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Platelet-rich plasma as treatment for persistent ocular epithelial defects

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ABSTRACT

Platelet- rich plasma (PRP) exhibits regenerative proprieties in wound healing but the biochemical mechanisms are unclear. In this study, autologous PRP with a mean value of 338×10^3 platelets/μL was used to treat corneal lesions of different aetiology, while homologous PRP with 1×10^6 platelets/μL was used to treat cornel lesions induced by a graft versus host disease. The impact of platelet count on the levels of PDGF AA and BB, VEGF, and EGF in the two PRPs was evaluated after a cycle of freezing/thawing. Treated corneal lesions healed or improved. The levels of PDGF AA and BB, VEGF, and EGF in the autologous PRP raised from 296 ± 61; 201.8 ± 24; 53 ± 14 and 8.9 ± 2 to 1017 ± 253; 924.7 ± 222; 101 ± 46.5 and 174 ± 15.5 pg/mL, while in the homologous PRP were 3.4, 4.5, 3.2 and 2 folds higher, respectively. High level of platelet counts seems not required to treat corneal lesions.

1. Introduction

Recently platelet derivates have been widely used in regenerative medicine to treat ulcerative lesions or recalcitrant chronic wounds [1] as well as filling biomaterial in maxillofacial and oral or orthopedic surgery [2–5]. Among the platelet derivates used in clinical practice, platelet (PLT) gel is of great scientific interest because of its wide potential clinical applications in tissue repair processes [6,7]. Nonetheless, the knowledge of the biochemical mechanisms underlying the regenerative processes induced by the platelet-derived growth factors (PDGFs) is scarce [8] and conflicting results have been reported [9].

PLT gel is usually obtained by adding thrombin and calcium chloride ($CaCl_2$) to platelet-rich plasma with consequent polymerization of the fibrinogen into a three-dimensional structure of stabilized fibrin and then platelet activation [9]. Other methods induce the coagulation cascade by adding substitutes of the thrombin such as batroxobina [10,11].

Inside the gel, the activated platelets release growth factors (GFs) from their α-granules into the structure of the fibrin [12,13] where you can find a variable amount of leucocytes depending on the method used [14].

According to the scientific literature, platelets release under different physiological and pathologic conditions a large amount of bioactive molecules with regenerative properties [8,12]; however, it is not clear how the platelets act in the architecture of the fibrin matrix; for example, in a specific blood component known as platelet rich fibrin matrix (PRFM) [15]. To this regard, some authors report that the PRFM releases GFs from activated platelets slowly and

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gradually over a period of 7 days from the application, whereas others claim that the GFs release is quick and occurs within the first hours from gel formation [9,15]. Furthermore, other studies show that the contextual presence of the leukocytes influences considerably the GFs release potential of the membrane [16].

Because of the poor knowledge of the biochemical mechanisms underlying the regenerative processes induced by these blood components, it is important to understand how the structure, the composition, and the methods used to make the membranes or gels may affect the clinical response to the blood-derived products applied locally [9,17,18]. For instance, it is important to consider the dilution of the GFs when released in the surrounding tissue in haemorrhagic sites [9].

The lack of a standardized method to make PLT gels [19], the absence of a consensus terminology that allows the identification of a specific product for that specific use [20], the individual variability of patients, and the different types of lesions to be treated [8] make it difficult to have commercial kits for each clinical need, though it is desirable to have a standardized PLT gel for each specific lesion.

In this study, platelet-rich plasma (PRP) which is a precursor of PLT gel and is defined as a high level of platelets in a small volume of plasma was used in the form of eye drops to treat a persistent epithelial defect in a series of patients admitted to the Ophthalmology Department of the University Hospital Tor Vergata. The objective of the study was to assess whether the number of platelets and the method used to activate them may affect the clinical outcome of the treated ocular lesions. It is known that the activated platelets release several biologically active agents and that the wound healing process is the result of a complex interaction between platelet-derived factors, soluble factors (cytokines, chemokines, hormones, plasma-derived proteins) and the extracellular matrix [8,21,22]. Bearing this in mind, we used a quantitative approach to assess whether the platelets number and the correlated GF levels influence the wound healing process through the interaction with the soluble factors already present in the damaged tissue without using fibrin matrix as support element or calcium chloride or thrombin for platelet activation.

