Plasma Rich in Growth Factors as a Therapeutic Agent for Persistent Corneal Epithelial Defects

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Objective: To evaluate the efficacy of topically applied autologous plasma rich in growth factors (PRGF) as a treatment for persistent epithelial defects (PEDs) of the cornea.

Methods: A series of prospective noncomparative cases.

Participants: Twenty eyes from 18 patients with PED with various underlying etiopathologies: neurogenic, iatrogenic, associated with burning or secondary to severe dry eye. Patients were treated with a PRGF eyedrop solution. Serial photographs of the cornea were taken until epithelialization was complete. We had previously characterized the levels of a panel of growth factors (platelet-derived growth factor, epithelial growth factor, vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor, and nerve growth factor) in the PRGF of 11 of these patients. The following variables were additionally recorded: (1) duration of PED before treatment, (2) previous treatments, (3) time for complete epithelialization, and (4) treatments required concomitantly with PRGF.

Results: Epithelial defects healed in 17 of 20 cases (85%), with a mean therapeutic time of 10.9 weeks (range 2–39 weeks). Mean progression time before treatment was 26.7 weeks (range 2–104 weeks). Growth factor concentrations were platelet-derived growth factor 12645.9 ± 690.0 pg/mL, epithelial growth factor 468.9 ± 97.6 pg/mL, vascular endothelial growth factor 204.5 ± 119.4 pg/mL, hepatocyte growth factor 149.5 ± 173.5 pg/mL, nerve growth factor 37.7 ± 18.6 pg/mL.

Conclusion: PRGF, when applied as eyedrops, is a highly effective therapeutic agent for the treatment of a broad etiopathological spectrum of corneal PEDs.

Key Words: persistent epithelial defect, neurotrophic corneal ulcer, growth factor, plasma rich in growth factors

(Persistent epithelial defect (PED) of the cornea is defined as a lesion that measures more than 2 mm in diameter, persists for more than 2 weeks, and is resistant to conventional treatments.¹² The etiopathology of PED can be very variable, but the 2 principal causes are alterations in the tear surface and neurogenic dysfunctions.¹³ Other causes include burns because of chemical agents, immunological alterations, dystrophies of the epithelium and basal membrane, metabolic alterations, iatrogenia, trauma, and infections.¹³

Not infrequently, PEDs do not respond to the application of conventional treatments such as artificial tears, therapeutic contact lenses, antiinflammatory agents, oral antibiotics, inhibitors of collagenolytic enzymes, or tarsorrhaphy. Resistant PEDs continue to degenerate, in many cases toward progressive stromal lysis and subsequent perforation; therefore new therapeutic alternatives are urgently being sought. Many of these consist of natural preparations, which are rich in growth factors or of these factors alone, derived by synthetic processes. Among the topically applied natural preparations, autologous serum has been used in recent years for the treatment of PED and dry eye with satisfactory results in most cases.¹²

More recently, a novel treatment has been proposed, consisting of plasma rich in growth factors (PRGF).¹⁴ This treatment involves the application of autologous platelet protein extracts, which are rich in growth factors. However, the use of PRGF has been limited up to now to the repair of lesions of the skin, mucous membranes, and subcutaneous tissue in oral, maxillofacial, and orthopedic surgery. Thus, the objectives of the present article are to (1) describe the manner of preparation of PRGF, (2) characterize PRGF in terms of its levels of a panel of growth factors, and (3) analyze the clinical response of PED to PRGF treatment, thereby evaluating the possibility that this treatment may serve as a new efficacious and safe alternative to enhance reepithelialization.

MATERIALS AND METHODS

Patients

This study included 18 patients (20 eyes) diagnosed with PED, studied between 2004 and 2007 at the Instituto Clínico-Quirúrgico de Oftalmología (Bilbao, Spain). The mean age of patients was 61.2 years (range 33–88 years). Thirteen were men and 5 were women.

All patients gave their written consent after being informed about the treatment. Our study was approved by the Ethics Committee of the Instituto Clínico-Quirúrgico de Oftalmología, and the principles of the Helsinki Declaration.

