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Effects of plasma rich in growth factors on wound healing of the tongue. Experimental study in rabbits

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Abstract

Objectives: To apply autologous Plasma Rich in Growth Factors (PRGF) in wounds provoked in the tongue of New Zealand albino rabbits and to study its effects in the epithelialization and inflammation of the wounds at 7 and 28 days after its application.

Study Design: A prospective study carried out on 20 adult rabbits. Two wounds were made on the midline of the dorsal surface of the tongue in each animal, one control, and the other in which PRGF was applied. A histological study of the epithelialization and inflammation of wounds at 7 and 28 days was made.

Results: At 7 days were not observed differences between the study group and the control, nevertheless at 28 days all the wounds in which we applied the PRGF were completely epithelialized and with resolution of the inflammatory process, finding significant differences with respect to the control (p=0.031) and (p=0.023).

Conclusions: The PRGF accelerates epithelialization and reduces inflammation at 28 days of provoking wounds in the oral mucosa.

Key words: *Plasma rich in growth factors, tongue, New Zealand rabbits.*

Introduction

The Plasma Rich in Growth factors (PRGF) is a mixture of autologous proteins concentrated from a determined volume of Platelet Rich Plasma (PRP). In the Alpha granules released by the platelets there are several growth factors, including: Platelet Derived Grow Factor (PDGF), Transforming Growth Factor β (TGF-β) and the Endothelial Growth Factor (VEGF), which are involved in the wound healing processes (1-6).

In the scientific literature the controversy surrounding this procedure is clear about the real effects of the PRGF. The main discrepancies are probably related to the lack of standardization of protocols of the different PRP and PRGF preparations and with the ignorance of the real effects of these proteins concentrations on the different surgical techniques in which they have been applied (2,7,8). The objective of this study was to study the effects of the autologous PRGF in the epithelialization and inflammation of the wounds provoked in the tongue of New Zealand albino rabbits.

Material and Methods

The animals used in this study were obtained from the Animalary of the Support Service to the Experimental Sciences of the University of Murcia (Spain), and the experiment was approved on October 31, 2006, by the Bioethics Committee of the same University.

Animals

A total of 20 adult male New Zealand albino rabbits, with a mean weight of 3.662 g (range 2700-5200 g) were used. Housing and care for the animals was in accordance with general advices of the National Research Council (9).

Surgical procedure

The animals were anesthetized with a mixture of ketamine (60%) and xylazine (40%) administering 1 mL/kg of body weight by intramuscular injection. To obtain PRGF a minimum of 10 mL of blood by animal are required. The blood was obtained via cardiac aspiration. Immediately after collection the blood was placed in two sterile extraction tubes with sodium citrate at 3.8% as anticoagulant (Biotechnology Institute® S.L, Álava, Spain). The tubes were placed in a centrifuge, BTI PRGF® Sytem III (Biotechnology Institute® S.L, Álava, Spain) and centrifuged at 460g (1800 rpm) for eight minutes, thus separating the different phases of the blood. The Plasma Poor in Growth Factors (the highest 500 µl of each tube) and the Plasma with Platelet (the following 500 µl of each tube) were eliminated. Finally, we obtained the last 500 µl of PRP that correspond to the PRGF and were activated using calcium chloride at 10%.

Two wounds were made on the midline in the middle third of the dorsal surface of the tongue in each animal, one as a control (mesial wound) and another (distal wound) which PRGF (Fig.1). The wounds were made using a 6 mm diameter biopsy punch (Stiefel Laboratories®, Madrid, Spain), to ensure that all wounds were the same size. Finally, all wounds were sutured with two simple 4/0 polypropylene (Propilorc®, Murcia, Spain) stitches.

Fig. 1. Two wounds were made on the midline in the middle third of the dorsal surface of the tongue, one as a control (mesial wound) and another (distal wound) which PRGF.

We therefore have a total of 40 wounds (20 control and 20 with PRGF). For the biopsy we used an 8 mm diameter biopsy punch, after the sacrifice of the animals (10 rabbits at 7 days and 10 at 28 días) by CO_2 inhalation.

Histopathological study

The specimens were immediately introduced in a wide-mouthed container and fixed in abundant 10% formalin-buffered saline. The specimens were finally embedded in paraffin and were cut into 4 µm sections and stained with hematoxylin and eosin. All samples were studied by the same experienced pathologist.

To measure the grade of epithelialization, the criteria established by Sinha et al. (10) were used; grade 0: epithelialization at the edge of the wound, grade 1: epithelialization covering less than half of the wound, grade 2: epithelialization covering more than half of the wound, grade 3: epithelialization covering the entire wound with irregular thickness, grade 4: epithelialization covering the entire wound with normal thickness.

The grade of inflammation was studied using the resolution phases of inflammatory processes described by Cotran et al. (11) and applied to the study of the wound healing in experimentation animals by other authors (12,13); grade 1: acute inflammation (pyogenic membrane is formed), grade 2: predominance of diffuse acute inflammation (predominance of granulation tissue), grade 3: predominance of chronic inflammation (fibroblasts beginning to proliferate), grade 4: resolution and healing (reduction or disappearance of chronic inflammation, although occasional round cells may persist).

