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RAPID COMMUNICATION

Effect of Allopurinol and Superoxide Dismutase on Indomethacin-Induced Gastric Lesions in the Rat

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Summary

Gastric lesions induced by indomethacin (20 $mg.kg^{-1}$ i.p.) were studied in rats after a 24 hour fast. The size of the lesions was correlated with gastric vascular permeability (determined from the Evans blue concentration in the stomach tissue after its i.v administration) and with the rate of gastric emptying (determined from the phenol red concentration after its intragastric application). These changes were correlated with the prevention of gastric lesions by allopurinol (50 $mg.kg^{-1}$) after a single dose or once daily for 3 days before indomethacin and by a single dose (15 000 $U.kg^{-1}$) of superoxide dismutase (SOD). Indomethacin significantly increases the rate of gastric emptying concomitantly with gastric vascular permeability. The pretreatment of animals with allopurinol and SOD inhibits gastric lesions as well as gastric vascular permeability without changing gastric emptying which was increased after indomethacin administration. The inhibition of gastric lesion formation and gastric vascular permeability was more marked in rats pretreated with allopurinol for 3 days when compared with rats treated with a single dose of allopurinol only. These results support the suggestion that oxygen-derived free radicals contribute to the pathogenesis of indomethacin-induced gastric lesions.

Key words

Gastric lesions – Gastric vascular permeability – Gastric emptying – Allopurinol – Superoxide dismutase

Although extensive studies on the pathophysiology of acute gastric mucosal lesions have been carried out, the pathogenesis of mucosal lesions is not fully understood. The ability of agents inducing gastric lesions to inhibit gastric prostaglandin synthesis is undoubtedly an important contributing factor (Rainsford and Willis 1982). Recently, much attention has been focused on the alterations in mucosal blood flow after nonsteroidal anti-inflammatory drug administration in the pathogenesis of gastric lesions (Ashley *et al.* 1985, Gana *et al.* 1987). The microvascular disturbances could be associated with increased vascular permeability. An increase in microvascular permeability may play an important role in the mechanisms of gastric lesions (Szabo *et al.* 1985, Kohút *et al.* 1992a,c). Takeuchi *et al.* (1990) showed the importance of the motility factor in the pathogenesis of gastric lesions induced by indomethacin. However, the mechanism by which gastric hypermotility induces damage in the mucosa still remains unclear. Takeuchi *et al.* (1991) reported that oxygen free radicals may play a role in the development of mucosal lesions associated with gastric hypermotility.

This study was designed to investigate the role of oxygen-derived free radicals in the gastric injury induced by indomethacin. The gastric lesions were correlated with gastric vascular permeability and gastric emptying. These changes were correlated with the prevention of gastric lesions by allopurinol (an inhibitor of xanthine oxidase) and superoxide dismutase (SOD) – agents affecting the production of oxygen free radicals.

Wistar rats of 220-270 g body weight of both sexes were used throughout. They were deprived of food for 24 h before the experiment but were allowed to drink tap water *ad libitum*. Stomach lesions were induced by an intraperitoneal injection of indomethacin in a dose of 20.0 $mg.kg^{-1}$ (Sigma Chemical Co.) dissolved in 2 % $NaHCO_3$ solution. The lesions were evaluated 4 h after injecting indomethacin. The damage

was expressed as the length of mucosal lesions in millimeters.

For the determination of gastric vascular permeability we used Evans blue (Fluka Chemia AG) according to the method described by Szabo *et al.* (1985). The Evans blue was injected into the tail vein (30 min after indomethacin) in a dose of 20 mg.kg^{-1} as a 0.5 % aqueous solution. Thirty minutes later, under ether anaesthesia, the animals were killed, the stomachs were removed and the amount of dye in the corpus mucosum was measured. Extraction of the dye was performed according to the method described by Lacy *et al.* (1990). The amount of dye recovered from the corpus mucosum was expressed as μg.100 mg^{-1} of wet tissue.

The rate of gastric emptying was measured by using phenol red according to the method described by Scarpignato *et al.* (1980). Phenol red was dissolved in methylcelulose (500 μg.ml^{-1}) and given just before indomethacin by stomach tube (1.5 ml/rat). Thirty minutes after administration of phenol red, rats were killed by exsanguination from the carotid artery, the stomachs were then removed, and the dye retention in the stomach was determined. The amount of dye was expressed as % when compared with the saline-treated control group.

