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HISTOPATHOLOGICAL STUDY OF PLATELET RICH PLASMA FOR PROMOTES CORNEAL RE-EPITHELIALIZATION FOLLOWING ABRASION IN THE RABBITS

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Abstract
The study aimed to evaluate the beneficial effect of platelets rich plasma (PRP) on corneal re-epithelialization in rabbit. Sixteen healthy male adult rabbit were used they were randomly divided into two equal groups (control group 8, treated group 8). A 3 mm diameter trephine was used to demarcate the central epithelial region of the right eye and the epithelium within the demarcated region was mechanically removed using an Alger brush II, treated group receive immediately post operation PRP (1ml) was subconjunctival injected on injured eye, while control group left without treatment. corneal healing process follow up 14 days post treatment by histopathological examination and clinically, the result display in treated group on the14 days post operation appear good epithelial layer, less infiltration of immune cells compare to the control group there is severe odema, ulceration , vascularization, hemorrhage and high infiltration of immune cells.

Introduction

The cornea is an avascular, optically transparent tissue that functions to filter incoming light and protect the rest of the eye from infection and blinding injuries. However, the cornea is highly susceptible to physical injuries, such as those occurring from epithelial abrasion or refractive surgeries. These injuries directly damage the stratified epithelial layer and cause keratocytes to die in the anterior region of the stroma beneath the affected site. While numerous studies have contributed to our current understanding of epithelial healing and keratocyte death (Li *et al*., 2007). In human medicine, extensive researches were conducted to treat corneal ulceration; recently, trials with platelet-rich plasma (PRP) were performed to assess its efficacy in healing corneal ulcers (Alizadeh *et al*., 2019). These trials were conducted after promising findings were recorded in experimental animals (Tanidir *et al*., 2010).

Autologous platelet-rich plasma (PRP) has also proven beneficial for cell proliferation and wound healing (Hartwig., *et al* 2004) The difference between autologous PRP and autologous serum is that platelets are preserved in the autologous PRP. Platelets are an excellent source of numerous growth factors such as platelet-derived growth factors (PDGFs) aa, bb, and ab, transforming growth factors (TGFs) β1 and β2, vascular endothelial growth factor, and epithelial growth factor. Platelets also adhere to the damaged vascular endothelium and initiate a healing reaction mediated by the release of numerous cytokines and growth factors(Alio., *et al* 2007) Autologous PRP is rich in growth factors with known roles in healing of epithelial and internal wounds. Clinically, some ocular surface defects, such as corneal ulcer and dry.

Material and Methods

Experimental animals:

A total of 16 apparently healthy adult local breed male rabbits were recruited for this study. All animals were evaluated clinically by physical examination before initiation of the experiments. The animals were housed in metal cages 30/70/60 cm in an air- conditioned room in the animal house along the period of the experiments. They were received free accesses to water and food. The animals were left 14 days for adaption with experimental condition with using of prophylactic drug, the animals were divided into two equal groups (control and treatment groups). Control group left without treatment, Specimens from the injured cornea were taken at 2 weeks post treatment for histopathological and clinical examination to evaluate the progress of corneal healing process.

PRP Preparation:

3ml of Blood were collected from each rabbits using a 3ml disposable syringe. The samples were transferred into anticoagulant tubes containing 0.35ml of 10% sodium citrate. The blood was initially centrifuged at 160 rpm, for ten minutes at room temperature. After the first centrifugation, two layers were observed in each sample. A red lower layer that consists of packed red blood cells and an upper straw-yellow layer that contains plasma component. The upper surface of packed red blood cells called Buffy coat is rich in platelets and leukocytes. Plasma and buffy coat were transferred to new sterile tubes. The retained component of blood samples was centrifuged again at 160 rpm for two minutes to obtain more concentrated platelets. Then, the plasma and Buffy coat was centrifuged for the second round at 400 rpm, for 15 minutes. Two layers eventually appeared: the upper two thirds of the sample was designated as platelet poor plasma (PPP) and was discarded, on the other hand, the lower third was PRP .Moreover, the platelets were activated by 0.05 ml of 10% calcium chloride solution to each 1 ml of PRP (Maghsoudi, o., *et al* 2015).

Wound protocol

Rabbits were anesthetized by administering xaylazine (10 mg/kg body weight) by intramuscular injection. And ketamine (135 mg/kg body weight) by intramuscular injection. The central corneal epithelium was abraded as previously described (Lam ., et al 2010). Briefly, a 2 mm diameter trephine was used to demarcate the central epithelial region). of the right eye and the epithelium within the demarcated region was mechanically removed using an Algerbrush II (Alger Equipment Co., Inc., Lago Vista, TX) while viewing the cornea under a dissecting microscope. The corneal epithelial abrasion injury model has been used for over a decade to evaluate corneal inflammation, epithelial healing, stromal recovery, and keratocyte death and recovery (Li ., *et al* 2010). Uninjured right corneas were obtained from separate sets of mice of each genotype for all baseline measurements.

