Global Veterinaria 13 (1): 10-16, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.01.8367

Histopathologyof Intestinalischaemia-Reperfusion Injury in Rats

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Abstract: The effect of intestinal ischemia-reperfusion injury (IIR) on gonads has been little investigated although the effects of ischemia-reperfusion injury on the lungs, liver, heart, kidneys and several other organs are well documented. This phenomenon, if it affects the gonads as other distant organs may well be a cause of iatrogenic infertility. The effects of ischemia-reperfusion in distant organs has been seen to be derangements in microvascular permeability and circulation leading to malfunction and in the gonads, if thisoccurs, the far reaching effects on the fertility and reproductive capability of the animal may be compromised.The effect of intestinal ischemia-reperfusion injury on distant organs and the gonads was investigated in 20 male Wistar rats aged 12weeks and weighing 254 ± 0.73grammes. They were divided into five groups A, B, C, D and E. Group A served as control while groups B, C, Dand E were the treatment groups. Within the treatment groups, the rats were subjected to either twenty minutes or one hour of ischemia and one hour of reperfusion and splenectomy. The intestines, lungs, heart, liver, kidney and testes were harvested and processed for histology. No gross lesions were observed. Histopathological lesions observed in all organs included graded congestion and change in architecture in the lungs, liver, heart and kidneys. Intestines were observed with villus matting, vacuolations and widening of crypts. The testicular architecture was severely changed by IIR but was well preserved in the splenectomy group. The histopathological picture of the effects of intestinal ischemia-reperfusion injury particularly in the gonads suggests the alteration of the ability to preserve and store sperm cells which may lead to a significant effect on fertility.

Key words: Histopathology · Intestinal ischemia-reperfusion injury · Rats · Infertility

INTRODUCTION

IIR injury is characterized by decreased intestinal barrier function [1]. Under normal physiological conditions, the intestinal barrier protects the body from the hostile environment within the bowel lumen. However, IIR disrupts this protective function, resulting in increased intestinal permeability and bacterial translocation into the portal and systemic circulations [2]. A devastating consequence of IIR is the development of remote organ injury and Multiple Organ Dysfunction Syndrome MODS which is a leading cause of death in critically ill patients in intensive care units (ICUs) with mortality directly correlating with the number of failed organ systems [3]. The pulmonary system is the most frequently injured organ in patients suffering from MODS and the onset of the syndrome is commonly preceded by the development of acute respiratory insufficiency within 24–72 h of the initiating ischemic event. Respiratory failure and development of acute respiratory distress syndrome (ARDS) may quickly ensue [4]. Respiratory failure is often followed by hepatic, renal, gastrointestinal and myocardial and central nervous (CNS) dysfunction. In addition to increased microvascular permeability, MODS is characterized by dysfunction of the coagulation and immune systems, leading to thrombosis, disseminated intravascular coagulation and immuno-compromise [1].

This is evidenced by dysfunction in almost all the organs of the body starting with cardiac contractile dysfunction, acute lung injury seen as leucosequestration, microvascular dysfunction and liver injury seen as neutrophil sequestration, hepatocellular

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enzyme release and renal dysfunction seen as impaired tubular function [5]. IIR injury activates a variety of circulating inflammatory mediators which induce a generalized microvascular injury and initiate a systemic inflammatory response resulting in multiple organ failure. This study aimed to evaluate the histopathological effects of IIR following ischemia-reperfusion injury on several vital distant organs.

MATERIALS AND METHODS

Twenty male Wistar rats aged twelve weeks and weighing 254±73grammes were used for this study. They were divided into five groups A, B, C, D and E. Group A served as control and groups B and C were the IIR groups while D and E animals were the splenectomy groups. Within the treatment groups, the animals underwent either twenty minutes and one hour ischemia and one hour of reperfusion and for groups D and E, this was done after splenectomy. After an overnight fast, the rats were anaesthetized using intramuscular injection of a mixture of ketamine hydrochloride and xylazine. A midline laparotomy was performed after skin shaving and preparation of the abdominal wall with chlorhexidine solution. The small intestine was reflected to the left of abdominal incision and the superior mesenteric artery (SMA) was exposed and clamped. Collateral arcades from the right colic artery and the jejunal arteries proximal to the site of occlusion were ligated to avoid the variable contribution of collateral circulation to the distal ileum as described byMegison *et al*. [6]. Intestinal ischaemia was confirmed when the mesenteric pulsations were lost and the intestine became pale. The bowel was returned to the abdominal cavity and the incision was closed with continuous 4/0 vicryl suture. After the period of ischemia depending on group, a re-laparatomy was performed and the microvascular clamp was removed. Reperfusion was confirmed with the restoration of pulsation and colour. The bowel was left within the abdomen during ischemia and reperfusion. Splenectomy was performed just before the occlusion of SMA in the splenectomy groups.

