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Research Article

Anatomopathological Findings in Deep Second Degree Burns Locally Treated with Allogeneic Platelet Lysate

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Abstract: Regenerative therapies with platelet concentrate are used as they are physiological reservoirs of growth factors (FCs) and proteins, capable of accelerating tissue regeneration. The purpose of this work has been to evaluate the regenerative effect of allogenic platelet lysate through pathological findings in deep dermal burns**.** A cross-sectional, observational, descriptive study was carried out to determine the clinical effect of platelet lysate on tissue regeneration in 30 patients with deep dermal burns, in the Caumatology and Plastic Surgery Service, Hospital General "Dr. Juan Bruno ZayasAlfonso", fromSantiago de Cuba, in the period from February 2013 to February 2014.The female sex prevailed in ages between 18 and 45 years, with the lower limbs standing out as the most affected region, in both groups of treatments. The application of platelet lysate favored healing and epithelialization in a shorter time than that described with conventional treatment, with minimal adverse events, without complications, achieving a better quality of the final. Histopathological findings showed that acts directly in the healing phases, stimulating early tissue regeneration.

Keywords: regenerative therapy, Platelet Lysate, burns, Anatomopathological findings.

INTRODUCTION

Burns bring with them physical disabilities, systemic disorders, and emotional and aesthetic repercussions [1-3].

Skin replacement continues to be an unmet challenge today. When the extent and depth of skin loss require the use of therapeutic coverage, auto graft is currently the gold standard for achieving full recovery of skin functionality. However, taking auto grafts is not exempt from certain morbidity and involves an additional injury that must be taken into consideration [4].

Many authors have spent decades investigating different alternatives of culture media and cell transport to improve the results of tissue growth and attachment. Rheinwald and Green 1957 described a methodology for the in vivo expansion of keratinocytes. However, little progress has been made in this field since then. Methodologies have been incorporated to provide a dermal analog to growing keratinocytes, but the truth is that given the structural complexity of the skin, with its attachments, it has not yet been possible to reproduce in the laboratory, in its histological integrity [4].

Burn wound repair processes are not always rapid and can lead to the appearance of scars, which are sources of biomechanical or psychosocial problems for most patients.

The persistence of the unhealed wound determines the final evolution of the patient. For this reason, the main need in patients with injuries that cause loss of skin integrity is their recovery in the shortest time, an aspect that is guaranteed with treatments that favor or accelerate healing, to which special attention is paid at present [1].

Molecular and cellular biology has made it possible to deepen the knowledge and development of a myriad of techniques for tissue regeneration in the last fifteen years. These regenerative techniques guarantee a higher morphological and functional quality of the regenerated tissue compared to that of the repaired or fibrotic tissue. Among these techniques we can mention the PRP (presentation of platelets for regenerative), which can be used for the treatment of different pathologies associated with acute injuries, chronic conditions and those that affect hard, soft and simple organs and tissues.

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In addition, this technique provides new concepts on the functional capacity of platelets because they influence the repair of wounds and the regeneration of tissues since they release tissue growth factors that are the universal initiators of almost the entire regeneration process [6].

The local application of high concentrations of growth factors with the use of concentrated allogenic platelet lysate by freezing - thawing has been used previously in order to accelerate the healing process of different lesions [5, 7].

The use of high concentrations of growth factor through platelet lysate is simple, safe, effective, inexpensive, induces minimal adverse effects, painless, easily accessible, and curative. This results in the improvement and well-being of the people who suffer these aggressions, so this therapeutic modality has become a fundamental pillar for the local treatment of burns [5, 7].

The purpose of this work has been to evaluate the regenerative effect of allogenic platelet lysate through pathological findings in deep dermal burns.

METHODOLOGY

A cross-sectional, observational, descriptive study was carried out to determine the pathological findings of Platelet Lysate (PL) in tissue regeneration in patients with deep dermal burns in the Caumatology and Plastic Surgery Service of the Hospital General Clínico-Quirúrgico "Dr. Juan Bruno Zayas Alfonso", in the period from February 2013 to January 2014. The research was carried out with a sample of 30 patients aged 18 years and over, who had recent deep AB dermal burns, produced by boiling water. Patients with a burned body surface equal to or less than 5% with adequate physical and nutritional status, without a history of chronic non-communicable diseases were included. Patients with diseases of the hemolymphopoietic system, collagen diseases and confirmed malignant neoplasms were not taken into account; those who consumed medications such as anticoagulants or non-steroidal anti-inflammatory drugs; pregnant and with referred humoral or cellular immunological deficit. In all cases, these procedures were done on an outpatient basis, spreading the LP over the lesion. The amount of allogeneic PL used in each lesion was determined by the frequency of cures and the extent of the lesion. The applications were made with an interval of 2 days until the total healing of the lesion.

Epithelialization was taken into account before 21 days after the injury occurred; absence of complications such as deepening and infection; Obtaining a final scar with a quality similar to the normal skin of the patient and the performance of histological studies by biopsy.

