

Applicability of platelet-rich plasma in dermatology

Aplicabilidade em dermatologia do plasma rico em plaquetas

ABSTRACT

Platelet-rich plasma has been proven to be promising in regards to its applicability in dermatology, especially in the healing of chronic ulcers and for soothing signs of aging. The autologous platelet-rich plasma is obtained through blood centrifugation, in a way that its components are separated by density gradient. The final product is a gel rich in growth factors that act in tissue repair, activating fibroblasts and inducing the extracellular matrix remodeling.

Keywords: platelet-derived growth factor; platelet-rich plasma; transforming growth factors; vascular endothelial growth factors; wound healing

RESUMO

O plasma rico em plaquetas vem-se mostrando promissor quanto a sua aplicabilidade em dermatologia, sobretudo na cicatrização de úlceras crônicas e na atenuação de marcas decorrentes do envelhecimento. O plasma rico em plaquetas autólogo é obtido através da centrifugação do sangue, de modo que os componentes são separados por gradiente de densidade. O produto final obtido é um gel rico em fatores de crescimento que atuam na reparação tecidual, ativando fibroblastos e induzindo ao remodelamento da matriz extracelular.

Palavras-chave: cicatrização; fator de crescimento derivado de plaquetas; fatores de crescimento transformadores; fatores de crescimento do endotélio vascular; plasma rico em plaquetas

INTRODUCTION

Cutaneous ulcers are characterized by tissue loss involving epidermis, dermis, and sometimes adipose tissue, as well as muscle fascia. The etiology of these lesions is diverse, including peripheral vascular disease, infectious diseases, and trauma. It can also be secondary to neurological, immune, neoplastic disorders and iatrogenic injury. Cutaneous ulceration is a rather common occurrence, with a current prevalence rate ranging from 0.18 - 0.32%, an incidence rate of 0.78% and a clear trend toward an increase in frequency as the average age of the global population increases. Additionally, there is an inevitable socio-economic cost impact. Chronic skin ulcers have a significant effect on a patient's quality of life, and also drive up public health costs in Brazil and worldwide. The European Union directs 2% of its annual health care budget for the treatment of such lesions.¹

Review Article

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Wound healing is a complex process brought about by signals of molecular interaction involving cellular mediators and events, and is followed by the recruitment of mesenchymal cells, and extracellular matrix proliferation and regeneration. The healing process is a response of innate immunity to restore tissue integrity. It is regulated by a standard sequence of events including coagulation, inflammation, granulation tissue formation, epithelialization, and tissue remodeling. These events are mediated and modulated by cytokines and growth factors that stimulate and modulate such cellular activities. More recently, platelet-rich plasma (PRP) has also drawn attention to the field of cosmia-try regarding skin rejuvenation. The aging of human skin results from a combination of a gradual decline in its function over time (intrinsic process) and the cumulative damage caused by environmental factors (extrinsic process), such as smoking and, in particular, exposure to ultraviolet B radiation (UVB). In the dermis, UVB exposure has been shown to stimulate the production of collagenase by fibroblasts. In skin continuously exposed to UVB rays, degradation of collagen and the altered elastic tissue deposition result in damage to the structural integrity of the dermal extracellular matrix, causing wrinkling of the skin. Cutaneous elasticity is also reduced. Given that PRP produces several growth factors linked to skin regeneration, it can be assumed that PRP is capable of inducing the synthesis of collagen and other extracellular matrix components through the stimulation of fibroblasts, thus leading to the rejuvenation of the skin. In this research field, however, studies that have confirmed the effect of PRP on aged fibroblasts are still considerably limited.²

A brief literature review

Studies already carried out have shown that PRP has been effective in several control-cases and uncontrolled clinical trials.³ Croveti et al.¹ published a prospective study on the efficacy of platelet gel in the healing of chronic skin ulcers. The lesions of the 24 patients involved in the study ranged in etiology, including vascular disease, infectious disease, post-traumatic ulcers, and also conditions related to diabetes mellitus, neuropathy, and vasculitis. The study's protocol consisted of weekly applications of platelet gel, and at the time of publication, nine patients had been completely cured, two had received skin grafts, four had halted the treatment, and nine had a partial response and continued to be treated.¹

McAleer et al.⁴ found that the use of autologous PRP worked satisfactorily in the healing of chronic ulcers of the lower extremities in a case study of a 57-year-old man with type II diabetes mellitus. The treatment with PRP was established after the unsuccessful application of a skin graft. The autologous PRP was synthesized at the assistant physician's practice, with inspection and debridement of the wound and an application of platelet gel performed weekly. The complete healing of the ulcer was achieved in the fourth week of treatment. Despite the fact that the study involved only one patient, this evidence nonetheless suggests that PRP can be used successfully in healing ulcers that do not heal with other treatment techniques.⁴

