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THERAPEUTIC HOTLINE

Benefits of plasma rich in growth factors (PRGF) in skin photodamage: Clinical response and histological assessment

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ABSTRACT: Skin ageing is characterized by small and fine wrinkles, roughness, laxity, and pigmentation as a result of epidermal thinning, collagen degradation, dermal atrophy, and fewer fibroblasts. Plasma rich in growth factors (PRGF) is an autologous plasma preparation enriched in proteins obtained from patient's own blood aimed at accelerating tissue repair and regeneration. To evaluate the benefits of PRGF in skin photodamage, 10 healthy volunteers were treated with three consecutive intradermal injections of PRGF in the facial area. Clinical outcomes and histological analysis were performed. A statistically significant increase in the epidermis and papillary dermis thickness was seen after PRGF treatment ($p < 0.001$). Skin thickening was observed in all patients studied, being more intense in the group of patients with photodamage ($p < 0.001$). After PRGF treatment, a reduction of the average area fraction of solar elastosis was observed in patients with clinical and histological signs of skin photodamage ($p < 0.05$). No changes were observed in the number of CD31, XIIIa factor, cKit, CD10, nor p53-positive cells. The improvement score after PRGF use was 0.75 (9/12) for the group of patients with signs of skin photodamage. Intradermal PRGF infiltration appears to be an effective treatment for the photodamaged skin.

KEYWORDS: clinical assessment, growth factors, histology, platelet-rich plasma, PRGF, skin photodamage

Introduction

Plasma rich in growth factors (PRGF) is an autologous plasma preparation enriched in platelets obtained from patient's own blood (1). Its goal is to enhance the body's innate ability of tissue repair and regeneration. The use of PRGF has been investigated in numerous fields of medicine (2–5), and more recently, PRGF has emerged as a new treatment modality in dermatology (6). The regenerative effect of PRGF can be attributable to the presence of a three-dimensional scaffold and many biologically active factors, especially EGF, PDGF,

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TGFb, and VEGF that are locally released after application (7). In the present study, we have tried to give some light on the insights of PRGF use for skin rejuvenation. To address this, 10 healthy volunteers with different degrees of skin photodamage were treated with autologous PRGF injected in the facial area. The clinical response and the histological assessment after PRGF infiltration were evaluated and the safety of the approach was assessed.

Material and methods

Study design

This was a clinical study to compare the clinical and histological features before and after treatment with PRGF. All patients were provided written informed consent before participating in the study, and the study was performed according to the declaration of Helsinki.

Patients

Participants were 10 healthy individuals (3 men and 7 women) aged 34–59 years. Photodamage was scaled in four categories ranging from 0 (*no signs of photodamage*) to 3 (*severe photodamage*) (8). Exclusion criteria included being receiving or have received in the last year any of the cosmetic treatment modalities commonly used for skin rejuvenation.

Treatment

PRGF was prepared according to the manufacture protocol (PRGF®-Endoret®, BTI Biotechnology Institute, Vitoria, Spain). Briefly, 36 mL of blood was collected into 9 mL tubes containing 3.8% (wt/vol) sodium citrate. The blood was centrifuged (BTI System IV) at 580 g for 8 minutes and then plasma column was fractioned into fraction 1 (F1) and fraction 2 (F2). Both fractions were activated with PRGF activator (BTI Biotechnology Institute, SL, Miñano, Spain). Activated F2 was injected in the deep dermis of the whole facial area whereas activated F1 was intradermically injected along patient's facial surface. Three treatments were given for each patient, with an interval of 3 weeks one from each other. For the histological analysis, two 3-mm punch biopsies were performed.

Assessment criteria

Patients were evaluated at four time points: T0 = beginning of study (first PRGF treatment and biopsy – pre); T1 = 3 weeks (second PRGF treatment); T2 = 6 weeks (third treatment); and T3 = 12 weeks (no treatment, biopsy posttreatment was performed). Each patient was digitally macrophotographed at all visits.