The quality of healing was measured 30 days after treatments using the Vancouver Scale (VSS; Vancouver Scar Assessment).

The Chi-square test was used to determine the association between the treatment and each of the variables analyzed. A significance level of 5% was established for all statistical tests. 95% confidence intervals were computed. The information was processed using the Minitab® 14 statistical system. The results were presented in tables and graphs.

RESULTS

Regarding the distribution of patients according to selected ages, a predominance of burn injury was evidenced in ages between 18 and 45 years, with an average age of 33.4 standing out. 73.3% belong to women for a male / female ratio (M / W) of 1: 2.7.

The most frequent location of burns was at the level of the lower limbs, representing 36.6% of the patients, followed by lesions located in the upper limbs, and in the anterior trunk, representing 26.7% for both regions. The mean of the injured area was 249.8cm2.

Table-1: Distribution of patients by time interval of burn healing

Healing time interval (days)	Nº	%*
7 – 13 days	20	66,7
14 – 20 days	10	33,3
21 days and more	0	0.0
Total	30	100
Mean	12.6	
Standard deviation	2.33	
confidence interval (95 %)	11.7 – 13.4	

The healing time between 7 and 13 days stands out, in 20 patients for 66.7% and 33.3%, 10 patients healed between 14 and 20 days, clinically translated by the appearance of firm and confluent epidermal islands, achieving faster healing time, being statistically highly significant, with a mean epithelialization time of 12.6 days.

No moderate or severe adverse events were observed in any of the patients. The adverse events that prevailed in the patients who received the treatment were pruritus and pain.

Furthermore, there were no complications with the application of the therapy. The evaluation of the applied therapy revealed that all patients reported successful treatment.

It is necessary to highlight the appearance of the first epidermal islets after the second application of the LP and the appearance of firm and confluent islands, after the third application; getting a thin and smooth-looking skin from the ninth day of receiving the application (Figure 1).

Fig-1: A: Anterior trunk second degree burn deep in first Lysate platelet application. B: second application of Lysate platelet. C: Third application of Lysate platelet

When analyzing the quality of each scar quantified using the Vancouver scale, 30 days after treatment we can see that after the treatment received with LP, the 30 cases move between a score of 1 and 5 with a median score of 3 representing 26, 6%. This is reflected by scars with normal pigmentation and vascularization, flexible, with adequate sensitivity and with a normal height or that does not exceed 2 mm.

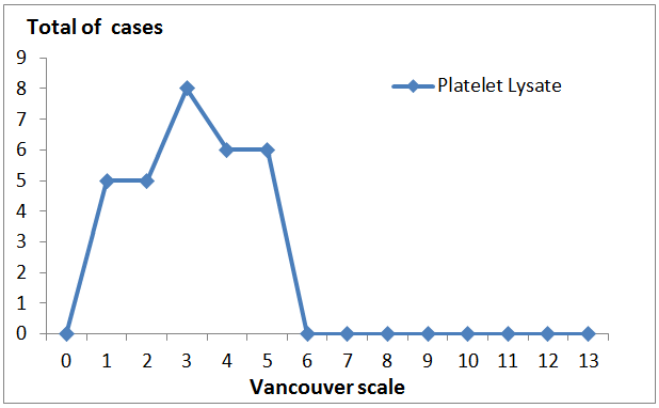

Fig-2: Evaluation of healing according to the Vancouver scale

Table-2: Histopathological characteristics of deep dermal burns treated with LP lesions

Acute inflammation	Discrete: presence of less than 25% of the polymorph nuclear lesion and neutrophils per field.
Oedema	Discrete: tissue cell separation was 8 µm to 14 µm.
Chronic inflammation	Moderate to severe: presence of mononuclear blood cells between 25 and 50% of the lesion per field.
Angiogenesis	Moderate to intense: presence of 10 or more vascular buds in the dermis.
Fibroblasts	Very abundant: presence of more than 30 fibroblasts per field.
Collagen	Abundant with grouping of collagen fibbers.
Neoformation Epithelium	Present. Atrophic or hypertrophic stratum corneum

DISCUSSION

The therapeutic use of platelets in regenerative medicine is based on the release, after platelet activation, of a large number of growth factors and bioactive molecules with the ability to improve tissue healing at the sites where they are implanted. Among platelet growth factors, platelet-derived growth factor is extremely important in tissue regeneration because it is bound to blood vessels, acts as a mesenchymal cell mitogen and enhances the activity of cells with receptors for the factors of platelet growth, which together helps to maintain and prolong the action initiated by the rest of the growth factors [5, 6, 8].

Current interest in the use of platelet concentrates in the modulation and acceleration of tissue regeneration and repair processes is based on: proliferative, chemotactic, anti-inflammatory and immunomodulatory action of growth factors, chemokines and cytokines; inducing, stimulating and attracting action of some factors on stem cells (CD34 +) and the hemostatic action of some factors [9-12].

