



Case report

Easy-to-prepare autologous platelet-rich plasma in the treatment of refractory corneal ulcers

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ABSTRACT

As platelets are rich in growth factors for tissue regeneration, autologous platelet-rich plasma (PRP) has been used to treat some refractory corneal defects. Although PRP is effective, the cost of its preparation is very high. This article presents three cases of refractory corneal ulcer under the prescription of autologous PRP. The autologous PRP used in these cases was easily prepared in the blood bank laboratory. In this paper, we collected three patients with refractory corneal ulcer who were unresponsive to conventional treatment. The patients presented with neurotrophic ulcer, exposure corneal ulcer, and limbal deficiency with corneal ulcer after hepatitic keratitis. Although we easily prepared autologous PRP eye drops using simple laboratory centrifugation, this preparation still had a clinical effect on corneal defect. The mean intervention time was 24 ± 6.9 days. The case with exposure corneal ulcer had significant wound healing and the other two cases felt subjective symptom relief. There were some clinical improvements of refractory corneal ulcers in our three cases. We present the clinical results of three cases and report an easy procedure for the preparation of autologous PRP. Autologous PRP prepared simply in the laboratory, it may be an alternative option for treating refractory corneal ulcer.

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1. Introduction

The treatment of refractive corneal ulcer still poses a challenge to many ophthalmologists. Previous therapeutic modalities include the use of bandage contact lenses, topical artificial tears, sodium hyaluronate, and autologous serum with fibronectin, substance P, or insulin-like growth factor 1.¹ Topical application of serum eye drops has been reported to accelerate healing of persistent ocular surface defects.

Autologous platelet-rich plasma (PRP) has also been proven to be beneficial in cell proliferation and wound healing.² The difference between autologous PRP and autologous serum is the platelets preserved in the autologous PRP. Platelets are great sources of

growth factors such as platelet-derived growth factors (PDGFs) $\alpha\alpha$, $\beta\beta$, and $\alpha\beta$, transforming growth factors (TGFs) $\beta 1$ and $\beta 2$, vascular endothelial growth factor, and epithelial growth factor. Platelets also adhere to the damaged vascular endothelium and start a healing reaction that includes the release of numerous cytokines and growth factors.³ Autologous PRP is rich in growth factors known for healing epithelial and internal wounds. Clinically, some ocular surface defects such as dormant corneal ulcer, dry eye, and ocular surface syndrome have been treated with autologous PRP.^{2–7} As a complement to tissue regeneration procedures, PRP also supports the wound healing process in other specialties such as oral and maxillofacial surgery, reconstructive surgery, orthopedics, cardiovascular surgery, and plastic surgery.^{8–15}

The conventional production of autologous PRP is complicated, involving large volumes of whole blood (500 mL), and a hospital environment with sophisticated and expensive equipment. This article presents three cases of refractory corneal ulcer under the prescription of autologous PRP that was easily prepared in the laboratory.

Conflicts of interest: The authors have no financial interests to disclose.

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2. Study design and methods

2.1. Participants

Four eyes of three patients with refractory corneal ulcers were treated at the Department of Ophthalmology Shin-Kong Wu Ho-Su Memorial Hospital in Taipei, Taiwan. This research was conducted with the approval of the Institutional Review Board.

2.2. Patients and examinations

In this study, we included three patients with refractory corneal ulcer. The mean age of the patients was 45.67 ± 30.17 years (range, 11–66 years). Their respective histories are briefly described as follows.

Case A was a 66-year-old woman who had underlying history of uremia on hemodialysis, diabetes mellitus, and hypertension for many years. She initially presented with bilateral refractory neurotrophic corneal ulcers: 9.3 mm $\times$ 5.2 mm diffuse corneal ulcer in the right eye and a 7.7 mm $\times$ 3.2 mm band form corneal ulcer in the left. Vision was only counting fingers at 30 cm in both eyes. The patient suffered from persistent ocular irritation and mucus discharge for about 1 month. Bacterial culture of the cornea showed no growth. We had already used nonpreserved artificial tears and antibiotic eye drops to protect the cornea. We also used therapeutic contact lenses for the neurotrophic ulcers. However, the corneal wound was persistent, so we added autologous PRP every 2 hours except sleep for 17 days.

Case B was a 60-year-old woman who had cataract surgery previously. Several months earlier she had received surgery for skull base meningioma, but she complained of an impairment in closing the right eye. The neurosurgeon prescribed lubrication for her. She visited our ophthalmology department because of vision change, severe eye congestion, and pain for 2 weeks. Her vision in the right eye was changed from 6/10 to 6/60. The right eye presented a diffuse corneal epithelial defect with severe infiltration almost in whole cornea. She refused tarsorrhaphy for exposure corneal ulcer. We used autologous PRP every 2 hours and topical antibiotic medication for 21 days.