Pre- and postmacrophotographs were given to three independent and blinded evaluators who were asked to identify the posttreatment image of each patient. The results of this clinical evaluation were scored in a three-category scale: 0 (if the masked evaluator found no differences between pre and postmacrophotographs); –1 (if the masked evaluator failed to recognize each image); and +1 (if the masked evaluator succeeded in recognizing the photographs).

Subjects were asked to complete a self-assessment questionnaire and rated their skin changes from 0 (*very unsatisfied*) to 4 (*very satisfied*).

Patient's clinical satisfaction.

- Date
- Patient number

Liquert scale	
Very unsatisfied	0
Unsatisfied	1
Indifferent	2
Satisfied	3
Very satisfied	4

- Satisfaction score

Histological analysis was made by an expert dermatopathologist who was not concerned about the schedule study protocol. The biopsies were analyzed using hematoxylin and eosin, Masson-Trichrome, orcein, colloidal iron and muramidase, CD 117, factor XIIIa, CD34, CD31, and p53. The epidermal and dermal lengths were measured. The quantity of collagen, solar elastotic, and elastic fibers were measured. CD31, p53-positive cells, CD34+ cells, and factor XIIIa-positive cells (deepest dermal dendrocytes and superficial dermal dendrocytes, respectively) were counted. The ImageJ program was used as imaging program to analyze histological figures.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). The parametric *t*-test for paired samples was used to perform before and after treatment comparisons. The statistical tests were two sided and a probability value of less than 5% was considered statistically significant.

Results

All subjects except one completed the study. No serious or persistent side effects occurred during the course of the study, and no subject withdrew from the study because of an adverse event.

Histological outcomes

In five of the nine patients, histological signs of photodamage (ranging from mild to severe) were seen. In four participants, no signs or very slight signs of photodamage were observed. A statistical significant increase in the epidermis and papillary dermis thickness was seen after PRGF treatment ($p < 0.001$) (Fig. 1). Skin thickening was observed in all patients, being more intense in those with photodamage ($p < 0.001$). The number of fibroblasts was higher and statistically different ($p < 0.001$) after treatment due to an increase in the amount of the deepest dermal dendrocytes (CD34+ cells), whereas the superficial ones showed no difference (Fig. 2A). The volume of collagen fibers in the papillary dermis was also greater and better organized in the posttreatment biopsy samples ($p < 0.05$) (Fig. 2B). After PRGF treatment, a reduction of the average area fraction of solar elastosis was observed in patients with signs of skin photodamage ($p < 0.05$) (Fig. 2C). Fig. 3A illustrates some photographs of patients suffering from moderate to severe skin photodamage before and after treatment with PRGF. Interestingly, no changes were observed in the number of CD31, XIIIa factor, cKit, CD10, nor p53-positive cells (data not shown).