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were complied with. The etiopathology of the epithelial defects was varied: neurotrophic ulcers (9; 6 nonherpetic and 3 herpetic), iatrogenic (6 cases; after ocular surgery), secondary to chemical burn (3), and secondary to severe dry eye (2).

The criterion for inclusion was PED with no improvement after conventional treatments, the latter including both medical (artificial tears, topical steroids and antibiotics, oral antibiotics or antiviral agents, therapeutic contact lenses, autologous serum, and so on) and surgical (amniotic membrane patch and eyelid surgery) treatments. Among the 20 eyes included in our study, 18 eyes (90%) had been receiving medical treatment consisting of artificial tears and an association of topical antibiotics and topical steroids. Two cases had also been treated with contact lenses and 2 with topical antiviral agents. Six patients had previously been treated with 20% autologous serum but without success. Surgical treatment had also been performed in 6 cases. Surgical approaches included lateral tarsorrhaphy, amniotic membrane patch, and lateral pexis of the inferior eyelid. Cases involving active corneal infection were excluded.

We decided that the application of PRGF did not need to preclude the use of other concomitant treatments (such as tarsorrhaphy, punctal occlusion, oral tetracyclines, antibiotics, antiinflammatory agents, and so on) when they were considered necessary. In addition, we included in the protocol the possibility of carrying out amniotic membrane transplant if at the end of a fortnight of treatment no objective improvement of the ulcer could be observed or if clinical features deteriorated at any stage.

**PRGF Preparation**

PRGF is a natural extract, which is obtained from the patient’s own blood. The preparation process involves a 2- to 3-fold concentration of platelets found in patient blood by centrifugation, resulting in a liquid, which can be used as eyedrops for surface application. Fifty milliliters of whole blood was collected by venipuncture in 5-mL sterile tubes containing 0.5 mL of 3.8% sodium citrate. Samples were centrifuged at 460g for 8 minutes at room temperature. The upper fraction immediately above the erythrocyte pellet was then transferred to a sterile tube. Platelet activation and fibrin matrix formation were induced by adding calcium chloride to a final concentration of 2.2 mM, and clots were allowed to retract for 2 hours at 37°C. The released supernatant is rich in growth factors. This supernatant (PRGF) was diluted 1:1 with 0.9% sodium chloride, and 2.5 mL of this diluted PRGF were transferred into 5-mL sterilized eyedrop bottles. All procedures were carried out under highly sterile conditions, operating inside a laminar flow hood. Before initiating treatment, patients were instructed to keep the bottles at −20°C until required; the bottle in use was to be stored at 4°C and discarded after 5 days.

**PRGF Treatment Regime**

A period of clearing before initiating PRGF treatment was not employed. PRGF was administered at a 1:1 dilution, initially at 1 drop every 2 hours (during daytime) for the first 3 days. Subsequently, the treatment regime was personalized as a function of the clinical evolution of each epithelial defect.

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**Ophthalmologic Evaluation**

Visual parameters were not recorded for this study.

**Measurement of Growth Factors in PRGF**

The concentration of a panel of growth factors, which could be considered to be potentially therapeutic, was measured in the PRGF of 11 patients. Platelet-derived growth factor (PDGF), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and nerve growth factor (NGF) were measured in undiluted PRGF using commercially available Quantikine colorimetric sandwich ELISA kits (R&D, Minneapolis, MN), according to the manufacturer’s instructions. All enzyme-linked immunosorbent assays were performed in triplicate.

**Evaluation Parameters**

The following variables were analyzed: duration of PED progression before PRGF treatment; previous treatments (medical and/or surgical); duration of treatment until complete epithelialization; need for PRGF-associated treatments (medical and/or surgical); levels of PDGF, EGF, VEGF, HGF, FGF, and NGF in the PRGF of these patients. Means and standard deviations were calculated using Windows NT Excel software.

**RESULTS**

PRGF was characterized in terms of the concentration of the growth factors indicated in Table 1. The most abundant growth factor was PDGF, and the least abundant one was NGF.

