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Case report

Autologous platelet-rich plasma in the treatment of refractory corneal ulcers: A case report

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ABSTRACT

Purpose: To evaluate the efficacy of a low-cost preparation of platelet-rich plasma (PRP) eye drops in the treatment of persistent non-infectious corneal ulcer.
Observations: A 67-year-old female presented to our clinic with a wide corneal ulcer and severe paracentral corneal thinning refractory to medical therapy with antibiotics, lubricant and contact lens bandage. The patient received a novel preparation of PRP solution. After 15 days of therapy, we observed complete resolution of the corneal ulcer with regrowth of the epithelium and a reduction in corneal opacity.
Conclusion and importance: Although the low-cost PRP preparation gives a lower platelet concentration than standard procedures, our work shows this preparation to be effective in the treatment of refractory non-infectious corneal ulcer.

1. Introduction

The treatment of refractive corneal ulcer remains a challenge for ophthalmologists. Current therapies include the use of ointments, bandages with contact lenses, artificial tears, and autologous serum with fibronectin, substance P, or insulin-like growth factor.[1] Topical application of serum eye drops has been reported to accelerate healing of persistent ocular surface defects.[2] Autologous platelet-rich plasma (PRP) has also proven beneficial for cell proliferation and wound healing.[3] 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.[4] 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.[4–7] 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.[8–15]

The conventional production of autologous PRP is complicated and requires large volumes of whole blood (500 mL) as well as a hospital setting with sophisticated and expensive equipment. This article presents a case of refractive corneal ulcer treated with autologous PRP prepared in the laboratory.

2. Case report

A 67-year-old woman presented to our clinic complaining of foreign body sensation, pain, redness and photophobia in her right eye for 3 months. The patient had previously been treated with non-preservative artificial tears, antibiotic eye drops and therapeutic contact lenses to protect the cornea. Therapy was subsequently changed to tobramycin-dexamethasone eye ointments, lubricants and ganciclovir ointment, without any improvement. The patient had a positive history of obesity,

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diabetes, symptomatic epilepsy secondary to bacterial meningoencephalitis, anxiety and depression syndrome, hypertension and cardiac hypertrophy.

Objective examination revealed a large paracentral corneal ulcer (2.67× 2.50 mm) and severe corneal thinning (245 μm) measured at the ulcer base without the presence of epithelium (Fig. 1). Visual acuity was 20/100 due to corneal opacity and irregular astigmatism. Bacterial, viral and fungal cultures showed no microbial growth. Corneal sensitivity, as assessed with a Cochet-Bonnet aesthesiometer, showed a corneal hypoesthesia (30 mm). Considering the patient's history and lack of responsive to antimicrobial therapy, a neurotrophic cause was suspected.

With consent, the patient accepted a novel PRP solution as treatment. To prepare the PRP, 9 mL of autologous blood was drawn in sterile Vacuette tubes (Greiner Bio-One, GmbH, Kremsmunster, Austria) without anticoagulant solutions and immediately centrifuged (Medifuge MF200, Silfradentsrl, Forlı, Italy) at the following conditions: 30 sec acceleration, 2 min 2700 rpm, 4 min 2400 rpm, 4 min 2700 rpm, 3 min 3000 rpm, and 36 sec deceleration and stop. This process resulted in three blood fractions: (1) the upper platelet-poor plasma (PPP) layer; (2) the middle fibrin-rich gel with aggregated platelets and concentrated growth factors (CGF); (3) the lower red blood cell (RBC) layer. The patient was instructed to keep the sample in the dark and under refrigeration at +4 °C. Eachweek, a new vial was collected to prevent accidental contamination by the patient. Autologous PRP was administered to the patient every 2 hours daily for 15 days, along with preservative-free lubricant eye drops. After just 15 days, resolution of the corneal lesion was observed, and all topical medications were gradually reduced. The OCT scan demonstrated resolution of the corneal ulcer with regrowth of corneal epithelium (Fig. 2). The subjective symptoms including burning, grittiness and ocular discomfort noticeably reduced, and the conjunctival congestion slowly resolved. (Fig. 3).

