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Pentoxifylline-associated Reduction of Indomethacin-induced Rat Gastric Mucosal Injury Is Supported by Decreased Lipid Peroxidation

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Summary

Pentoxifylline pretreatment protects rat gastric mucosa against indomethacin-induced damage. Lipid peroxidation after indomethacin treatment (determined as thiobarbituric acid reactants) was significantly reduced by a single dose of pentoxifylline. The same was true for pentoxifylline administration for 6 days. There is a relationship between reduced lipid peroxidation, decreased number of circulating activated neutrophils and diminished disposition for acute gastric mucosal lesions induced by indomethacin in pentoxifylline-pretreated rats.

Key words

Pentoxifylline – Indomethacin – Gastric lesions – Activated neutrophils – Lipid peroxidation – Rat

Introduction

Adhesion and activation of circulating polymorphonuclear neutrophils (PMNs) contribute to the cell and organ injury *via* oxygen free radical formation (Hernandez *et al.* 1987, Vedder *et al.* 1988, Kraemer *et al.* 1990). Experimental evidence suggests a connecting link between indomethacin-induced gastric mucosal injury in rats and the increased lipid peroxidation (Takeuchi *et al.* 1991). Pharmacological intervention with pentoxifylline directed towards reducing the pool of circulating activated PMNs may be effective in preventing different organ damage (Barroso-Aranda and Schmid-Schönbein 1991). We have recently shown that pretreatment with pentoxifylline for 6 days lowered the activated PMNs count and decreased gastric mucosal lesions in rats after indomethacin administration (Kohút *et al.* 1992).

In the context of our interest in gastroprotection against drug-induced injury we wanted to verify, in the present study, whether the reduced number of activated PMNs by pentoxifylline pretreatment also correlates with the lowering of lipid peroxidation reactants in the rat gastric mucosa after indomethacin medication. A comparison between a single dose and six-day pentoxifylline pretreatment was also made.

Material and Methods

Wistar rats of both sexes weighing 220 – 270 g were deprived of food for 24 h before the experiment but with free access to water. Acute stomach lesions were induced a single intraperitoneal injection of indomethacin in a dose of 20 $mg.kg^{-1}$ (Sigma, St. Louis Mo) dissolved in a 2 % $NaHCO_3$ solution. The animals were killed under ether anestesia 4 h after indomethacin administration. The number and length of stomach lesions were expressed in mm, counting the length of spot lesions as 1 mm.

Pentoxifylline (Hoechst-Roussel) dissolved in distilled water was administered intragastrically in a dose of 45 $mg.kg^{-1}$ to the first group and daily for six days to the other group, in both cases 30 min before indomethacin administration.

The estimation of the number of circulating activated PMNs was carried out by the nitroblue tetrazolium test (NBT) according to the method of Barroso-Aranda and Schmid-Schönbein (1990). PMNs that showed stippled cytoplasmic deposits of formazan or a single dense clump of formazan were counted as NBT positive cells (NBT(+)PMNs).

Blood samples for estimation of the circulating neutrophil count and NBT-test were taken from a tail vein. Four hours after indomethacin injection lipid peroxidation in the gastric mucosa was determined as thiobarbituric acid (TBA) reactants (Ohkawa *et al.* 1979, Takeuchi *et al.* 1991). The control rats received saline in a comparable volume by the same route. The results were evaluated by Student's t-test for unpaired data.

Results

As shown in Fig. 1, pentoxifylline pretreatment 30 min before indomethacin significantly reduced acute gastric mucosal lesions in the rat. There were no differences between a single dose and six days' administration of pentoxifylline.

A moderate decrease in the number of circulating neutrophils was observed after pentoxifylline pretreatment as compared to the rats with indomethacin but without pentoxifylline.

The percentage of activated circulating PMNs measured by a reduction of nitroblue tetrazolium (NBT(+)PMNs) was significantly lower in pentoxifylline-pretreated rats than in animals with indomethacin alone or the control group. Again, one dose of pentoxifylline was sufficient to lower the number of activated PMNs.

