

## Prevention by aluminium phosphate of gastric lesions induced by ethanol in the rat: role of endogenous prostaglandins and sulfhydryls

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**Summary.** This study was designed to demonstrate the cytoprotective effect of an antacid containing aluminium phosphate (Phosphalugel®) against ethanol-induced gastric injury in the rat and to determine whether this cytoprotective effect is mediated by endogenous prostaglandins and sulfhydryls. We have quantitatively evaluated gastric mucosal lesions using macroscopic and histological techniques one hour after ethanol administration. Two ml of aluminium phosphate given orally one hour before administration of 2 ml of 100% ethanol significantly ( $p < 0.01$ ) reduced the area of macroscopic lesions induced by ethanol ( $3.3 \pm 0.9\%$ ) when compared to distilled water ( $20 \pm 4.8\%$ ). The histological study showed that aluminium phosphate prevented deep tissue necrosis. However, it did not protect surface epithelial cells against ethanol injury. Pretreatment with indomethacin, 5 mg/kg sc one hour before aluminium phosphate, slightly but significantly ( $p < 0.05$ ) reduced the cytoprotective effect of aluminium phosphate. Macroscopic lesions occupied  $4.3 \pm 0.94\%$  and  $1.88 \pm 0.41\%$  of total mucosal area in indomethacin group and in vehicle group, respectively. On the other hand, the sulfhydryl blocker, N-ethyl-maleimide, 10 mg/kg sc, given one hour before aluminium phosphate, completely abolished the cytoprotective effect of aluminium phosphate ( $32.92 \pm 4.85\%$  in N-ethyl-maleimide group versus  $3.78 \pm 1.41\%$  in vehicle group;  $p < 0.01$ ). These results show that aluminium phosphate has a cytoprotective effect against ethanol injury in the rat. This property appears to be mediated by both endogenous prostaglandins and sulfhydryls.

**Key words:** Aluminium phosphate, Cytoprotection, Prostaglandins, Sulfhydryls, Rat

### Introduction

Gastric cytoprotection is defined as prevention of gross gastric mucosal injury by mechanisms other than inhibition or neutralization of gastric acid secretion. The cytoprotective effect of prostaglandins has been extensively studied (Robert et al., 1979; Lacy and Ito, 1982; Guth et al., 1984; Schmidt et al., 1985; Tarnawski et al., 1985). As reviewed by Guth (1987), such a cytoprotective effect has also been described for a large number of other agents, including antacids. Antacids containing aluminium or magnesium hydroxide or calcium carbonate have been shown to prevent gastric mucosal damage induced by ethanol (Tarnawski et al., 1985; Quadros et al., 1986; Szelenyi et al., 1986) or sodium taurocholate (Szelenyi et al., 1983). Acid neutralisation does not explain the efficacy of antacids in preventing gastric mucosal damage induced by ethanol, which is known to be acid-independent (Tarnawski et al., 1985). Moreover, when antacids are acidified with hydrochloric acid, they still protect the gastric mucosa against ethanol damage (DiJoseph et al., 1989). The cytoprotective effect of antacids was reported to be mediated through endogenous prostaglandins (Quadros et al., 1986; Szelenyi et al., 1986). In addition, the cytoprotective effect of aluminium hydroxide has been shown also to be mediated through endogenous sulfhydryl-containing compounds (Szelenyi et al., 1986).

Phosphalugel®, an aluminium phosphate-containing antacid was reported to be cytoprotective in the rat (Hagel et al., 1982). However, the mechanism by which this property is mediated was not studied. In the present study, the cytoprotective effect of Phosphalugel® was confirmed macroscopically and histologically in the rat. This cytoprotective effect was shown to be mediated by endogenous prostaglandins and sulfhydryl-containing compounds.

### Materials and methods

Male Wistar rats weighing between 170 and 205 g

were fasted for 24 hours in individual cylindrical wire-mesh cages with flat bottoms in order to limit coprophagy. They were allowed free access to water, except during the last 4 hours.

