

Research Article

Histomorphometry of Rat Testes Following Intestinal Ischaemia-Reperfusion Injury and Splenectomy

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Summary: Intestinal Ischaemia-Reperfusion Injury as distinct from Ischaemia-Reperfusion Injury is what occurs when blood supply to the intestines is cut off either inadvertently in some gastrointestinal emergencies or during abdominal surgical interventions. The phenomenon leads to formation of reactive oxygen species and subsequent up-regulation upon reaching the spleen and consequent effects in organs of the body such as increased sperm cell abnormalities. The changes which occur in the intestine and some distant organs due to remote effects of intestinal ischaemia-reperfusion have been demonstrated but there is paucity of information on these effects on the testes with implications on fertility which was investigated in this study. Fifteen adult male Wistar rats were used for this study. Group A was the control in which a sham laparotomy was done, Group B, in which intestinal ischaemia-reperfusion was set up and C in which splenectomy was done before setting up intestinal ischaemia-reperfusion by clamping the superior mesenteric artery. The ischaemia was for an hour and reperfusion also for an hour. Following reperfusion, portions of the intestines, epididymis and testes were harvested. Histomorphometry of intestines revealed a villus height of 93.98 μ m, 91.44 μ m, and 110.48 μ m in control, intestinal ischaemia-reperfusion and splenectomised rats and villi width of 24.5 μ m, 24.6 μ m and 38.5 μ m respectively. Testicular histomorphometry revealed seminal tubular diameter as 189 μ m, 197 μ m and 215 μ m and luminal diameter of 58 μ m, 59 μ m and 62 μ m. Histopathology revealed congestion, desquamation and defoliation of germinal epithelium in all three groups but control rats had sperm cells present. In IIR group tubular damage and oedema were present and sperm cells were absent but in splenectomised rat, sperm cells were present in some tubules and absent in others but no tubular damage was observed. Splenectomy does exert a protective effect on intestinal ischaemia-reperfusion by moderating the effects in remote organs, as seen in testes.

Keywords: Intestinal Ischaemia-Reperfusion Injury, Rat Testes Histomorphometry, Splenectomy

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INTRODUCTION

Intestinal ischaemia-reperfusion (IIR) injury is a severe disease associated with high mortality (Nadatani *et al.*, 2018). Even though it affects several organs, the intestine is the most sensitive organ to IIR injury (Sasaki and Joh, 2007). 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 (Kong *et al.*, 1998). IIR injury may also be associated with impaired gut motility and absorption. Activation of complement and circulating leucocytes by translocated bacteria may eventually lead to the development of Systemic Inflammatory Response Syndrome (SIRS) following IIR injury (Eltzschig and Collard, 2004). 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 (Neary and Redmond, 1999). Remote organ injury following IIR has been shown to be due to neutrophil mediated reactions, and Kupffer cells of the Mononuclear Phagocyte System (MPS) play a pivotal role in this sequence of events (Tullis *et al.*, 1996).

Sperm abnormalities as a result of remote effects of intestinal ischaemia-reperfusion may be an iatrogenic cause of infertility in animals (Olatunji-Akioye and Fayemi, 2014). The ability of the intestine to serve as a Reactive Oxygen Species (ROS) generator and the unique position of the gastro-intestinal system to distribute metabolites round the body lends credence to a study by Ogbuewu *et al.* (2010) who found that excessive generation of ROS by abnormal spermatozoa and contaminated leucocytes is one of the few defined aetiologies for male infertility. Male infertility is most often investigated after the female has been investigated and no abnormalities are found. Histomorphometry of testes is an indication of normalcy and changes should be consistent with changes in fertility.

This study attempts to determine the histomorphometric changes that occur with intestinal ischaemia-reperfusion injury in the testes and epididymis and how splenectomy will mitigate the effect by removal of reactive metabolites that are implicated in the pathogenesis.

MATERIALS AND METHODS

Animals and groupings: Fifteen adult male Wistar rats were divided into three groups, A, B, and C. Group A served as control, group B was the IIR group and were subjected to intestinal ischaemia by clamping the mesenteric artery

while group C were splenectomised prior to clamping the mesenteric artery to set up ischaemia.