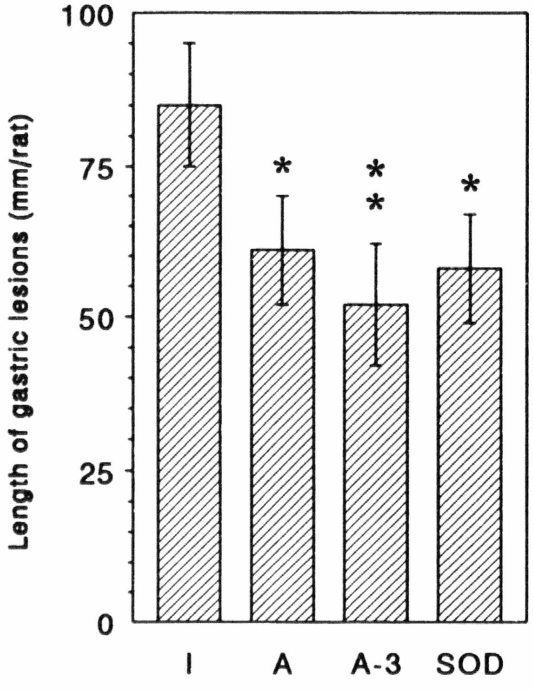

Fig. 1
Gastric lesions after indomethacin (I), indomethacin + allopurinol – single dose (A), indomethacin + allopurinol – once daily for 3 days (A-3), indomethacin + SOD (SOD). Mean values ± S.E.M. + $p<0.05$, + + $p<0.01$ when compared with the value after indomethacin.

To assess the effect of superoxide dismutase (Sigma Chemicals Co.), the dose of 15 000 U.kg^{-1} was injected intravenously just before indomethacin administration. Allopurinol (Sigma Chemical Co.), dissolved in distilled water (pH 10.8) was administered intraperitoneally in a dose of 50.0 mg.kg^{-1} 1 h before indomethacin or, in other groups, once daily for 3 days before indomethacin. The results were statistically evaluated using Student's t-test; a value of $p<0.05$ was regarded as significant.

Fig. 1. shows that allopurinol decreases indomethacin-induced gastric lesions ($p<0.05$). It can be seen that the effect is significantly greater ($p<0.01$) in the group of rats treated with allopurinol for 3 days. The administration of SOD also decreases indomethacin-induced gastric lesions ($p<0.05$).

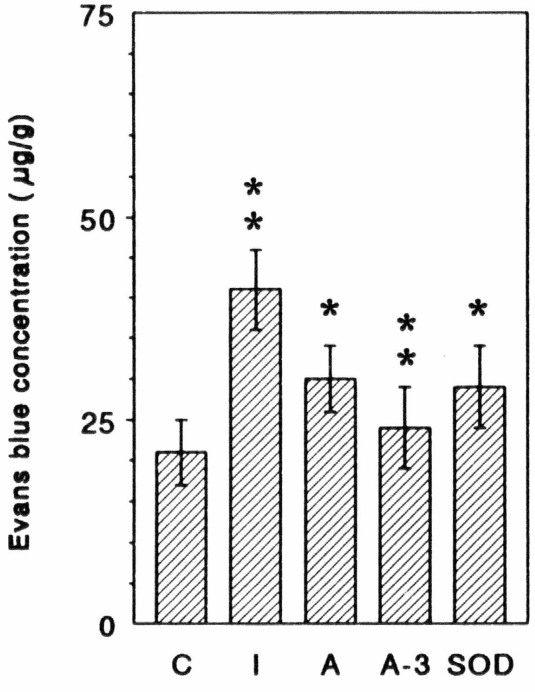

Fig. 2
Evans blue gastric concentration in control rats (C), rats given indomethacin (I), indomethacin + allopurinol – single dose (A), indomethacin + allopurinol once daily for 3 days (A-3), indomethacin + SOD (SOD). Mean values ± S.E.M. + $p<0.05$, + $p<0.01$ when compared with the value after indomethacin (indomethacin compared with the control).

Fig. 2. shows that the Evans blue concentration in the stomach tissue (as a criterion of permeability of the gastric blood vessels) is increased after indomethacin as compared with control rats ($p<0.01$). Allopurinol decreased ($p<0.05$) the concentration of Evans blue which was increased after

indomethacin. A more distinct decrease of vascular permeability was observed in the rats treated with allopurinol for 3 days ($p<0.01$). The increase of Evans blue concentration was inhibited by pretreatment with SOD ($p<0.05$).

