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Effect of Stobadine on Gastric Mucosal Injury After Ischaemia/Reperfusion

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Summary

The ability of stobadine to prevent gastric mucosal injury was tested in rat gastric ischaemia induced by 30 min clamping of the coeliac artery with subsequent 30 min reperfusion. Serious injury of gastric mucosa (macroscopic and microscopic) and the increase of microvascular permeability was found after ischaemia/reperfusion in rats without stobadine. After oral pretreatment with stobadine (5 mg.kg^{-1}, 30 min before surgery), the development of gastric mucosal lesions and changes of vascular permeability were significantly decreased.

Key words
Stobadine – Ischaemia/reperfusion – Free radicals – Gastric mucosal injury

Introduction

Gastric mucosal injury has been associated with ischaemia in different clinical settings. Although early restitution of blood flow is essential to prevent further hypoxic injury, it was found that mucosal injury was greater when the ischaemia was followed by a period of reperfusion. In the last decade, evidence has been accumulated that oxygen radicals, appearing during reperfusion of ischaemic tissue, play an important role in gastric mucosal injury (Andrews *et al.* 1992, Mojžiš *et al.* 1993). Xanthine oxidase and polymorphonuclear leucocytes are the main source of free radicals. It is proposed that xanthine oxidase-derived oxidants induce the production of inflammatory mediators which attract and activate polymorphonuclear leukocytes into postischaemic tissue (Zimmerman and Granger 1994). Neutrophils adhere to the endothelium and cause further tissue injury *via* the release of oxygen radicals and some proteases (Andrews *et al.* 1994). Stobadine (−)-cis-2,8 dimethyl- 2,3,4,4a,5,9b-hexahydro-1H- pyrido(4,3b) indole dihydrochloride, is a drug with potent antiarrythmic, antihistaminic (Štolc *et al.* 1982) and antioxidant properties (Santrůček and Křepelka 1988).

The aim of this study was to establish the ability of stobadine to prevent gastric mucosal injury in ischaemia induced by occlusion of the coeliac artery with subsequent 30 min reperfusion.

Material and Methods

Animal preparation

Thirty male Wistar rats weighing 250–300 g were used for this study. They fasted for 24 h before the experiments but were allowed free access to water. They were then anaesthetized by an intraperitoneal injection of pentobarbital sodium at a dose of 50 mg.kg^{-1}. The animals were subjected to 30 min ischaemia induced by occlusion of the coeliac artery that was followed by 30 min reperfusion. The coeliac artery was clamped using an atraumatic microvascular clamp. The first experimental group was considered as the control (sham operation). The animals in the second group were subjected to ischaemia/reperfusion (I/R) without pretreatment with stobadine. The third group was pretreated with 5 mg.kg^{-1} of stobadine, administered orally 30 min before occlusion of the coeliac artery.

Measurement of mucosal lesions

At the end of the experiment, the extent of gastric lesions was measured, and their length was expressed in millimeters.

Assessment of microscopic injury

The animals were killed immediately after 30 min of reperfusion. The stomach was removed, opened along the greater curvature and fixed in a 10 % phosphate-buffered formaldehyde solution. Afterwards, the stomach was embedded in paraffin wax and cut into section. The specimens were stained with toluidin blue. The absence of cells or their disruption were regarded as a criterion of gastric mucosa damage.

Vascular permeability

Vascular permeability was established according to Ma *et al.* (1993) in our modification. Evans blue dissolved in saline (0.9 % NaCl), was given as an intravenous bolus dose of 20 $mg.kg^{-1}$ into a tail vein at the start of the experiment. At the end of reperfusion period, the stomach was removed and the oesophagus and pylorus were ligated. After this, the stomach was filled with distilled water (2 ml). After 5 min of incubation the above fluid was again collected and centrifuged at 3600 rpm for 10 min. The clear supernatant was measured at wavelength of 620 nm using a Perkin-Elmer spectrophotometer. The amount of extravascular dye leaking from the mucosa into distilled water was finally calculated from a standard curve. The experimental data were analyzed using ANOVA. $P<0.01$ was considered as significant.