Surgical procedure: After an overnight fast, the rats were anaesthetized using intramuscular injection of a mixture of ketamine hydrochloride (10mg/kg) and xylazine (100mg/kg). 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. 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 by Megison *et al.*, 1990. An atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int., St. Paul, Minn., USA) was then placed across the SMA just after its origin from the aorta, avoiding occlusion of the superior mesenteric vein (SMV). Intestinal ischaemia was confirmed after fifteen minutes 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-laparotomy 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 ischaemia and reperfusion. Splenectomy was performed just before the occlusion of SMA in the splenectomy group.

Organ harvest included the intestine, and sections of the epididymis and testes. Samples of the small bowel tissue were fixed in 10% formalin solution, embedded in paraffin wax and 5 μ m sections were cut and stained with hematoxylin and eosin. Testicular tissues were fixed in aqueous Bouin's 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 microtome at 7 μ thick. The slides were stained with haematoxylin - eosin (H and E) for histological evaluations.

Histological evaluation: Histological evaluation of the intestinal segment was undertaken by an independent pathologist who was blinded to the experimental groups from which the specimens were derived. The microscopic assessment was performed using the following grading scores: + (mild congestion and no changes in architecture), ++ (moderate congestion and minimal change in architecture) and +++ (moderate congestion and significant change in architecture) adapted from Geboes, 2003. 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.

A semi-quantitative grading system was applied to the testes histological variables based on the approximate percentage of the total sample involved with the individual process. 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% (Creasy *et al.*, 2002).

RESULTS

Intestinal morphometry: Histomorphometric analysis of intestines following IIR and Splenectomy (Table 1), revealed an average villus height of 93.98 μ m and 91.44 μ m in control and IIR rats while the villus height in splenectomised rats was significantly higher, 110.48 μ m. The villus width followed a similar pattern with control and IIR rats measuring 24.5 μ m and 24.6 μ m while splenectomised rats had a significantly higher measurement of 38.5 μ m.

Testicular morphometry: Comparing the diameter of the seminal tubule and luminal diameter, it was observed that IIR rats had the least seminal diameter of 189 μ m, while control rats had measurements of 197 μ m (Table 2) and splenectomised rats had significantly higher measurements of 215 μ m compared to control and IIR rats. Luminal diameter was smallest in IIR (58 μ m) and splenectomised (59 μ m) rats respectively while control rats had a significantly higher luminal diameter of 62 μ m.

Table 1:

Histomorphometric analysis of intestines following Intestinal ischaemia-reperfusion and splenectomy in rats

	Control	IIR	Splenectomy
Villus	93.98	91.44	110.48
Height	± 2.71	± 4.34	$\pm 8.01^*$
Villus	24.5	24.6	38.5
Width	± 1.20	± 2.10	$\pm 3.26^*$

Table 2:

Histomorphometry of Testes following IIR and splenectomy.

	Control	IIR	Splenectomy
Diameter of seminal tubule	198.0	189.0	215.0
	± 12.06	± 11.01	$\pm 10.50^*$
Luminal diameter	62.0	58.0	59.0
	$\pm 2.59^*$	± 5.32	± 1.18

Values with * are significantly different from values on that row

Table 3:

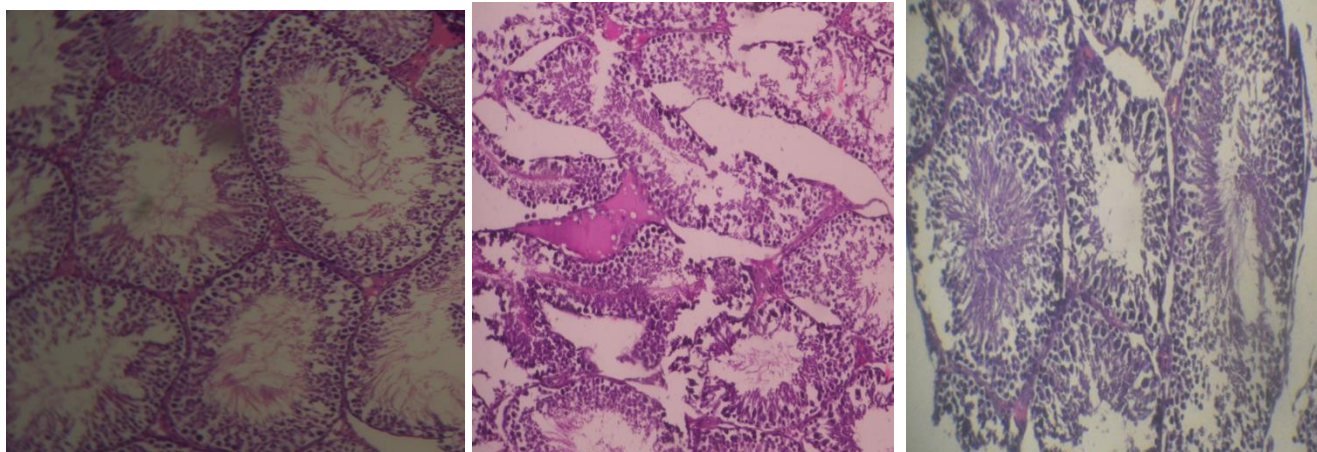
Histopathology scores of Testes following IIR and splenectomy.