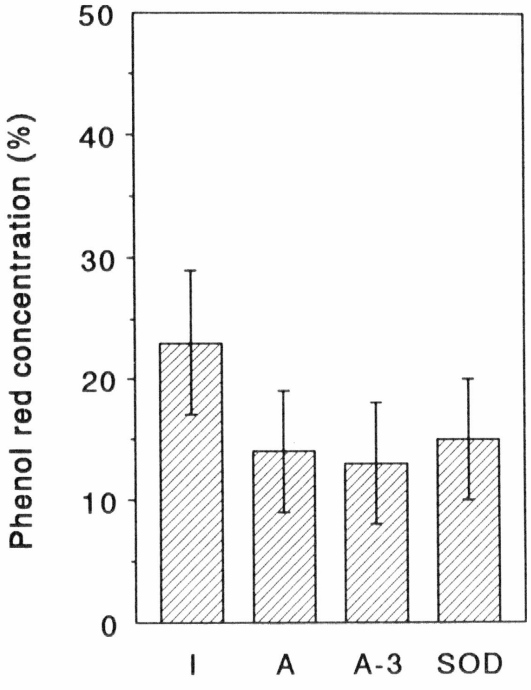

Fig. 3
Phenol red gastric concentration in % of decrease in rats after indomethacin (I), indomethacin + allopurinol – single dose (A), indomethacin + allopurinol – once daily for 3 days (A-3), indomethacin + SOD (SOD). Mean values ± S.E.M.

Indomethacin increases the gastric emptying rate (Fig. 3). The results illustrate that the increased gastric emptying rate after indomethacin was not significantly influenced by treatment with allopurinol and/or SOD.

We previously reported that gastric emptying after indomethacin is enhanced (Kohút *et al.* 1992b,c). Others have found that indomethacin increases gastric motility (Takeuchi *et al.* 1990, 1991, Ueki *et al.* 1988). The present results are consistent with the above mentioned data, when we take into consideration that gastric emptying is an indirect index of gastric motility. The observed increase in vascular permeability was associated with increased indomethacin-induced gastric lesions. On the other hand, the decrease of vascular permeability after allopurinol or SOD treatment was connected with decreased gastric lesions. We did not observe any relation between gastric ulceration and gastric emptying after allopurinol or SOD. These results show that the preventive effects of allopurinol or SOD on indomethacin-induced gastric lesions are associated with decreased vascular permeability without affecting gastric motility. This is consistent with other findings (Soldato *et al.* 1985, Takeuchi *et al.* 1991).

Kauffman *et al.* (1980) reported vascular injury and reduced mucosal blood flow, as an early event after indomethacin treatment. It is assumed that these vascular injuries could be associated with increased gastric motility observed after indomethacin. It is known that gastric permeability may result in a temporal restriction of blood flow to the mucosa and decreases the mucosal resistance to injury. Livingston *et al.* (1990) reported that reduced gastric mucosal blood flow after strong stomach contractions was followed by a period of hyperaemia. In general, the contracted stomach would block the small vessels, decrease blood flow and impair mucosal microcirculation. The stomach contraction is usually followed by relaxation, and reperfusion of the ischaemic part of the mucosa. It is well known that reperfusion is followed by the production of oxygen-derived free radicals (Takeuchi *et al.* 1991, Yoshikawa *et al.* 1990). Free radicals have been shown to play an important role in various models of gastrointestinal lesions (Itoh and Guth 1985, Perry *et al.* 1986, Smith *et al.* 1987, Vaananen *et al.* 1991).

We found that allopurinol and SOD prevented gastric lesions and decreased vascular permeability after indomethacin treatment. Since these agents, affecting the production of oxygen free radicals, prevented indomethacin-induced gastric lesions, it is reasonable to speculate that oxygen free radicals are implicated in the pathogenesis of these gastric lesions. This suggestion is supported by the observation that indomethacin stimulates lipid peroxidation (Pennington and Smith 1979, Takeuchi *et al.* 1991) and that indomethacin inhibits gastric peroxidase activity (Banerjee 1990).

The results of the present study suggest that oxygen free radicals contribute to the formation of indomethacin-induced gastric lesions. Pretreatment with either allopurinol or SOD markedly inhibits the indomethacin-induced increase of gastric vascular permeability, and the formation of gastric lesions.

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Reprint Requests

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