Statistical analysis

The data were processed using the SPSS version 12.0 (SPSS® Inc, Chicago, USA). A descriptive study was made of each variable. The associations between the different qualitative variables were studied using Pearson's $\chi 2$ test. Statistical significance was accepted for p-value ≤ 0.05.

Results

Seven days after provoking the wounds in the tongue, the majority of the samples presented a grade 3 of epithelialization, irrespective of the application or not of PRGF. Nevertheless, at 28 days, where PRGF had been applied, all the wounds had completed the epithelialization process, with statistically significant differences to the control (p=0.031) (Table 1) (Fig. 2 and 3).

With respect to the resolution of the inflammatory process, at 7 days we found no significant differences between the two groups. Nevertheless, at 28 days, all samples in which PRGF had been applied, demonstrated complete resolution of the inflammatory process, finding statistically significant differences with respect to the controls (p=0.023) (Table 2).

Table 1. Grade of wound epithelialization at 7 and 28 days after surgical intervention on the tongue (total values) (Pearson's $\chi 2$ test).

Day	Groups	Total	Histopathologic scale to evaluate epithelialization*					*p-value*
			Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	
7	Wound+PRGF	9	0	0	0	7	2	0.166
	Control	9	0	0	3	6	0	
28	Wound+PRGF	9	0	0	0	0	9	0.031
	Control	9	0	0	1	4	4	

*Grade 0: epithelialization at the edge of the wound, Grade 1: epithelialization covering less than half of the wound, Grade 2: epithelialization covering more than half of the wound, Grade 3: epithelialization covering the entire wound with irregular thickness, Grade 4: epithelialization covering the entire wound with normal thickness. Note: One sample was lost during the processing phase in the laboratory.

Table 2. Grade of wound inflammation at 7 and 28 days after surgical intervention on the tongue (total values) (Pearson's $\chi 2$ test).

Day	Groups	Total	Histopathologic scale to evaluate inflammation*				*p value*
			Grade 1	Grade 2	Grade 3	Grade 4	
7	Wound+PRGF	9	0	2	5	2	0.637
	Control	9	1	3	3	2	
28	Wound+PRGF	9	0	0	0	9	0.023
	Control	9	0	0	4	5	

* Grade 1: acute inflammation (pyogenic membrane is formed), Grade 2: predominance of diffuse acute inflammation (predominance of granulation tissue), Grade 3: predominance of chronic inflammation (fibroblasts beginning to proliferate), Grade 4: resolution and healing (reduction or disappearance of chronic inflammation, although occasional round cells may persist). Note: One sample was lost during the processing phase in the laboratory.

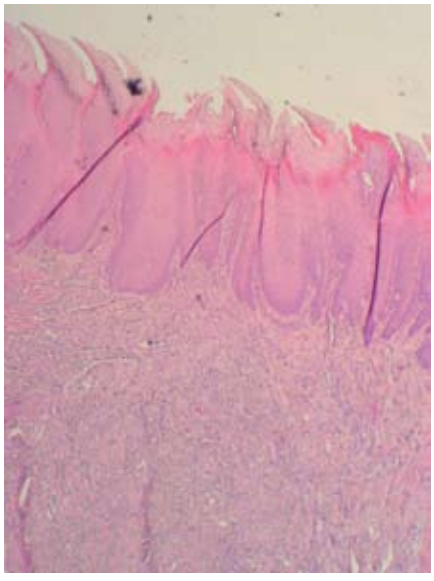

Fig. 2. Wound provoked on tongue mucosa where PRGF had been applied, at 28 days we can observe epithelialization covering the entire wound with normal thickness.

Fig. 3. Control wound at 28 days, we can observe epithelialization covering the entire wound with irregular thickness.

Discussion

Growth factors accumulate in the β granules of platelets and it is generally accepted that they play an essential role in the wound healing. Growth factors applied to wounds can accelerate healing by stimulating angiogenesis, tissue maturation and epithelialisation (1,2,6,14).

PRGF is an autologous product, and thus avoids the risk of transmitting disease. In our study, in the oral mucosa the epithelialization and inflammation was not completely resolved until 28 days after surgery; this may be explained by the fact that the mouth is a moist area, where the saliva and maceration of the tissue (due in part to mastication) may initially interfere with the healing process.

To obtain PRGF, we have followed the protocol described by Anitua in 1999 (2); in this protocol the activator is calcium chloride at 10%, this eliminates the risk of immunological reactions and the transmission of diseases associated with the use of exogenous bovine thrombin. Furthermore, in this protocol the PRGF can be obtained in a single centrifuging at 460g (1800 rpm) for eight minutes; in contrast with other protocols that use double centrifuge technique to obtain PRP and requires a higher blood volume (minimum 50 mL), which is unfeasible in rabbits (15).

In conclusion, our results suggest that the application of PRGF (obtained by means of this protocol) accelerates epithelialization and reduces inflammation at 28 days of provoking wounds in the tongue. However, the regenerative effects of PRGF in soft tissue are unclear, and in this respect we should continue investigating.

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