#### Chemicals

The following compounds were used: aluminium phosphate gel (Phosphalugel®, Boehringer Ingelheim, Paris, France), carboxymethylcellulose (Fluka, Buchs, Switzerland), dimethyl sulfoxide (Sigma, St. Louis, Mo), 100% ethanol (Merck, Darmstadt, Germany), N-ethylmaleimide (Fluka), indomethacin (Sigma). Indomethacin was dissolved at a concentration of 20 mg/ml in dimethyl sulfoxide and diluted to 1 mg/ml with distilled water. N-ethylmaleimide (2 mg/ml) was prepared in 1% carboxymethylcellulose. Indomethacin and N-ethylmaleimide were prepared just before the experiments and administered subcutaneously in volumes of 0.5 ml/100 g.

#### Effect of aluminium phosphate on ethanol-induced gastric lesions

Two groups of 30 rats were studied. The rats received 2 ml of either aluminium phosphate or water by gavage one hour before intragastric administration of 2 ml of 100% ethanol. The rats were killed one hour after instillation of ethanol.

**Gross lesion scoring.** The stomachs of 15 rats in each group were removed, opened along the greater curvature, pinned flat on cork board, and fixed by glutaraldehyde 2.5% for 24 hours. They were coded in such a way that the observer was unaware of the treatment given. Each stomach was wiped to remove mucus which concealed the lesions and photographed with film for colour slides (Kodak Ektachrome, 200 daylight). The slides were projected onto a screen. The border of the fundic mucosa and the contours of gross lesions of the fundic mucosa were traced on clear plastic sheets.

These sheets were used to measure the surface of the fundic mucosa and of the lesions by computerized image analysis (System MOP, Digiplan Kontron). The surface of macroscopic lesions was expressed as percent of total fundic mucosa surface.

**Histological study.** The stomachs of 15 other rats in each group were fixed *in situ* by intragastric administration of 3 ml of alcoholic Bouin's fluid immediately before the sacrifice. The stomachs were removed, and opened along the greater curvature. They were pinned flat on cork board, immersed in alcoholic Bouin's fluid, and assigned a code number. Two strips of gastric wall parallel to the limiting ridge were cut from each stomach, one strip each from the left and right upper corpus. The strips were dehydrated, embedded in paraffin and cut into 4  $\mu$ m-thick sections. The sections were stained with periodic acid-Schiff, and

observed at  $\times 62.5$  with a light microscope.

The histological lesions were graded as follows. Type 1 damage consisted of superficial lesions characterized by the erosion of interfoveolar surface mucous cells on a length at least equal to that between two gastric pits (Fig. 1). Type 2 damage consisted of lesions including interfoveolar surface mucous cells and extending to 2/3 the depth of the gastric gland, or lesions characterized by erosion of surface mucus cells, gastric pit cells, and superficial glandular cells (Fig. 2).

Using an ocular micrometer, we measured the length of each section, and the length of type 1 and type 2 lesions. Values for the two strips from each stomach were then totalled and the length of the injured mucosa (type 1 lesions and type 2 lesions) and of type 2 lesions were expressed as a percentage of the overall length of the mucosa assessed.

#### Effect of indomethacin on cytoprotection afforded by aluminium phosphate

Two groups of 15 rats were subcutaneously administered with either vehicle or 5 mg/kg of indomethacin, a prostaglandin cyclooxygenase inhibitor, 1 h before the administration of 2 ml of aluminium phosphate. This was followed 60 min later by the administration of 2 ml of 100% ethanol. The rats were killed 1 h after being given ethanol. The macroscopic lesions were assessed according to the procedure previously described.

#### Effect of N-ethylmaleimide on cytoprotection afforded by aluminium phosphate

To evaluate whether the protection afforded by aluminium phosphate is related to sulfhydryls, two groups of 15 rats were given a subcutaneous injection of either vehicle or 10 mg/kg of N-ethylmaleimide, a sulfhydryl-alkylator, 1 h before the administration of 2 ml of aluminium phosphate. Sixty minutes later, 2 ml of 100% ethanol were administered intragastrically. The rats were killed 1 h later. The macroscopic lesions were assessed according to the procedure previously described.

#### Statistical analysis

For each group, results were expressed as means  $\pm$  SEM. Statistical differences between the macroscopic scores were evaluated using the nonparametric Mann-Whitney U-test. After analysis of variance to assess overall significance, the histological scores were compared by the Student's t-test. A p-value less than 0.05 was considered statistically significant.