Organ harvest included the intestine, heart, lungs, liver and kidneys. Samples of the small bowel tissue were fixed in10% formalin solution, embedded in paraffin wax and 5ìm sections were cut and stained with hematoxilin and eosin. Testicular tissues were fixed in aqueous Bouins' fixative for 24 hours after which they were dehydrated in graded levels of ethyl alcohol, cleared in chloroform, embedded in paraffin and sectioned in a microtone at 7µ thick. The slides were stained with haematoxylin - eosin (H and E) for histological evaluations.

Histological evaluation of the intestinal segment was undertaken by an independent pathologist who had no knowledge of the experimental groups from which the specimens were derived. The microscopic assessment was performed using the following grading scores according to Geboes [7]: + (mild congestion and no changes in architecture), ++ (moderate congestion and minimal change in architecture) and +++ (moderate congestion and significant change in architecture). The abnormalities detected in 20 random sites in every animal plate were subjectively scored and compared with the controls. The sections were carefully examined microscopically and the best observed areas were selected for morphometric studies. The images were captured in 20 selected areas per histological section of the small bowel mucosa with a digital camera.

Although the grading system is a reflection of the extent of the change, it generally also correlates with the severity of the change. Grade - showed nil effects seen, grade + showed moderate effects from 5-25% involvement of the overall sample, grade ++, 6-55%; grade +++, above 55%.

RESULTS

Observation of Organ Changes: All organs assessed showed no gross lesions after the period of ischaemia during which the intestines experienced loss of pulsation and change in colour from pink to blue occurred and restoration of blood supply evidenced as restoration of pulsation and a change in colour from blue, back to pink.

Histopathology of Organs: Histologically, the intestines in control rats had a mild congestion and minimal change in the normal architecture, mild and sustained IIR rats had moderate to severe congestion and significant change in architecture but the splenectomized rats appeared like the control rats with only moderate congestion and moderate changes in normal architecture.

Lungs, liver, heart, kidney and testes in control rats showed mild congestion and no change in architecture. Lungs in all the groups of rats showed moderate congestion and minimal changes in architecture. In the liver, there appeared to be moderate congestion in mild IIR rats but there was a moderate congestion with a significant change in architecture in the sustained IIR rats.

Table 1: Histopathology scores of tissues following IIR and splenectomy

	Control	Mild IIR	Sustained IIR	Mild IIR+Splenectomy	Sustained IIR+Splenectomy
Intestine	++	+++	+++	++	++
Lungs	+	++	++	++	++
Liver	+	++	+++	+	++
Heart	+	++	++	+	++
Kidney	+	+	++	+	+
Testes	+	++	+++	+	+

Legend-
+ mild congestion and no change in normal architecture
++moderate congestion and minimal change in architecture
+++moderate congestion and significant change in architecture

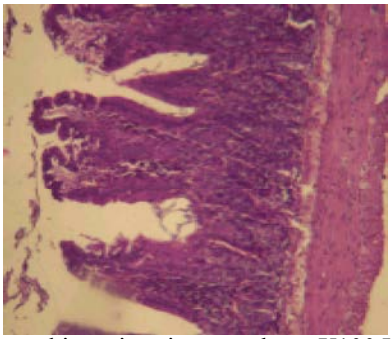

Fig. 1: Normal intestines in control rats X100 H and E

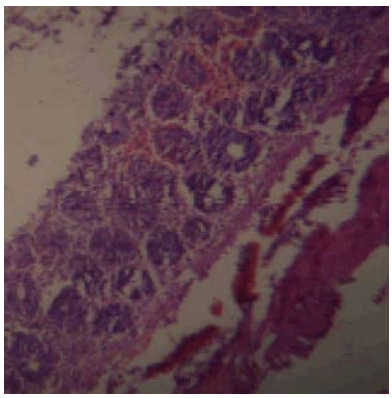

Fig. 2: IIR- Congestion and separation of basement membrane and vacuolations. X100 H and E