Driver et al.⁵ carried out the first multicenter prospective,

randomized, and controlled trial in the U.S., on the use of autologous PRP for the treatment of diabetic foot ulcers. Among the participants were 72 patients with type I and type II diabetes, aged between 18 and 95 years, and with lesions having set on at least four weeks prior. In this study, researchers compared the autologous PRP's effectiveness to that of the usual saline gel, for 12 weeks. The study's primary objective was to evaluate PRP's safety and the incidence of the complete closure of the wound (defined as 100% re-epithelialization), as compared to the treatment administered to the control group. The secondary objective concerned the closing rate. At the end of the study, it was found that patients treated with PRP had their ulcers healed in 68.4% of cases when compared with 42.9% for the saline group. Furthermore, in the PRP group, healing occurred in about 42.9 days, while it took 47.4 days on average in the control group.⁵

Salemi et al.⁶ recently conducted a study evaluating the effectiveness of the combination of PRP and autologous adipose tissue in an ulcer in the lower extremity of a 65-year-old non-diabetic patient, whose condition had been in development for three years. This study lasted for four weeks, with follow-up events after one, three, six, and fifteen months. Despite the absence of statistical analysis in this study, the researchers noted that the graft integrated well and the patient suffered no local infection or other complications. In the fifteenth month of follow-up, the wound was completely healed and there was restoration of the limb's function.⁶ Other authors have used this technique for the treatment of ulcers in the lower limb.⁷ Cho JM et al.⁸ led a study involving genetically modified mice, with naked skin, photoaged through exposure to UVB radiation. They were divided into three groups (untreated, injected with saline, and injected with PRP). After four weeks the degree of wrinkle formation was compared between the three groups using replica analysis; skin biopsies were also performed. An additional *in vitro* trial with growth factors neutralizing antibodies has also been developed to assess whether the growth factor contained in PRP could accelerate fibroblast proliferation and collagen production. The promising results of the study indicated that PRP is effective in rejuvenating photodamaged skin.⁸

PRP composition

Plasma plays an important role in creating an appropriate microenvironment for tissue repair.⁹ The presence of some leukocytes in PRP lends some natural resistance to the infectious processes of this compound, improving the treatment's prognosis. The platelets, in turn, correspond to the most important component regarding the modulation of tissular healing due to its ability to release growth factors. They are responsible for regulating a number of cellular events such as DNA synthesis, chemotaxis, cytodifferentiation, and matrix synthesis. The alpha granules of the platelets release numerous growth factors, which act by joining cellular receptors located on the cell membrane and which transmit the signal from the exterior to the interior of the cell, by coupling different proteokynases, which in turn phosphorylate and activate a cascade of signals that end with the activation of one or several genes (signal transduction). The platelet-derived

growth factor (PDGF) was one of the first identified growth factors. It is the main factor contained in platelets, due to the fact that it is the first to be present in the wound and to guide the revascularization, collagen synthesis, and tissue repair processes. The PDGF is probably produced in megakaryocytes and is stored in the platelets' alpha granules. Macrophages, endothelial cells, and osteocytes are additional sources of PDGF. They are released from their original granules when platelets adhere to the sites of vessel breakage and/or basal membranes area. The platelets' PDGF start the repair process, while that present in the macrophages continues to heal the wound. Moreover, the PDGF stimulates DNA synthesis, chemotaxis, and collagen synthesis, processes that are essential to wound healing and tissue repair.

The beta transforming growth factors (TGF- β) constitute a superfamily of local mediators that regulate the proliferation and functions of most cells in the body. This family is composed of β 1, β 2, β 3, β 4 and β 5 TGFs. The TGFs most commonly present in the PRP are TGF- β 1 and TGF- β 2, which are factors related to connective tissue healing. Their most important functions regarding tissue repair are the chemotaxis of inflammatory cells and extracellular matrix synthesis. Also known as somatomedins, insulin-like growth factors (IGF) are produced in the liver and circulate when bound to proteins and such substances. Types 1 and 2 (IGF1 and IGF2) are involved in the tissue repair process, by regulating the availability of amino acids for protein synthesis, collagen and other connective tissue molecules. The epidermal growth factor (EGF) is a peptide that produces a variety of biological responses – most of them involving replication, movement, and cell survival regulation. It belongs to a family of related ligands (which includes the alpha transforming growth factor, TGF- α), which share a homologous amino acid sequence with high affinity for the same receptor, the EGFR. The vascular endothelium growth factor (VEGF) plays an important regulatory role in the physiological vascular development, increasing the tissue's vascularization, hence improving the supply of oxygen and nutrients to a particular site.

