

Involvement of neutrophils in postischaemic damage to the small intestine

M H Schoenberg, B Poch, M Younes, A Schwarz, K Baczako, C Lundberg, U Haglund, H G Beger

Abstract

Haemorrhagic mucosal lesions are produced during intestinal ischaemia and after reperfusion probably mediated by oxygen radicals. Oxygen radicals react with cell membrane lipids and induce cell damage by peroxidation and induce accumulation of polymorphonuclear leucocytes in the tissue. The aim of the study was to elucidate the involvement of polymorphonuclear leucocytes in post-ischaemic intestinal damage. Intestinal ischaemia was induced in cats by partial occlusion of the superior mesenteric artery. Samples from the small intestine were excised before and at the end of the two hour hypotensive period as well as 10 minutes and 60 minutes after reperfusion. Conjugated dienes, myeloperoxidase, and the purine metabolites were determined in the samples. The tissue was also examined histologically. Seven cats were treated before reperfusion with a monoclonal antibody (IB4) which inhibits leucocyte adherence to endothelial cells and its subsequent activation. After reperfusion myeloperoxidase activity increased and the ischaemic mucosal lesions worsened significantly. IB4 treatment prevented an increase in posthypotensive myeloperoxidase activity and attenuated the normally observed severe mucosal lesions. We conclude that the severe postischaemic lesions are induced by polymorphonuclear leucocytes. Such mucosal injury may be appreciably reduced by blocking leucocyte adherence with IB4.

Department of Surgery
M H Schoenberg
B Poch
A Schwarz
H G Beger

and Department of
Pathology, University
Ulm, Ulm, Germany
K Baczako

Department of
Toxicology,
Bundesgesundheitsamt,
Berlin, Germany
M Younes

Department of
Inflammation Research,
Pharmacia AB, Uppsala,
Sweden
C Lundberg

Department of Surgery,
University Uppsala,
Uppsala, Sweden
U Haglund

Correspondence to:
Dr H G Beger, Department of
Surgery, University Ulm,
Steinhöfelstrasse 9, 7900 Ulm,
Germany.

Accepted for publication
25 September 1990

Haemorrhagic mucosal injury in the small intestine is often seen in animals subjected experimentally to various shock protocols.¹ Similar lesions have been observed in the intestine of patients suffering from shock and hypotension.² The mucosal damage is believed to be important for the further development of shock.^{3,4} There is evidence that the damaged intestine releases cardiodepressive substances into the circulation, thus initiating a vicious circle which ultimately leads to irreversible circulatory deterioration. Morphological studies show that after a period of hypoxia of the intestinal tissue mucosal injury is produced not only during intestinal ischaemia but also after reperfusion.^{5,6}

There are now substantial experimental data indicating that the postischaemic tissue damage is caused by oxygen derived free radicals.⁵ These highly reactive oxygen metabolites may initiate the peroxidation of membrane lipids and the subsequent release of chemoattractants, leading

to the accumulation of polymorphonuclear leucocytes in the tissue.⁷

Two possible disease mechanisms are suggested to explain the generation of oxygen radicals after ischaemia and reperfusion. The enzyme xanthine oxidase is believed to be the primary source of these reactive oxygen metabolites. This concept was supported by experimental studies showing that inhibition or inactivation of xanthine oxidase protected the intestinal mucosa from the postischaemic damage normally observed.⁸⁻¹⁰

The second potential source of oxygen radicals are activated polymorphonuclear leucocytes (neutrophils) which accumulate after reperfusion in the capillaries and venules of the tissue.¹¹ Stimulation of these neutrophils – for example, by phagocytosis – induces the release of large amounts of reactive oxygen species and other non-oxidative toxins.

Romson and coworkers were the first to propose a role for neutrophils in generating oxygen radicals after ischaemia and reperfusion of the myocardium.¹² They were able to reduce the infarct size appreciably either by pretreatment with superoxide dismutase and catalase or by neutrophil depletion with antisera.

Recently, Grisham *et al* showed that reperfusion of the ischaemic intestine resulted in a pronounced increase in neutrophil accumulation and subsequent activation. This influx of neutrophils seems to depend on oxygen radicals generated by xanthine oxidase since pretreatment with superoxide dismutase and allopurinol prevented this response.¹³

In order to determine the involvement of neutrophils in the development of microvascular injury, Hernandez *et al*¹⁴ pretreated cats with antineutrophil serum or a monoclonal antibody specific for the β chain of the CD11/CD18 complex (MoAb 60.3) preventing neutrophil adherence and extravasation. Both neutropenia and inhibition of neutrophil adherence attenuated the increase of microvascular permeability otherwise induced by ischaemia and reperfusion. These findings suggest that activated neutrophils which accumulate in the intestine after reperfusion might mediate the microvascular damage normally observed.¹⁴

The purpose of this study was (i) to determine to what extent adherence and accumulation of polymorphonuclear leucocytes are involved in the development of intestinal mucosal damage and (ii) to assess whether preventing neutrophil adherence influences the increase of lipid peroxidation and the changes in the purine metabolism normally observed during ischaemia and after reperfusion.