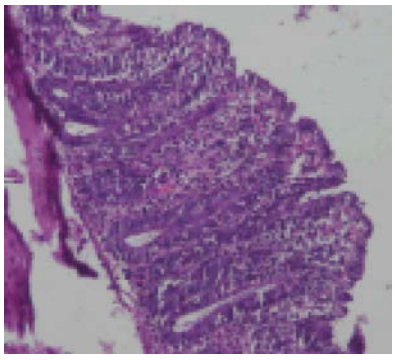

Fig. 3: Splenectomised rat intestine-Villus matting, vacuolation and widening of crypts. X100 H and E

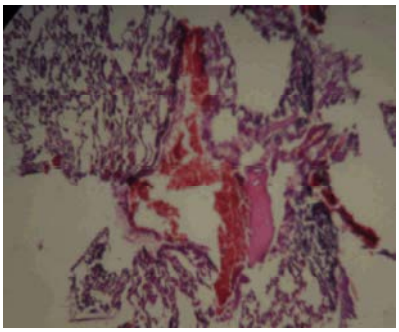

Fig. 4: IIR-congestion and oedema observed in the lungs X100 H and E

In splenectomized rats, those that were subjected to mild IIR had mild congestion and no change in architecture while those that were subjected to sustained IIR had moderate congestion and minimal architectural change. In the heart, control rats had only mild congestion and no change in architecture while the rats subjected to IIR showed moderate congestion, splenectomized rats subjected to mild IIR showed mild congestion with no change in architecture while those subjected to sustained IIR showed moderate congestion and minimal changes to architecture. In the kidneys, only the rats subjected to sustained IIR showed moderate congestion and minimal changes to architecture, all other rats had no change to mild congestion and architecture. In the testes, control and splenectomized rats showed no change to mild congestion and architecture, rats subjected to mild IIR showed moderate congestion and minimal architectural change while those subjected to sustained IIR showed moderate congestion with significant change in architecture (Table 1).

Normal intestinal morphology was observed from control rats (Fig. 1) while splenectomised rats had villus matting and vacuolation with widening of crypts (Fig. 2) and IIR rats had congestion, separation of basement membrane (Fig. 3). In the lungs, congestion and oedema was observed with IIR (Fig. 4) while splenectomised rats

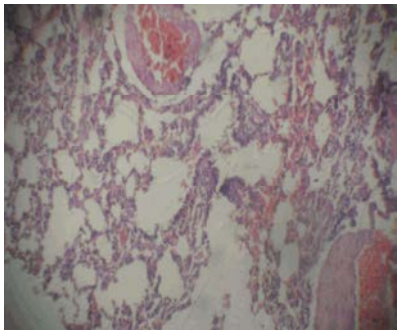

Fig. 5: Splenectomised rats-congestion and high cellular infiltration in lungs. X100 H and E