The extent of pentoxifylline-associated protection against indomethacin-induced rat gastric mucosal injury seems to be directly proportional to the reduction of activated circulating polymorphonuclear neutrophils.

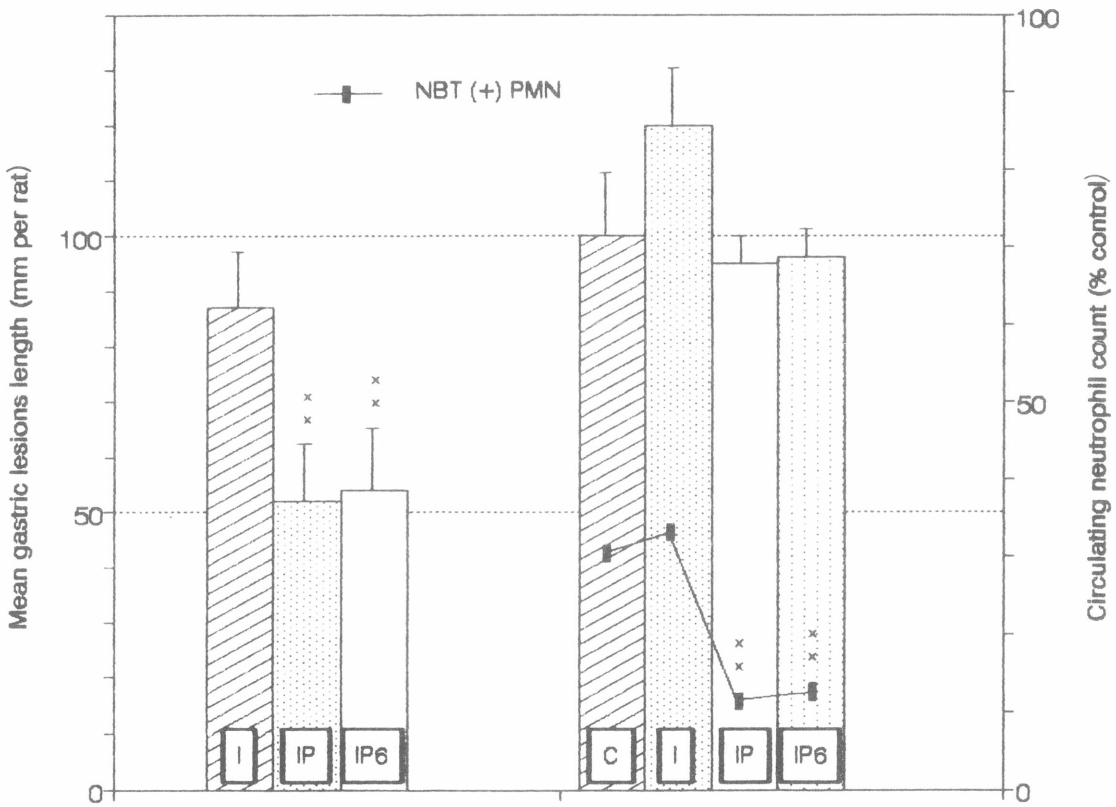

Fig. 1
Left side – gastric lesions induced by indomethacin (I), in rats pretreated by single dose of pentoxifylline (IP) and after six days administration of pentoxifylline (IP_6). Right side: number of circulating polymorphonuclear neutrophils (PMNs) and the percentage of their activated forms expressed as NBT(+)PMN in control (C), after indomethacin medication (I), in rats pretreated with one dose of pentoxifylline (IP) and after six days' administration of pentoxifylline (IP_6). In both groups pebtoxifyllin was given 30 min before administration of indomethacin. Values are means ± S. E. M. of 8 rats *per* group. Statistically significat differences + + = $p<0.01$