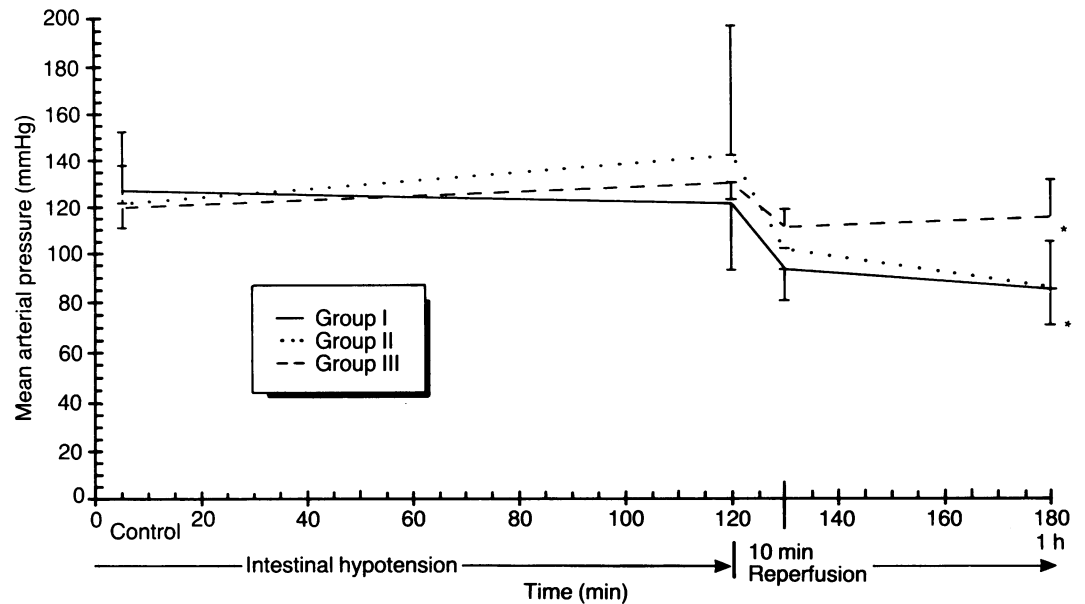


Figure 1: Development of the systemic mean arterial pressure during intestinal ischaemia and reperfusion. * $p < 0.01$ compared with control mean arterial pressure.

This study was performed using a feline model of two hours of intestinal ischaemia and one hour of reperfusion. The adherence of neutrophils was inhibited by a monoclonal antibody (IB4) directed against the β chain (CD18) of the leucocyte glycoprotein CD11/CD18. The monoclonal antibody was administered during intestinal ischaemia and shortly before reperfusion.

Methods

Fourteen cats of both sexes weighing 1.5–3 kg were used. They were fasted for 24 hours before the experiment. After anaesthesia with ketamine-HCl (Ketanest, Parke-Davis, Munich, Germany) at a dose of 10 mg/kg body weight and xylazine (Rompun, Bayer AG, Leverkusen, Germany) at a dose 2.5 mg/kg body weight, the cats were placed in a supine position on a heating pad. After cannulation of the left femoral vein and artery the animals were tracheotomised and ventilated artificially. The duodenum, the spleen, the omentum, and the colon were extirpated to ensure that the small intestine was only perfused via the superior mesenteric artery and vein. Thereafter an adjustable clamp was placed around the artery close to the aorta. The ileocecal artery was cannulated to determine the blood pressure distal to the clamp. To minimise evaporation from the tissue, the small intestine was covered with sterile gauze pads soaked with 37°C saline. A 10% glucose solution containing 10 mmol $\text{NaHCO}_3/100$ ml was slowly infused intravenously (6 ml/h) during the experiment to minimise the effects of dehydration, surgical trauma, and anaesthesia. Systemic arterial and intestinal arterial pressures were measured by Statham pressure transducers (p23A) and recorded on a Hellige recorder (330T).

EXPERIMENTAL PROTOCOL

After the operative preparation and a stabilisa-

tion period of 30 minutes the intestinal arterial inflow pressure was reduced to about 25–30 mm Hg by means of the adjustable clamp around the superior mesenteric artery. After two hours the clamp was released and the ischaemic intestine was reperfused. Then the cats were observed for one hour.

The systemic and intestinal flow pressures were recorded continuously. During the pre-hypotensive period, after two hours of intestinal ischaemia, and 10 minutes and one hour after reperfusion, intestinal tissue samples were excised. Some were randomly selected for histological examinations, others were immediately frozen in liquid nitrogen to determine purine metabolite, myeloperoxidase, and conjugated diene levels in the tissue.