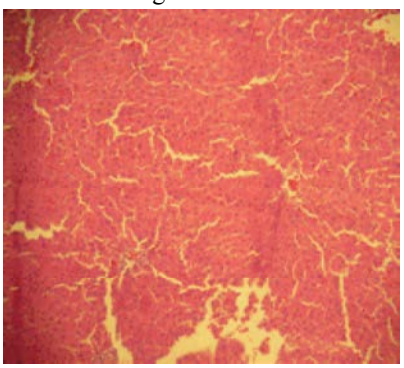

Fig. 6: Control rats-normal liver architecture observed X100 H and E

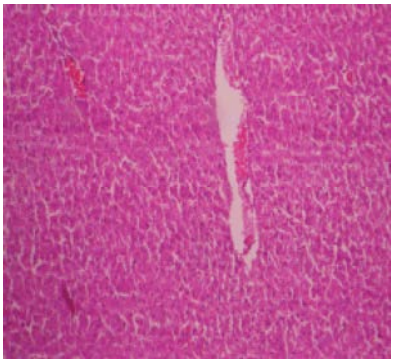

Fig. 7: IIR-congested Liver X100 H and E

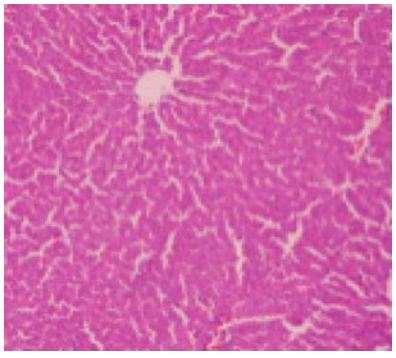

Fig. 8: Splenectomised rats- congestion within the liver parenchyma X100 H and E

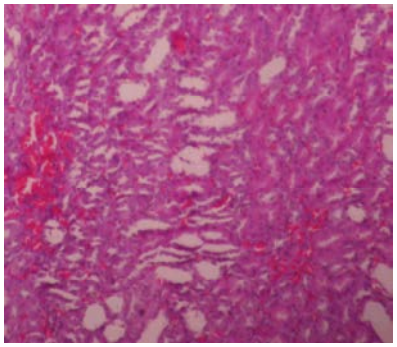

Fig. 9: IIR-kidney with areas of congestion X100 H and E

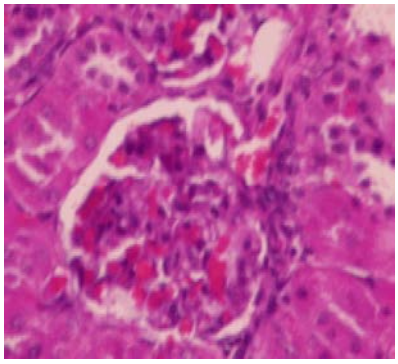

Fig. 10: One bowman's capsule from above to emphasize congestion in the kidney. X400 H and E

had congestion and a high cellularity in lungs (Fig. 5). Normal lung architecture was observed with control rats. Liver architecture did not alter in control rats (Fig. 6) but with IIR, congestion was noted (Fig. 7) and this was also observed within the parenchyma in splenectomised rats (Fig. 8). Congestion was observed in the kidneys with IIR (Fig. 9) and massive PMN infilteration (Fig. 10). The heart was observed to have normal architecture in control animals (Fig. 11) and show congestion following IIR and splenectomy (Fig. 12). In the testes, the control animals had normal testicular architecture with the presence of germinal epithelium and sperm cells within the tubular lumen (Figure 13). IIR caused changes in architecture with disruption;oedema and congestion of the tubules and germinal epithelium were in place but withno particular pattern while there were hardly any intact lumens to appreciate presence of sperm cells within (Figure 14). In splenectomised rats, congestion within the inter-tubular areas of the testes appeared but the architecture of the testes was not altered and the germinal epithelium and sperm cells within the tubules were present (Figure 15).

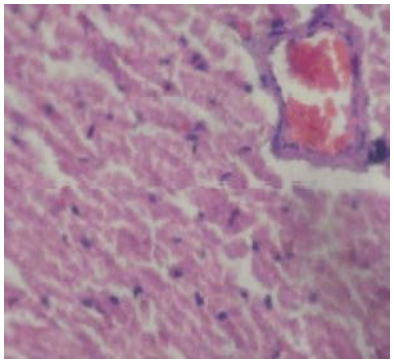

Fig. 11: Control rats- heart showing normal architecture X100 H and E

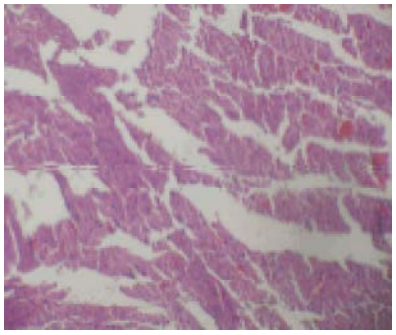

Fig. 12: Splenectomised rat- heart showing congestion X100 H and E

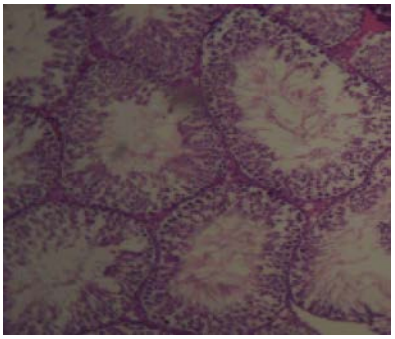

Fig. 13: Control rats -testes showing germinal epithelium and sperm cells within the lumen X100 H and E

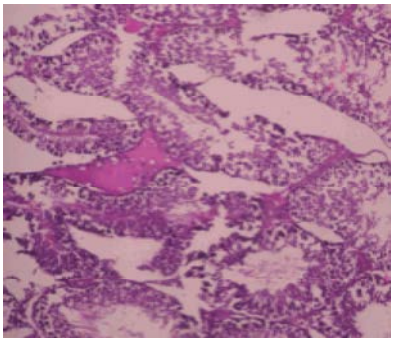

Fig. 14: IIR-testes showing oedema, congestion, destruction of germinal epithelium and sperm cells within the lumen X100 H and E

