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Full Length Research Paper

Effects of Intestinal Ischaemia-Reperfusion Injury and Splenectomy on Haematological and Biochemical Parameters in Rats

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ABSTRACT

Splenectomy has been proposed to alleviate mild and sustained ischemia reperfusion injury (IR). Intestinal ischaemia reperfusion injury (IIR) appears to have remote effects from the gastrointestinal tract. The effects of splenectomy on haematology and serum biochemical parameters in mild and sustained intestinal ischemia reperfusion injury was evaluated in male Wistar rats. The rats were splenectomized and then subjected to either 20minutes or one hour of superior mesenteric artery and collateral supply ischemia and one hour of reperfusion. Control rats were subjected to either 20minutes or one hour of ischemia and one hour of reperfusion only. Blood samples were collected before and after surgery for control animals and the other groups, for analysis. Mild IIR significantly reduced packed cell volume ($32.4 \pm 2.6\%$), haemoglobin concentration ($9.7 \pm 0.1 \text{ g/dL}$), red blood cell count ($5.3 \pm 0.3 \times 10^6/\text{L}$) and leucocytosis ($16.3 \pm 0.28 \times 10^9/\mu\text{L}$) due to lymphocytosis (78%), postoperatively. Sustained IIR significantly lowered values of neutrophils (29%). Mild and sustained IIR did not significantly alter biochemical parameters. Significantly lower albumin values were observed in sustained IIR ($3.4 \pm 0.55 \text{ g/dL}$). Splenectomy did not significantly alter hematological parameters, a measure of protection. Elective splenectomy may thus be useful in moderating haematological changes that may occur following an incident in which intestinal ischaemia-reperfusion injury may follow as a palliative measure.

Keywords: splenectomy; haematology; serum chemistry; Wistar rats; intestinal ischaemia-reperfusion injury

INTRODUCTION

The spleen is the largest lymphoid organ, found within the omentum closely adhered to the greater curvature of the stomach in dogs. It is a dark red haematopoietic organ that is non-essential for life. Its functions include storage of red blood cells and platelets, destruction of

worn out red blood cells, removal of particulate matter from the circulation and production of lymphocytes (Aspinall and O'Reilly, 2004). Indications for splenectomy include malignant neoplasia, ischaemic obstruction (in splenic torsion), and generalized splenic enlargement secondary to infiltrative diseases of the spleen (Tillson, 2002).

A decrease in the blood flow to an organ results in ischemic damage. But when blood flow is restored, a

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more pronounced damage, called ischemia-reperfusion injury (IR) occurs (Savas *et al.*, 2003). Reperfusion of ischemic tissues results in both a local and a systemic inflammatory response that, in turn, may result in widespread microvascular dysfunction and altered tissue barrier function (Eltzschig and Collard, 2004). If severe enough, the inflammatory response after IR may even result in the systemic inflammatory response syndrome (SIRS) or multiple organ dysfunction syndrome (MODS), which account for up to 30–40% of intensive care unit mortality (Neary and Redmond, 1999).

Thus IR injury may extend beyond the ischemic area at risk to include injury of remote non-ischemic organs. Intestinal ischaemia reperfusion (IIR) injury is characterized by decreased intestinal barrier function (Eltzschig and Collard, 2004). 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). This clinical scenario may be presented with derangements of haematology and serum chemistry following intra-abdominal surgery and may result in death even though visual assessments of intestinal viability precluded resection and anastomoses (Olatunji-Akioye and Akinrinmade, 2006). Remote organ injury following IIR has been shown to be due to neutrophil mediated reactions, and Kupffer cells of the mono phagocytic system (MPS) play a pivotal role in this sequence of events (Tullis *et al.*, 1996; Akinrinmade and Olatunji-Akioye, 2007). Savas *et al.*, (2003) demonstrated an attenuation of remote organ injury with splenectomy in dogs.