BIOCHEMICAL DETERMINATIONS

Purine metabolites

The tissue samples were stored at -70°C until assayed. They were homogenised at -40°C in 1 M HCl in MeOH and then at 0°C in 0.4 M perchloric acid. The concentrations of adenosine triphosphate, diphosphate, and monophosphate, adenosine, inosine, hypoxanthine, and uric acid were measured by high pressure liquid chromatography using adenosine N-oxide as an internal standard.¹⁵ Protein was determined according to Lowry *et al.*¹⁶

Myeloperoxidase assay

To measure myeloperoxidase activity tissue samples were ground in 10 ml of 0.5% hexadecyltrimethylammonium bromide, freeze thawed, and centrifuged ($1700 \times g$, 30 min, 4°C). Myeloperoxidase, a marker enzyme of polymorphonuclear leucocytes and suitable for determining the accumulation of these leucocytes,¹⁷ was determined in the supernatant by measuring the H_2O_2 dependent oxidation of 3,3',5,5'-

Adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, energy charge, and hypoxanthine levels in the intestinal tissue before (control) and after ischaemia and reperfusion. Values are mean (SD) nmol/mg protein

	ATP	ADP	AMP	Hypoxanthine	Energy charge
Control group:					
Control	7.93 (0.22)	2.75 (1.01)	0.79 (0.31)	0.08 (0.07)	0.82 (0.05)
2 hours after ischaemia	2.35 (0.31)	2.24 (0.58)	2.56 (0.48)	0.82 (0.35)	0.49 (0.06)
10 minutes after reperfusion	4.37 (1.83)	2.50 (1.58)	1.05 (0.22)	0.66 (0.43)	0.70 (0.06)
1 hour after reperfusion	5.95 (0.91)	2.60 (0.86)	0.99 (0.23)	0.15 (0.20)	0.76 (0.02)
IgG group:					
Control	6.50 (0.16)	2.41 (0.01)	1.10 (0.37)	0.09 (0.08)	0.77 (0.03)
2 hours after ischaemia	2.94 (1.60)	1.71 (0.18)	2.24 (2.18)	0.65 (0.67)	0.57 (0.28)
10 minutes after reperfusion	5.56 (1.46)	1.85 (0.17)	0.81 (0.66)	0.38 (0.33)	0.79 (0.11)
1 hour after reperfusion	5.95 (1.93)	2.10 (0.86)	0.80 (0.05)	0.28 (0.18)	0.79 (0.01)
IB4 group:					
Control	6.25 (1.19)	2.00 (0.68)	0.59 (0.39)	0.09 (0.13)	0.82 (0.05)
2 hours after ischaemia	3.74 (0.42)	1.89 (0.33)	1.38 (0.40)	0.71 (0.55)	0.61 (0.14)
10 minutes after reperfusion	5.03 (0.91)	1.96 (0.62)	0.81 (0.32)	0.34 (0.33)	0.77 (0.05)
1 hour after reperfusion	4.79 (1.33)	1.72 (0.50)	0.89 (0.59)	0.25 (0.32)	0.77 (0.05)

tetramethylbenzidine (TMB)¹⁸ spectrophotometrically at 650 nm. The reaction mixture for analysis consisted of 25 µl tissue sample, 25 µl TMB (final concentration 0.16 mM; Sigma, St Louis, MO, USA) dissolved in dimethylsulphoxide and 200 µl H₂O₂ (final concentration 0.24 mM; Merck, Darmstadt, Germany) diluted in 0.08 M phosphate buffer with a pH 5.4. The mixture was incubated at 37°C for five minutes and the reaction stopped with 25 µl bovine catalase (final concentration: 13.6 µg/ml; Boehringer-Mannheim, Mannheim, Germany). To ensure linearity of the reaction during this time period, myeloperoxidase standards were included in each assay (myeloperoxidase of human leucocytes, 0.004–0.5 U/ml; Green Cross Corporation, Osaka, Japan). One unit of myeloperoxidase activity was defined as the amount of enzyme reducing 1 µmol peroxide/min.

Conjugated dienes

Before and after intestinal hypotension and 10 minutes and one hour after reperfusion tissue concentrations of conjugated dienes were deter-

mined using the method described by Buege and Aust.¹⁹

HISTOLOGICAL EXAMINATION

After fixation in 5% formalin, intestinal tissue samples were embedded in paraffin, cut at 4 µm, and stained with haematoxylin and eosin. The slides were coded and examined for morphological changes in a blinded manner. The mucosal lesions of the small intestine were graded according to Chiu *et al.*²⁰

Treatment

The animals were divided randomly into three groups and treated 10 minutes before the stenosing clamp around the superior mesenteric artery was released by an intravenous injection as follows: group I: 10 ml of saline (n=7); group II: 1 mg/kg body weight of mouse IgG (Sigma, St Louis, MO, USA) diluted in 10 ml of sterile saline (n=3); group III: 1 mg/kg of IB4 diluted in 10 ml of sterile saline (n=7). IB4 is a murine monoclonal antibody (IgG_{2a}), directed to the β chain (CD18) of the human leucocyte adhesion glycoprotein complex CD11/CD18. This monoclonal antibody was produced as described previously.^{21,22}

Endotoxin levels in the IB4 and mouse IgG solution were analysed using the Coatest (Kabi-Vitrum, Stockholm, Sweden) and were 0.8 and 10 energy units/mg protein, respectively.