The mean duration of PED progression before PRGF treatment was 26.7 weeks (range 1–104 weeks, with a median of 9 weeks). The epithelial defect was restored in 85% of cases (17 of 20 eyes), with a mean epithelialization time of 10.9 weeks (range 2–39 weeks, median of 5 weeks). Table 2 summarizes the relevant characteristics of the patients.

For the 3 cases in which the epithelial defect did not heal, follow-up time was 10, 12, and 22 weeks. In these cases, the etiopathology was neurotrophic; 1 was iatrogenic (case 9), and 2 were nonherpetic (posttrigeminal ablation, case 6 and postradiotherapy, case 18). Two cases required conjunctival flap (cases 9 and 18), and another was treated with a Boston keratoprosthesis (case 6).

PRGF was applied alone to 4 eyes (20% of cases). In the remaining cases, other medical and/or surgical treatments were employed during follow-up: topical antibiotics, topical corticoids, oral antiviral agents, oral tetracyclines, topical antiviral agents, amniotic membrane transplant, and lateral tarsorrhaphy.

The aim of the treatment with PRGF was epithelialization; once it was achieved, the treatment was discontinued except in cases 1, 2, and 3. Cases 2 and 3 correspond to PED associated with severe dry eye; when the PED was resolved, PRGF treatment was maintained in a prophylactic manner at concentration of 20%. In case 1, PED was secondary to surgical damage to both facial and trigeminal nerves; after various episodes of PED successfully treated with PRGF, we finally decided to maintain treatment continuously at a concentration of 20%.
No change in corneal sensation was reported, except for patient 12, for whom anesthesia before PRGF treatment was replaced by a hypesthesia once epithelialization was complete. No differences were found regarding Schirmer test measures.

PRGF tolerance was good in 95% of cases (19 of 20). In case 18, PRGF had to be discontinued because of the patient’s discomfort (redness and itching). No other complications associated with its use were detected. In particular, we had examined for the presence of overt infection and neovascularization because of the high content of VEGF in PRGF.

Slit-lamp images of representative cases are shown in Figures 1 and 2. A nonherpetic neurotrophic PED was observed in an 83-year-old man (case 15); the duration of the defect before PRGF treatment was 2 weeks. The defect measured \(5.6 \times 4.4\) mm. Time to complete healing was 6 weeks, with PRGF being applied in the absence of other treatments (Fig. 1). An 84-year-old woman (case 17) developed a posttrigeminal ablation PED. The duration of the defect was 22 weeks. Time to complete healing was 7 weeks. PRGF was applied in conjunction with a topical antibiotic (Fig. 2).

## DISCUSSION

PED is a serious eye condition because it frequently progresses to perforation of the cornea and can eventually lead to irreversible loss of vision. Despite having various causes, these defects share in common the incapacity of the cornea to close its damaged epithelium and thus constitute an important therapeutic challenge in daily clinical practice.\(^1,2,7,8\) The forms of PED with worse prognosis have been reported to be those associated with severe dry eye and neurotrophic ulcers.\(^1\) In the case of dry eye, the lack of tears results in growth factor deprivation; in the case of neurotrophic ulcer, factors necessary for neural metabolism, such as substance P and NGF, are absent.

The inefficacy of different treatments, such as artificial tears, topic antiinflammatories, oral tetracyclines, bandage contact lenses, or tarsorrhaphy, for the treatment of PED has driven the search in recent decades for new therapeutic approaches. Thus, the effects of individually applied synthetic growth factors, neurogenic factors, and natural extracts rich in both growth and neurogenic factors have been studied.\(^9\) The growth factors used to favor epithelialization include EGF,\(^10\) FGF,\(^9\) PDGF,\(^11\) NGF,\(^9\) and fibronectin, a protein associated with wound healing.

### TABLE 1. Concentrations of Growth Factors in Undiluted PRGF

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>PDGF</th>
<th>EGF</th>
<th>VEGF</th>
<th>HGF</th>
<th>FGF</th>
<th>NGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRGF</td>
<td>12645.9 ± 1690.0</td>
<td>468.9 ± 97.6</td>
<td>204.5 ± 119.4</td>
<td>149.5 ± 173.5</td>
<td>82.6 ± 95.9</td>
<td>37.7 ± 18.6</td>
</tr>
</tbody>
</table>

Concentrations expressed as picograms per milliliter.

