Effects Of Topical Heterologous Blood Serum And Bovine Thrombin Activated Platelet Rich Plasma On Experimental Corneal Stromal Ulcers In Rabbit

Kaffashi Elahi, R, Mousavi, Gh, Mohajeri, D
1Assistant Professor of Small Animal Internal Medicine, Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz-Iran
2Assistant Professor of Veterinary Surgery, Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz-Iran
3Associate Professor of Veterinary Pathology, Department of Pathobiology, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz-Iran

ABSTRACT

Nowadays there is an increasing prevalence of the use of autologous blood products to facilitate healing in a variety of applications. Corneal ulcers due to trauma and burns are one of the most common ocular disorders in animals that if not cured might progress to vision impairments, ocular perforation and blindness. In this research we tried to evaluate a better method to replace the current therapies in purpose to decrease the healing period, and to improve the quality of vision retained after healing. We decided to evaluate the effects of platelet derived growth factors in combination with heterologous serum droplets on the rate and quality of healing. We adopted of negative control (5) positive control (10) treatment (10) groups totally comprised of 25 adult male rabbits weighing 2±200 g. Then by means of a probe able to produce uniform corneal ulcers in cornea made an ulcer on central cornea of each animal. Then we placed pre prepared activated PRP on the ulcer and administered daily heterologous serum. In positive control group we used chloramphenicol, ciprofloxacin and nafazoline ophthalmic drops. In negative control group stilled water drops were administered. The remaining days every 3 groups were evaluated for 35 days for the vision retainement and took daily photos. Finally in all photos ulcer diameter and ulcerated area opacity, vision, conjunctivitis and corneal edema were evaluated. Statistical analysis showed that treatment group in compare with other two groups significantly has repaired and shortened diametrically sooner and gained better vision and least oedema and least scar production. It seems this new method for treating ulcers in the body specially those on the cornea will do better than other traditional methods and help our animals back to sweet life with clearer vision.

Key words: corneal ulcer, platelet rich plasma, PRP

Introduction

In Europe and the United States, there is an increasing prevalence of the use of autologous blood products to facilitate healing in a variety of applications. Recently, we have learned more about specific growth factors, which play a crucial role in the healing process. With that knowledge there is abundant enthusiasm in the application of concentrated platelets, which release a supra-maximal quantity of these growth factors to stimulate recovery in non-healing injuries [2,20]. The application of PRP has been documented in many fields. First promoted by M. Ferrari in 1987 [7] as an autologous transfusion component after an open heart operation to avoid homologous blood product transfusion. There are now over 5200 entries in fields from orthopedics, sports medicine, dentistry, ophthalmology etc. Corneal ulcers especially with traumatic causes are one of important and severe disorders in small animal paediatric ophthalmology that if not cured might progress to vision impairments, ocular perforation and blindness due to added secondary infections and other deteriorative factors. There are many effective therapeutic trials [21] but in veterinary medicine, Patient Corporation and hard practicality of treatment, results in low functional healing. It seems that topical application of heterologous platelet jelly and blood serum may shorten the healing period of ulcerated area and causes healing of better quality. A study from Chen et al. demonstrated that PRP might potentially play a role in prevention of disc degeneration. They demonstrated that PRP can act as a growth factor cocktail to induce proliferation and differentiation and promote tissue-engineered nucleus formation.
regeneration via the same pathway [4]. Autologous plaetelet-rich plasma promoted healing of dormant corneal ulcers even in eyes threatened by corneal perforation and was accompanied by a reduction in pain and inflammation[1]. Tsubota k, et al applied daily topical blood serum drops over superficial corneal excoriations and reported its positive effects on their healing [22,23]. The aim of this research was to identify a possible better method to help cornea with healing of better quality and retaining vision faster with least scar formation and finally more clear vision.

Materials and Methods

25 healthy adult male rabbits weighing 2±200 kg were randomly selected, fed with standard ration and water ad libitum. After 2 weeks adaptation period, randomly divided into 3 groups of negative control (n=5) positive control (n=10) and treatment (n=10) respectively. All animals had access to free walking and fresh air 1 hour per day.

A fresh whole blood in Citrate Phosphate Dextrose (CPD) bags was centrifuged (200 G for 5 minutes) by double centrifugation method [15], packed cell portion were extracted and second centrifugation (1000 G for 15 minutes) were done to concentrate platelets as much as possible. Platelet concentrate were kept with no harsh handling for preventing accidental platelet degranulation. Platelet aggregate was derived and 20 microliter activator (1000 IU/ml bovine thrombin mixed with 1 ml 0.05calcium chloride) added to each 200 microliter of platelet aggregate.

All rabbits handled with extreme caution to decrease environmental stress and given oral diazepam (0.5 mg/kg) 2 hour before anesthesia, each animal were anesthetized by ketamine (80mg/kg) diazepam (0.5 mg/kg) mixture. To produce uniform ulcers, by means of a probe, on central corneal area on left eye of each animal a round stromal ulcer was created.

In Treatment group, 0.05cc of pre-prepared thrombin activated PRP placed on the ulcer and administered daily heterologous serum drops (2 drops 3 times per day). The remaining days we evaluated the repair period and captured daily standard photographs. In positive control group we used chloramphenicol, ciprofloxacin and nafazoline ophthalmic drops each one 2 drops 3 times per day. In Negative control group we used distilled water drops 2 drops 3 times per day. The remaining days every 3 groups were evaluated for 35 days for the vision retainment and took daily photos (standard and fixed settings by fixed distance between cornea and camera lens, by means of a specially designed photography tuning apparatus and a canon G7 digital camera).