3. Discussion

PRP is defined as a portion of the plasma fraction of autologous blood with an enriched platelet concentration. Autologous PRP has proven effective for tissue regeneration and wound healing.[2–7,16-18]

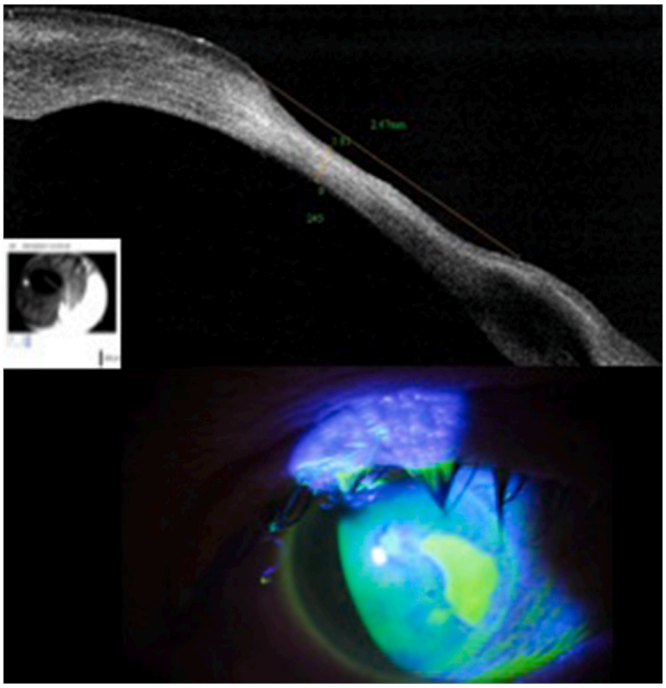

Fig. 1. Slit lamp image (lower) shows a large ulcer filled with fluorescein. The OCT image (upper) demonstrates extreme corneal thinning (245 μm) and a lack of epithelium.

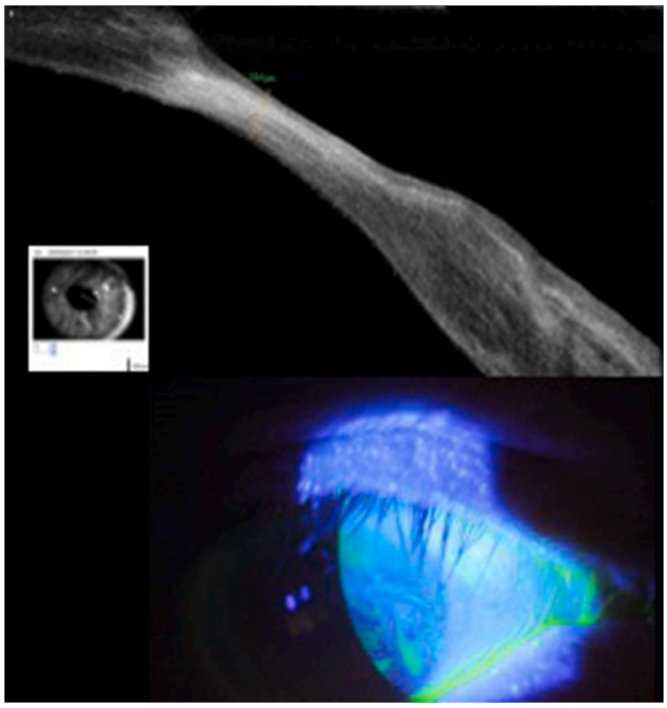

Fig. 2. Slit lamp image (lower) shows resolution of the ulcer and fluorescein pooling. The OCT image (upper) demonstrates an increase in corneal thickness (284 μm) and clear epithelial regrowth.