PRP Collection

Platelet-rich plasma is obtained from autologous blood via a process that uses the cell separation principle of differential centrifugation.¹⁰ At least 16 PRP preparation systems are currently available.¹¹ The resulting PRP volume and final platelet and leukocyte concentrations differ in the divergent preparation systems. According to Saucedo et al.,¹¹ Dohan Ehrenfest et al. classified PRP according to the concentration of leukocytes and fibrin while Mishra et al. accomplished this according to the concentration of platelets, presence of leukocytes, and the inclusion of an activator.¹¹ In order to create PRP, whole blood is usually collected in the presence of an anticoagulant that binds to calcium and prevents the start of the coagulation cascade, thus preventing the conversion of prothrombin into thrombin. Although there are various anticoagulants available, only two were deemed suitable for the process so as not to damage the platelets: acid citrate dextrose A, and citrate phosphate dextrose.⁹ Once the whole blood sample is obtained, it is subjected to one or two

centrifugation stages, depending on the desired characteristics of the final product. An amount of 10–20 ml of blood is then collected and distributed in 5 ml tubes containing a 10% sodium citrate solution. The tubes are then centrifuged at room temperature, resulting in three basic components: red blood cells, PRP and PPP, from the lowest to the uppermost portion, respectively. After this process, plasma is collected. The volume of about 1.2 ml per tube is associated with 5 ml of 10% calcium chloride, and approximately five minutes after, the gel is formed.¹²

Safety in the use of PRP

Given the autologous nature of PRP, safety concerns are minimal at this time. The complications reported in most clinical series have been limited to transient pain and inflammation at the site of application. A laboratory study showed that PRP has an antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli*, potentially reducing the risk of infection by these organisms.¹³

Dose-response effect

In vitro studies demonstrated that the majority of the growth factors for dose-response curves is non-linear, meaning that from a certain point, increasing the concentration of growth factors brings no additional effects, as cell surface receptors would be fully occupied. On the other hand, some growth factors can effectively exert an inhibitory effect on cell functions once a sufficiently high concentration is reached. Thus, not only is there a presence of growth factors that dictates the level of the healing response, but also the presence and ability of target-cells to use these factors appropriately.¹⁴

DISCUSSION

Due to increased life expectancy and hence the significant aging of the global population, the prevalence of chronic skin ulcers is expected to become higher, mainly those resulting from atherosclerotic and microangiopathic processes. The same can be said of skin aging, given the longer cumulative exposure to UVB rays. In this respect, there is a need to develop techniques that will assist in the process of wound healing and skin repair. The PRP arises as a tool that allows the application of large amounts of growth factors that stimulate the production of collagen and extracellular matrix through minimal amounts of plasma. Growth factors promote a rapid increase in the number of undifferentiated mesenchymal cells in the healing site during the repair and healing processes. Thus, PRP has the advantage of accelerating the regenerative process via the amount of growth factors present in platelets. Conversely, it has the disadvantages of short platelet life (about three to five days), and the fact that the growth factors expire in seven to ten days. Despite the platelets' short life span, it was proved that PRP is able to promote a faster and qualitatively better skin repair. However, there are many potential confounding variables in the studies – both regarding the variation in patients' and in the PRP's characteristics. Hence the difficulty in carrying out standardized studies.¹⁵

CONCLUSION

Based on the literature referred to here, it is possible to conclude that the use of PRP in dermatology, while recent, is a very promising technique. PRP is a preparation of organic, non-immunoreactive, non-toxic, and low morbidity, with production costs that are reasonably low. Regarding the healing of chronic skin ulcers, treatment with PRP can result in a shorter healing and recovery time of the limb's function, and decrease the amputation rate, thereby improving the patient's quality of

life. Regarding its use in cosmiatry, one may suggest that stimulation with growth factors is able to promote skin rejuvenation. Finally, further studies are necessary on PRP's mechanism of action and ideal preparations standardization, either for the healing of chronic ulcers or for the mitigation of signs resulting from aging. Many questions remain unanswered regarding the use of PRP in dermatology, however, despite these remaining issues, PRP promises to be an effective treatment modality. ●

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