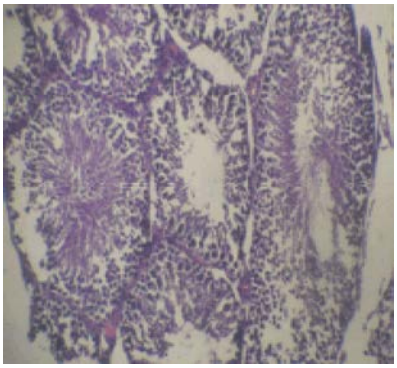

Fig. 15: Splenectomised rats testes showing congestion and intact germinal epithelium and sperm cells within the lumen. X100 H and E

DISCUSSION

The observations of this study show the lack of gross lesions in IIR injury in the immediate post-surgical period. There were no gross lesions seen in any of the treatment groups. Histological changes which occurred also appear to be non-specific and will probably cause impairment in organs based on the specific function which was beyond the scope of this study. The histological picture of the intestines, heart, liver, spleen, kidneys and testes appear similar withischemia-reperfusion in remote organs. The histological picture of congestion and cellular infilteration occurs in all organs and increased cellularity which is in agreement with the findings of non-specific signs of oedema and congestion observed following IIR and evaluation by electron microscopy bySavas *et al*. [5]. This should actually provide improved oxygen delivery for the organ needs but the damaged nature of the blood cells makes this impossible suggesting the cells are trapped and destroyed by the phagocytic cells or they end up in the spleen [5]. Savas *et al*. [5], also report that experiments with leucocyte antisera proved the efficacy of splenectomy in reducing IIR injury by reducing the metabolites like malondialdehyde (MDA) that was generated in that experiment. The splenectomised rats in this study, showed a similar histology to control animals with an amelioration of the effects of both mild and sustained IIR. This suggests that the cells stored in the spleen probably contribute to the injury seen in IIR following reperfusion and splenectomy removed them leading to the milder histological changes seen.

The implications of the oedema and congestion on the gonads observed in this study may be far reaching and whileShalaby and Afifi [8] talks about infertility as a clinical outcome of testicular torsion which establishes IR

in the testicles, the mechanism of action is based on ROS rather than oedema and congestion. Remote organ injury following IIR has been shown to be due to neutrophil mediated reactions and Kupffer cells of the MPS play a pivotal role in this sequence of events [9]. Okutan *et al*. [10] demonstrated an attenuation of remote organ injury with splenectomy. When splenic monocytes/macrophages were removed from the body with splenectomy, the total number of MPS cells contributing to the remote organ injury was reduced. Various toxic substances in the reperfusate, including free oxygen radicals and other inflammatory mediators generated during IIR are first released into the liver via the portal circulation, then to the lungs. Finally when this reperfusate reaches the heart, it is distributed to the systemic circulation and resulting in a systemic inflammatory response [11, 12]. When this reperfusate reaches the splenic circulation, a second wave of inflammatory mediator release might take place, contributing to the remote organ injury which can be avoided with splenectomy [10]. The other mechanism that may be responsible for the remote organ injury is the haemodynamic and microcirculatory derangements seen after IIR [13]. Cellular effects seen are a result of molecular effects like ATP depletion, defective ATP resynthesis, increase in hypoxanthine, activation of xanthine oxidase and generation of ROS, antioxidant depletion, intracellular sodium and calcium overload. Cellular effects seen include endothelial cell dysfunction/swelling, leucocyte recruitment, polymorphonuclear cell presence, nomediated impaired vasodilation, enhanced vasoconstriction, endothelial barrier disruption [14].

The effects of IIR on the testes as evidenced by the change in architecture and destruction of germinal epithelium may adversely impact on the fertility of the animal as the epithelium provides the precursor cells for sperm cells. The effects are consistent with damage that occurs in ischaemia-reperfusion in the testes. There is a need to assess the implications of the histopathology observed on fertility and ascertain the clinical consequences in animals. This may well be an iatrogenic cause of infertility in animals.

In conclusion, IIR injury may be a silent cause of infertility in animals through destruction of germinal epithelium and should be considered when evaluating for breeding problems in animals especially when there appears to be no evidence for such infertility.

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