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EFFECT OF CATECHOLAMINE TOLERANCE ON INTESTINAL ISCHEMIA-REPERFUSION: AN EXPERIMENTAL STUDY

By
Mahmoud F. Sakr[1], M.D., Nahed M. Baddour[2], M.D.
Department of Surgery[1] and Department of Pathology[2] Faculty of Medicine, University of Alexandria, Egypt

Background: *Adrenaline tolerance improves survival in animal models of shock. The purpose of the present study was to determine the effects of adrenaline tolerance on intestinal ischemia-reperfusion (IIR) in a rat model.*

Materials and Methods: *Adrenaline tolerance was developed by injecting adrenaline intravenously (IV), gradually increasing the dose from 0.05 mg/kg to 2 mg/Kg at 5 days. In experimental animals, the intestine was subjected to ischemia for one hour and then reperfusion for 3 hours. Evans blue was given IV to all animals to quantify pulmonary microvascular injury. After reperfusion, animals were sacrificed and the effects of reperfusion were assessed by measuring tissue myeloperoxidase (MPO), malonyldialdehyde (MDA), liver neutrophil sequestration and serum alanine aminotransferase (ALT). Intestinal injury was also assessed by a histological mucosal injury score.*

Results: *Evans blue dye concentrations were significantly higher in animals with IIR than in those having sham IIR or in AT-rats having IIR or sham IIR (P<0.01). MPO levels were significantly lowered in the lung by adrenaline tolerance (P<0.05). MDA levels significantly increased in the lung, liver and intestine of the IIR group compared with those in the sham IIR groups (P< 0.01), whereas there was no difference in adrenaline-tolerant animals. Adrenaline tolerance significantly reduced PMN sequestration within the liver and serum ALT levels as compared to the IIR group (P<0.05), which were significantly higher than the sham IIR group. The intestinal mucosal injury scores increased significantly in IIR animals as compared with sham IIR animals (P<0.01). Adrenaline tolerance did not reduce this injury.*

Conclusions: *The present study indicates that IIR leads to local and remote organ dysfunction, catecholamines are involved in the IIR insult, and induction of adrenaline tolerance has a possible protective effect, though the local injury appears to be independent of catecholamines.*

Key Words: *Catecholamine – ischemia – reperfusion – small bowel – tolerance.*

INTRODUCTION

The release of catecholamines during various shock states reduces mesenteric vascular perfusion. Reperfusion of ischemic bowel after hemorrhagic shock or intestinal ischemia as in small bowel transplantation, has been shown to cause both microvascular and mucosal dysfunction within the small bowel[1-3] and remote organ injury[4], mainly by neutrophil priming, and release of free radicals. It has previously been reported in several studies that adrenaline-tolerant dogs had improved survival from hemorrhagic, endotoxic and cardiogenic shock[5-9]. It is conceivable that adrenaline tolerance may also affect intestinal ischemia-reperfusion (IIR) injury by interfering with neurtrophil priming or free radical formation.

The purpose of the present study was to investigate the effect of catecholamine (adrenaline) tolerance on both the local and remote injury that result from intestinal ischemia and reperfusion (IIR) in a rat model.

MATERIALS AND METHODS

Animal Preparation: The present study was performed on 40 adult male Lewis rats weighing 200-250 g. All animals received humane care as defined by The Guide for the Care and Use of Laboratory Animals prepared by

the National Academy of Sciences and published by the National Institute of Health, USA (NIH Publications no. 80-23, revised 1978). Animals were acclimatized to the animal research laboratory for five days before being utilized, and were allowed free access to standard rodent show and water both before and after operation.

Development of Adrenaline Tolerance: Twenty animals were injected with intravenous adrenaline via the tail vein, starting with 0.05 mg/Kg which, was gradually increased to 2 mg/Kg over 5 days. The drug was diluted in 0.4ml saline. Control animals (n=20) were injected with an equivalent volume of saline for 5 days.

