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SUPEROXIDE DISMUTASE (SOD) AND THE PAF-ANTAGONIST (BN 52021) REDUCE SMALL INTESTINAL DAMAGE INDUCED BY ISCHEMIA-REPERFUSION

M.T. DROY-LEFAIX,* Y. DROUET,** G. GERAUD,*** D. HOSFORD,* and P. BRAQUET*

**IHB/IPSEN Research Laboratories, Le Plessis Robinson; **University of Medicine Pitié Salpétière, Paris; ***Laboratory of Cellular Pathology, Paris; France*

Oxygenated free-radicals appear to play a prominent role in mediating damage associated with gastrointestinal diseases. Production of reactive oxygen metabolites in ischemia-reperfusion involves oxidases found in resident phagocytic cells and microvascular and mucosal epithelial cells. Platelet activating factor (PAF), a phospholipid associated with inflammatory disorders, has been shown to both prime and amplify the release of superoxide anion and hydrogen peroxide from polymorphonuclear neutrophils and macrophages stimulated by FMLP or PMA. To further elucidate the involvement of free radicals in intestinal damage and the potential role of PAF in their production, we examined the effect of superoxide dismutase (SOD) and BN 52021 (ginkgolide B) on ischemia-reperfusion induced damage in the small intestine.

The study involved 32 Sprague-Dawley rats (100-200 g) divided into four groups. Three of these groups were subjected to occlusion of the mesenteric artery 30 mins followed by 24 h reperfusion. On 2 groups SOD (15,000 U/kg/iv) and BN 52021 (20 mg/kg/po) were administered 45 mins before arterial occlusion. Following the 24 h reperfusion, the rats were sacrificed after overnight fasting. The jejunum and ileon were removed and fixed for morphological examination. Lesions in the small intestine were quantified.

The results showed extensive necrosis, hemorrhage, oedema and neutrophil invasion in the jejunal and ileal mucosa. This injury was significantly reduced by SOD (15,000 U/kg/iv) and BN 52021 (20 mg/kg/po) pretreatment. In conclusion, free-oxygenated radicals appear to mediate reperfusion damage in the small intestine and PAF appears to be involved in the genesis of these toxic products. Thus, SOD and BN 52021 may be considered as protectors against ischemic disorders.

KEY WORDS: Free-radicals, PAF, ischemia-reperfusion, intestine.

INTRODUCTION

Experimental data suggested that oxygen-derived free radicals such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) mediate microvascular and parenchymal injury, observed during ischemia-reperfusion.[1-7] The major source of oxygen free-radicals are oxidases found in resident phagocytic cells and microvascular and mucosal epithelial cells.[8-10]

There is substantial evidence of a role for a phospholipid, the Platelet-Activating-Factor (PAF) as a mediator of inflammatory disorders.[11-14] Recent studies reported that PAF, in low concentrations (10^{-10} to 10^{8} M), primes neutrophils or macrophages for enhanced release of superoxide and hydrogen peroxide in response to subsequent stimulation by FLMP or PMA.[15,16]

Correspondence should be addressed to: M.T. Droy-Lefaix, Research & Development Dept., Institut Henri Beaufour, 17 avenu Descartes, 92350 Le Plessis Robinson, France.

The purpose of this investigation was to determine the contribution of reactive oxygen metabolites and the potential role of PAF in their release in the pathogenesis of rat intestinal mucosal lesions during ischemia-reperfusion. The experiments were performed using a rat model subjected to 30 mins of ischemia and 1 h of reperfusion. The effect of superoxide dismutase (SOD) and a PAF-antagonist, ginkgolide B (BN 52021), on the morphological alterations following the reperfusion was tested.

MATERIALS AND METHODS

Surgical Procedure

Thirty-two female Sprague-Dawley rats, 180 to 200 grs (Charles Rivers, France) were fasted overnight with access to water "ad libitum". The animals were anesthetized with pentobarbital (50 mg/kg/ip) and divided into four groups. Three of these groups were subjected to ischemia. After a midline abdominal incision, the mesenteric artery was occluded for 30 mins, the reperfused for 24 h. During the experiment, to minimize evaporation and tissue dehydration, all exposed tissues were moistened with saline-soaked gauze and rats were placed in a plastic bag. Body temperature was maintained at 37° with a lamp. At the end of the experiment, all the animals were sacrificed by prolonged ether anesthesia. Segments of jejunum and ileon were harvested. Each segment was opened at the antimesenteric border and rinsed with saline. Tissue samples were fixed for morphological observations.