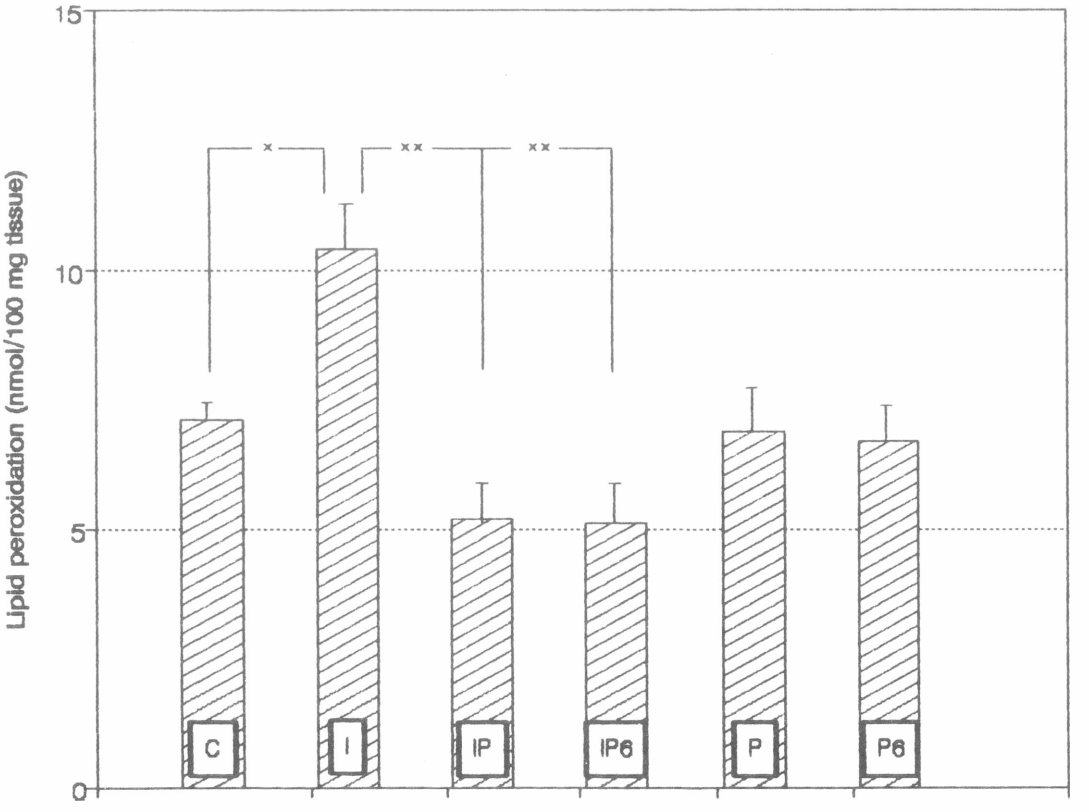

Fig. 2
Lipid peroxidation expressed as nmol.100^{-1} mg tissue of TBA reactants in control group (C), 4 h after injection of indomethacin (I), in rats pretreated with single dose of pentoxifylline (IP) and after six days' administration of pentoxifylline (IP_6) 30 min before administration of indomethacin. Lipid peroxidation after one dose (P) and after six days' administration of pentoxifylline (P_6) without indomethacin. Data are presented as means ± S.E.M. of 7 rats per group. Statistically significant differences + = $p<0.05$, ++ = $p<0.01$

To confirm further the protective effect of pentoxifylline against indomethacin, we investigated lipid peroxidation in the gastric mucosa by measuring the TBA reactants (Fig. 2). As expected, gastric mucosal lipid peroxidation was significantly higher after indomethacin administration compared to the control. The pentoxifylline pretreatment was very effective in reducing the increased amount of TBA reactants in the rat gastric mucosa after indomethacin administration 10.4±0.9 (I), 5.2±0.8 (IP) and 5.1±0.6 (IP6) μmol 100 mg^{-1} tissue. The differences in reactants were statistically significant ($p<0.01$).

The lipid peroxidation after a single dose of pentoxifylline was 6.9±0.8 and 6.7±0.7 after six days administration of pentoxifylline without injection of indomethacin which was similar to the control group with the value of 7.1±0.4 nmol.100 mg^{-1} tissue.