Experimental Protocol: After 72 hours of completion of injection, control animals and adrenaline-tolerant rats were each divided into two equal groups: intestinal ischemia-reperfusion (IIR) and sham IIR. Animals that died during induction of adrenaline tolerance or during the surgical procedure were excluded and replaced by others. Thus, eventually, there were four groups with 10 rats in each.

Surgical Procedure / Induction of Intestinal Ischemia-Reperfusion: Following an overnight fast, animals were anesthetized with intraperitoneal pentobarbital sodium (65 mg/Kg). Evans blue dye (30 mg/Kg) was injected intravenously via the tail vein. A midline laparotomy was performed. Collateral vessels from the caudal mesenteric and celiac axis were ligated and the superior mesenteric artery (SMA) was occluded with A Schwartz microvascular non-crushing clip (Roboz Surgical Instruments, Washington, DC). The laparotomy incision was closed and reopened one hour later, then the microvascular clip was removed. Reperfusion was confirmed by the return of pulsations of the mesenteric vascular arcade. The incision was again closed, and all animals were maintained supine for an additional 3 hours of monitoring during reperfusion. At the end of the observation period, the animals were sacrificed with an overdose of pentobarbital sodium. In the sham ischemia-reperfusion groups, the same procedure was performed except for SMA clamping.

Measurement of Pulmonary Permeability: Pulmonary microvascular dysfunction was assessed by measuring the concentration of Evans blue dye within the lung. Evans blue dye binds avidly to albumin and is used as a marker of protein extravasation and inflammatory tissue injury[10,11]. After the 3-hour period of reperfusion and animal sacrifice, tissues were harvested. Pulmonary vessels were emptied of blood by infusing saline into the right ventricle. The lungs were then weighed, placed in 5 ml formamide at 37°C for 16 hours, after which the dye concentration was measured by spectrophotometer at 620 nm and expressed in ng/Kg of wet lung weight.

Determination of Reperfusion Organ Injury: After reperfusion, a portion of the right lobe of the liver, the left lung, and a segment of small intestine were used for analysis. Tissue myeloperoxidase (MPO) was assessed to determine neutrophil infiltration as a marker of severity of intestinal ischemia-reperfusion (IIR). Tissue malonyldialdehyde (MDA), an end-product of lipid peroxidation, was measured as a marker of free radical-induced lipid destruction. Both MPO and MDA were determined spectrophotometrically according to the methods described by Suzuki et al[12] and Mihara and Uchiyama[13], respectively.

Liver Neutrophil Sequestration: Liver biopsies, taken from both right and left lobes were fixed in 10% formalin, dehydrated, embedded in paraffin, cut at 5 μm and stained for histological examination with hematoxylin and eosin (H&E). Microscopic sections were interpreted in a blind fashion. Neutrophil sequestration was expressed as the mean number of PMN per 50 high-power field (HPF). The MPO assay was not used to quantify liver PMN sequestration because liver tissue has been reported to inhibit MPO activity[14].

Assessment of Hepatocellular Injury: The hepatic injury after the 3 hours of reperfusion was quantitated by measurement of serum alanine aminotransferase (ALT) level. Blood (3 ml) was drawn from the right ventricle and assayed for serum ALT concentrations by use of standard clinical automated analysis based on the method of coupling lactic dehydrogenase and reduced nicotinamide adenine nucleotide to the aminotransferase reaction. Results are expressed as international units per liter (U/L).

Assessment of Intestinal Injury: At the end of each experiment, intestinal biopsies were taken at five equidistant points along the length of the bowel. They were immediately fixed in 10% formaldehyde-saline solution (Baxter Health Products, Malvern Park, CA). The fixed tissue was embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The injury was assessed by a mucosal injury score[15]. In a blinded fashion, a minimum of 200 villi per animal were graded histologically for mucosal damage as follows:

1. Grade 0: normal mucosal villi.
2. Grade 1: development of a subepithelial space, usually at the tip of the villus, with capillary congestion.
3. Grade 2: extension of the subepithelial space with moderate lifting of the epithelial layer.
4. Grade 3: massive epithelial lifting down the sides of villi.
5. Grade 4: denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of the lamina propria.