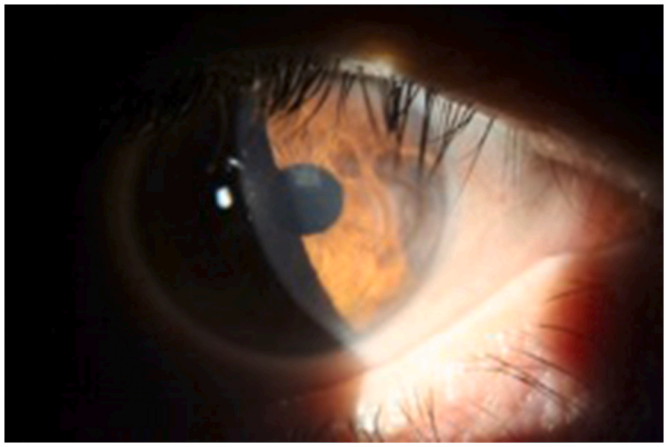

Fig. 3. Normal slit-lamp view of the eye after treatment. A mild paracentral opacity can be appreciated over the area of ulcer.

As in other tissues, corneal wound healing is regulated by a multitude of factors that modulate the proliferation, differentiation and migration of corneal epithelial cells. With PRP, an increased number of platelets and growth factors are delivered to the pathological area, including PDGFs aa, bb, and ab, TGF-β1 and-β2, VEGF, and epithelial growth factor.[16–19] Epithelial growth factor supports the proliferation and migration of epithelial cells and has been suggested to inhibit apoptosis. PDGF, hepatocyte growth factor, and fibroblast growth factor stimulate cell proliferation, whereas TGF-β seems to inhibit corneal epithelial cell proliferation and enhance apoptosis.[14,15]

When manufacturing a biologic PRP, it is essential to adhere to established specifications to assure safety and effectiveness. Conventional PRP production is complicated, requiring large volumes of whole

blood (500 mL) and a hospital environment with sophisticated and expensive equipment, which can potentially restrict its availability for socioeconomically disadvantaged poulations.[23] To the best of our knowledge, there is only one other report describing a preparation of autologous PRP in the laboratory that is not performed with the conventional kit and manufacturing cabinet.[26]

We analysed our easy-to-prepare autologous PRP, which showed in a 2-3-fold enrichment of platelets with respect to levels in blood, which is sufficient to reach therapeutic levels. Costs were also lower, as the procedure did not require specific equipment or an expensive disposable kit.[18]

The mechanism of action of autologous PRP is similar to autologous serum. However, PRP has a higher concentration of growth factors that can stimulate the growth of epithelial cells, thus leading to faster healing, from a limited volume of plasma. Moreover PRP has fewer leucocytes.[24,25]

For topical ocular surface defects, an easy preparation of growth factor-rich PRP from a blood bank laboratory is attractive. However, there are concerns about safety and traceability given the risk of bacterial contamination. To lower the risk of infection, we stored PRP in the refrigerator at 4 °C, and the patient was instructed to do the same.[19] At each check, we also monitored the condition of the PRP, including a platelet count and bacterial culture.

In a total of 10 PRP preparations, only one positive bacterial culture was found, which showed a positive *Steno-trophomonas maltophilia* culture; Although it is possible that this was a false-positive culture result, no data are available to support this conclusion. It is crucial to perform aseptic procedures to prevent sample contamination in the laboratory, and patients require health education for PRP usage at home.[17,18]

PRP has a higher concentration of platelets compared to normal plasma (approximately 150–400 $\times$ 10^9/L), and it also contains a full complement of clotting factors, which usually remain at normal physiologic levels. Lee et al. compared whole blood and PRP and found that the platelet count in PRP was approximately 4.25-fold higher than whole blood.[20,21] As demonstrated by the clinical results, our easy-to-prepare autologous PRP remained effective. This treatment is useful in the management of refractive non-infectious corneal epithelial and stromal defects, excluding infectious ulcers.

Although this is an isolated case, autologous PRP maybe a useful tool in the treatment of corneal ulcers.[22] The platelet concentration of our easily prepared autologous PRP is lower than with specialized procedures, but the autologous PRP from our laboratory could be useful in situations where the manufacture of biologic-grade PRP is not practical.

Patient consent

The patient gave written informed consent. This report does not contain any personal information that could lead to the identification of the patient.

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Authorship

All authors attest that they meet the current ICMJE criteria for authorship.

Declaration of competing interest

The following authors have no financial disclosures: MR, FR, LG, VT, MF.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajoc.2020.100838.

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