis located predominantly in the glandular portion, which showed black or reddish brown lines accompanied by hemorrhage. The lesions were usually in the form of elongated bands and parallel to the long axis of the stomach. When PGE₂ at doses ranged from 0.01 to 3.0 mg/kg was given s.c. 0.5 or 6 hr prior to the necrotizing agent, the total length of lesions was dose-dependently reduced by gross inspection (Fig. 1). When PGE₂ was given 6 hr before HCl-ethanol administration, the degree of protection was diminished, but the inhibitory effect was significant at 1.0 or 3.0 mg/kg. The inhibitory rate was 30% at 1.0 mg/kg and 75% at 3.0 mg/kg. PGE₂ given s.c. at 3.0 mg/kg at 12 hr

before the necrotizing agent still showed a significant protective effect, the inhibitory rate being 44%.

Effects of PGE₂ on gastric secretion are summarized in Table 1. Ligation of the pylorus led to an accumulation of about 4.5 ml of gastric contents 4 hr later. When PGE₂

Fig. 1. Effects of PGE₂ at various doses on the gastric mucosal lesions induced in rats by giving 60% ethanol in 150 mM HCl (HCl-ethanol). Prostaglandin E₂ was given 0.5, 6 and 12 hr before giving 1 ml of HCl-ethanol. Animals were killed 1 hr after HCl-ethanol administration. Data are presented as the mean $\pm$ one S.E.M. from 8 rats per group. *Statistically significant difference from the control at $P < 0.05$.

Table 1. Effects of PGE₂ on gastric secretion in pylorus-ligated and intact rats

Doses of PGE ₂ (mg/kg)		Pylorus-ligated rats			Unoperated rats	
		Volume (ml/rat)	Acidity (meq/L)	Acid output (μeq/hr)	Volume (ml/rat)	Acid content (μeq/rat)
-0.5 hr						
	Control	4.5±0.4	70.9±5.0	79.8± 8.4	0.61±0.10	29.9±3.8
	PGE ₂ 0.1	5.0±0.5	71.3±9.3	96.0±20.0	0.87±0.10	27.7±2.4
	PGE ₂ 1.0	5.4±0.3	47.8±6.2*	69.6±11.4	0.74±0.09	11.3±1.2*
	PGE ₂ 3.0	4.1±0.5	37.6±6.1*	41.0± 9.5*	0.59±0.05	9.0±2.5*
-6 hr						
	Control	4.7±0.8	65.0± 8.1	84.3±26.0	0.65±0.05	24.5±2.1
	PGE ₂ 3.0	4.4±0.7	74.5±10.0	88.2±21.0	0.71±0.06	25.1±2.9

All values are presented as the mean $\pm$ S.E.M. from 8 rats per group. PGE₂ was given subcutaneously 0.5 or 6 hr before ligating the pylorus, and animals were killed 4 hr after the ligation. In the case of unoperated rats, animals were killed 0.5 or 6 hr after PGE₂ treatment. *Statistically significant difference from the controls at $P < 0.05$.

at different doses was given s.c. 0.5 hr before ligation of the pylorus, the volume was not affected, and gastric acidity was decreased in a dose-dependent manner, the decrease being significant at 1.0 and 3.0 mg/kg. Acid output was not affected at doses ranging from 0.1 to 1.0 mg/kg of PGE₂, but a significant reduction was observed at the dose of 3.0 mg/kg. Administration of PGE₂ 6 hr prior to ligation of the pylorus failed to affect the volume, acidity and acid output. Similarly, PGE₂ given s.c. to intact rats 0.5 hr prior to the execution dose-dependently and significantly inhibited gastric acid secretion. However, the inhibitory action disappeared 6 hr after administration of PGE₂.

The present study confirmed previous observations of other investigators (3) that the formation of gastric mucosal injury caused by HCl-ethanol in rats was significantly prevented by administration of PGE₂ 0.5 hr prior to the necrotic agent. It was reported by Robert et al. (2, 4) that the cytoprotection afforded by PGE₂ diminished when it was given earlier than 1 hr before ethanol. Gastric lesions had returned to 50% of the control values at 4 hr after p.o. administration of PGE₂, and about 2 hr after parenteral administration at non-antisecretory doses. On the other hand, the duration of cytoprotection induced by 16,16-dimethyl PGE₂ and U-68, 215, a long-acting analog of prostacyclin, was about 4 and 8 hr after p.o. administration, respectively (5). In the present study, we further found that PGE₂ at the antisecretory dose given s.c. 6 and 12 hr before the necrotic agent still showed the cytoprotective effect on the lesions induced by HCl-ethanol. It was found that after s.c. administration of PGE₂, the cytoprotective action lasted for 12 hr, but the inhibitory effect on gastric acid secretion

rapidly disappeared. PGE₂ at the antisecretory dose (3.0 mg/kg) failed to affect the gastric secretory ability in pylorus-ligated and intact rats 6 hr after administration of the agent, although the protective property significantly persisted. These findings strongly suggest that the cytoprotection by PGE₂ is unrelated to the inhibition of gastric secretion. The reason that the gastric cytoprotection with PGE₂ lasted as long as 12 hr remains to be demonstrated.

We conclude that PGE₂ given s.c. 6 or 12 hr prior to HCl-ethanol still possesses an appreciable gastric cytoprotective action independent of its inhibitory effect on acid secretion.

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