6. Grade 5: digestion and disintegration of the lamina propria, hemorrhage, and ulceration.

Statistical Analysis: Statistical analysis was performed using the SPSS/PC version 8 Computer Software (Prentice Hall, Chicago, IL). Data were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to assess differences in data between the different groups. A "P" value of less than 0.5 was considered to be statistically significant.

RESULTS

Pulmonary Permeability: Pulmonary permeability was significantly greater in rats subjected to IIR without previous induction of adrenaline tolerance (mean Evans blue concentration 229.4 ± 42.6 ng/mg) as compared to other groups (P<0.01) (Fig. 1).

Lung and Intestinal MPO: As shown in Fig. 2, the mean MPO levels in the lung tissue were significantly (P<0.05) higher in the IIR group (190.5 ± 41.6 U/g) than in the sham IIR group (111.4 ± 50.9 U/g) or the adrenaline-tolerant (AT) IIR group (140.2 ± 33.5 U/g). Although MPO levels were also higher in the intestine in the IIR group, the difference was not statistically significant in, or between, normal or AT-animals.

Lung, Liver and Intestinal MDA: The mean MDA levels (nmol/g) were significantly increased (P<0.01) in the lung (65.4 ± 12.1), liver (59.4 ± 7.6), and small bowel (82.6 ± 5.7) in animals belonging to the IIR group, as compared to sham IIR animals (44.6 ± 9.6, 42.6 ± 7.0 and 51.3 ± 7.6 nmol/g, respectively). Both AT-groups had significantly higher MDA levels (P<0.05) than the sham IIR group. In addition, the mean MDA levels in the liver of both AT-groups were significantly higher than in the IIR group (Fig. 3). There was no significant difference, however, in MDA levels between the two AT-groups for all organs.

Liver Leukosequestration: Liver specimens of animals belonging to the IIR groups showed dilated, congested vascular spaces in the portal tracts (Fig. 4a), with increased number of sequestrated neutrophils (Fig. 4b). PMN sequestration within the liver increased from 90±10 PMN/50 HPF in the sham IIR group to 270 ± 30 PMN/50 HPF in the IIR group (P<0.05). In the AT-IIR animals, the PMN sequestration (210 ± 20 PMN/50 HPF) was significantly lower than in the IIR animals (P<0.05) as shown in Fig. 5.

Hepatocellular Injury: After a 60-minute period of intestinal ischemia and three hours of reperfusion, the serum ALT levels rose to 567 ± 67 U/L in animals subjected to IIR compared to 56 c 34 U/L in sham IIR animals (P<0.05). This index of liver injury was significantly reduced to 348 ± 77 U/L in AT-IIR animals (P<0.05) as shown in Fig. 6.

Intestinal Mucosal Injury: After intestinal ischemia and 3 hours of reperfusion, the intestinal mucosal injury scores increased to 2.3 ± 0.16 compared with 0.32 ± 0.04 in sham IIR animals (P<0.01) (Fig. 7). The sections in the IIR group showed extensive development of the subepithelial space with lifting of the epithelial layer from the lamina propria involving both the tips and sides of the villi. Fig. 8a shows denuded villi with exposure of the dilated capillaries in the lamina propria, which showed increased cellularity; changes corresponding to grade 4 injury. These changes were totally absent in sham IIR group (Fig. 8b), and adrenaline tolerance did not reduce this injury (mean score 2.1 ± 0.23 in the AT-IIR group).