Assays of Drugs

Superoxide dismutase (SOD, bovine liver, Sigma Chemical Co., St. Louis, MO USA) was administered intravenously into the tail (15,000 U/kg), 45 minutes before arterial occlusion.

Ginkgolide B, BN 52021 (IHB/IPSEN Research Laboratories, Le Plessis Robinson, France) was given by gastric intubation, in suspension in distilled water with 1% tragacanth at a dose of 20 mg/kg, 45 minutes before arterial occlusion.

The untreated ischemia group and control groups received only 1 ml of distilled water.

Morphological Technique

For histological examinations, tissue samples were rapidly fixed in Bouin liquid, embedded in paraffin, cut at about 7 μm and stained with Masson trichrome.

For scanning electron microscopy, each sample was fixed using Trump liquid.[17] They were then dried under CO_2, covered with a gold-palladium mixture and examined on a Jeol Scan 100 CX.

Morphologic Evaluation

Coded histopathologic sections were examined for ischemic damage using morphologic criteria, adapted from Granger *et al.* 1986.[5] This evaluation was done without knowledge of the groups. Alterations of the intestine mucosa were graded as follows:

0: normal mucosa

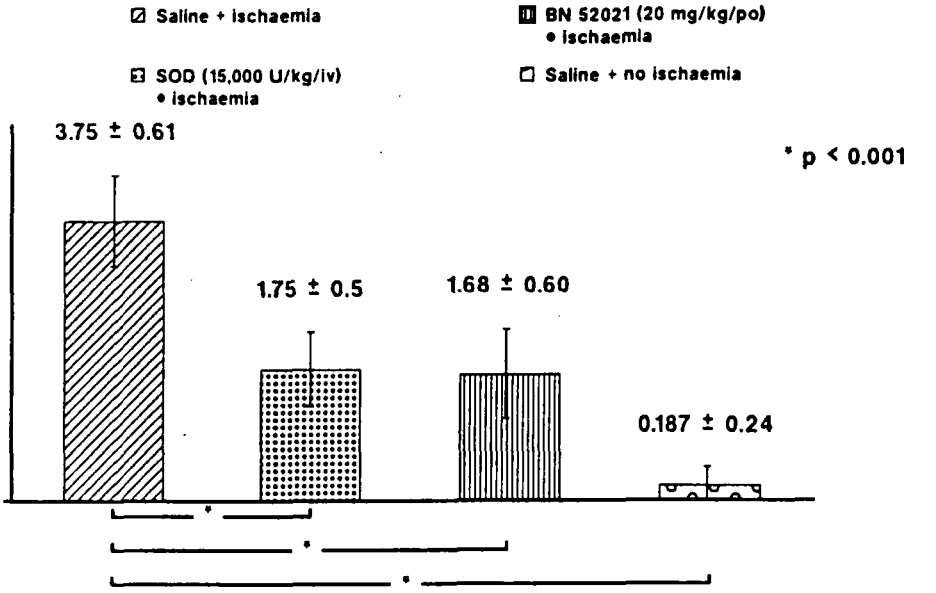

FIGURE 1 Protective effects of SOD and BN 52021 on histological lesions of the rat jejunal mucosa induced by ischemia-reperfusion (Using score 0–5).

1: separation of epithelium from lamina propria

2: occasional necrosis of tip of villi and crypts; minor cell exfoliation

3: necrosis of the majority of tips and of mid-portion of the villi and of many crypts, oedema, hemorrhagic lakes