When splenic monocytes/macrophages were removed from the body with splenectomy, the total number of mononuclear phagocytic system (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 were first released into the liver via the portal circulation, then to the lungs. Finally when this reperfusate reached the heart, it was distributed to the systemic circulation resulting in a systemic inflammatory response (Bradbury *et al.*, 1993; McMillen *et al.*, 1993). When this reperfusate reached the splenic circulation, a second wave of inflammatory mediator release might take place, contributing to the remote organ injury (Savas *et al.*, 2003).

The other mechanism that may have been responsible for the remote organ injury is the haemodynamic and microcirculatory derangements seen after IIR (Yao *et al.*, 1996). Brief periods of mesenteric

ischemia led to an increase in microvascular permeability, whereas prolonged ischemia led to disruption of the intestinal mucosal barrier, primarily through the actions of reactive oxygen metabolites and polymorphonuclear neutrophils (Oldenburg *et al.*, 2004).

In veterinary medicine, the most common syndromes include gastrointestinal emergencies like gastric dilatation volvulus (GDV), mesenteric torsion, diaphragmatic hernia, intestinal incarceration following intussusceptions, arterial thrombo-embolism (ATE), and resuscitation from hemorrhagic shock, organ transplantation, head trauma, and spinal cord trauma (McMichael and Moore, 2004; Olatunji-Akioye and Akinrinmade, 2006). This study was carried out to evaluate the effects of splenectomy prior to IIR on haematology and serum chemistry in both mild and sustained intestinal ischemia reperfusion injury to assist the clinician in deciding to include splenectomy in therapy following conditions in which IIR may present.

MATERIALS AND METHODS

Twenty male Wistar rats aged twelve weeks and weighing 207 ± 22.3 grammes were used for this study. They were divided into groups A, B, C and D. Groups A and B served as control and underwent twenty minutes and one hour of ischaemia respectively followed by an hour of reperfusion. Groups C and D animals underwent splenectomy and then twenty minutes and one hour ischaemia respectively, followed by one hour of reperfusion.

After an overnight fast, the rats were anaesthetized using intramuscular injection of a mixture of ketamine hydrochloride (10mg/kgbw) and xylazine (100mg/kgbw). Preparation of the ventral abdominal wall for surgery was done by skin shaving and cleansing with chlorhexidine solution. A midline laparotomy incision was made on the abdomen and the small intestine was reflected to the left of the incision. Splenectomy was performed before the occlusion of SMA in groups C and D. SMA was identified along with collateral arcades from the right colic artery and the jejunal arteries proximal to the site of occlusion. These 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-statsII, 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 when the mesenteric pulsations were lost and the intestine became pale. Following ischaemia, the bowel was returned to the abdominal cavity and the incision was closed with continuous 4/0 vicryl suture. After the period of ischemia, a re-laparotomy was performed and the microvascular clamp was removed. Reperfusion was confirmed with the restoration of pulsation and colour.

Bowels were left within the abdomen during ischaemia and reperfusion. Blood was obtained immediately following reperfusion. It was obtained from the portal vein of each rat into EDTA containing sample bottles were placed on ice and then taken to the research laboratory for evaluation. Blood samples were spun at 1000g for 10 minutes and the serum decanted. Serum samples were stored for later blood biochemistry. Then, the animals were sacrificed by cervical dislocation. Haemoglobin (Hb) was determined using the cyanmethaemoglobin method of Jain (1986). Red blood cell count (RBC) and white blood cell counts (WBC) were determined using the improved Neubauer haemocytometer. Differential WBC counts were carried out by staining blood smears with Giemsa (Gulye, 1988). Serum levels of urea were determined by the Colorimetric method, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were analyzed using a commercially available diagnostic kit based on the method of Hoder and Rej, (1983). Total protein (TP) was determined by the Biuret method and Albumin was determined by the Bromocresol Green (BCG) method (Tietz, 2005).

RESULTS

The pre-operative haematology values are presented in Table 1. Postoperatively, in the MIIR group, PCV, Hb, RBC, eosinophil and monocyte count decreased, WBC, platelets and lymphocytes increased while neutrophils decreased significantly (Table 2). In the SIIR group, PCV, Hb and RBC increased, WBC and platelets decreased, lymphocytes decreased significantly and neutrophils increased significantly (Table 2). There was a minimal increase in urea, ALT, AST and albumin, only TP increased minimally in this group of rats (Table 3). In the SIIR splenectomy group, PCV, Hb, and RBC decreased, WBC and platelets decreased while lymphocytes and neutrophils did not change (Table 2). There was no change in the Urea values but ALT, AST, TP and albumin decreased non-significantly in SIIR group. There was no change in urea, ALT, a slight decrease in TP and albumin and decrease in TP in SIIR splenectomy group (Table 3).