### TABLE 2. Characteristics of Patients With PED

<table>
<thead>
<tr>
<th>Eye</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Duration Before PRGF (Wk)</th>
<th>Time to Complete Healing (Wk)</th>
<th>Associated Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>Petic neurotrophic</td>
<td>29</td>
<td>23</td>
<td>AB, TS</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>F</td>
<td>PED associated with dry eye</td>
<td>78</td>
<td>11</td>
<td>AB, TS, OT</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>F</td>
<td>PED associated with dry eye</td>
<td>78</td>
<td>4</td>
<td>OT</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
<td>M</td>
<td>Iatrogenic</td>
<td>3</td>
<td>10</td>
<td>AB, TS</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>M</td>
<td>Chemical burn</td>
<td>10</td>
<td>39</td>
<td>AB, TS, AMT</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>M</td>
<td>Petic neurotrophic</td>
<td>33</td>
<td>No epithelialization</td>
<td>AB, OT, AMT</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>M</td>
<td>Petic neurotrophic</td>
<td>104</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>M</td>
<td>Petic neurotrophic</td>
<td>104</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>F</td>
<td>Iatrogenic</td>
<td>11</td>
<td>No epithelialization</td>
<td>AB, TS, OAV, AMT</td>
</tr>
<tr>
<td>10</td>
<td>68</td>
<td>M</td>
<td>Postherpetic neurotrophic</td>
<td>2</td>
<td>5</td>
<td>AB, TS, OT</td>
</tr>
<tr>
<td>11</td>
<td>68</td>
<td>M</td>
<td>Postherpetic neurotrophic</td>
<td>20</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>55</td>
<td>M</td>
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<td>AB, TS, AMT, LT</td>
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<tr>
<td>13</td>
<td>45</td>
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<td>3</td>
<td>4</td>
<td>No</td>
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<tr>
<td>14</td>
<td>40</td>
<td>M</td>
<td>Iatrogenic</td>
<td>1</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>15</td>
<td>83</td>
<td>M</td>
<td>Petic neurotrophic</td>
<td>2</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td>M</td>
<td>Traumatic</td>
<td>3</td>
<td>1</td>
<td>AB</td>
</tr>
<tr>
<td>17</td>
<td>84</td>
<td>F</td>
<td>Petic neurotrophic</td>
<td>22</td>
<td>7</td>
<td>AB</td>
</tr>
<tr>
<td>18</td>
<td>61</td>
<td>M</td>
<td>Iatrogenic</td>
<td>2</td>
<td>No epithelialization</td>
<td>AB, TS, OT, AMT, LT</td>
</tr>
<tr>
<td>19</td>
<td>64</td>
<td>F</td>
<td>Postherpetic neurotrophic</td>
<td>8</td>
<td>2</td>
<td>TAV</td>
</tr>
<tr>
<td>20</td>
<td>63</td>
<td>F</td>
<td>Iatrogenic</td>
<td>20</td>
<td>10</td>
<td>No</td>
</tr>
</tbody>
</table>

AB, topical antibiotics; AMT, amniotic membrane transplantation; F, female; LT, lateral tarsorrhaphy; M, male; OAV, oral antiviral; OT, oral tetracycline; TS, topical steroids; TAV, topical antiviral.
Despite having achieved good initial results in animals, results in human patients have been somewhat contradictory and controversial to date.

One neurogenic factor that has been explored for PED therapy is substance P. This is a neurotransmitter, which is liberated by the peripheral autonomous nervous system and is present at high levels in nerve endings in the cornea. It acts in synergy with insulin-like growth factor 1 (IGF-1) and NGF.8,15–18 Results from experiments with substance P applied with IGF-1 in humans are scarce. Recently, a study has been published reporting promising findings regarding the use of tetrapeptides derived from substance P and IGF-1.19 NGF has been used in neurotrophic ulcers since 1998, with quite promising results; however, NGF treatment has still not yet been approved for use in humans. Nevertheless, the essential limitation of these treatments is that because of their mechanism of action, they are only applicable to PEDs of neurogenic origin.