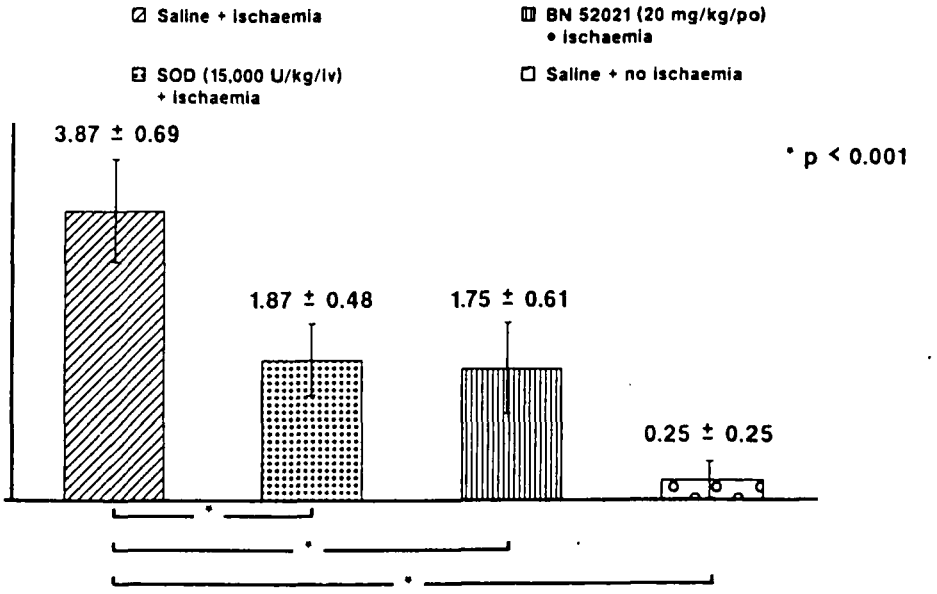

FIGURE 2 Protective effects of SOD and BN 52021 on histological lesions of the rat ileal mucosa induced by ischemia-reperfusion (Using score 0–5).

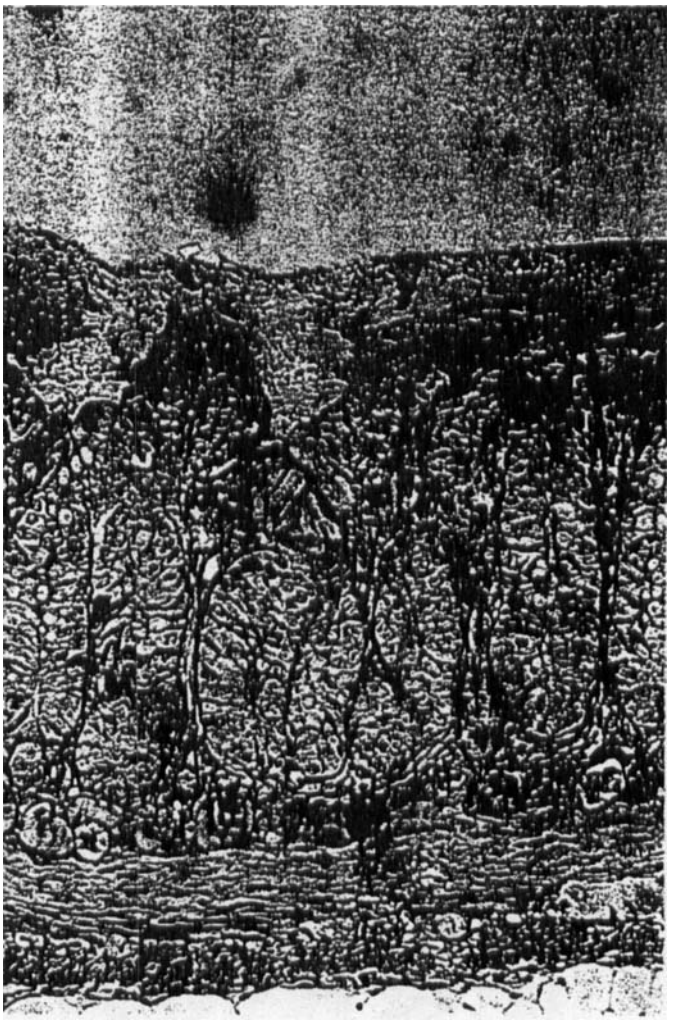

FIGURE 3 Histologocial section of a jejunal mucosa showing in ischemic group the abrasion of the tips of the villi with oedema and hemorrhagic necrosis (X 400).

4: necrosis of the majority of tips and of mid- portion of the villi and of many crypts, oedema, hemorrhagic lakes

5: necrosis of tips, mid and lower portions of the villi and of the majority of crypts, hemorrhagic lakes, oedema and polymorphonuclear leukocyte infiltration.

Statistical Analysis

Analysis of variance was used to determine if the treatment effects were significant.

FIGURE 4 Ileal mucosa after ischemia-reperfusion. Noted on this histological section, the abrasion of the villi, the major hemorrhagic necrosis of the crypts and the neutrophil invasion (X 250).