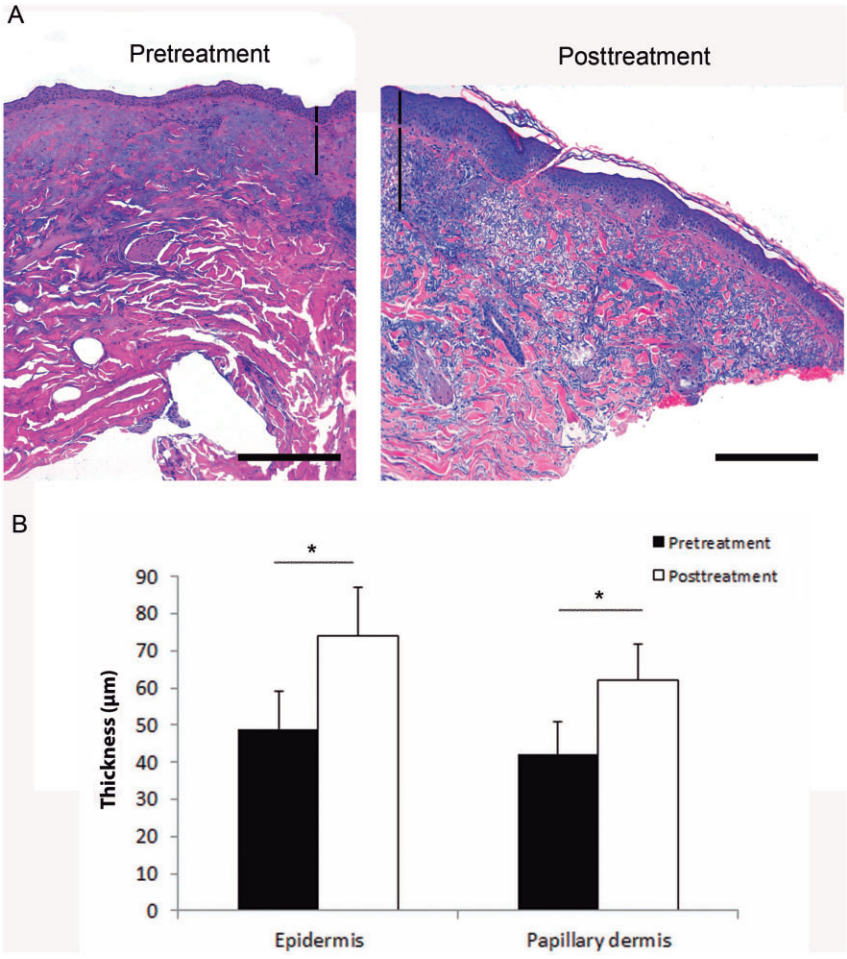

FIG. 1. **(A) Histological staining of skin biopsies (hematoxylin and eosin) was analyzed in order to measure the width of the epidermis and papillary dermis before and after the PRGF treatment. (B) A statistically significant increase in the epidermis and papillary dermis thickness was observed after PRGF treatment ($p < 0.001$) for all the patients. Original magnification of both images: 5×. Scale bar: 400 µm.**