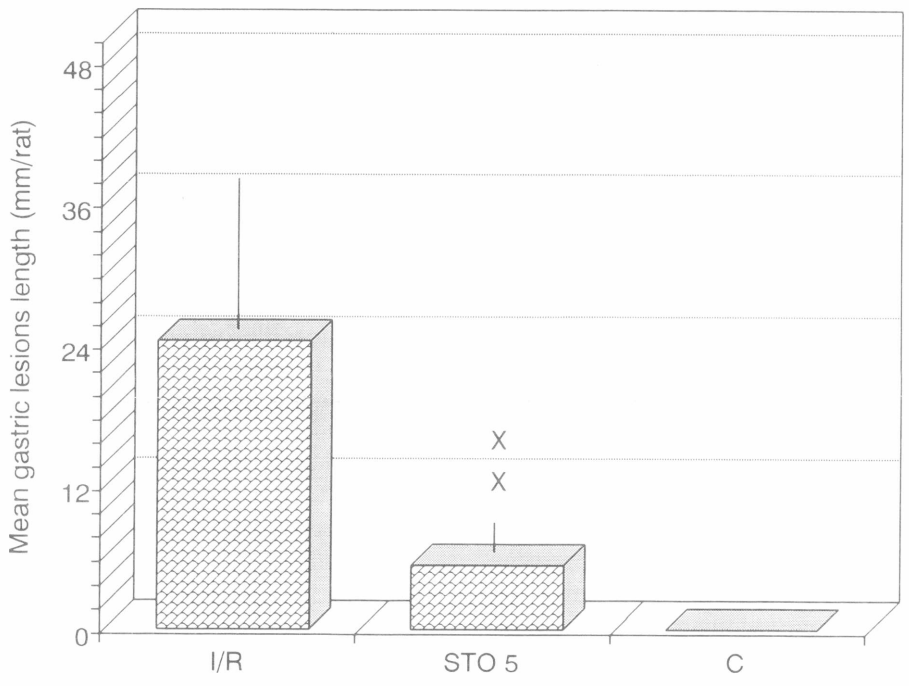

Fig. 1

Effect of stobadine (STO5) on the average length of ischaemia/reperfusion-induced gastric mucosal lesions in mm/rat. I/R – ischaemia/reperfusion without pretreatment, C – sham-operated animals. Significance of differences xx $P<0.01$

Results

Macroscopic observations

After I/R, without pretreatment with stobadine, serious injury of gastric mucosa was found. The areas of discoloration of gastric mucosa were caused by pooling of extravasated blood. Pretreatment of rats with 5 $mg.kg^{-1}$ of stobadine 30 min prior to ischaemia significantly mitigated these changes of gastric mucosa. No changes in the gastric mucosa were found in sham-operated rats. These findings were closely related to the microscopic picture. Figure 1 demonstrates the extent of mucosal damage in rats of all groups.

Microscopic injury

No histological changes were found in sham-operated rats (Fig. 2, X and X'). Figure 2, (Y and Y') illustrates the changes of gastric mucosa after I/R. The main histological findings in the damaged area were: a)

reduction and destruction of the mucosal layer, b) dilatation of capillaries, c) leakage of red blood cells and polymorphonuclear leukocytes into gastric tissue. Figure 2, (Z and Z') illustrates the effect of stobadine on the gastric mucosal damage induced by I/R. Only minimal histological changes were found in this group (a small erythrocyte leak and a few small surface lesions).