RESULTS

Histological Analysis

Figures 1 and 2 summarized the results obtained after ischemia-reperfusion on jejunal and ileal segments. The values clearly showed that both SOD and BN 52021 pretreatments significantly reduced the severity of the mucosal damage induced by 30 minutes of ischemia ($p < 0.001$). The morphological changes were characterized by hemorrhagic necrosis of the majority of the villi. This injury was accompanied by abrasion of the tips of the villi, gross epithelium exfoliation and desintegration of the lamina propria (Figure 3). Frequently, necrosis of the crypts, oedema and neutrophils in-

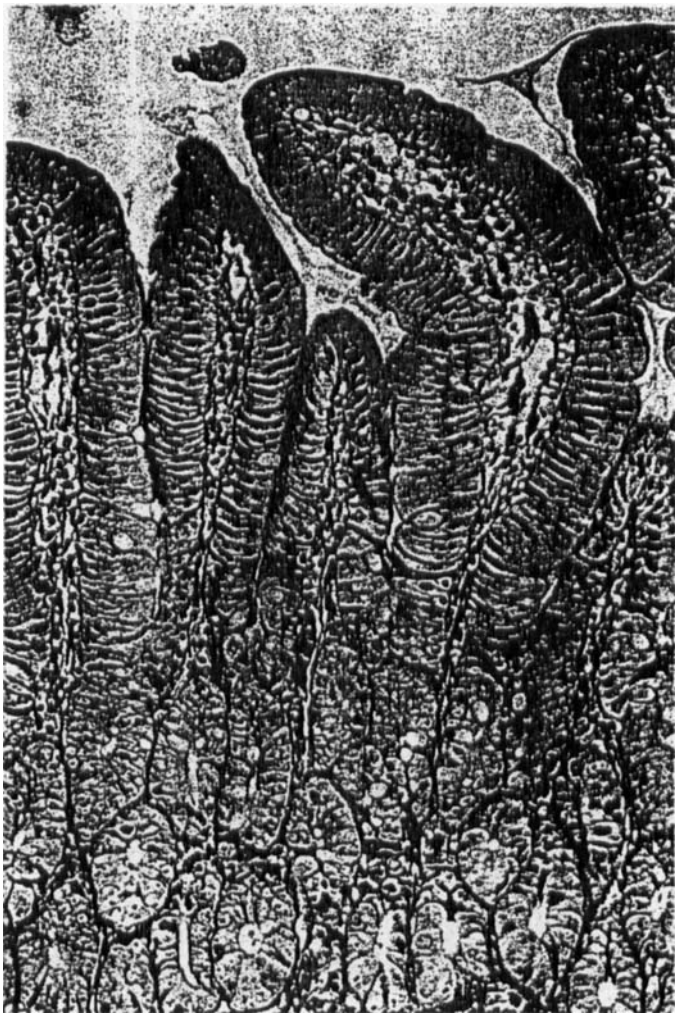

FIGURE 5 Jejunal mucosa: a pretreatment with SOD (15,000 U/kg/iv) 45' before arterial occlusion protected against ischemia-reperfusion damage. The histological section presented a normal aspect of the villi (X 250).

vasion were noted (Figure 4). With SOD (15,000 U/kg/iv) (Figure 5) and with BN 52021 (20 mg/kg/po) (Figure 6), the jejunal and ileal mucosa retained a normal aspect. Necrosis and hemorrhages were prevented and only minor erosion was occasionally observed.

Scanning Electron Microscopy

In the experimental non-treated group, severe ischemic injury of the jejunal and ileal mucosa was noted on scanning electron microscopy. The lesions were characterized by marked reduction of villi height, denudation of the villi epithelium, marked