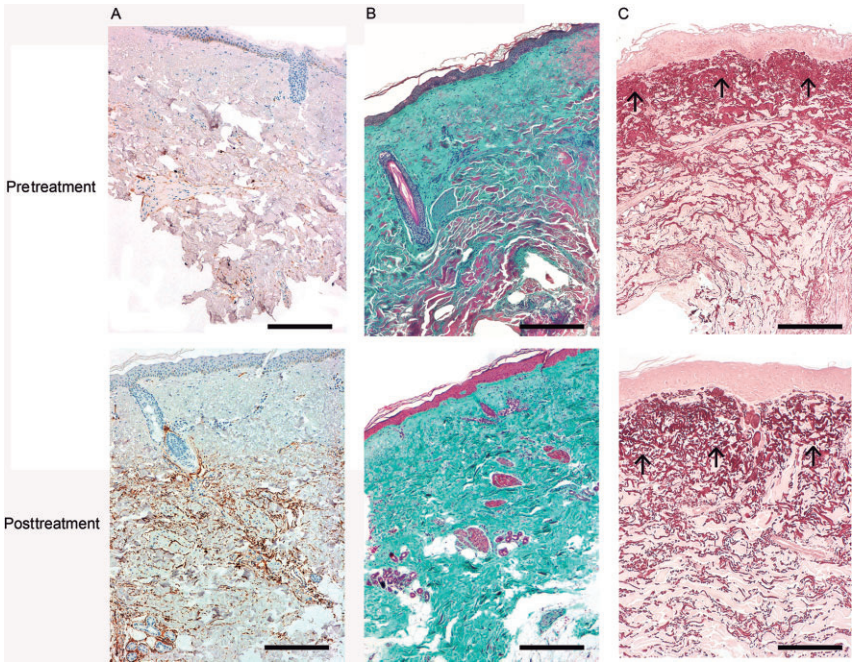

FIG. 2. **(A) Immunohistochemical staining of CD34+ cells showed a significant increase in the population of deep dermal dendrocytes ($p < 0.001$) but no differences were found among superficial ones. (B) Masson-Trichrome staining of pretreatment and posttreatment biopsies showed a statistically significant increase in the amount and organization of collagen fibers ($p < 0.05$). (C) Orcein staining of elastic fibers (arrows) showed a great actinic elastotic area among photodamaged patients (moderate to severe) whereas statistically significant reduction ($p < 0.05$) of solar elastosis was achieved after PRGF treatment. Original magnification of images: 5×. Scale bar: 400 μm.**

Subjective satisfaction scale

Fig. 3B shows that among the nine patients who finished the study, two referred to be very satisfied, five were satisfied, and the remaining two patients answered to be indifferent about the treatment.

Evaluator's clinical image comparison

The overall improvement score for the appearance of the face was 0.3 (9/27). The improvement score was 0.75 (9/12) for the group of patients with signs of skin photodamage and 0 in patients with no photodamage (Fig. 3C). Differences between groups were statistically significant ($p < 0.05$).

Discussion

In the present study, an increase in the epidermal and dermal thickness, with a higher amount and better organized collagen fibers, has been observed in PRGF injection. Our results suggest that PRGF acts on cell proliferation and probably on the deepest dermal compartment (deepest dermal dendrocytes). Interestingly, a reduction of solar (actinic) elastosis was observed after PRGF treatment. Sun-related changes in the skin involve the appearance of accumulated abnormal elastic fibers in the papillary dermis (9). To our knowledge, this is the first time that the reduction of solar elastosis has been demonstrated to be a PRGF mechanism of action for skin rejuvenation.

We also found histological changes after PRGF use. All patients experienced uniform increase in the epidermal and dermal thickness with independence of patient's age or the severity of skin photodamage they presented at baseline. We venture to suggest that efficacy of PRGF mostly depends on the photoageing phenomenon rather than on chronological ageing. Greater changes were observed in the posttreatment biopsy of a 55-year-old woman with severe clinical and histological signs of photodamage, i.e., that of a 55-year-old woman with slight signs of photodamage. Most of the patients felt satisfied with the study. Surprisingly, the group of no-photoaged patients were as satisfied as those with photodamage (in whom clinical and stronger histological changes were observed).

The two main weaknesses of the present study are to be uncontrolled and the relatively short time