Figure 1. Comparison of Pulmonary Permeability

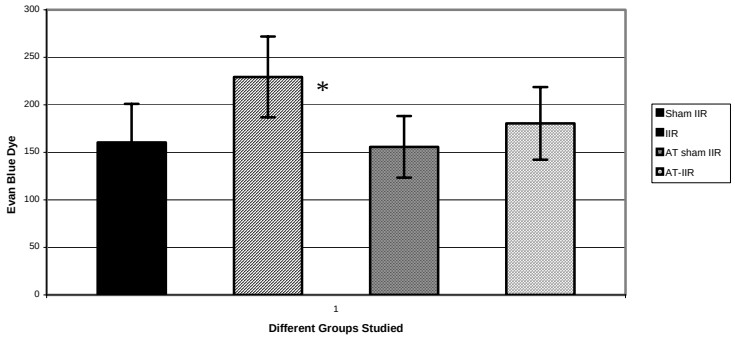

Fig.(1): Comparison of pulmonary permeability.
Values are mean (sd). IIR, intestinal ischemia-reperfusion. AT, adrenaline-tolerant rats. * P<0.01 versus other groups.

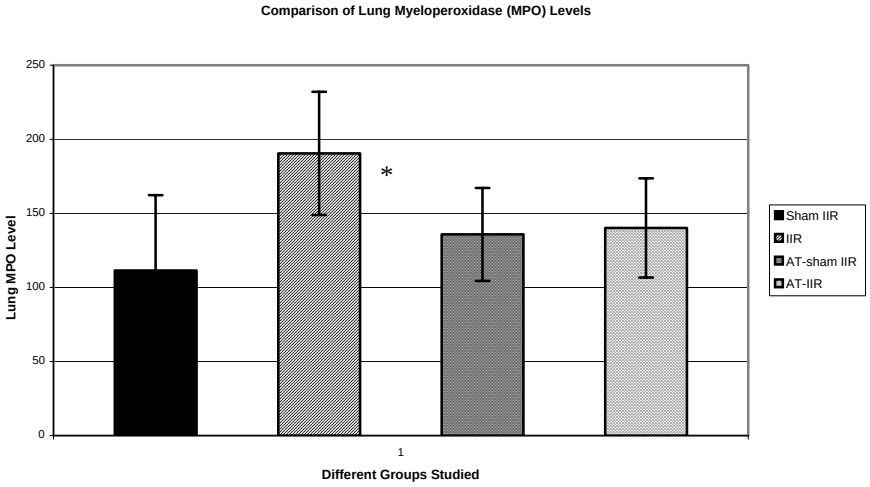

Fig.(2): Comparison of lung myeloperoxidase (MPO) levels.
*Values are mean (sd). IIR, intestinal ischemia-reperfusion. AT, adrenaline-tolerant rats. * P<0.05 versus other groups.*

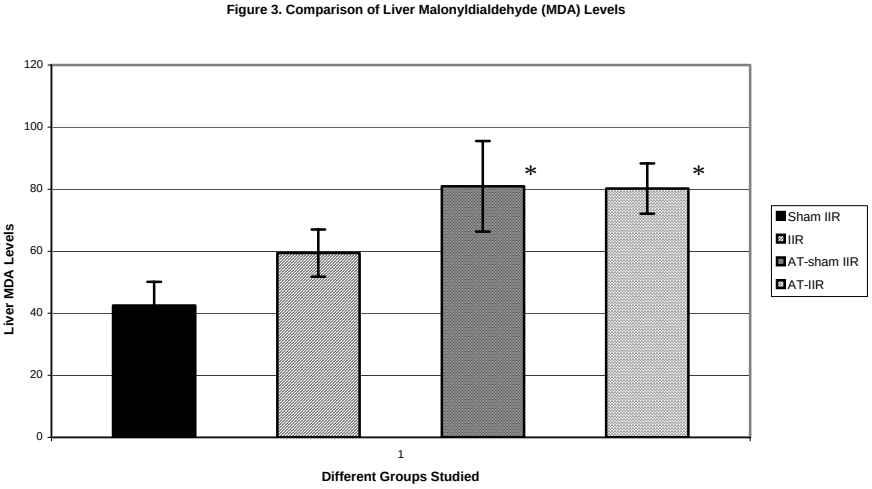

Fig.(3): Comparison of liver malonyldialdehyde (MDA) levels.
*IIR, intestinal ischemia-reperfusion. AT, adrenaline-tolerant rats. * P<0.05 versus sham IIR.*

