Histopathological Effects of Ischemia - Reperfusion on Intestinal Anastomotic Wound Healing in Dogs

Efectos Histopatológicos de Reperfusión Isquemia en la Anastomosis Intestinal de Heridas Curadas en Perros

*Akinrinmade, J. F. & **Olatunji-Akioye, A. O.

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SUMMARY: The histopathological effects of three levels of ischemia-reperfusion (IR) on healing of intestinal anastomotic wound were investigated in dogs. Three groups of dogs in which superior mesenteric artery (SMA) and collaterals were isolated but not occluded (A -control), isolated and occluded immediately distal to the aorta with collateral interruption using an atraumatic arterial clip for fifteen minutes (B) and for forty-five minutes (C) prior to ileal resection and anastomosis after return of normal perfusion in occluded groups was done. Within each group, animals were anaesthetized on third and seventh post-operative day. Grossly, impaired abdominal wound healing, intra-abdominal adhesions and partial and complete anastomotic dehiscence occurred in groups B and C commensurate with intensity of ischemic injury. Histologically, light cellular infiltration with loose granulation tissue was observed in the control group with moderate neovascularization and epithelialization by the seventh day. With mild IR, moderate cellular infiltration was observed four days post operation, but haemorrhage and oedema persisted with minimal mucosal regeneration at seven days post operation. With profound IR, sloughing of the mucosa with cellular infiltration at four days with absence of mucosal regeneration and persistence of inflammatory cellular infiltrates minimal neovascularization and dense granulation tissue. Neutrophilia in the immediate post operative period appears significant to impaired healing due to ischemia-reperfusion.

KEY WORDS: Histopathology; Ischemia-reperfusion injury; Intestinal anastomoses; Wound healing.

INTRODUCTION

Healing of intestinal tissue wounds are affected by various factors, of which intrinsic vasculature is paramount as this determines the early movement of mediators of inflammatory process into the wound and commencement of wound cleansing to prevent infection which also affects healing significantly (Hunt *et al.*, 1969).

Ischemia-reperfusion injury significantly impairs healing of intestinal anastomosis either directly or as a systemic phenomenon (Kuzu *et al.*, 1998; Kuzu *et al.*, 2000). It has been established that damage from an ischemic event is a product of two phases of injury with the most significant occurring after reperfusion, the severity being dependent on the intensity of the insult (Grace, 1994; Schiller *et al.*, 1993; Olatunji-Akioye & Akinrinmade, 2006). When blood flow is restored to ischemic tissues, a local inflammatory response begins, which is then enhanced

by the production of bioreactive oxygen radicals (Koksoy *et al.*, 2000), which promote increased mitochondrial membrane permeability (Qian *et al.*, 1997). Also, production and release of pro-inflammatory agents lead to polymorphonuclear leukocyte adherence and emigration causing microvascular leakage leading to cell death in remote organs by necrosis or apoptosis, which results in systemic immune response syndrome (MacLellan & Schneider, 1997; Austen *et al.*, 1999).

The interplay of cells during inflammation and wound healing is macrophages and fibroblasts are predominant during early inflammation, whereas lymphocytes peak later (Witte & Barbul, 1997). Neutrophil infiltration is the primary mediator of reperfusion induced tissue damage through its effects on microvascular permeability and bacterial contamination when present (Megison *et al.*, 1990).

*Department of Veterinary Surgery & Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

**Veterinary Teaching Hospital, University of Ibadan, Nigeria.

Collagen production, which determines the gain in strength of anastomoses is by both fibroblasts and smooth muscle cells in the gastrointestinal tract (Graham *et al.*, 1987) and influence of xanthine oxidase derived specie on collagen matrix is inhibitory (Arisawa *et al.*, 1996).

MATERIAL AND METHOD

Ten dogs (six female and four male aged between nine months and a year) were used for this study. They were housed at the Department of Veterinary Surgery kennel. They were fed a meal of rice and fish pre-operatively and pap and milk peri- and post-operatively. They were brought to condition base-line blood samples were collected to establish normal parameters. Operation day was determined as day 0.

The dogs were pre-medicated using atropine sulphate (atropine injection B.P., Hamex Medica Ltd, UK) at a dose rate of 0.1mg/kgbw intra-muscularly and xylazine hydrochloride (Xylazine 20 inj. Kepro, Holland) at a dose rate of 2mg/kgbw intramuscularly. Anaesthesia was induced and maintained using Pentobarbitone sodium (6% solution, Kyron Laboratories, South Africa) at a dose rate of 25mg/kgbw, intravenously.