STATISTICAL METHODS

The results are presented as means (SD). The data were evaluated statistically by the Institute of Biomathematics and Statistics, University of Ulm. Differences in purine metabolites, myeloperoxidase, and conjugated dienes were tested pairwise for significance according to the Mann-Whitney U test and re-evaluated by Student's *t* test. The histological data were tested according

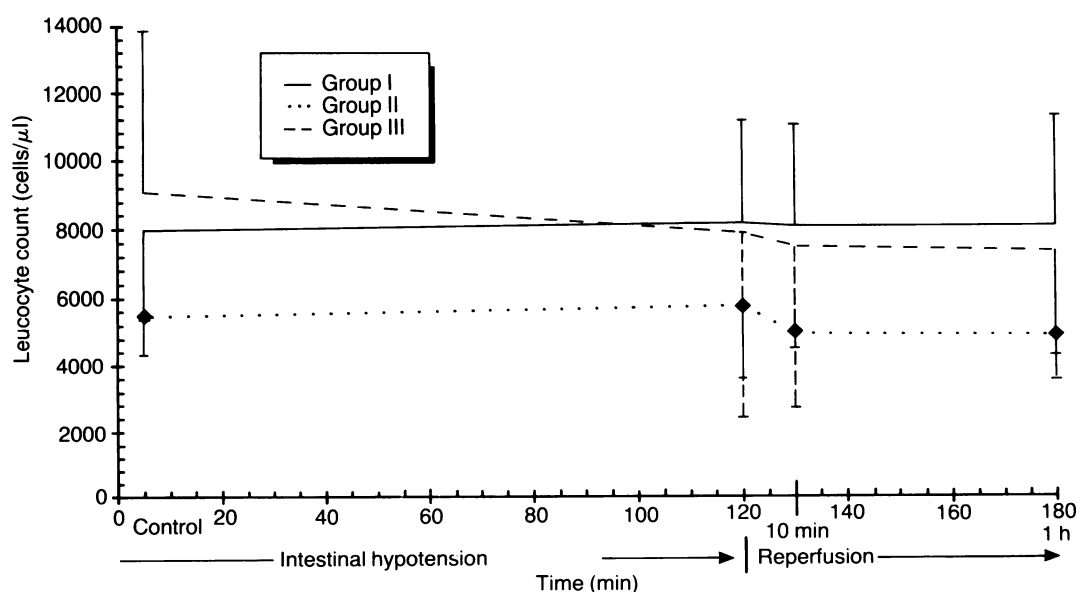


Figure 2: Peripheral blood leucocyte count in untreated, IB4 treated, and IgG treated cats during the experiment.

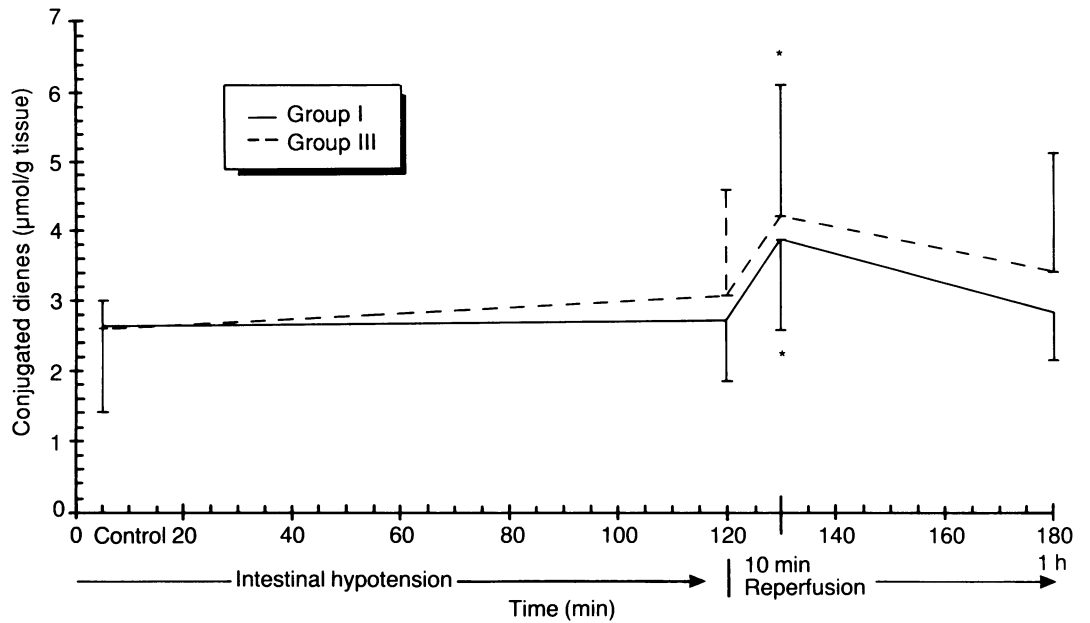


Figure 3: The changes in conjugated diene formation during ischaemia and reperfusion in groups I and III. * $p < 0.05$ and $p < 0.02$ compared with the corresponding control.

to the Wilcoxon unpaired signed rank test. In all cases p values less than 0.05 were considered significant.

In all three groups the number of circulating neutrophils in the blood were in the same range and did not change during the experiment (Fig 2).

Results

The basal mean (SD) arterial pressure in group I was 128 (31) mm Hg. It remained stable throughout the phase of intestinal ischaemia but decreased significantly after reperfusion ($p < 0.01$). One hour after reperfusion it amounted to 84 (14) mm Hg (Fig 1). The development of mean arterial pressure in group II was similar. Basal pressure in the IB4 treated cats (group III) was 120 (9) mm Hg. In contrast to the other groups, the pressure in this group did not decrease during reperfusion. One hour after reperfusion mean arterial pressure reached 115 (16) mm Hg (Fig 1).