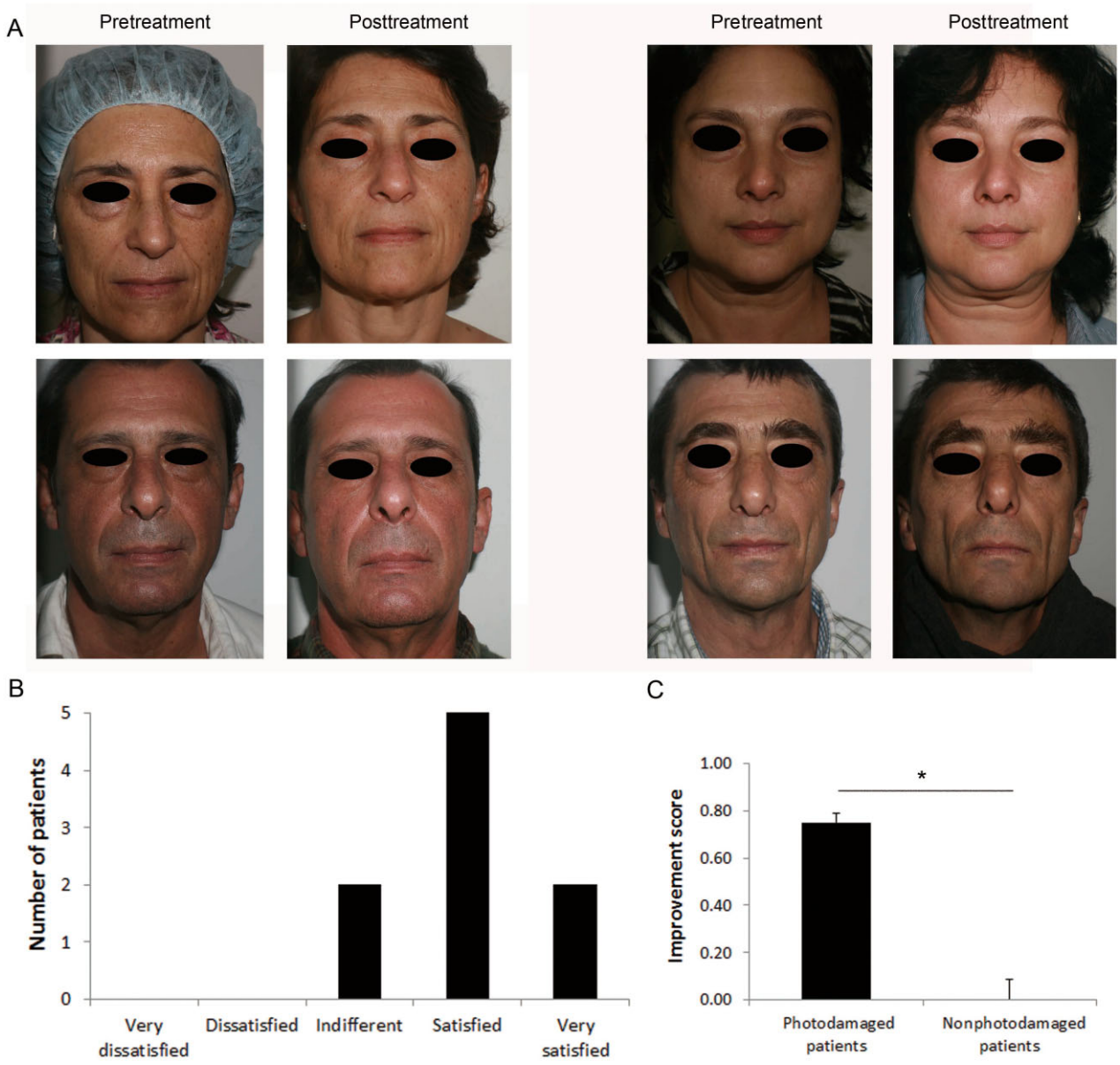

FIG. 3. **(A) Overall improvement of four photodamaged patients before and after PRGF treatment. (B) According to a self-assessment questionnaire completed by nine patients, none of them was very unsatisfied or unsatisfied after PRGF treatment. Two patients were indifferent and five answered to be satisfied whereas two referred to be very satisfied. (C) The improvement score obtained after the analysis of pre- and post-images showed a significant difference between nonphotodamaged and photodamaged patients ($p < 0.05$). Photodamaged patients achieved a greater improvement score compared with the nonphotodamaged ones.**

of follow-up. Although long-term and controlled placebo studies are needed in the future, our preliminary data suggest that PRGF is a potential approach to improve the appearance of the skin face, and overall a scientific field that deserves our special attention. Last but not least, these results should not be extrapolable to other platelet-rich products whose composition may dramatically change, leading maybe to other conclusions and lack of biological effects.

Declaration of interest

EA is a scientist of BTI Biotechnology Institute, a company that investigates the potential of PRGF.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Fig. S1. (A) Histological staining of skin biopsies (hematoxylin and eosin) before and after PRGF treatment. 40×. Scale bar: 50 μm. (B) Masson-Trichrome staining of pretreatment and posttreatment biopsies showing organization of collagen fibers at higher magnification. 40×. Scale bar: 50 μm.