Subconjunctival injection of PRP

After the corneal alkali burn, the16 rabbits were randomly divided into PRP (n= 8) and control (n= 8) groups. Rabbits in the PRP group received a subconjunctival injection of 0.5 ml PRP immediately and repeat after 7 days post corneal injury. In the control group, rabbits left without treatment. All rabbits in each group were clinically evaluated.

Result

1- Clinically

A- Control group: 14 days post treatment the figure shows the dry eyelid skin with pus-like discharge and a white or grey spot on the iris, this refers to beginning of opaque corneal ulcer (fig. 1A). Also the corneal opacity appears at grade 3, corneal odema and the bulbar conjunctiva appear a fully hyperemic pattern was observed in the control.

B- Treatment group: also 14 days post treatment the figure shows elicits the cornea have partial opacity grade 1 and have no ulceration and less vascularization on corneal surface. The bulbar conjunctiva adjacent to the injury was mildly hyperemic, (fig. 1B).

Histologically:-

A- Control group: 14 days post treatment we observed that the corneal odema and the stroma contain large number of inflammatory cells, especially lymphocytes, macrophages and neutrophils and plasma cells interspace of collagen was irregular (fig. 2A)

B- Treatment group: 14 days post treatment we observed that The cornea has scattered infiltration of inflammatory cells, normal stroma which composed of regular arranged collagen, and complete epithelial repair with normal stratified squamous epithelium, there is mild corneal odema and the interspace of collagen was regular (fig. 2B)

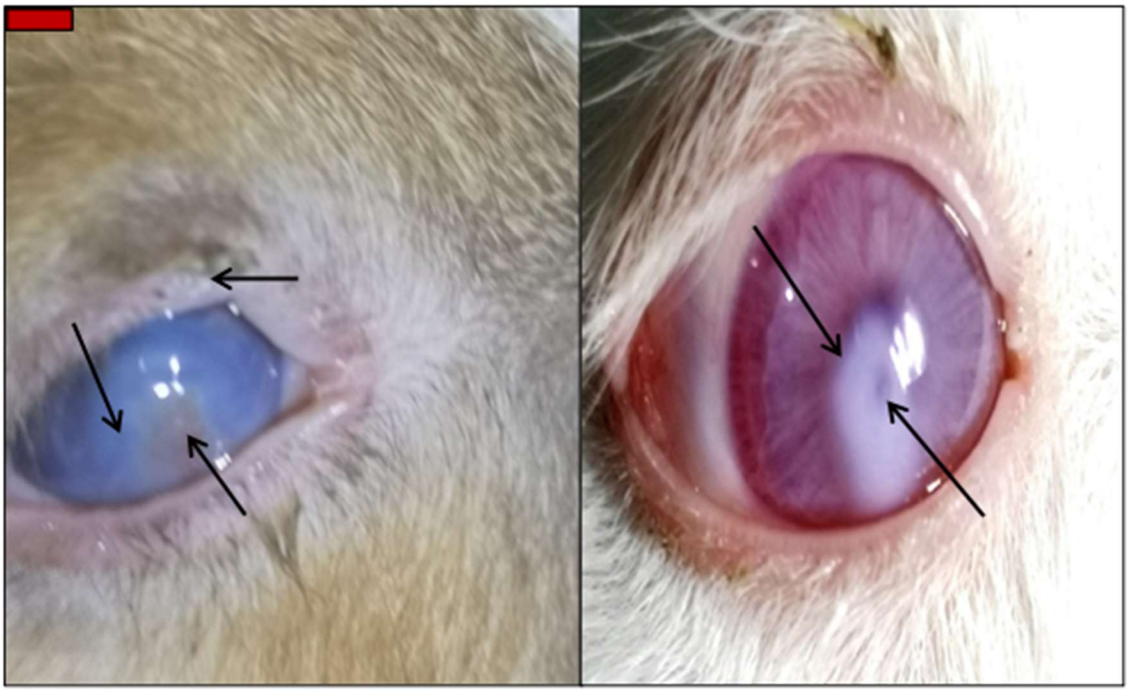

Fig (1): A- Rabbit cornea in control group 14 days post injury. There is dry eyelid skin (E) with pus-like discharge (P) and a white or grey spot on the iris (S), this refers to beginning of opaque corneal ulcer (U). B- Rabbit cornea in treatment group 14 days post injury. There is few or mild purulent exudate (P) with a very small spot on the iris and less vascularization and mild hyperemic.