BIOCHEMICAL DETERMINATIONS

During the regional hypotension the adenosine triphosphate concentration in the intestinal tissue in control cats (group I) dropped from (mean (SD)) 7.93 (0.22) nmol/mg protein to 2.35 (0.31) – that is, 30% of the initial level. At the same time the intestinal adenosine monophosphate concentration increased from 0.79 (0.31) to 2.56 (0.48), whereas the adenosine diphosphate concentration remained unchanged (Table). Consequently, the energy charge²³ dropped to 60% of its initial value.

During intestinal hypotension the concentra-

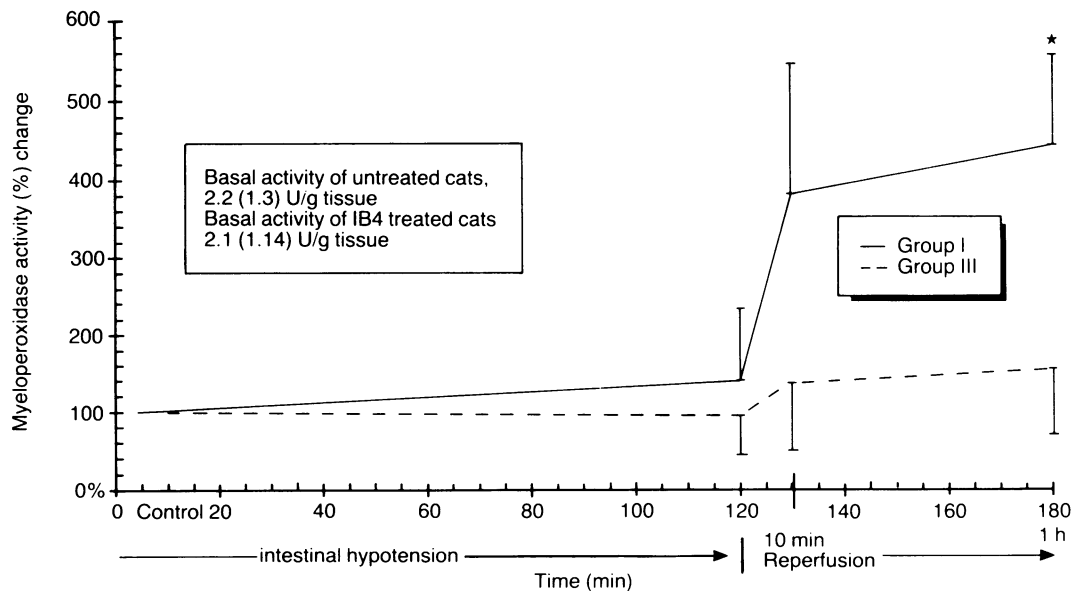


Figure 4: Relative changes of myeloperoxidase activity in untreated and IB4 treated cats. * $p < 0.01$ compared with the corresponding control levels.

tions of hypoxanthine and uric acid in the tissue increased from 0.08 (0.07) to 0.82 (0.35) and from 0.09 (0.04) to 1.71 (0.57) nmol/mg protein, respectively. After reperfusion the hypoxanthine levels decreased significantly and reached 0.15 (0.20) nmol/mg protein one hour after release of the clamp.

The uric acid concentrations remained high. Concomitantly, adenosine triphosphate increased and adenosine monophosphate decreased towards the control concentrations without reaching them, leading to an increase of the energy charge to almost 90% of its control level.

The adenosine triphosphate, diphosphate, and monophosphate concentrations in the tissue of cats treated with IB4 showed the same changes during intestinal hypotension as those of group I. The energy charge decreased to 74% of its initial level. Concomitantly, the concentrations of hypoxanthine and uric acid increased eightfold and 18 fold, respectively. After reperfusion the adenosine triphosphate and monophosphate concentrations returned to their basal concentrations and the energy charge increased to 94% of its control value. At the same time the hypoxanthine concentrations decreased appreciably to 0.25 (0.32) nmol/mg protein one hour after reperfusion (Table).

The purine metabolites in the intestinal tissue of group II showed the same changes during intestinal ischaemia and reperfusion as described for groups I and III (Table).

The tissue concentrations of conjugated dienes in group I remained unchanged during intestinal ischaemia (Fig 3). Ten minutes after reperfusion, however, the conjugated diene levels increased slightly, yet significantly, from 2.64 (0.35) to 3.88 (1.29) $\mu\text{mol/g}$ tissue ($p < 0.05$) and returned to their basal levels one hour after reperfusion (Fig 3). A similar development was observed for the cats in group II (data not shown). Also in the IB4 treated cats the conjugated diene levels increased from 2.6 (1.2) during the prestenotic phase to 4.2 (1.9) $\mu\text{mol/g}$ tissue ($p < 0.02$) shortly after reperfusion and returned thereafter towards the basal concentrations again (Fig 3).

The tissue associated myeloperoxidase activity in groups I and III did not change during intestinal hypotension. Ten minutes after release of the clamp, however, the activity in group I increased significantly from 2.17 (1.3) to 4.6 (1.2) and reached 7.2 (2.5) U/g tissue one hour after reperfusion (Fig 4). Myeloperoxidase activity in the cats treated with mouse IgG (group II) increased approximately sixfold after reperfusion compared with their control activity (data not shown).