Discussion

It is well known that the generation of oxygen-derived free radicals is greatly increased under pathological conditions and is implicated in tissue injury (Yoshikawa *et al.* 1990).The cytotoxic effect of oxygen free radicals is the result of their ability to react with unsaturated lipids and to initiate lipid peroxidation reactions in target cell membranes leading to cell injury (Fantone and Ward 1985, Pihan *et al.* 1987, Babbs and Steiner 1990). One source of oxidants is the xanthine oxidase system, another the polymorphonuclear leukocyte (PMN). The xanthine oxidase-based free radical generating system is present within the endothelial cell itself, even in the absence of neutrophils. But in some organs it may only act as an initial trigger to atract and activate PMNs which themselves actually cause the damage (Reilly *et al.* 1991).

Experimental studies have indicated that gastric mucosal injury induced by non-steroidal anti-inflammatory drugs in rats is a neutrophil-dependent process (Wallace *et al.* 1990, Lee *et al.* 1992). Drugs decreasing the number of activated PMNs also reduce tissue injury. Barroso-Aranda and Schmid-Schönbein (1990) reported that pentoxifylline pretreatment

decreased the pool of activated neutrophils, *in vivo* adhesion to endothelium and improved survival from hemorrhagic shock. In a previous study, we found out that six-day pretreatment with pentoxifylline decreased the circulating activated PMNs count and at the same time lowered the development of indomethacin-induced gastric mucosal lesions in rats. Moderately but not significantly improved results were observed after ethanol administration (Kohút *et al.* 1992). Ethanol- and indomethacin-induced gastric mucosal lesions differed from each other, because of the direct necrotizing effect of intragastrically administered ethanol (Mirossay and Kohút 1991). The pentoxifylline protective effect on gastric mucosa against ethanol is likely to be due to increased tissue oxygen tension and improved microcirculatory blood flow (Tominaga *et al.* 1988).

The results of our present study provide further support for the role of activated PMNs and the formation of oxygen free radicals in indomethacin-induced gastric injury (Takeuchi *et al.* 1991, Vaananen *et al.* 1991, Wallace *et al.* 1991, Kohút and Mojžiš 1993). A protective effect of pentoxifylline on the indomethacin-exposed rat gastric mucosa was confirmed in our present study by reduced lipid peroxidation and was associated with the decreased activated circulating PMN count. Our experimental model permitted to compare the protective effect of pentoxifylline after a single dose and six-day administration. There were no significant differences. The values of circulating neutrophils, their activated form and lipid peroxidation were very similar after one and six-day administration of pentoxifylline. The experimental studies indicate that leukocyte adherence to the endothelium is the essential step for its further migration and activation which leads to cell and tissue damage (Granger *et al.* 1989, Kraemer *et al.* 1990, Vadas and Gamble 1990, Arnould *et al.* 1993). Pentoxifylline is a methylxanthine interacting with several types of cells, including red blood cells, neutrophils, blood monocytes and endothelial cells (Kulmann *et al.* 1993). Pentoxifylline has been shown to decrease neutrophil adhesion and to be able to block numerous destructive agents released by degranulating neutrophils (Bone 1992, Weiss *et al.* 1992). It is possible that a single dose of pentoxifylline is sufficient to decrease leukocyte adherence to the endothelium as a trigger for further reactions. Hammerschmidt *et al.* (1988) reported that the adherence of unstimulated PMNs to cultured endothelial cells was not inhibited by pentoxifylline. The mediators and mechanisms of increased leukocyte adhesion and activation are of imminent research interest (Kubes *et al.* 1990, Zimmerman *et al.* 1990, Watanabe *et al.* 1991, Arnould *et al.* 1993). Some studies have indicated that pentoxifylline metabolites are able to decrease the stimulation of neutrophils and superoxide anion formation (Crouch and Fletcher 1992, Sullivan *et al.* 1992).

In conclusion, the results of our present study indicate that the protective effect of pentoxifylline on indomethacin-induced gastric mucosal lesions in rats is mediated through the reduction of circulating activated PMNs and followed by reduced lipid peroxidation. A single dose of pentoxifylline administered 30 min before indomethacin is sufficient to exert a beneficial effect on gastric mucosa.

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Reprint Requests

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