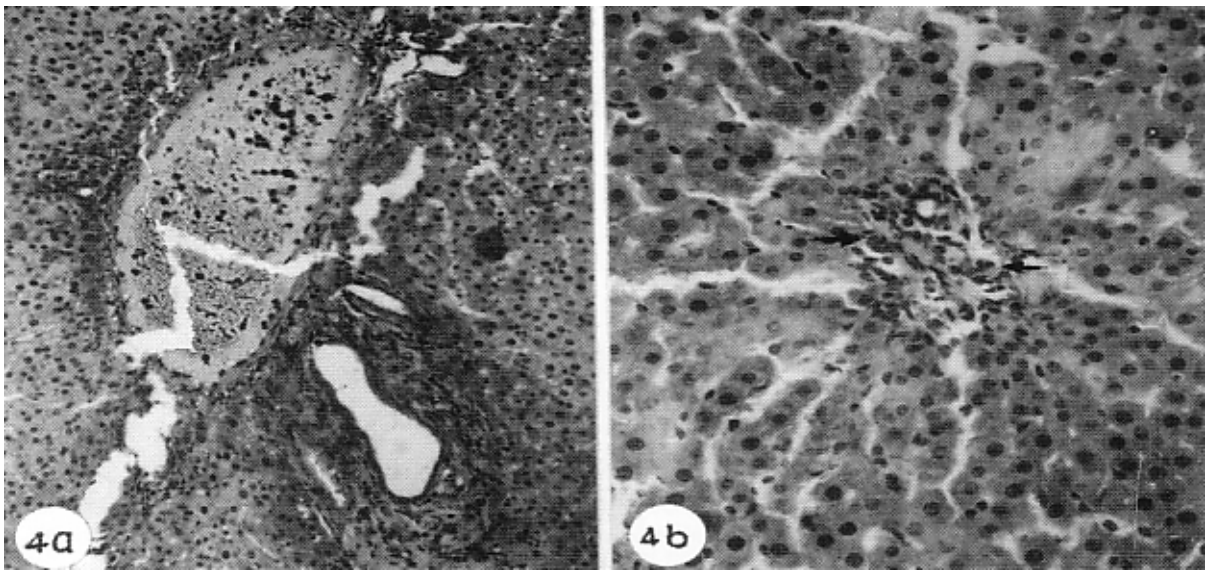

Fig.(4): (a) Section of a liver of a reperfused animal (IIR group) showing dilated congested vascular spaces in the portal tracts with (b) increased number of sequestrated neutrophils.