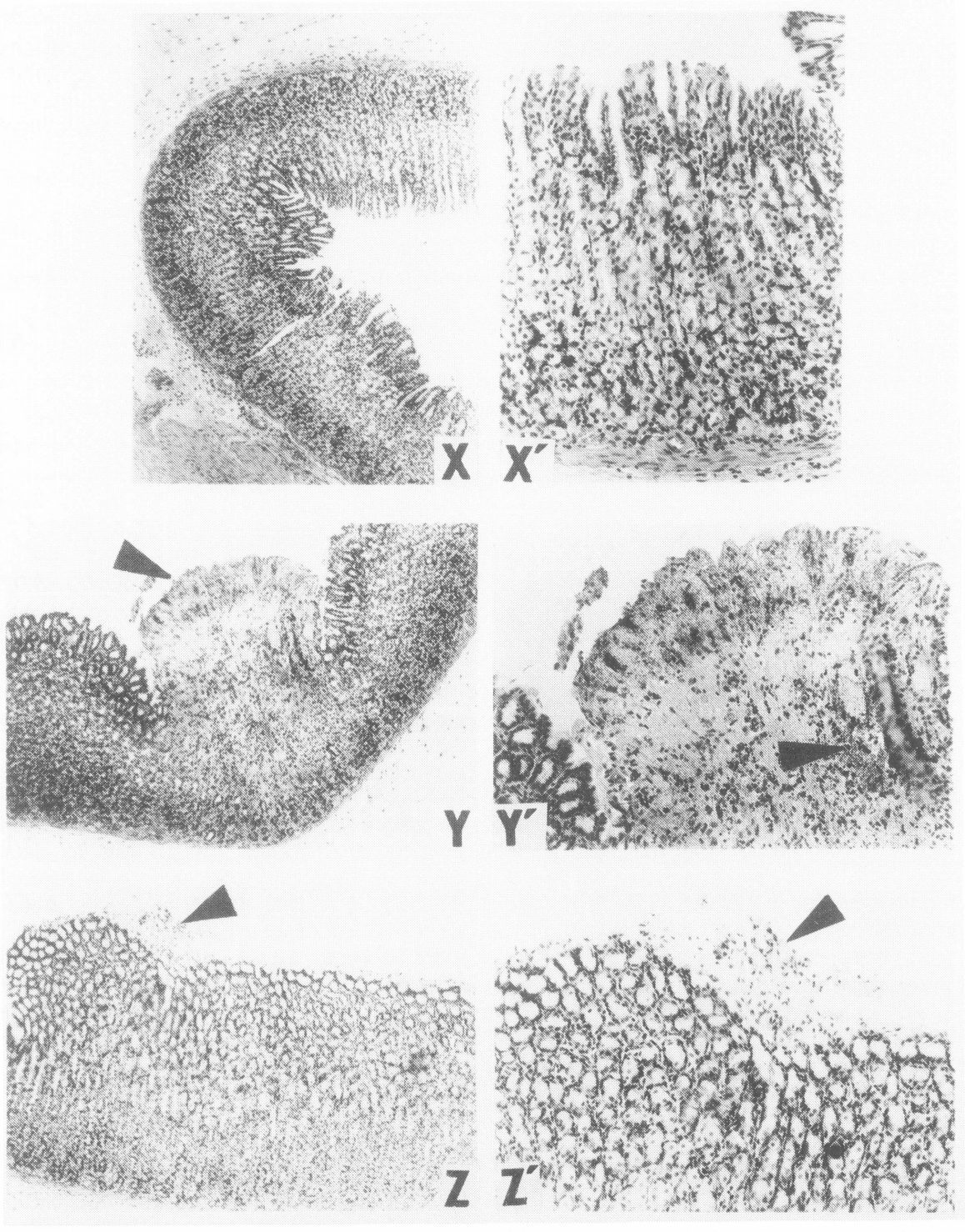

Fig. 2

Histological findings induced in gastric mucosa by ischaemia/reperfusion. Control sample (sham operation) appeared normal (X,X' magnification x 250 and x 375, respectively). Serious mucosal damage was found in samples obtained after I/R (Y,Y' magnification x 250 and x 375, respectively). Main histological findings were: destruction of mucosal layer and leakage of red blood cells into gastric tissue (arrowheads). Minimal epithelial damage was found in samples after stobadine pretreatment (Z,Z' magnification x 250 and x 375, respectively). Main histological findings were the small surface mucosal lesions (arrowheads).