	Control	IIR	Splenectomy
Congestion	+	++	++
Desquamation and defoliation of germinal epithelium	+	++	+
Presence of sperm cells	+	-	-/+
Damaged tubules	-	+	-
Oedema	-	+	+

- nil effects seen

+ moderate effects (5-25%)

++ significant effects (26-55%)

**Plate 1**

Testicular histology. A - Normal Testes showing discrete germinal epithelium and sperm cells within the tubules; B- Testes with damaged germinal epithelium and absence of sperm cells within the lumen; C- Splenectomised Rat testes showing germinal epithelium and sperm cells within the lumen. X100 H & E

Testicular histopathology: Histopathological evaluation of testes (Table 3) showed that control rats had congestion; desquamation and defoliation of germinal epithelium (figure 1), with sperm cells present and no damaged tubules. There was desquamation, defoliation, damaged and oedematous tubules with no sperm cells in the germinal epithelium (figure 2) in IIR rats. Tubules were damaged and oedema was also observed. In the splenectomised rat group, germinal epithelium was congested, desquamated, defoliated with scanty sperm cells (figure 3)

DISCUSSION

The histological picture of congestion and cellular infiltration in the organs should ordinarily be associated with improved oxygen delivery for the organ needs. However, the damaged nature of the blood cells makes this impossible. Although there is increased cellularity, the cells are trapped and destroyed by the phagocytic cells or they end up in the spleen. Savas *et al.* (2003) reported that experiments with leucocyte antisera proved the efficacy of splenectomy in reducing Ischaemia-Reperfusion injury by reducing the metabolites that were generated in that experiment. The splenectomised rats in this study, showed a similar histology to control animals with an amelioration of the effects of IIR. This suggests that the cells stored in the spleen probably contribute to the injury seen in IIR following reperfusion. Splenectomy removes them leading to the milder histological changes seen.

The histomorphometric changes observed suggest a response in the immediate post ischaemic period of the villus to the ischaemia and subsequent reperfusion by a width and height increase. This was probably facilitated by the chemo attraction of neutrophils which is seen to be up-regulated in IIR injury. Savas *et al.*, (2003) also reported ultrastructural changes in remote organs. The changes in diameter of the seminal tubule and luminal diameter which became significantly reduced with IIR but significantly increased in splenectomised rats suggests that splenectomy may enhance the storage of sperm cells. The implications of the oedema and congestion on the gonads observed in this

study suggest that IIR may be detrimental to sperm cell storage.

Shalaby and Afifi, (2008) described infertility in terms of clinical outcome of testicular torsion which establishes IR in the testicles. The mechanism of action was linked to ROS rather than oedema and congestion. However, ROS are directly responsible for the oedema and congestion that was seen in this study. The splenectomised rats were thus afforded a measure of protection as seen by the measurements as well as histology. O'Connor *et al.*, 2002 detailed the effect of oestrogen on the morphology of testes and its ability to reabsorb fluid in the rete testis, efferent ducts and initial segment of the epididymis. This effect which is triggered by inflammation may be responsible for oedema seen in the IIR group of rats.

The protective effect of splenectomy in the rats lends credence to the removal of cells which would have contributed to attraction of phagocytic cells and heightened the inflammatory response. As the spleen is described as non-essential for life, it does perform storage functions and thus acts as a reservoir for blood cells in cases of urgent need. Removing the spleen in health may cause no untoward effects; it may thus be protective to splenectomise breeder animals to preclude adverse reactions in the event of an ischaemia-reperfusion injury. The importance of male fertility in breeding is such that whatever can be done to enhance it will improve the health of the entire herd.

In conclusion, splenectomy mitigates the effect of ischaemia-reperfusion injury and confers protection to the germinal epithelium and sperm cells present in the testes and epididymis

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