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Life Science Archives (LSA)

ISSN: 2454-1354

Volume – 7; Issue - 4; Year – 2021; Page: 2179 – 2184

DOI: 10.22192/lsa.2021.7.4.1

Research Article

EFFICACY OF AUTOLOGOUS PLATELET-RICH PLASMA ON CORNEA WOUND HEALING AFTER ALKALI BURN IN RABBIT

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Abstract

The present study was aimed to evaluate the beneficial effect of Platelets Rich Plasma (PRP) on corneal healing in Rabbit. Sixteen healthy male adult Rabbit were used they were randomly divided into two equal groups (Control group 8 and Treated group 8). A piece of 3 filter paper (3mm diameter) soaked in 4 ml NaOH (1 mol/l) was applied to the centre of the cornea for 40 seconds. The cornea was then rinsed with 60 ml of saline for 1 minute treated group receive immediately post operation.PRP (0.5ml) was sub-conjunctival injected at the site of the eye, while control group left without treatment. Corneal healing process follow up 21 days post treatment by histopathological examination and clinically the result display in treated group on the 21 days post operation development of mild Odema, less infiltration of immune cells compared to the control group there is severe Odema, Ulceration, Vascularization and High infiltration of immune cells.

Article History

Received : 16.04.2021
Revised : 13.05.2021
Accepted: 06.06.2021

Key words: Platelets Rich Plasma (PRP), Rabbits, Cornea wound healing and Odema.

1. Introduction

The cornea, the transparent part of the eye, performs a significant function in eyesight by refracting the light to focus a visual image. As the cornea is indispensable for vision, corneal inflammation may induce visual disturbance and blindness. Several investigations have reported that various corneal inflammatory diseases cause visual impairment and chronic inflammation of the cornea can lead to blindness (Wang *et al.*, 2014).

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It is well documented that chronic inflammation of Human Corneal Fibroblasts (HCFs) leads to several corneal diseases including corneal opacity and ulceration, and these conditions lead to vision impairment in severe cases (Xi *et al.*, 2011).

Autologous platelet-rich plasma (PRP) has also proven beneficial for cell proliferation and wound healing (Chidambaram, 2007). 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. 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 eye, have been treated with autologous PRP (Alizadeh *et al.*, 2019). As a complement to tissue regeneration procedures, PRP can also support the wound healing process in other specialties such as oral and maxillofacial surgery, reconstructive surgery, orthopaedics, cardiovascular surgery, and plastic surgery. This present study was aimed to assess histologically the effect of subconjunctival injection of autologous PRP in the treatment of corneal alkali burn in rabbit.

2. Materials 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 3 weeks post treatment for histopathological and clinical examination to evaluate the progress of corneal healing process.

PRP Preparation

Three ml of Blood were collected from each rabbits using a 3 ml disposable syringe. The samples were transferred into anticoagulant tubes containing 0.35 ml 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 *et al.*, 2015).

Animal model of Corneal alkali burn

A corneal alkali burn was generated in the right eye of each Rabbit (16 Rabbits). The rabbits were anesthetized by a mixture of xylazine ketamine given by IM injection (6 mg/kg. B.W. ketamine, and 10 mg/kg. B.W. xylazine). A piece of 3 filter paper (3 mm diameter) soaked in 4 ml NaOH (1 mol/l) was applied to the center of the cornea for 40 seconds. The cornea was then rinsed with 60 ml of saline for 1 minute.

Sub-conjunctival 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.

3. Result

Clinically

- A - Control group: 21 days post treatment the photograph taken show the corneal opacity grade 3, ulceration and corneal vascularization on surface of cornea and the bulbar conjunctiva a fully hyperemic pattern was established in the control group (Figure - 1).

- B - Treatment group: Also 21 days post treatment the photograph taken show the cornea has partial opacity grade 1 and have no ulceration and less vascularization on corneal surface. The bulbar conjunctiva adjacent to the injury was mildly hyperemic (Figure - 2).

Histologically

- A - Control group: 21 days post treatment we observed that the corneal odema and the stroma contain large number of immune cells, especially lymphocyte, macrophage and neutrophils (Figure - 3).
- B - Treatment group: Also 21 days post treatment we observed that the mild corneal odema and the stroma contain immune cells but low number compare with control group (Figure - 4).