## Results

#### Effect of aluminium phosphate on ethanol-induced gastric lesions

**Macroscopic mucosal necrosis.** Results are shown in

Figure 3. In the control group, macroscopic necrotic lesions occupied  $20 \pm 4.8\%$  of the total mucosal area. Pretreatment with aluminium phosphate significantly ( $p < 0.01$ ) reduced the extent of these lesions to  $3.3 \pm 0.9\%$ .

**Mucosal histology.** The average total length of tissue examined from each stomach was  $34.74 \pm 1.00$  mm and  $34.75 \pm 0.52$  mm in control and aluminium phosphate treatment groups, respectively. In control group,  $93.32 \pm 0.92\%$  of the length of mucosa examined showed evidence of injury. Type 2 lesions involved  $41.42 \pm 4.99\%$  of the mucosal length. In aluminium phosphate treatment group, the length of mucosa injured ( $90.37 \pm 1.65\%$ ) was similar in extent to that seen in control group. However, type 2 lesions were significantly ( $p < 0.001$ ) reduced to  $13.43 \pm 2.23\%$ .

*Effect of indomethacin on cytoprotection afforded by aluminium phosphate*

As shown in Figure 3, indomethacin slightly but significantly ( $p < 0.05$ ) increased the area of macroscopic lesions from  $1.88 \pm 0.41\%$  in vehicle group to  $4.3 \pm 0.94\%$  in indomethacin group.

*Effect of N-ethylmaleimide on cytoprotection afforded by aluminium phosphate*

Administration of N-ethyl-maleimide counteracted the cytoprotective effect of aluminium phosphate (Fig. 3). The area of macroscopic lesions was significantly ( $p < 0.01$ ) increased in N-ethyl-maleimide-pretreated rats ( $32.92 \pm 4.85\%$ ) as compared with vehicle-pretreated rats ( $3.78 \pm 1.41\%$ ).

## Discussion

This study demonstrates that an antacid containing aluminium phosphate significantly reduces macroscopic gastric lesions induced by 100% ethanol in the rat. The histological study clearly shows that protection is afforded against deep necrotic lesions but not against superficial cell damage. These results are in agreement with a previous study using an antacid based on aluminium and magnesium hydroxide (Tarnawski et al., 1985). The cytoprotective effect of prostaglandins has also been shown to be restricted to the gastric mucosa under the surface epithelium (Lacy and Ito, 1982; Guth et al., 1984; Schmidt et al., 1985; Tarnawski et al., 1985).

Conclusions regarding the mechanisms underlying the gastric cytoprotective effect of aluminium phosphate



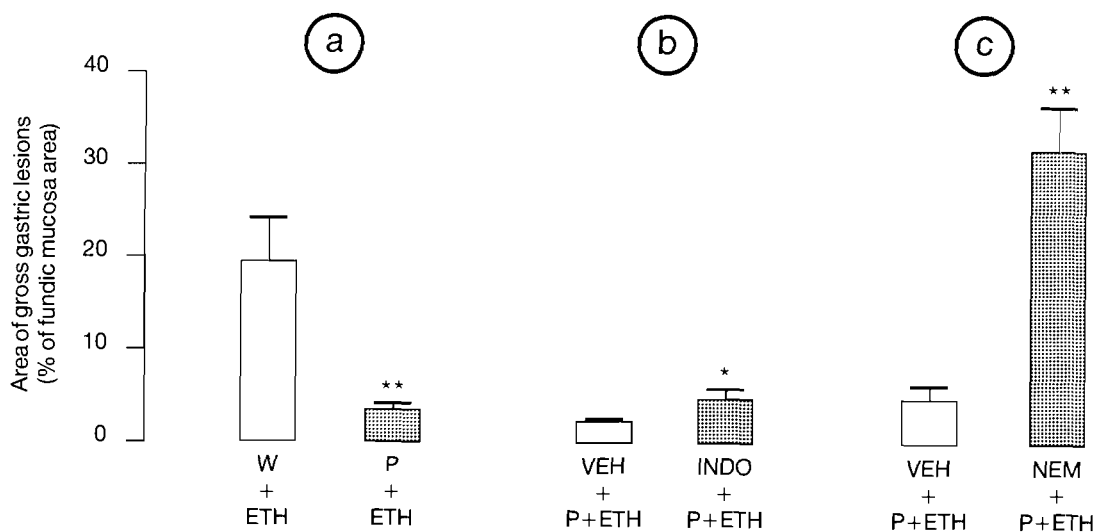
**Fig. 1.** Light micrograph of gastric mucosa from a rat pretreated with aluminium phosphate one hour prior to intragastric administration of 100% ethanol. Features of type 1 damage are seen. Interfoveolar surface mucous cells are exfoliated. A coat of mucus and cellular debris is present on the luminal surface. Glandular cells and mucous cells in the lower part of the gastric pit are spared. Periodic acid-Schiff stain.  $\times 730$