In contrast, IB4 treatment prevented the increase of myeloperoxidase after reperfusion. The activity remained in the range of the control level (basal activity 2.1 (1.2) U/g tissue; one hour after reperfusion 2.6 (0.6) U/g tissue) (Fig 4).

MORPHOLOGICAL CHANGES

The specimens taken before hypotension showed a normal small intestinal mucosa in all cats. Astonishingly little damage was observed

after two hours of intestinal hypotension (Fig 5). The villous layer seemed slightly oedematous in four out of seven cats in group I and in three out of seven cats in group III. The other animals presented a normal intestinal mucosa. Significant differences between the experimental groups were not found (Fig 5).

Ten minutes after reperfusion the mucosal layer in six of seven cats in group I became severely oedematous and lifting of the epithelial layer from the tips of the villi was found in five cats.

One hour after release of the stenosis a further aggravation of damage was evident in group I. The villi were completely denuded from their epithelial layer; in five of seven animals haemorrhagic ulceration in the mucosa was observed. This degree of injury corresponds to grade 4 or 5 on the scale designed by Chiu *et al*²⁰ (Fig 5). A fairly similar development of mucosal lesions was observed for group II (data not shown).

The IB4 treatment in group III did not prevent the mucosal alterations seen after intestinal hypotension and 10 minutes after reperfusion (Fig 5). One hour after release of the adjustable clamp the mucosal lesions were moderately worse. They did not, however, show the same severe damages as seen in the untreated cats ($p < 0.01$) (Fig 5). The villi were oedematous and the epithelial layer had lifted from the tips of the villi. Complete loss of the villi and haemorrhagic ulcerations of the mucosal layer, however, were not seen. The median grade in this phase was 2 to 3 (Fig 5).

Discussion

Reactive oxygen metabolites are considered to be important mediators of microvascular injury and mucosal damage associated with reperfusion after a short duration of and incomplete intestinal ischaemia.^{7, 24, 25} The initial source of oxygen radicals is believed to be the hypoxanthine-xanthine oxidase system. In fact competitive inhibition of this enzyme by treatment with allopurinol, pterin aldehyde, folic acid, and a tungsten supplemented molybdenum deficient diet attenuated the morphological and functional alterations of the intestine normally observed after intestinal ischaemia and reperfusion.^{9, 26, 27}

The reactive oxygen metabolites, especially the hydroxyl radical, which is produced by the superoxide radical and hydrogen peroxide in the presence of active catalytic iron, attack most readily polyunsaturated fatty acids thus inducing lipid peroxidation.⁷ Since polyunsaturated fatty acids are present in high concentrations in the cellular membrane peroxidation of these membrane constituents lead to disintegration of the cells and irreversible cell and tissue damage.⁷ In addition, in the endothelial layer of the capillaries and venules the radical reactions may result in microvascular injury leading to an increased permeability with extravasation of plasma and even erythrocytes.²⁸

In our experiments the increased formation of conjugated dienes, an indirect measure for lipid peroxidation, 10 minutes after reperfusion supports this concept. Moreover, this increase in lipid peroxidation products was paralleled by a