DISCUSSION

The most widely recognized long-term risk of splenectomy is overwhelming bacterial infection and even though the spleen was considered non-essential for life, it clearly serves important haematologic and immunologic functions (Crary and Buchanan, 2009). Systemic sepsis due to asplenia is however infrequently reported (Allen, 1991; Couto & Hammer, 1995). In this study, it was observed that following mild IIR, PCV, Hb, and RBC decreased in the control group which did not occur in the splenectomy group. Packed cell volume is routinely monitored postoperatively in patients (Tillson, 2002) and it could be that there was a diminished ability to carry oxygen by red blood cells thereby stimulating peripheral chemoreceptors as reported by Abdallah and Abdelatif, 2010. The damage to the lipid membrane of the red cells could have led to an increased trapping and destruction in the spleen and reduction in the number of the cells which was seen as a decrease in PCV and haemoglobin concentration.

Savas *et al.*, 2003 reported that an intestinal ischaemic event is categorized under conditions where red blood cells are altered or dysfunctional and require removal from circulation. Changes in PCV and WBC was detectable within five minutes as reported in a study by Qu *et al.*, 2011 and therefore explain the decrease observed in mild rather than severe IIR. Whereas in severe IIR, the decrease in RBC, PCV and Hb might have been sufficiently low to trigger a release of immature red cells from the spleen. The neutropenia observed following sustained IIR was probably due to the movement of neutrophils into the liver following mild IIR and the subsequent tethering, rolling and adhesion which was similar to what Abdallah and Abdelatif, (2010) observed in a study on haemorrhaged goats compared with splenectomised goats. The dynamics of the WBC count also establish that the spleen is the major storage organ and its removal is important in limiting increases in WBC counts because the splenectomised groups (C and D) have no recorded increases in WBC counts.

Platelet numbers may be excessive after splenectomy because of loss of splenic storage (Tillson, 2002) as was evidenced in the control group of both mild and sustained IIR in this study, which was contrary to what was observed in the treatment groups. Serum proteins and albumin changes is clearly related to haemodilution in this study may have been due to the flux of interstitial fluid as observed by Abdallah and Abdelatif, 2010.

Splenectomy and blood indices

Commented [DS01]: Footnotes explaining what A,B,C and D represents is required here

Table 1:

Pre-operative Haematological Parameters of Mild and Severe IIR and Splenectomy in Wistar Rats

	PCV%	Hb g/dL	RBc10 ⁶ /μL	WBc 10 ³ /μL	Platelets10 ⁵ /μL	Lym %	Neut %	Eos%	Mono%
MIIR	40.4 ^a ± 1.6	12.6 ^a ±0.3	6.3 ^a ±0.3	14500 ^a ± 376.8	138000 ^a ± 4000.0	39 ^a ± 4.6	53 ^a ±5.8	3 ^a ± 1.2	5 ^a ± 1.1
Splenectomy+MIIR	40.0 ^a ± 1.8	12.8 ^a ±0.4	6.8 ^a ±0.5	9300 ^a ± 507.0	148000 ^a ± 2966.4	71 ^a ± 4.6	26 ^a ± 2.2	1 ^a ± 1.1	2 ^a ± 1.7
SIIR	45 ^a ± 4.1	14.5 ^a ±0.5	7.7 ^a ±0.6	6200 ^a ± 587.4	52000 ^a ± 3162.3	64 ^a ± 4.6	32 ^a ± 2.9	2 ^a ±1.5	1 ^a ± 1.2
Splenectomy+SIIR	48 ^a ± 1.1	15.2 ^a ±0.4	7.4 ^a ±0.6	12800 ^a ± 609.9	184000 ^a ± 3847.0	83 ^a ± 4.9	14 ^a ± 1.7	1 ^a ± 0.5	2 ^a ± 1.5

IIR = Intestinal ischaemia reperfusion injury

Table 2:

Post-operative Haematological Parameters of Mild and Severe IIR and Splenectomy in Wistar Rats.