*Cytoprotection by aluminium phosphate*



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**Fig. 2.** Light micrograph of gastric mucosa from a rat pretreated with water prior to intragastric administration of 100% ethanol. Features of type 2 damage are seen. The thick arrow shows a necrotic lesion with edema and severe cellular damage extending through the middle third of the mucosa. Adjacent parts (arrows) of this necrotic lesion exhibited sloughing of surface and pit mucous cells with extensive glandular damage. Periodic acid-Schiff stain.  $\times 290$



**Fig. 3. a)** Effect of aluminium phosphate on gross gastric lesions induced by 100% ethanol in the rat; **b)** Effect of indomethacin on the cytoprotective effect of aluminium phosphate; **c)** Effect of N-ethyl maleimide on the cytoprotective effect of aluminium phosphate. Each bar represents the mean  $\pm$  SEM of gastric damage for each group (n = 15 per group). ETH: 100% ethanol, INDO: indomethacin, NEM: N-ethyl-maleimide, P: aluminium phosphate, VEH: vehicle, W: distilled water. In each experimental, asterisks denote statistical difference with the corresponding control group: \* p < 0.05; \*\* p < 0.01 (Mann-Whitney U-test)

can be drawn from the present study. Pretreatment with indomethacin at a dose causing about 77% inhibition of gastric cyclooxygenase activity (Wallace, 1988) slightly but significantly decreased the gastric cytoprotection afforded by aluminium phosphate. This suggests that the cytoprotective effect of aluminium phosphate is due at least partially to stimulation of endogenous prostaglandin synthesis. Such a mechanism has been proposed for several other antacids. The cytoprotective effect of aluminium hydroxide was reported to be blocked by indomethacin pretreatment (Szelenyi et al., 1986). In addition, antacids containing either aluminium hydroxide, magnesium hydroxide (Milk of Magnesia®), calcium carbonate (Titalac®), or aluminium hydroxide plus magnesium hydroxide (Mylanta®) were shown to stimulate endogenous prostaglandin synthesis in human gastric biopsies (Quadros et al., 1986; Preclik et al., 1989).

Endogenous sulfhydryl-containing compounds have been previously shown to mediate, at least partially, the effect of aluminium hydroxide (Szelenyi et al., 1986). The present study showed that these compounds may also be involved in the cytoprotection by aluminium phosphate. Although the role of sulfhydryl-containing compounds in gastric cytoprotection is still controversial, several studies indicated that these compounds may be involved in the maintenance of gastric mucosal integrity. Szabo et al. (1981) and Miller et al. (1985) have reported that ethanol causes depletion of endogenous nonprotein sulfhydryls in the gastric mucosa. On the other hand, various sulfhydryl-containing drugs were reported to exert a protective effect against the macroscopic gastric lesions induced by ethanol (Szabo et al., 1981; Konturek et al., 1987; Salim, 1987; Trier et al., 1987; Rogers et al., 1988). Furthermore, sulfhydryl blockers antagonize mucosal protection by protective agents (Szabo et al., 1981; Dupuy and Szabo, 1986; Konturek et al., 1987; Evangelista and Meli, 1988).

In conclusion, the present study demonstrates that aluminum phosphate is a cytoprotective agent against 100% ethanol in the rat. This property appears to be mediated through at least two different mechanisms. The primary mechanism is a sulfhydryl- and N-ethylmaleimide-sensitive process, the secondary one is dependent on endogenous prostaglandins.

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*Acknowledgements.* Financial support for this work was provided by Boehringer Ingelheim Laboratories, Biotherax Division.

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Accepted September 18, 1989