As a result of the wide range of etiologies of PEDs, an ideal treatment would be that which provides the largest quantity and variety of growth factors. The search for natural extracts that are rich in these substances began as early as in 1940, with the application of amniotic membrane for conjunctival defects.20 Amniotic membrane has since then been used for the treatment of PED with notable success. However, it has a number of disadvantages; on the one hand, it involves a surgical procedure, with all of the inconveniences that this implies for both the ophthalmologist and the patient, particularly when cases are chronic and involve long-term treatments. On the other hand, it is unknown if growth factors survive the process of cryoconservation implicit in this technique. Moreover, because amniotic membrane is not an autologous tissue, it also has the risk of being a source of currently undetectable pathogens.21

In contrast, autologous serum, obtained from the patient’s own blood, has none of the aforementioned disadvantages. The use of autologous serum for the treatment of dry eye was reported in 1984. In 1999, it was proposed for the treatment of PED and dry eye, with satisfactory results.1,2,22,23 More recently, the use of serum from fetal umbilical cord has also been reported.3,24 The results are encouraging, but the technique presents the same disadvantages as amniotic membrane because such serum is a heterologous substance.

In more recent times, a novel treatment based on PRGF is being examined for its therapeutic potential. The technique involves the use of autologous platelet protein extracts, which are rich in growth factors.4–6 However, it has been applied only to the repair of lesions of mucous membranes, skin, and subcutaneous ulcers in oral, maxillofacial, and orthopedic surgery. Our group reported the first case of PRGF application in ophthalmology,25 and Alió et al26 reported the use of a similar treatment, which was called autologous plasma rich in platelets. The principal difference between both treatments resides in platelet activation. Thus, in PRGF treatments, the physiological activation of platelets is achieved by adding calcium, whereas a controlled activation of platelets is not induced in treatments based on autologous plasma rich in platelets; instead, the platelet extract is frozen. After defreezing, platelets will lyse, releasing their growth factors. However, these are accompanied by lysosomal enzymes responsible for their degradation. We have measured the concentration of some growth factors in PRGF to attempt to correlate their presence with PED improvement. PDGF and EGF were found to be the most stable between patients; other factors were also
found to be present, but at highly variable levels (some factors were even undetectable in some patients), making their association with the therapeutic effect somewhat more difficult to correlate.

In our experience, the application of 50% PRGF is effective as a treatment for the reestablishment of the corneal epithelial surface in patients with PED of various etiologies, even in cases in which prior treatment with autologous serum was ineffective, as occurred with 6 patients in our study. PRGF can also be considered to be a cost-effective treatment because it avoids or at least diminishes the frequency of surgical interventions, which PEDs often require. Infection (mainly because of manipulation of the product by the patient) and neovascularization are 2 of the potential side effects of PRGF, but in our hands, neither of these was detected.

PRGF healing appears to be because of the presence of elements, which contribute to epithelial closure. Liu et al quantified a variety of substances in different blood derivatives and concluded that the concentration of growth factors is higher when platelets are activated. Platelet activation may thus be the relevant difference between our treatment and that based on autologous serum. In our study, the concentration of growth factors in the extracts was found to be highly variable among patients, with HGF and FGF presenting most variation (Table 2). Despite the theoretical importance of this finding, its significance remains to be determined.

The diversity of the clinical types of PED involved in our study (such as age, etiology, severity, prior treatments, and associated treatments) makes it difficult to analyze in more detail the clinical efficacy of PRGF. Differences in etiology and clinical characteristics and the need for other additional therapeutic interventions in some cases, limited the homogeneity of our study group. For this reason, it is not possible in our study to establish a correlation between the etiopathology of the PEDs and their response to PRGF treatment. For the same reason, it is not possible to compare results among different treatments, as has been shown in previous studies.

Nevertheless, the fact that our sample of patients was so heterogeneous and that healing took place in the majority of treated cases clearly indicates that PRGF is a promising and effective therapeutic agent for the treatment of a wide range of PEDs in ophthalmology.

REFERENCES