	PCV %	Hb g/dL	RBc10 ⁶ /μL	WBc 10 ³ /μL	Platelets10 ⁵ /μL	Lym %	Neut %	Eos%	Mono%
MIIR	32.4 ^b ± 2.6	9.7 ^b ± 0.1	5.3 ^b ± 0.3	16250 ^b ± 288.1	186000 ^b ±12328.8	78 ^b ± 6.1	20 ^b ± 3.0	1 ^a ±0.8	1 ^b ± 0.8
Splenectomy+MIIR	42 ^a ± 1.7	13.6 ^a ±0.5	6.9 ^a ± 0.4	8450 ^a ± 466.9	132000 ^a ±9939.8	55 ^b ± 5.2	39 ^b ± 2.6	2 ^a ± 2.4	4 ^b ± 1.7
SIIR	45 ^a ±2.6	14.4 ^a ±0.7	7.5 ^a ± 0.5	11000 ^b ±612.4	111000 ^b ± 3646.9	68 ^a ± 4.8	29 ^a ± 3.0	1 ^a ± 0.7	2 ^a ± 2.0
Splenectomy+SIIR	39 ^b ± 1.9	12.8 ^b ±0.3	6.2 ^b ± 0.2	9100 ^b ±904.4	124000 ^b ±8648.7	83 ^a ± 4.1	15 ^a ± 2.4	0 ^a ± 0.5	2 ^a ±1.5

Table 3

Serum Chemistry values following Mild and Severe IIR and Splenectomy in Wistar Rats

	Urea Pre Mg/dL	Post	ALT Pre U/L	Post	AST Pre U/L	Post	TP Pre g/dL	Post	ALB Pre g/dL	Post
Control MIIR	12 ^a ± 0.8	12 ^a ± 1.6	35 ^a ± 4.8	20 ^a ± 2.2	43 ^a ± 4.2	31 ^a ± 0.8	8.5 ^a ± 1.9	7.3 ^a ± 1.0	4.8 ^a ± 0.6	4.3 ^a ± 0.9
Splenectomy+MIIR	9 ^a ± 1.5	11 ^a ± 0.9	21 ^a ± 0.9	30 ^a ± 1.6	24 ^a ± 2.8	32 ^a ± 3.0	7.8 ^a ± 0.6	7.2 ^a ± 0.6	3.8 ^a ± 1.1	4.2 ^a ± 0.4
Control SIIR	11 ^a ± 0.8	8 ^a ± 1.2	28 ^a ± 3.7	26 ^a ± 2.8	30 ^a ± 4.0	29 ^a ± 2.2	7.8 ^a ± 0.6	7.6 ^a ± 0.8	3.2 ^a ± 0.2	3.4 ^a ± 0.2
Splenectomy+SIIR	10 ^a ± 1.6	11 ^a ± 0.8	18 ^a ± 1.9	19 ^a ± 1.5	19 ^a ± 1.8	29 ^a ± 2.2	8.0 ^a ± 0.2	6.8 ^{ab} ± 0.5	3.9 ^a ± 0.8	3.4 ^b ± 0.5

*Pre and Post values with same superscripts are not significantly different

The significant changes in albumin may be due to its contribution to overall osmotic pressure. The most significant finding was that changes in haematology and serum chemistry in the SIIR group were the reverse of those in the MIIR group while those in MIIR and splenectomy group showed a picture of recovery and combined with splenectomy in the SIIR and splenectomy group, we noted that the haematological picture was similar to a long term regenerative anaemia.

In conclusion, Intestinal Ischaemia Reperfusion Injury alters haematology in at-risk animals as a consequence of MPS cells which are primarily responsible for the upregulation of ROS formation. Inclusion of elective splenectomy may serve as a useful tool for limiting those effects in conditions of vascular impairment and its common sequelae.

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