Finally in all photos ulcer diameter evaluated by computer and using Corel software. Degree of vision evaluated by means of obstacle course and motion detection tests. All parameters were categorized in four degrees: loss of vision=0, weak vision=1, semi complete vision=2, complete vision=3. This experiment was conducted as blind and in all parts of research period, all animals treated with extreme gentle, mixed with healthy females, 1 hour daily access to fresh air and free walking.

Statistical analysis made by variance analysis (ANOVA) and Tukey test, at a significance level of 5% (p<0.05).

Results:

Statistical analysis showed that treatment group in compare with negative and positive control groups had significantly achieved the desired vision in shorter period of time, in other words, in day 16, treatment group achieved full vision, while in negative control vision score in the same day was not desirable and around day30 has been able to reach more than 90 percent of that vision. At the end of day35, this situation has continued. Negative control group until the end of research did not reach the complete vision (figure 4). And between groups, treatment group had significantly less inflammatory reaction and infectious process (figure 1and 3) and negative control group had maximum inflammation and purulent discharge. Corneal opacity of non-ulcerated area (figure 2) and ulcerated area (figure 6) are of important factors denoting better corneal wound healing and goes parallel with sooner vision retainment. Also Corel software aided ulcerated area diameter measurements showed significant diameter reduction in treatment group in compare to other two, but also positive control group showed better performance in compare to negative control group (figure5). Statistical analysis made by variance analysis (ANOVA) and Tukey test, at a significance level of 5% (p<0.05).

Discussion and Conclusion:

This study showed that vision retaining, certainly is faster, clearer with minimum scar production, minimum local inflammation and least infectious complications when using PRP Jelly and ophthalmic serum drops. Zhijie et al [24] in a study after cutting and removing a 6 mm diameter circle in the rat cornea, without inducing damage to limbus and stroma (superficial epithelial excoriation), investigated the re-epithelialization status and invasion of neutrophils. They found that platelets accumulate in limbal vessels and neutrophils emigrated to the damaged place. They found that the induction of thrombocytopenia in mice significantly endangered the healing process and re-transfusion of platelets acts conversely. The results show that local platelets in corneal limbus and near the wound are essential in epithelialization. The research subject of
this study is similar to both impacts on importance and role of platelet in the corneal healing. Reid and colleagues [19] found that local administration of autologous blood serum droplets are effective in the treatment of ocular superficial disorders it seems our research findings are similar with their results. Man D and colleagues [13] and Federico and colleagues [6] used of PRP sticky properties in tissue transplantation, in the present research also characteristics of stickiness and actions of remaining platelet gel products and exerting there local effects were evaluated. Pierce and colleagues [18] in a study found that the administration of PRP can reduce the final wound diameter; our results showed the same results. Nishijima and colleagues [16] and [17] found that the major role of the platelet in recalling and localization of leukocytes in acute corneal inflammation, this can be the reason for lesser local inflammation and purulent discharges in our study. Cerwinka and colleagues [3] and Tailor and colleagues [21] found that anti-neutrophilic serum, administration interferes with platelet localization in mesenteric venules up to 50 percent, it shows the important role of platelets in corneal healing and shows that our research may had concomitant results with mentioned research. Cooper D et al [5] also found that the leukocytes themselves also play a role to platelet aggregation. So in our research recalled neutrophils and PRP and serum droplets have done two important role, first, leukocytes play role in fight with microorganisms (so that treatment group had minimal to lack of any local infection) and secondly more recalling of platelets into the wounded area(5). Gawaz [9] and Kuligowski [12] and Tailor [21], found sharing and cooperation of platelet releasates in adjustment of inflammatory response and acceleration of healing process. In our study showed that the inflammation and other complications of ocular inflammation was in the lowest degree in treatment group (early elimination of corneal edema and neurological reflexes) are results that concluded from this article. In another study, two groups were chosen; one of them received routine treatment of corneal ulcers and other group received routine treatment with subcutaneous autologous platelet concentrates, Significant differences between the two mentioned groups occurred in shorter treatment period and time of discharge that reduced the time needed to complete the treatment group after discharging from hospital [14], during a study found that the applying other growth factors derived from platelets on the corneal epithelium, increase their development and trophism, these findings are consistent with our results that showed rapid wound healing and fast return of corneal transparency [11,16,17]. Far has not been established that blood platelet isolates are the only materials that contain corneal epithelial growth incentives, and Goto [10], Tsubota [22] and Fox [8] found that the other blood segments also contain corneal growth incentives, also this reveals that serum droplet have been effective in rapid wound healing. It seems that autologous serum as the non-platelet segment, have role in ocular surface damage regeneration and epithelialization. Finally with the above interpretations and study of vision ranks in different days after PRP administration results shows that treatment group gained faster and sooner healing in compare with other two groups.
It seems that there might be better indications for PRP effects on corneal ulcer healing that might do better for proving our results, like pathologic evaluations, but it is second step for next research project. Before knowing PRP positive effects we are not allowed to induce remaining defects on an animal’s cornea.

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References