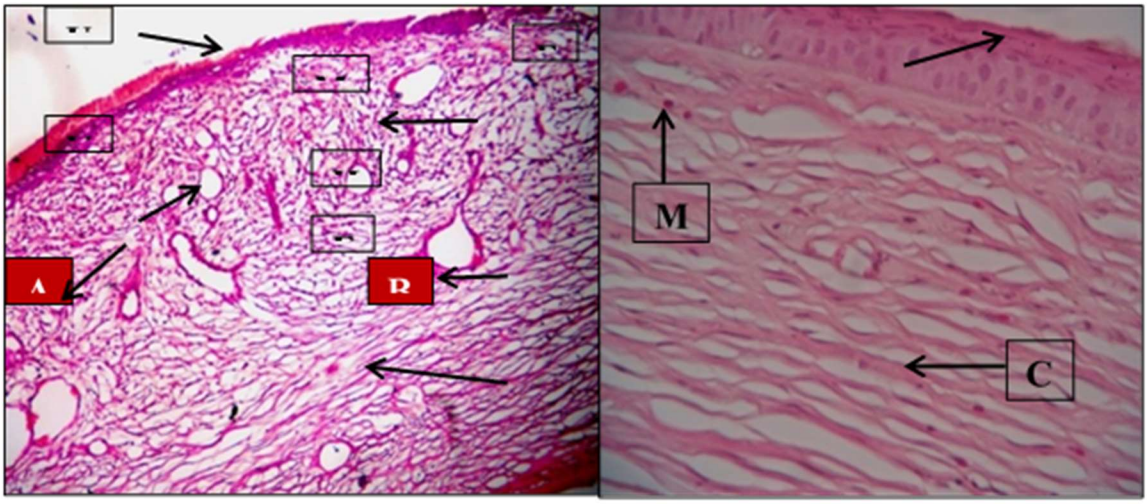

Figure (2): A- Rabbit cornea in control group 14 days post injury. There is neovascularization, complete sloughing and ulceration of the corneal epithelium (U), hemorrhage (H), high infiltration of inflammatory cells (M) and stromal fibrosis may be seen (F) and interspace of collagen was irregular, 10X H&E. B- Rabbit cornea in treatment group 14 days post injury. The cornea has scattered infiltration of inflammatory cells (M), normal stroma which composed of regular arranged collagen (C), and complete epithelial repair with normal stratified squamous epithelium (E), 10X H&E.

Discussion

The eyes' position in the body makes them continuously exposed to numerous agents and traumatic events (Donatti., *et al* 2013). These circumstances make them react to any insult constrained and may lead to vision loss (Cremonini., *et al* 2007). In recent years, PRP was used in the regeneration and reconstruction of tissues, plastic, and cardiovascular surgeries as well as it aids in corneal lesion healing (Acosta ., *et al* 2014).

PRP, especially E-PRP, was advocated for usage in human medicine for its cost-effectiveness, ability to heal the corneal wound as sole/adjuvant therapy, and relative safety (Arnalich., *et al* 2016); it was used successfully in the treatment of dormant corneal ulcer and was proven to help reduce inflammation and pain in these patients (Alio., *et al* 2007). The absence of clotting factors and the presence of many growth factors as the main advocate for its usage (Arnalich., *et al* 2016). In one human study, PRP was used as eye drops, the healing was improved significantly, and complete healing was possible in most of the participant cases (Alio., *et al* 2007).

in the current study the PRP showed that the initiated re-epithelialization of injured corneal after 14 days after treatment this compatible with Alio *et al.,* 2007 concluded that autologous PRP enhanced healing of dormant corneal ulcers and was accompanied by reduction in inflammation. Also our study showed that PRP contain growth factor important for cell regeneration that lead to promoting of tissue repairing this consistence with Hartwig *et al.,* 2004 reported a superior effect on cell growth in platelet releases than in serum owing to its high content of growth factors and concluded platelet release could be a novel treatment option for ocular surface disorders. in the

present study there is inflammatory cells, keratocyte and fibroblast it is important for tissue healing this is compatible with Wong TTL, who found throughout the corneal defect, wound healing is started by recruitment of leukocytes, fibroblasts, and vascular endothelial cells to begin healing phases, including inflammation, angiogenesis, re-epithelialization, granulation tissue formation, and ECM deposition in response to MMPs and other proteinases (Wong TTL., *et al* 2007).

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