Operative procedure. The ventral abdomen was shaved and prepared for aseptic surgery. A ventral mid-line incision was made on the skin from the xiphoid to the pubic region through the skin. The intestines were exteriorized and covered with sterile towel soaked in saline at 37°C in order to minimize fluid evaporation and heat loss. Maintenance of body temperature was achieved by using a heating lamp, and warm Lactated Ringer's solution is given intravenously during the operation, which also helps prevent dehydration of the animal.

The superior mesenteric artery (SMA) was located beneath the first lumbar vertebra close to the celiac artery within the root of the mesenteric lymph node just distal to the aorta.

In group A, the SMA and collateral vessels were not occluded; a 5cm length of the ileum was resected and anastomosed.

In group B, the SMA is clamped with an a-traumatic arterial clip along with the collateral supply for 15 minutes.

In group C, the SMA was similarly clamped along with the collateral blood supply for 45 minutes.

When declamped, restoration of vascular supply was assessed based on the color of the intestines, return of pulsation

and peristalsis. After declamping, a 5cm length of the ileum about 5cm from the ileoceccocolic junction in both groups B and C.

Following return of normal blood supply, anastomosis of the resected ends of the intestine was achieved by end-to-end anastomotic technique. The anastomosis was tested for leakage by placing a syringe filled with 5mls normal saline, using fingers to occlude both ends of the intestine and delivering the normal saline therein.

The laparotomy incision was closed in 3 layers- the linea alba was closed using chromic cat-gut (size 2-0 Hospibrand), in a simple continuous suture pattern (Ahchong *et al.*, 1996), the subcutis using a subcuticular suture pattern while the skin was closed using nylon (size 2-0 Hospibrand) in a horizontal mattress suture pattern.

Post-operative care. which was given routinely consisted of antibiotic therapy (penicillin and streptomycin in a 2:1 mixture, (Streptopen, Kepro, Holland) at a dose rate of 5ml/10kg bwt intramuscularly. The dogs were fed a diet of pap and milk and housed in individual kennels.

Sample collection. Re-operation of the animals was done on either day 4 or 7 postoperatively in all the groups in order to assess the intestinal anastomotic site after which the anastomotic site was removed and preserved in formalin. The samples were then embedded in paraffin for serial longitudinal sections to be cut and stained with Heamatoxylin and Eosin (H and E).

Histological Assessment. This was done by using a double blind method and it involves scoring done according to De Roy van Zuidewijn *et al.*, 1992:

The examination of wound healing procedure was based on re-epithelialization of the mucosa and the repair of the muscularis propria and this was scored on a seven point scale while the repair of the muscularis propria was considered as positive or negative.

Necrosis, inflammatory exudates, granulation tissue and the level of granulocytes, macrophages and fibroblasts present in the granulation tissue was evaluated and scored on a four-point scale as 0-negative, 1+-low, 2+- moderate, 3+-high.

RESULTS AND DISCUSSION

In this study, it was observed that at four days post-operation, loose granulation tissue was present in the control group (Fig. A1), intact mucosa with foci of cellular

infiltrates was present in the mild ischemia group (Fig. B1) whereas there was sloughing of canine intestinal mucosa with cellular infiltration, which was significantly more than the other groups (Fig. C1 and Table I). it is however important to note the increased inflammation and cellular infiltration, which is evident even in adipose tissue (Fig. C1b) and the presence of macrophages to the same degree, fibroplasia is least in the profound group (Table I) and this may be responsible for the impaired healing later observed (Olatunji-Akioye & Akinrinmade) as fibroblasts are responsible for collagen formation as well as smooth muscle cells in the intestines and this lays the framework for granulation tissue formation (Graham *et al.*). Also, the extent of fibroplasia is proportional to oxygen tension, which is dependent on vascularization.

Table I. 4 days post-operation

Histopathological features	Control A	Mild B	Profound C
Inflammatory	1+	1+	3+
Inflammatory cells:			
Neutrophils	1+	3+	4+
Macrophages	2+	2+	2+
Lymphocytes	1+	1+	1+
Plasma cells	2+	0	0
Granulation tissue	3+	3+	3+
Fibroplasia	3+	2+	1+
Neovascularization	3+	3+	2+
Reepithelialization	Moderate	Moderate	Bad

Table II. 7 days post-operation.