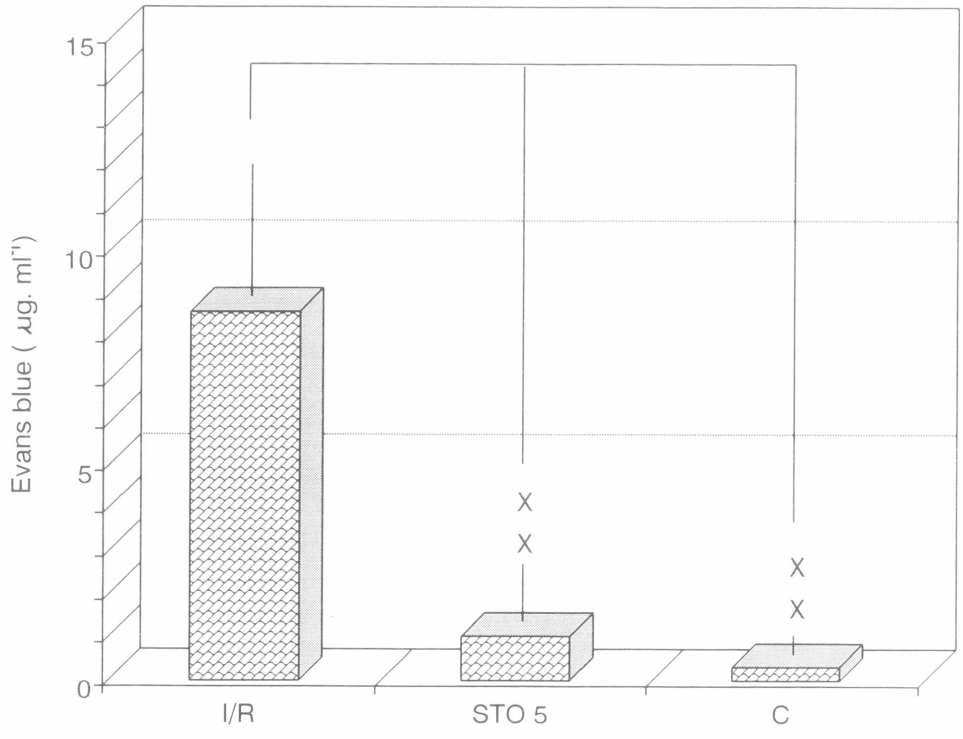

Fig. 3
Effect of ischaemia/reperfusion on mucosal vascular permeability. In stobadine-pretreated animals (STO5) the vascular permeability was significantly reduced in comparison with ischaemia/reperfusion (I/R) without stobadine. No significant differences between sham-operated (C) and stobadine-pretreated animals were found. Significance of differences xx $P<0.01$.

Vascular permeability

I/R markedly increased the amount of Evans blue escaping from the mucosa in comparison with sham-operated controls ($P<0.01$). Stobadine in the dose of 5 $mg.kg^{-1}$ significantly reduced the dye leakage from vessels in comparison with the I/R group ($P<0.01$). There were no significant differences between sham-operated and stobadine-pretreated animals (Fig. 3).

Discussion

Acute gastric injury is the result of an acute imbalance between mucosal defence and aggressive factors. The main aggressive factors responsible for this imbalance in ischaemia/reperfusion injury are oxygen free radicals, arachidonic acid cascade products, and activation of neutrophil-mediated endothelial injury (Welbourn *et al.* 1991).

There are many potential sources of superoxide radical generation in the course of ischaemia/reperfusion. One of these is the conversion of xanthine dehydrogenase to xanthine oxidase. Degradation of hypoxanthine by xanthine oxidase is accompanied by superoxide generation. A very important source of xanthine oxidase is the microvascular endothelium and loss of the endothelial barrier plays an important role in the development of ischaemia/reperfusion injury (Korthius *et al.* 1985). Stobadine did not affect the activity of xanthine oxidase (Horáková *et al.* 1991) and it is not a scavenger of the superoxide radical (Kagan *et al.* 1993). It is supposed that stobadine maintains the activity of glutathione peroxidase by holding glutathione in its reduced state (Horáková *et al.* 1990). On the other hand, stobadine is a very potent scavenger of hydroxyl radicals and a singlet molecular oxygen quencher (Štefek and Beneš 1991, Steenken *et al.* 1992).

In addition to its direct antioxidant effect, stobadine could protect the gastric mucosa by another mechanism. During detoxification of lipid hydroperoxides, aldehydes and H_2O_2, lipid peroxidation products, the dysfunction of Ca^{2+}-ATPase appears as a consequence of a decrease of the glutathione-content and oxidation of proteins (Pruijn and Bast 1989). The dysfunction of Ca^{2+}-ATPase may result in a calcium overload and activation of Ca^{2+}-dependent enzymes that lead to the deterioration of oxidative damage (Bast *et al.* 1991). As was found by Clinch *et al.* (1993), stobadine protected the ionic pump ATPase from free radical inhibition.

The results of this study show that the amount of extravasated Evans blue in the gastric lumen was significantly increased after reperfusion following 30

min ischaemia. This dye binds mainly to albumin and its measurement in the gastric content is considered to reflect vascular permeability to proteins (Takeuchi *et al.* 1987). Pretreatment with stobadine significantly decreased the reperfusion-induced increase in permeability of microvessels. We therefore suggest that vascular damage plays an important role in the development of gastric injury induced by I/R, and that stobadine probably exerts its effect by protecting endothelial cells.

Our data thus show that stobadine in the dose 5 $mg.kg^{-1}$ decreases ischaemia/reperfusion-induced increase of microvascular permeability and protects gastric mucosa against mucosal injury in ischaemia/reperfusion.

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