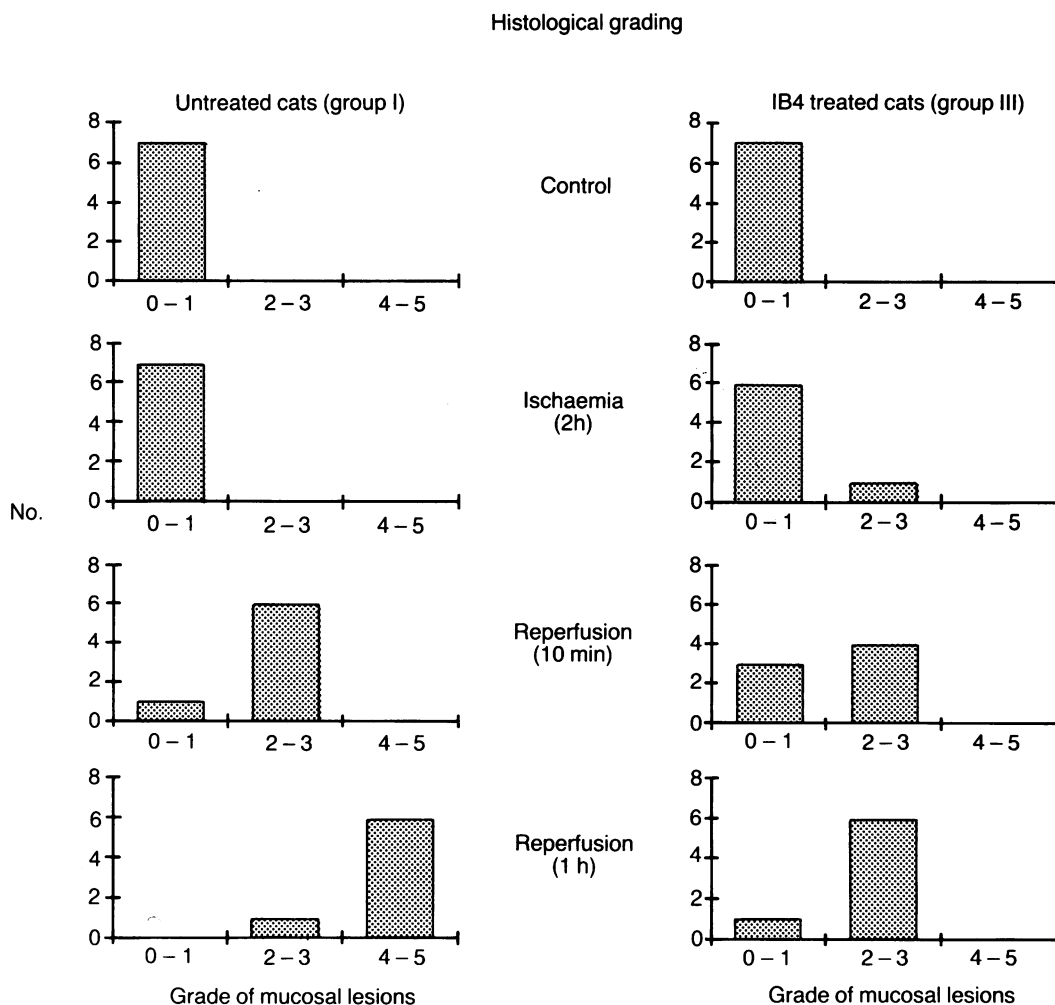


Figure 5: Grade of small intestinal mucosal damage before and after two hours of ischaemia, as well as 10 minutes and one hour after reperfusion (grades according to Chiu *et al.*²⁹).

moderate worsening of the histological damage in the intestinal mucosa. One hour after reperfusion the conjugated dienes had returned to the normal range whereas the histological lesions worsened, showing pronounced epithelial lifting, disintegration of the lamina propria, and haemorrhagic ulcerations.²⁹

Another potential source of oxygen radical generation during reperfusion of ischaemic tissues is activated neutrophils. It has been shown that during ischaemia and after reperfusion neutrophils accumulate in the capillaries and venules of the damaged tissue.¹¹ These neutrophils adhere to the capillary wall ('leucocyte sticking') and in some cases even plug the entire vessel, which adds to microcirculatory derangements observed in the reperfusion phase.³⁰ The adherence of the neutrophils to the endothelium of the vessels is a prerequisite for accumulation of these cells and subsequent damage in microvasculature.³¹ If activated, neutrophils secrete various enzymes such as myeloperoxidase, elastase, neutral and acid proteases, and prostaglandins and leucotrienes. Furthermore, neutrophils liberate oxygen radicals as byproducts of phagocytosis or of an interaction with certain ligands with plasma membrane receptors.³² These various enzymes and metabolites can induce severe tissue injury. It has been shown that the enzyme myeloperoxidase catalyses the oxidation of Cl^- by

H_2O_2 to yield hypochlorous acid (HOCl), a highly cytotoxic oxidant.³³ Moreover, tissue associated myeloperoxidase activity is directly proportional to the number of neutrophils seen in histological sections of the intestinal mucosa and is regarded therefore as a reliable index for neutrophil infiltration.³⁴

In our study the myeloperoxidase activity in the mucosa remained unchanged during ischaemia but increased three to fourfold after reperfusion. Concomitantly, the mucosal damage was significantly worse. This observation reconfirms partly the results previously reported by Grisham *et al.*¹³ These workers showed that after reperfusion of the ischaemic gut the myeloperoxidase activity in the mucosa increased 18-fold compared with control levels. In an attempt to determine the relation between enhanced oxygen radical generation and polymorphonuclear leucocyte accumulation Grisham and coworkers pretreated the cats with superoxide dismutase or allopurinol¹³ and, more recently, with catalase, deferoxamine, or dimethylthiourea, a hydroxyl radical scavenger.^{27,35} Each pretreatment attenuated the increase of myeloperoxidase in the mucosa, suggesting that post-ischaemic accumulation of polymorphonuclear leucocytes was almost completely prevented. Furthermore, the results suggest that oxygen radicals generated by the hypoxanthine-xanthine oxidase metabolism induce the post-

ischaemic infiltration of neutrophils in the intestine.

The exact pathogenetic mechanism governing the interaction between oxygen radicals and granulocyte infiltration, however, is still unknown. Petrone *et al* showed in vitro that superoxide radicals generate or activate a 'chemotactic factor' in human plasma.³⁶ The structure of this chemotactic factor, however, has not been elucidated yet. Moreover, these in vitro results could not be reproduced in vivo. Exposing feline plasma to superoxide by various methods, Zimmerman *et al* could not show enhanced chemotactic activity of the feline extracellular fluid thereafter.³⁵

It has been shown recently that the membrane associated glycoprotein complex CD11/CD18 plays a crucial part in the adherence of neutrophils to the endothelium.³¹ Monoclonal antibodies recognising CD18 inhibit neutrophil adhesion to and chemotaxis across cultured endothelium.³¹ Arfors *et al* showed that pretreatment of rabbits with an anti-CD18 monoclonal antibody (MoAb 60.3) prevented neutrophil accumulation and extravasation of albumin induced by intradermal injection of the chemoattractants C5a and *N*-formyl-methionyl-leucyl-phenylalanine.³⁷ Moreover, the same workers showed by intravital microscopy of the tenuissimus muscle in the rabbit that monoclonal antibody treatment prevented leucocytes sticking in the venules after topical application of leukotriene B4 and zymosan activated plasma.