Histopathological features	Control A	Mild B	Profound C
Inflammatory exudates	1+	4+	1+
Inflammatory cells:			
Neutrophils	1+	2+	1+
Macrophages	2+	2+	1+
Lymphocytes	1+	1+	3+
Plasma cells	2+	0	1+
Granulation tissue	3+	3+	1+
Fibroplasia	3+	2+	3+
Neovascularization	3+	2+	2+
Reepithelialization	Moderate	Bad	Bad

Seven days postoperation, neovascularization and granulation tissue was observed in the control group (Fig. A2) which suggests that re-epithelialization is commencing as epithelial cells move and replicate most rapidly in hydrated and well oxygenated tissues. In the mild ischemia group, there's absence of mucosal regeneration with minimal neovascularization and cellular infiltrates (Fig. C2).

Wound healing is a complex process involving different biologic and immunologic systems and despite improvements in diagnostics and therapy, wound failures remain a critical problem (Witte & Barbul).

Fig. A1. Control. Day 4-canine intestines: loose granulation tissue with diffuse mononuclear cell infiltration.

Fig. A2. Control. Day 7-canine intestines: neovascularization granulation tissue mostly plasma cells observed.

The histopathology of IR is based on hemostasis, which precedes inflammation where the fibrin clot serves as scaffolding for invading cells, such as neutrophils, monocytes fibroblasts, plasma cells and endothelial cells (Kurkinen *et al.*, 1980). Chemotaxis of cells into wound is followed by functional activation, which is the phenotypic altering of cellular, biochemical and functional properties induced by local mediators. This induces new cell surface antigen expression, increased cytotoxicity, increased production and release of cytokines and other effects.

Fig. B1. Mild ischemia. Day 4-canine intestine: intact mucosa, loose submucosal granulation tissue with foci of cellular infiltrates.

Fig. B2. Mild ischemia. Day 7-canine intestine: loose, oedematous and heamorrhagic granulation tissue, minimal mucosal regeneration.

Fig. C1. Profound ischemia. Day 4-canine intestinal mucosa: sloughing of mucosa with cellular infiltration.

Fig. C1b. Profound ischemia. Adipose tissue infiltrated by polymorphonuclear cells. Loose collagenous fibrous connective tissue and neovascularization observed.

Fig. C2b. Profound ischemia. Day 7-canine intestine: absence of mucosal regeneration, dense granulation tissue with minimal neovascularization and inflammatory cellular infiltrates.

Fibroblasts in surrounding tissues need activation from their quiescent states and then migrate into the wound site. Granulocytes are responsible for a significant degree of collagenolytic activity and contamination and tissue necrosis increases their presence. They are however almost totally absent from ileal anastomoses by day seven (Hesp *et al.*, 1985).

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RESUMEN: Se estudiaron, en perros, los efectos histopatológicos de tres niveles de reperusión isquemia en la curación de anastomosis intestinal. Fueron utilizados tres grupos de perros, en los cuales las colaterales de la arteria mesentérica superior fueron aisladas, pero no ocluidas (A- control), aisladas y ocluidas inmediatamente distal a la aorta con interrupción colateral usando un clip arterial no traumático, por 15 minutos (B) y por cuarenta y cinco minutos (C), previo a la resección ileal y la anastomosis después de retornar a la perfusión normal en los grupos ocluidos. Dentro de cada grupo, los animales fueron anestesiados al tercer y séptimo días postcirugía. En la cicatrización del daño abdominal se observaron adhesiones y dehiscencias parcial y completa en los grupos B y C, correspondiéndose con la intensidad del daño causado por la isquemia. Histológicamente, se observó en el grupo control, baja infiltración celular con tejido de granulación laxo, con moderada neovascularización y epitelización, desde el séptimo día. Con una suave IR, también se observó moderada infiltración celular cuatro días postcirugía, pero la hemorragia y el edema persistieron con una mínima regeneración mucosa hasta los siete días después de la operación. Con una profunda IR, la mucosa se desprendió con infiltración celular hasta los cuatro días con ausencia de regeneración mucosa y persistencia de infiltrado celular inflamatorio con mínima neovascularización y tejido denso de granulación. Se observó una significativa neutrofilia en el postoperatorio inmediato lo que perjudicó la cicatrización debido a la reperusión-isquemia.

PALABRAS CLAVE: Histopatología; Reperusión isquemia; Anastomosis intestinal; Cicatrización de heridas.

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Correspondence to:
Dr. Adenike O. Olatunji-Akioye
Veterinary Teaching Hospital,
University of Ibadan
NIGERIA

Tel: 234-803-409-1407

Email: bonik2001@yahoo.com

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