Figure 5. Comparison of Sequestration of PMN within Liver Parenchyma

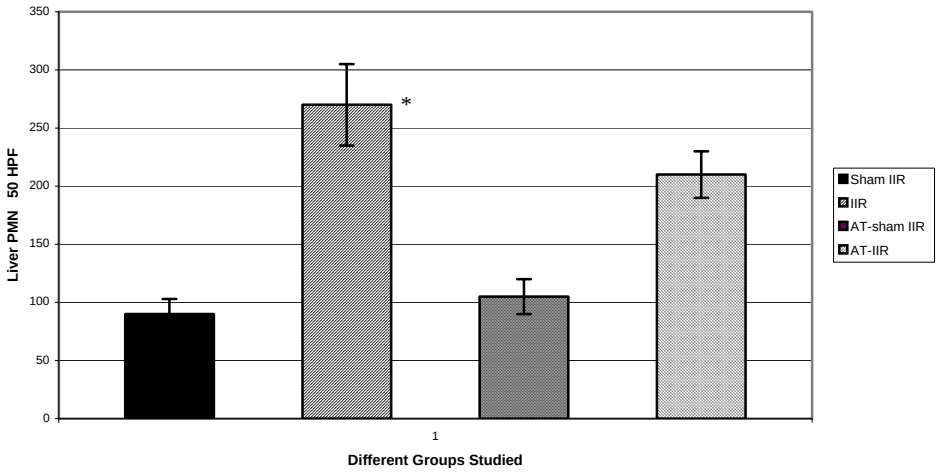

*Fig.(5). Comparison of PMN sequestration in the liver parenchyma as expressed by number of PMN/50-HPF. Values are mean (sd). IIR, intestinal ischemia-reperfusion. AT, adrenaline-tolerant rats. * P<0.05 IIR versus AT-IIR, and both versus other groups.*

Figure 6. Comparison of Serum ALT Levels

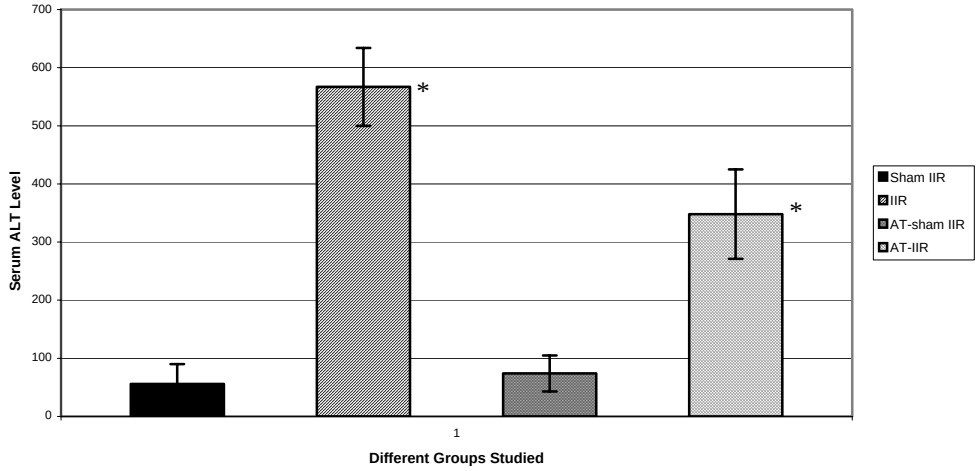

*Fig.(6): Comparison of serum Alanine aminotransferase (ALT). Values are mean (sd). IIR, intestinal ischemia-reperfusion. AT, adrenaline-tolerant rats. * P<0.05 IIR versus AT-IIR, and both versus other groups.*

Figure 7. Comparison of Mucosal Injury Score

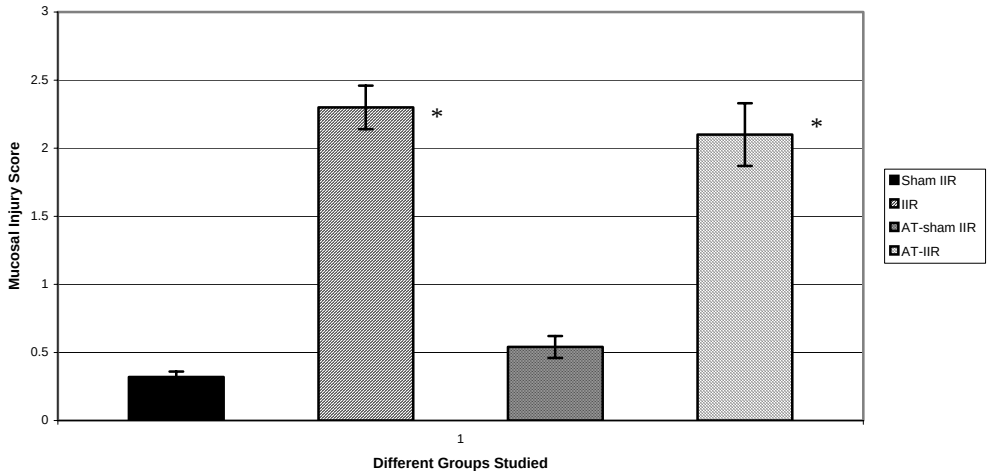

*Fig.(7): Comparison of intestinal mucosal injury score. Values are mean (sd). IIR, intestinal ischemia-reperfusion. AT, adrenaline-tolerant rats. * P<0.05 IIR versus AT-IIR, and both versus other groups.*