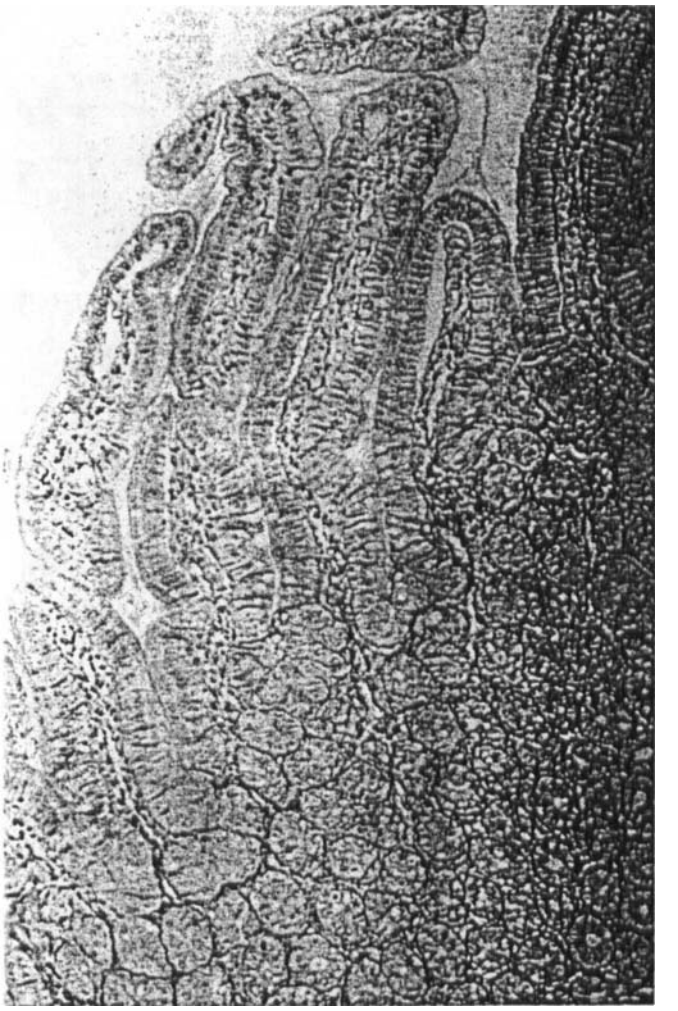

FIGURE 6 Ileal mucosa: a pretreatment with BN 52021 (20 mg/kg/po) administered 45' before arterial occlusion preserved from the lesions induced by ischemia reperfusion (X 250).

necrosis of the lamina propria and inflammation. Some villi appeared totally disintegrated (Figure 7).

In the ischemic preparations pretreated with either SOD or BN 52021, the mean villi height was normal and the erosion was only superficial with partial cell exfoliation at the top of the villi (Figure 8).

DISCUSSION

There is considerable evidence supporting a role for oxygenated free radicals in the mucosal changes during ischemia-reperfusion in the stomach[1,2,3,5,18,19] and the intestine.[4,20,21,22,23]

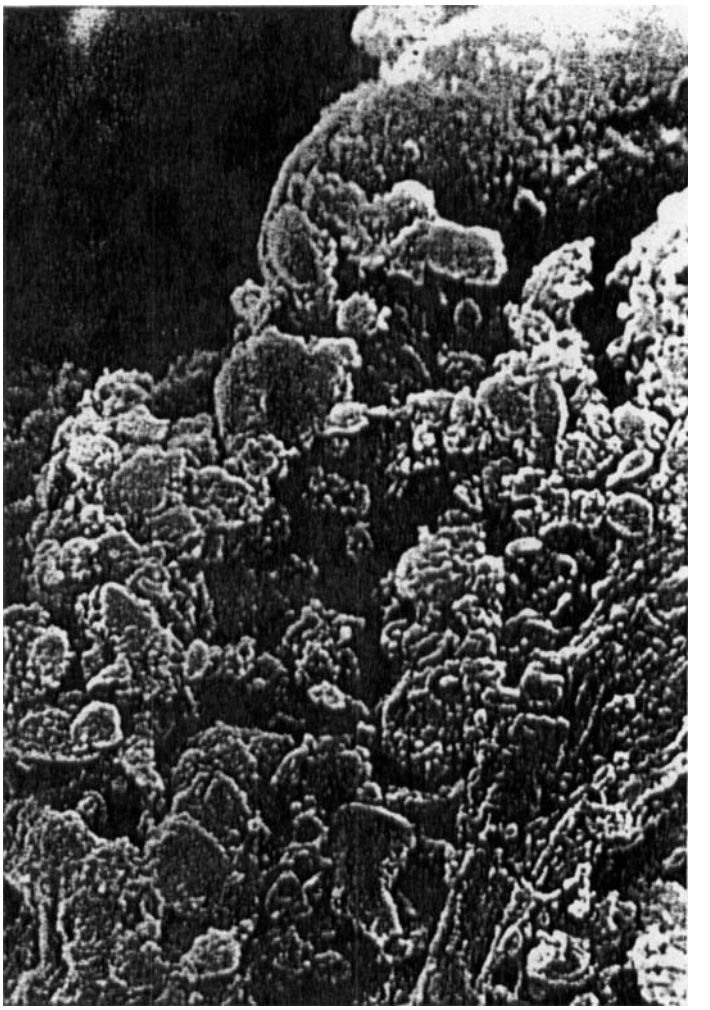

FIGURE 7 Scanning electron microscopy of ileal mucosa. Part of a villi showed the loss of epithelium and the major necrosis of the lamina propria (MEB X 1000).