Hernandez *et al* pretreated cats with an anti-CD18 monoclonal antibody (MoAb 60.3) and induced intestinal hypotension for one hour. They showed that this treatment significantly prevented the postischaemic increase in microvascular permeability.¹⁴ Similar results were found earlier by neutrophil depletion, superoxide dismutase, catalase, and other oxygen radical scavengers.²⁷

In our study we used the monoclonal antibody IB4 which, similar to MoAb 60.3, recognises the β chain (CD18) in CD11/CD18 adhesion complex and inhibits neutrophil adhesion. In contrast to Hernandez *et al*¹⁴ we treated the cats at the end of the hypotensive period but shortly before reperfusion, which more closely corresponds to the clinical situation.

It was shown recently by intravital microscopy in cats that IB4 attenuated the adherence of neutrophils to the mesenteric venules after intestinal ischaemia and reperfusion³⁸ and after intra-arterial infusion of the platelet activating factor into the superior mesenteric artery.³⁹ Moreover, IB4 significantly prevented the concomitant increase in microvascular permeability of the intestinal mucosa due to infusion of platelet activating factor.³⁹

In our study IB4 treatment prevented the increase of myeloperoxidase during the reperfusion phase, indicating that neutrophils did not accumulate in the intestinal mucosa. The conjugated diene levels, however, increased 10 minutes after release of the clamp. Apparently, the formation of lipid peroxidation products in this early phase of reperfusion seems to be induced by oxygen radicals generated independently from accumulated neutrophils. The

source of oxygen radicals in this phase is most likely the hypoxanthine-xanthine oxidase system.²⁴ In support of this it was shown previously that the competitive inhibition of the latter enzyme by allopurinol prevents the increase of conjugated dienes in the intestinal tissue 10 minutes after reperfusion.⁹

In the early reperfusion phase the mucosal lesions were moderately worse in the IB4 treated animals but to the same extent as in the untreated controls. One hour after reperfusion, however, IB4 treatment prevented the development of severe mucosal lesions normally seen. But, in contrast to previous reports,¹⁴ treatment with the monoclonal antibodies IB4 given shortly before reperfusion did not provide the same degree of protection as superoxide dismutase treatment in our earlier experiments.⁶

Available data suggest that the early formation of oxygen radicals induce moderate mucosal lesions by lipid peroxidation. The subsequent more severe alterations of the mucosa may be mediated by the influx and activation of neutrophils. Moreover, the present results suggest that neutrophil adherence is instrumental in the development of postischaemic mucosal injury to the intestinal tissue.

Two mechanisms are suggested: (i) the accumulation and adherence of granulocytes enhance the ischaemic insult by plugging the microvasculature, as mentioned above,¹¹ and (ii) the release of cytotoxic substances by granulocytes upon activation.³²

Treatment with the monoclonal antibody IB4 was given at the end of the hypotensive phase, which excludes a possible influence during ischaemia. During reperfusion the fast and almost identical replenishment of energy rich phosphates in the untreated, IgG treated, and IB4 treated animals suggests that the microvascular perfusion of the intestine did not differ among the three groups. It seems unlikely, therefore, that the protective effect of IB4 in this series could be attributed to an improved microcirculatory perfusion and reoxygenation during the reperfusion period, thus favouring the release of oxidative and non-oxidative toxins on activation of neutrophils as the most important mechanism. Further studies are needed to try to answer this question. Moreover, it remains unclear if IB4 exerts its beneficial effects by protecting the microvasculature or by preventing neutrophil accumulation in the mucosal interstitium.

The treatment with IB4 not only attenuated the mucosal damages, it also prevented the decrease in systemic mean arterial pressure which is usually observed after reperfusion in untreated animals. In a similar experimental model Haglund *et al* have shown that both the decrease in mean arterial pressure and impairment of cardiac function seen after reperfusion correlated with the extent of mucosal lesions in the gut.⁴ They attributed these macrocirculatory derangements to the release of cardiodepressant factors from the damaged gut. The results in this series suggest, therefore, that IB4 treatment, possibly due to the protection against the severe postischaemic mucosal injury, prevented the release of cardiodepressant substances from the

intestine after reperfusion. Moreover, Vedder *et al* recently showed that pretreatment with MoAb 60.3 resulted in an appreciable reduction in multiple organ failure and improvement in survival rates after haemorrhagic shock.⁴⁰

Many questions remain unanswered and therefore warrant future studies. Nevertheless, treatment with monoclonal antibody inhibiting the adherence and subsequent accumulation of granulocytes is a new and promising concept for treatment of intestinal and other postschaemic disorders without inducing neutropenia.

The technical assistance of Mrs M Habdank and Mr M Marzinzig is gratefully acknowledged. This study was supported by grants of the Deutsche Forschungsgemeinschaft (DFG) project SCHO 309/1-3.

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