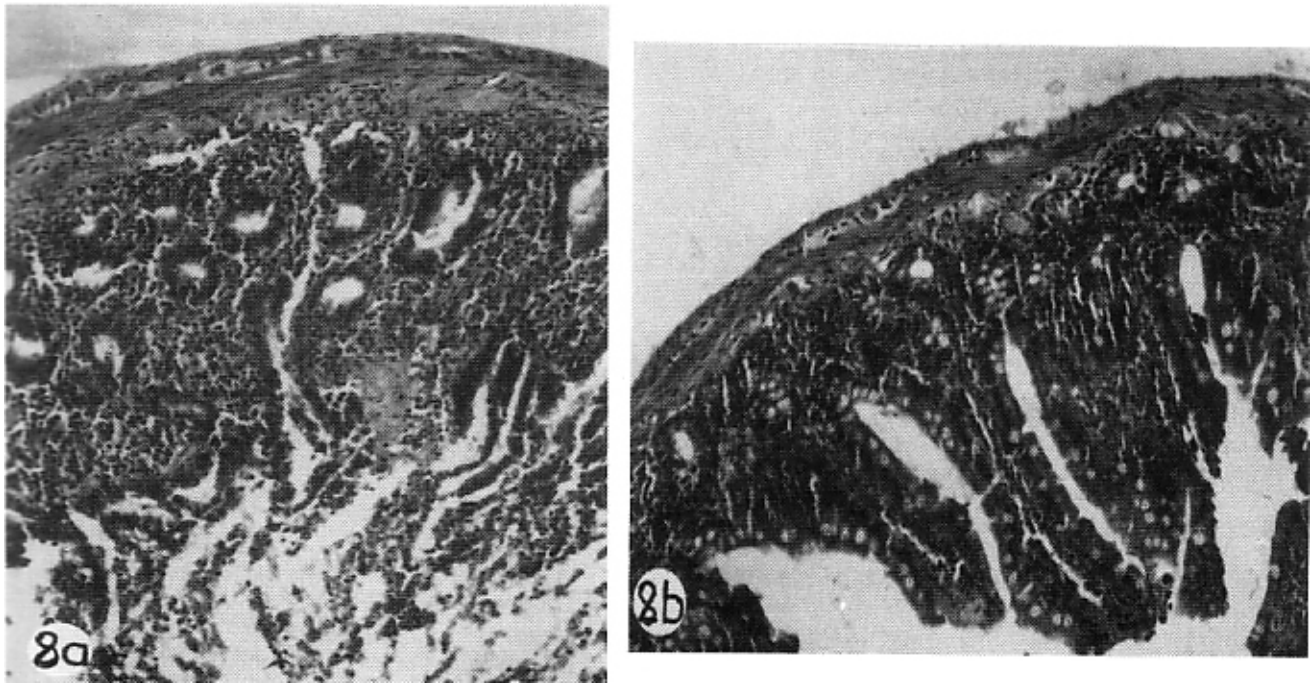

Fig.(8):

- ***(a) Section of a reperfused small intestine massive destructive manifested by denuded villi with exposure of the dilated capillaries in the lamina propria. Increased cellularity of the lamina propria is a prominent feature. Changes correspond to grade 4 injury;***
- ***(b) These changes resulting from ischemia / reperfusion injury are totally absent in sham IIR animals.***

