Effect of Gefarnate on Endogenous Prostacyclin, Prostaglandin E₂ and Thromboxane in Water-Immersed Rats

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KOBAYASHI, K., ARAKAWA, T., NAKAMURA, H., CHONO, S., YAMADA, H., SATOH, H. and ONO, T. *Effect of Gefarnate on Endogenous Prostacyclin, Prostaglandin E₂ and Thromboxane in Water-Immersed Rats.* Tohoku J. exp. Med., 1983, 140 (2), 181-185 — The level of endogenous prostacyclin (PGI₂), prostaglandin E₂ (PGE₂) and thromboxane A₂ (TXA₂) in rat gastric mucosa was determined by radioimmunoassay to examine whether gefarnate, an antiulcer agent, maintained the endogenous prostaglandin (PG) level in rats subjected to water-immersion stress. Seven-hr immersion induced gastric lesions and a marked reduction in PGI₂ and PGE₂. When gefarnate was injected subcutaneously before stress exposure, the mean ulcer index was reduced and the PGI₂ and PGE₂ levels were maintained. Our results suggest that the reduction of endogenous PGI₂ and PGE₂ is a major factor in water-immersion-induced ulcers in rats, and that gefarnate inhibits this ulcer formation by inhibiting a reduction in those PGs induced by water-immersion stress ——— prostacyclin (PGI₂); prostaglandin E₂ (PGE₂); thromboxane A₂ (TXA₂); gefarnate; gastric mucosa

Exogenous prostaglandins (PGs) stimulate defensive factors in gastric mucosa (Ferguson et al. 1973; Bolton et al. 1978; Chaudhury and Jacobson 1978; Garner and Heylings 1979; Johansson et al. 1980). In rats, the administration of nonsteroidal anti-inflammatory compounds (Fitzpatrick and Wynalda 1976; Kobayashi et al. 1981b), exposure to water-immersion stress (Arakawa et al. 1981a), and CCl₄-liver damage (Nakamura et al. 1981) reduce the endogenous PG level in gastric mucosa, suggesting that PGs play an important role in susceptibility or resistance to gastric mucosal injury. Therefore, the maintenance of normal endogenous PG levels which are reduced by some ulcerogens seems to inhibit ulcer formation.

We subjected rats to water-immersion stress, thereby reducing the endogenous PG levels in the gastric mucosa, and examined whether an antiulcer agent, gefarnate, could inhibit this PG reduction.

MATERIALS AND METHODS

Male Wistar rats weighing 200–250 g were used throughout this investigation. The animals were divided into three groups of 10 rats each. Group I received a subcutaneous (s.c.) injection of peanut oil and was not subjected to water-immersion; Group II received peanut oil s.c. and was subsequently stressed by water-immersion; Group III received gefarnate s.c. and was subsequently subjected to water-immersion. Peanut oil (0.4 ml) or gefarnate (100 mg/kg) dissolved in 0.4 ml peanut oil was administered twice a day for 7 days. Half of the rats from each group were used to examine the presence of gastric mucosal lesions, the other half was used for radioimmunoassay of PGs and thromboxane (TX).

The animals were fasted for 17 hr preceding the last injection. Group I was not further treated, Groups II and III rats were restrained and immersed for 7 hr in a 23°C water bath after the last injection.

Determination of gastric mucosal lesions

The rats were bled on head and the stomach was quickly removed, instilled with 1% formalin and immersed for 5 min in a 1% formalin bath. Then it was opened along the greater curvature and the length of the mucosal lesions was measured. The sum of individual measurements was taken to represent the ulcer index (UI).

Determination of gastric mucosal PGE₂, PGE₂ and TX

Gastric mucosal tissue was weighed and homogenized with a glass homogenizer in 5 ml methanol containing 3×10^{-4} M sodium meclofenamate (Waner-Lambert Co., Ltd.) to stop PG synthesis during the following procedure. Before homogenization, ³H-PGE₂ (1,000 cpm) and ³H-6-keto-PGF_{1α} (New England Nuclear) were introduced into the homogenizer as recovery markers. The homogenizer was rinsed with 10 ml chloroform and the resulting solution was vigorously admixed with the homogenate and allowed to stand for 30 min at room temperature. PGs and TX were extracted using a modification of our previously reported method (Kobayashi et al. 1981a): The solution was filtered and washed with Folch's solution (CHCl₃-CH₃OH; 2:1 by volume) and evaporated to dryness under a nitrogen stream at 40°C. The residue was dissolved in 5 ml CCl₄, 10 ml of 10% methanol-phosphate buffer were added, followed by vigorous shaking and 10-min centrifugation at 2,000×g. The upper phase was adjusted to pH 3 with 1 N HCl, 10 ml of ethylacetate were added, followed by vigorous shaking and 5-min centrifugation at 2,000×g. To the upper phase we added 200 μl of 1 N NH₃OH and 2 ml methanol, this was followed by mixing and evaporation to dryness under a nitrogen stream at 40°C. The residue was subjected to thin-layer chromatography (TLC) to separate PGE₂-TX and 6-keto-PGF_{1α} with Analtech 0.25 mm silica gel G precoated plates. An organic phase consisting of ethylacetate: acetic acid: isooctan: H₂O (110:20:50:100) was used for developing (Sun et al. 1977). PGE₂, 6-keto-PGF_{1α} and TXB₂ were determined by radioimmunoassay using ³H-PGE₂, ³H-6-keto-PGF_{1α}, ³H-TXB₂ (New England Nuclear) and antisera. PGE₂-antiserum was obtained from Institut Pasteur Production; 6-keto-PGF_{1α}- and TXB₂-antiserum were from Ono Pharmaceutical Co., Ltd.