Case C was a 9-year-old girl who had history of right eye amblyopia. She also had suffered from persistent herpetic keratitis 4 years earlier on the same-side right eye. After the infection, the right eye developed macular cornea, corneal scar, and neovascularization. Vision was only light perception in the right eye. Because of limbal deficiency, she suffered from recurrent ocular irritation, corneal ulcer, and stromal infiltration. Although we have used many lubricants to the irregular ocular surface, she still often complained of burning pain and discharge. Trying to relieve the symptom, she received autologous PRP every 2 hours for 35 days.

All the three patients were refractory to conventional treatment for corneal ulcer, such as preservative-free artificial tears, sodium hyaluronate eye drops, patching, or soft therapeutic contact lens applications.

2.3. Preparation of autologous PRP

In our blood bank, 20 mL of whole blood from each patient was taken by venipuncture. Before venipuncture, we used Sindine solution (povidone iodine 10% 200 mL/BT) and alcohol swab (isopropyl alcohol 70%) to scrub the area at least 4 cm in all directions from the intended site of venipuncture three times. Blood samples were drawn into 20-mL syringes. Then we transferred 2 mL into a tube (K2-EDTA) for complete blood count (Sysmex XE5000), and 18 mL blood into sterile glass tube with 2 mL of anticoagulant

CPDA-1 in preparation for autologous PRP. Samples were gently agitated to mix the anticoagulant thoroughly with the whole blood and centrifuged for 10 minutes at 800 rpm (KA1000A Kubota). After centrifugation, the upper fraction of each sample was 2–3 mL PRP. Plasma was separated carefully in a sterile manner under clean bench conditions. Then 1.0 mL plasma was counted for platelet data with Sysmex XE5000 (Sysmex Corporation, Europe GmbH, Bornbarch 1, 22848 Norderstedt, Germany), and the remaining 1.0–2.0 mL plasma was collected as the final autologous PRP product. To minimize contamination, we discarded all 5 mL of antibiotic eye drops from its sterile bottle and used this emptied bottle for storage of autologous PRP.

Patients were instructed to keep their current bottles in a dark, cool place under refrigeration at $+4^{\circ}\text{C}$. Every week, we prepared a new bottle for each patient to keep autologous PRP fresh and avoid patient infection.

Autologous PRP was administered to patients once every 2 hours daily as required in addition to preservative-free artificial tears for at least 1 week. All topical medications were discontinued with the resolution of the corneal lesions or limited response after 35 days.

3. Results

3.1. Laboratory test of autologous PRP

The results of laboratory tests are shown in Table 1. The result of microbiology tests were: the autologous PRP of Cases B and C was normal, but autologous PRP eye drops of Case A on second examination were found to contain *Stenotrophomonas maltophilia*.

3.2. Clinical features

Refractory corneal ulcer of diabetic Case A had chronic stromal infiltration and corneal scarring for a long time. The neurotrophic ulcer showed limited response to autologous PRP. Clinically, the epithelial defect still persisted and stromal infiltration had little improvement after 17 days treatment with autologous PRP. However, the patient mentioned that foreign body sensation improved subjectively. The mucus discharge and ocular congestion were decreased after autologous PRP treatment (Fig. 1).

The diffuse epithelial defect in Case B was almost healed after 21 days of PRP treatment (Fig. 2). The patient reported a gradual decrease of ocular discomfort and felt that vision improved week-by-week. Her vision recovered to 6/10. The exposure keratopathy subsided without the need for a more aggressive surgical procedure.

The refractory corneal ulcer of Case B with stromal infiltration induced by herpetic keratitis shows mild improvement (Fig. 3). The subjective symptoms such as burning pain and discharge were relieved. Meanwhile, the corneal neovascularization and conjunctival congestion declined moderately. The girl was happy to return to her daily activities because of ocular symptom improvement.

Table 1
Platelet count in whole blood and platelet-rich plasma (PRP).

	Platelet count in whole blood	Platelet count in PRP
Case A	$134.67 \pm 6.11 \times 10^9/\text{L}$	$377.67 \pm 76.79 \times 10^9/\text{L}$
Case B	$215.67 \pm 5.86 \times 10^9/\text{L}$	$580.653 \pm 52.56 \times 10^9/\text{L}$
Case C	$366 \pm 42.22 \times 10^9/\text{L}$	$795.33 \pm 16.04 \times 10^9/\text{L}$

All patient data including prothrombin time and activated partial thromboplastin time tests were normal.

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