In the present study, ischemia-reperfusion in the jejunal and ileal mucosa was associated with the formation of severe lesions.

The cytotoxic effects of the release of free radicals resulted in a substantial alteration of the villi with gross epithelial lifting, hemorrhage, oedema, blunting and necrosis of the lamina propria and the crypts.[22] Acute inflammation was observed with major invasion of the intestine by neutrophils.[21,22]

Pretreatment of the ischemic rat by superoxide dismutase (15,000 U/kg/iv) and the ginkgolide PAF-antagonist, BN 52021 (20 mg/kg/po), significantly protected against these morphological alterations. No infiltration of neutrophils was observed and the erosion was only superficial.

Although a number of studies have implicated superoxide radicals in the ischemic injury, the sources of superoxide remains unclear. Studies using xanthine oxidase

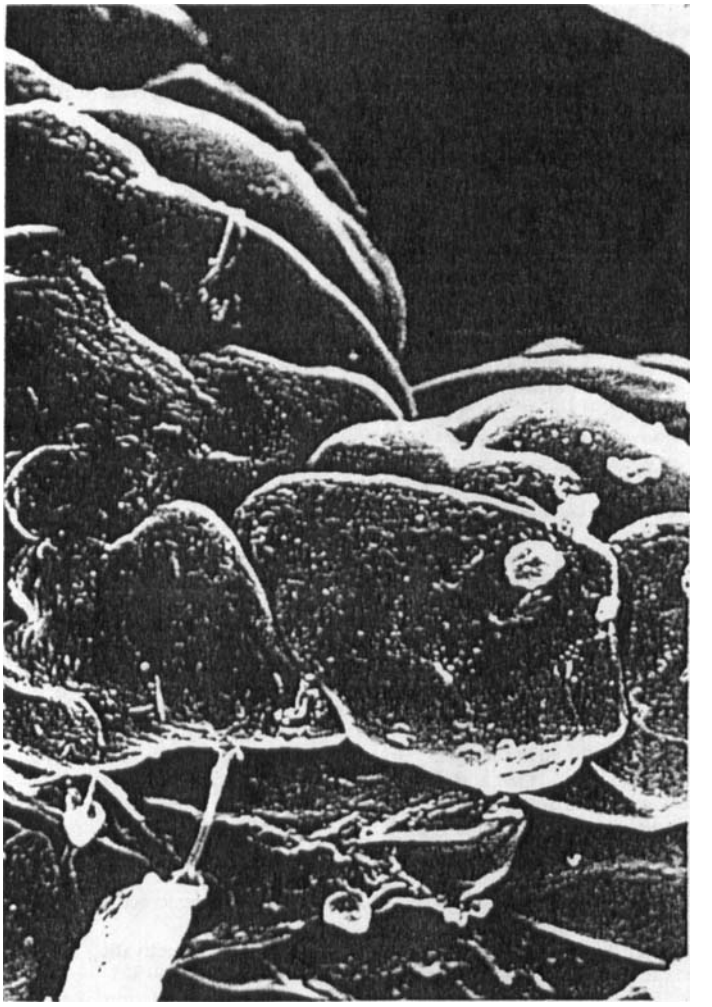

FIGURE 8 Scanning electron microscopy of jejunal mucosa. Part of the villi showed the protective effect of a treatment with BN 52021 (20 mg/kg/po). Only a slight cell exfoliation occured (MEB X 800).

inhibitors such as allopurinol or antioxidants enzymes such as superoxide dismutase have identified xanthine oxidase as a source of oxygen free radicals.[9,23,24]

Another source appears to be the extensive neutrophil influx. Data indicate that neutrophils may contribute to the injury in ischemic disorders. Activated neutrophils release superoxide and hydrogen peroxide which rapidly interact in the presence of transition metals such as iron to form hydroxyl radicals which induce lipid peroxidation and injury of the mucosa.[9,25-27]

PAF, a potent agent of inflammation could play a major role in the ischemic mucosa both by increasing the neutrophil infiltration and by priming the release of superoxide and hydrogen peroxide by these cells.[16]

Our findings showing a substantial protective effect with both SOD (15,000 U/kg/iv) and BN 52021 (20 mg/kg/po) provide support for the role of neutrophils in the

injury process. Inhibition of the inflammatory reaction appears to prevent the intestinal damage induced by ischemia-reperfusion.

In conclusion, these results confirm the role of oxygen-derived free radicals in the pathophysiology of ischemia-reperfusion.

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