Figure – 1: Treatment group photograph taken 21 days post treatment exhibited local opacity and have no ulcer and vascularization

Figure – 2: Control group photograph taken 21 days post treatment exhibited complete opacity and have ulcer and vascularization

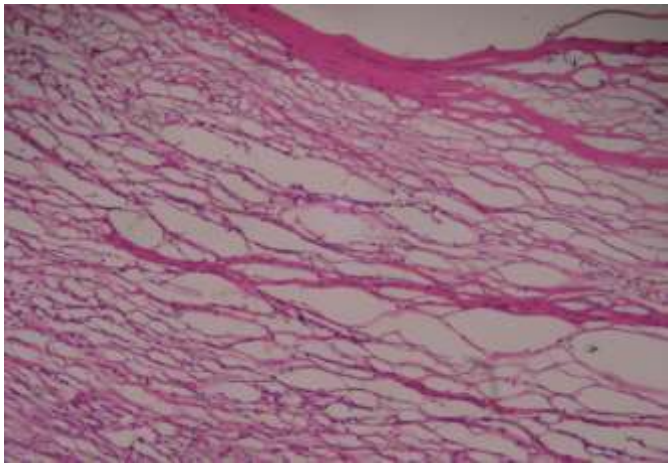

Figure – 3: Control group, H&E staining showing that the corneas of PRP there is odema, with high infiltrated of immune cells

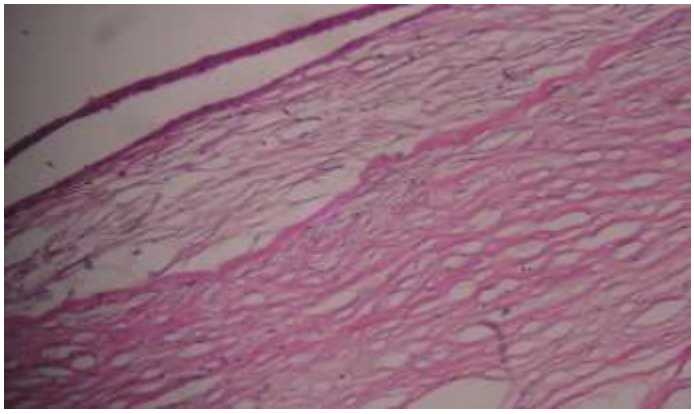

Figure – 4: Treatment group, H&E staining showing that the corneas of PRP there is mild odema with low infiltrated of immune cells

4. Discussion

Corneal alkali burn is a major health burden disrupting the overall functioning and quality of life, with continuous influx of recruited leukocytes and inflammatory cytokines (Horwitz, 1996). As major endogenous counter-regulatory mechanisms of inflammatory responses and therapeutic efficacy in treating various tissue injuries (Dombrowski *et al.*, 2017; Cho *et al.*, 2019). Tregs-treatment has also been studied in facilitating wound repair for eye diseases (Sharabi *et al.*, 2018). Blood-derived products are known to be powerful, effective, and safe remedies for various wound-healing processes (Marx *et al.*, 1998). Similarly, eye drops derived from serum have been shown to be effective and safe for the treatment of various ocular surface diseases, such as severe dry eye syndrome, persistent epithelial defects, and neurotrophic keratitis (Tsubota *et al.*, 1999). Autologous serum has a number of epitheliotrophic factors including EGF, TGF-β, fibronectin, and vitamin A. These factors are essential for the proliferation and maintenance of corneal epithelial cells and are also useful in treating ocular surface disorders (Gupta *et al.*, 1996; Nishida *et al.*, 1996). In the current study, the PRP observed that the initiated re-epithelialization of injured corneal after 21 days post treatment this compatible with Alio *et al.* (2007) concluded that autologous PRP promoted healing of dormant corneal ulcers and was accompanied by reduction in pain and inflammation. Also in our study we thought that PRP contain growth factor important for cell

regeneration that lead to accelerating of tissue healing 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 defects. in the present study there is immune cells and fibroblast it is important for tissue healing this is compatible with Wong TTL, who found during the corneal injury, 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 *et al.*, 2007). Also, we found in this study in treatment group there is no scar tissue compare to control due to contain growth factor that modulate in healing process this consistence with Donatti *et al*. (2013). In one study conducted in Rabbits, PRP contains growth factors that were directly applied on the injured surface of the cornea and in turn modulate repair and reduce formation of scars (Donatti *et al.*, 2013).

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Website	**www.jpsscientificpublications.com**
DOI Number	**DOI: 10.22192/lsa.2021.7.4.1**
Thomson Reuters Researcher ID	**L – 5547 – 2016**
ISI Impact Factor	**4.206**

How to Cite this Article:

Hayder Hussein Abd. (2021). Efficacy of Autologous Platelet - Rich Plasma on Cornea Wound Healing after Alkali Burn in Rabbit. *Life Science Archives*, 7(4): 2179 – 2184.

DOI: 10.22192/lsa.2021.7.4.1