RESULTS

Effect of gefarnate on gastric ulcer formation

None of the control rats injected with peanut oil developed gastric lesions. Water-immersion stress in the absence of gefarnate (Group II) induced gastric lesions mainly in the fundic gland area; the mean (±s.e.) ulcer index was 10.9 (±1.5). Gefarnate administration prior to stress (Group III) reduced the mean (±s.e.) ulcer index to 5.5 (±0.8); this value was significantly different ($p < 0.01$) from Group II.

Effect of gefarnate on gastric mucosal PGI₂, PGE₂ and TX

The gastric mucosal PGI₂, PGE₂ and TXA₂ levels of the 3 groups are shown in Table 1. Seven-hr water-immersion reduced PGI₂ by about 95%, PGE₂ by about 80%, and TXA₂ by about 70% in the fundic mucosa and PGI₂ by about 95%, PGE₂ by about 70%, and TXA₂ by about 70% in the antral mucosa of gefarnate-untreated rats (Group II). In gefarnate-treated, water-immersed rats (Group III), the reduction of mucosal PGI₂ and PGE₂ was significantly inhibited.

TABLE 1. *The gastric mucosal prostaglandin and thromboxane levels of controls, water-immersed rats, and gefarnate-treated, water-immersed rats*

	Control (Group I) (n=5)	Water-immersion (Group II) (n=5)	Gefarnate + Water-immersion (Group III) (n=5)
PGI ₂ (6-keto PGF _{1α})			
Fundic mucosa	3815 ± 370	165 ± 74 [†]	843 ± 202 [§]
Antral mucosa	7308 ± 1388	467 ± 230 [†]	1711 ± 305 [§]
PGE ₂			
Fundic mucosa	3125 ± 270	667 ± 106 [†]	903 ± 98 [‡]
Antral mucosa	4607 ± 584	1390 ± 236*	2404 ± 226 [§]
TXA ₂ (TXB ₂)			
Fundic mucosa	73 ± 56	24 ± 8	50 ± 11
Antral mucosa	334 ± 104	93 ± 14	93 ± 31

The values are the mean ± S.E., shown as ng/g wet weight of mucosal tissue of 5 rats.

* $p < 0.025$, $† p < 0.01$ significantly different; Group I vs. Group II.

$‡ p < 0.05$, $§ p < 0.01$ significantly different; Group II vs. Group III.

DISCUSSION

Various PG compounds and their synthetic analogues protect the gastric mucosa against various ulcerogens by mechanisms other than the inhibition of acid secretion (Robert 1976; Whittle et al. 1978). PG cytoprotection may be ascribable to the stimulation of mucus production and secretion (Bolton et al. 1978; Johansson et al. 1980), the stabilization of the cell membrane (Ferguson et al. 1973), the reduction in gastric mucosal permeability (Chaudhury and Jacobson 1978), the stimulation of HCO₃⁻ secretion (Garner and Hyelings 1979), and an increase in gastric mucosal blood flow (Konturek et al. 1980).

We previously reported that PGE₂, the most potent gastric protector among PGs (Robert et al. 1979), was the predominant form of primary PGs in the rat gastric mucosa (Kobayashi et al. 1981a). PGI₂, an unstable, potent vasodilator and inhibitor of platelet aggregation, was first shown to be produced by vascular tissue (Moncada et al. 1976), and PGI₂-like material has been detected in the gastric mucosa of several species (Moncada et al. 1977; Whittle et al. 1978). Exogenous PGI₂ was shown to be more potent than exogenous PGE₂ in stimulating gastric mucosal blood flow (Kauffman et al. 1979; Konturek et al. 1979). Furthermore,

PGI₂ exerts gastric cytoprotection even at a non-antiseecretory dose (Konturek et al. 1981). TXA₂ induces platelet aggregation and vasoconstriction (Hamberg et al. 1975). However, the physiological role of TX in the gastric mucosa remains to be elucidated.

Our present study indicates that in the rat gastric mucosa, the PGI₂ level is the highest, followed by that of PGE₂ and TXA₂ (Table 1).

As nonsteroidal anti-inflammatory compounds such as aspirin and indomethacin, inhibitors of PG synthetase, reduce the endogenous PG level in rat gastric mucosa, their ulcerogenicity may be attributable to this reduction (Kobayashi et al. 1981b). Robert et al. (1978) found that in rats, mild irritants prevented gastric necrosis and hypothesized that it was due to stimulation of PG synthesis. Arakawa et al. (1981b) demonstrated that the intragastric instillation of diluted HCl increased gastric mucosal PGs. These findings suggest that an increase in endogenous PGs inhibits the formation of gastric mucosal lesions induced by a necrotizing agent.

Arakawa et al. (1981a) have shown that water-immersion stress reduced the gastric mucosal PGE₂ level in rats, suggesting that this reduction was one of the factors involved in the pathogenesis of ulcers induced by water-immersion stress. In the present study, 7-hr water-immersion induced a marked reduction of gastric mucosal PGI₂ and PGE₂. Gefarnate inhibited this reduction and the concomitant development of ulcers in water-immersed rats. The mechanisms by which gefarnate assists in maintaining the endogenous mucosal PG levels is presently under study in our laboratory.

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