2. Materials and methods

From September 2011 to March 2012, 10 patients affected by persistent ocular epithelial defect were enrolled at the Ophthalmologic Department of the University Hospital Tor Vergata, Rome, Italy. Nine patients received autologous treatment while one received homologous treatment. The mean age of the patients was 53.7 years old. Of these patients, 5 were males and 5 were females.

Sixteen ml of whole blood was withdrawn by venipuncture and placed into two sterile 8 ml RegenKit BCT tubes (REGENLAB CH – 1052 Mont-sur-Lausanne Switzerland) containing sodium citrate and a separation gel. After centrifugation at 1500 g for 5 min, at room temperature, the obtained PRP was stocked in 1 ml aliquots using the COL 10 system (BCT Medical Europe S.r.l. VR Italy) and stored at –20°C. All manipulations were done under strict sterile conditions in a laminar flow hood using dedicated materials and devices. Of the obtained PRP, two small aliquots of 150 µl each were used for the dosage of platelet-derived growth factors (PDGF AA and PDGF BB), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) using the Quantikaine colorimetric sandwich ELISA kit (R&D System ELISA, Minneapolis, MN, USA), following the manufacturer's instructions. In one aliquot, the GFs were measured after centrifugation of PRP at 10,000 g for 10 minutes to remove the platelets, while in the second one the GFs were measured after a cycle of freezing/thawing to activate the platelets followed by centrifugation at 10,000 g to remove them.

In the homologous PRP, obtained from platelet-apheresis procedure, and used for one patient who developed Graft Versus Host Disease (GVHD), the GFs dosage was performed using the same method described above.

The SISMEX instrument (Sysmex XT 1800J – DASIT via Merendi 200010,Cornareti, MI, Italy) was used to determine the platelet counts in the peripheral blood and in the autologous and homologous PRP preparations. The basal mean platelet counts in the peripheral blood of patients who used the autologous PRP was 242.2×10^3/µl, while in the corresponding PRP it was 338.3×10^3/µl. In the homologous PRP, platelets were 1×10^6/µl.

2.1. Patients' treatment

Thirteen eyes from 10 patients affected by severe corneal pathology were treated with topical instillation of autologous or homologous PRP: 1 drop/eye, 3 times/day. Artificial tears were used when needed.

During the follow-up visits, patients underwent ophthalmological examination including corrected visual acuity and photography of the anterior chamber after fluorescein staining. Subjective ocular symptoms of dry eye were recorded. Patients were asked to describe their symptoms as mild, moderate, severe or very severe. In addition, it was asked to describe if their ocular symptoms improved, worsened or remained stable.

Patients learned to use autonomously the PRP preparations, keeping the aliquots at –20°C until use.

Informed consent was obtained from all patients enrolled in the study according to the Ethics Committee of our Institution.

3. Statistical analysis

The amount of the released GF was expressed as mean value in pg/mL ± the standard deviation, and as fold increase between the 2 categories tested: the pre-freezing PRP compared to the post-freezing PRP. Means and standard deviations were calculated using the Windows Excel software version 2003.

4. Results

The mean value of PDGF AA, PDGF BB, VEGF and EGF, expressed in pg/mL±DevSt, in the fresh autologous PRP was 296 ± 61; 201.8 ± 24; 53 ± 14 and 8.9 ± 2, respectively. After a cycle of freezing/thawing the values of PDGF AA, PDGF BB, VEGF and EGF were 1017 ± 253; 924.7 ± 222; 101 ± 46.5 and