DISCUSSION

Intestinal ischemia and reperfusion (IIR) is a common, clinical event with a high complication rate, characterized by adult respiratory distress syndrome (ARDS), hypotension, and renal failure, as well as a high mortality rate varying between 44% and 89%[16,17]. In the reperfused intestine, there is a massive influx of PMN[18], an increase in microvascular permeability[2], and evidence of mucosal barrier dysfunction[19] which results in translocation of endotoxins from the bowel lumen into lymphatic vessels[20] and portal blood[21]. In addition to local injury, the appearance of these circulating agents has been related to remote organ injury in the lungs and liver. This injury is also associated with PMN sequestration and generation of proteolytic enzymes and toxic free radicals in these organs[22-24]. Manipulating PMN numbers or antagonizing the products of activation, that is, free radicals or elastase lead to significant reduction in liver[25,26] and lung[27] injury, but not intestine possibly due to the extreme sensitivity of this organ to even small concentrations of PMN-derived elastase or free radicals[27].

IIR is also associated with raised catecholamine levels caused by impairment of uptake and storage[28], and has been reported to cause pulmonary vasoconstriction[29] and pulmonary hypertension[30], which in turn, leads to accumulation of interstitial lung water by increasing the microvascular filtration pressure[31]. In the present rat model, decreased pulmonary permeability could be the result of an attenuated catecholamine effect.

MPO levels increase in lung[22,27] and intestine[32] during IIR, and act as a marker for neutrophil infiltration. In the present study, MPO levels were also increased by IIR in both organs studied, although significantly only in the lung. In this model, adrenaline tolerance did not have any effect on IIR-induced changes in MPO levels in the intestine. Similar results in an IIR model in mice were reported by Baykal et al[33]. It is conceivable that catecholamines do not play a major role in neutrophil infiltration in that organ.

Increased lipid peroxidation in the lung, liver and small intestine in animals with IIR was evidenced in this study by significantly increased MDA levels in all three

organs. This has been demonstrated previously by Van Ye and colleagues[32]. Conversely, there was no difference in MDA levels in AT-animals having IIR or sham IIR. However, MDA levels in the sham AT-group were significantly higher than in the sham IIR animals probably as a result of the oxidation products of adrenaline, which is known to generate free oxygen radicals[34,35]. Another study[36] showed that after induction of ischemia, inhibition of monoamine oxidase (MAO) attenuates production of hydrogen peroxide, revealing the importance of catecholamine oxidation in ischemia-reperfusion injury. In the liver, the difference between MDA levels in normal and AT-animals was more evident as both AT-groups had significantly higher MDA levels than the IIR group. This might be because catecholamines are mostly metabolized in the liver and MAO has its highest concentrations in the liver and kidney[37]. These findings support the hypothesis that oxidation of catecholamines leads to accumulation of oxygen free radicals.

In the present model, IIR-induced lipid peroxidation has been attenuated by adrenaline tolerance. There was no difference between the two AT-groups, showing that the IIR insult did not lead to additional lipid peroxidation. This suggests that catecholamines are involved in IIR injury, possibly having a positive modulatory role.

The present study has shown that rendering animals adrenaline tolerant not only reduces lung permeability but also significantly prevents liver leukosequestration and hepatocellular injury, however, it leaves unaltered the local intestinal mucosal injury. One likely possibility is that the resident population of granulocytes is situated in closer proximity to mucosal epithelium and thus is capable of inflicting greater damage to the mucosal barrier than newly recruited granulocytes[38]. Another possibility is that the resident population of peroxide-positive cells can produce larger quantities of oxidants and proteases than their newly recruited counterparts. This view is consistent with the work of Zimmerli et al[39] who showed that the interstitial PMNs produce larger quantities of oxidants in response to pro-inflammatory agents than PMNs derived from the whole blood.

In conclusion, the present study indicates that (1) IIR leads to local and remote organ dysfunction, (2) catecholamines are involved in the IIR insult, and (3) induction of adrenaline tolerance has a possible protective effect, though the local injury appears to be independent of catecholamines.

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