The use of allogeneic platelets or ABO group from transfusion services, which maintain current safety in pre-transfusion practice, has been a modality used in

regenerative medicine. The latter allows the use of platelets that have hemostatically decreased their action, but that retain their ability to secrete plasma growth factors (FCP) and other bioactive materials. This makes it possible to take advantage of the product that has been discarded until now [13, 7, 14].

In the literature consulted that justify the use of preserved platelets for the healing of skin lesions, it has been pointed out that keratinocytes and fibroblasts of the skin and those exposed in wounds do not express molecules of the major histocompatibility complex, which allows the use of platelets allogeneic without immune reactions occurring. This offers certain advantages over autologous PRP, such as: the high proliferation potential and higher concentration of FCs [5, 13, 14]. Deep dermal burns have an average healing time of 21 days as described by many authors since this process occurs from the bottoms of the sweat and sebaceous glands as well as the bulb of the hair follicle that are located deep in the dermis and who are unharmed when this type of injury occurs. Under proper care conditions, these burns usually recover in 3 to 4 weeks, although without respecting the normal architecture of the epidermis. If they reach the deep dermis, they give rise to hypertrophic scars, keloids and significant retractions [1, 3, 15].

In our study with LP, the re-epithelialization time of these burns was reduced by between 40 and 55% if it is assumed that they usually recover between 3 and 4 weeks, respectively. A rapid evolution of healing with early epithelialization was observed in all the areas treated with allogeneic LP after the third application.

From a clinical point of view, healing was characterized by the appearance of firm and confluent epidermal islands. In addition, the appearance of the first epidermal islets was after the second application of allogeneic LP and the appearance of firm and confluent islands, after the third application, resulting in a skin with a fine and smooth appearance.

Similar results were found in other studies where PRP was used in burn patients [16, 17]. The ideal treatment for the burned patient has not been established so far and it is the one that induces the healing of the lesion in the shortest time with minimal adverse events in the patient. With the application of LP, the minimal appearance of adverse effects was observed, with itching and pain prevailing in the patients. No infection is reported during the observation period. In addition, this study did not report adverse reactions and urticaria produced by hematopoietic preparations containing a large amount of platelets obtained from allogeneic plasma.

The absence of complications in patients treated with LP coincides with the reports in a previous study where it is clinically evidenced that the use of any form of PRP facilitates the natural healing and healing processes of wounds [16, 17].

The final result of the scar should not be considered a trivial part of the type of treatment that is used, since the healing of poor quality injuries can constitute a disabling pathology and have implications, such as social exclusion, with contractures being of special importance scarring that cause significant alterations in mobility.

The results of the Vancouver scar scale show better quality of healing with the application of LP, if we compare with the final healing obtained after conventional local treatment, by using topical first-line medications. The usefulness of the scale has been corroborated by other authors, showing quality in the healing of the donor areas [18, 19]. Other authors document the use of this scale to assess the quality of the scar after the use of two treatments. Topics in cosmetic surgery patients [20].

The favorable evaluation and degree of satisfaction of patients treated with allogeneic LP reported in this study are consistent with those documented in other studies [17].

All the patients belonging to 100.0% of the sample, evolved favorably, which one would expect that they demonstrated to have been very satisfied with the treatment received, since their problem was solved in the shortest possible time with better aesthetic results and without leaving disfiguring sequelae.

Histopathological examinations are valuable tools for estimating the evolution of tissue repair. The healing of the skin is a process of complex cellular, molecular, physiological and biochemical mechanisms, which through chemotaxis, proliferation, deposition and reorganization of the extracellular matrix lead to the closure of the injured tissues. Therefore, it is essential to reveal the different histopathological findings of the lesions treated with a type of therapy.

The alterations that burn induces in the tissue hinder the arrival of nutrients and regenerative factors from the vascular bed to the injured area [16]. These regenerative factors and bioactive molecules facilitate tissue repair and are stimulated when allogeneic LP is administered directly into the injured tissue.

In this study, the histopathological findings found on the ninth day, after three applications of LP, indicate the high proliferation of fibroblasts with a significant level of early vascular proliferation. In addition, the acceleration of the fibrogenesis process is observed, given by the deposits of collagen fibers.

When using first-line drugs such as silver sulfadiazine, the inflammatory process is much more

intense and persists for a longer time. Consequently, macrophages and neutrophils produce a large number of chemical mediators, enzymes, free radicals and reactive oxygen species, which determine the extent of tissue damage and enlargement of the burned bed, in agreement with other authors [21, 22].The histopathological findings of this investigation show an absent or mild acute inflammatory response, which is replaced by the chronic inflammatory response. This may be related to the release of growth factors, which have direct influences on the remodeling of the extracellular matrix and the release of vasoactive substances that help the later phases of inflammation that modulate the inflammatory response, as reported in a previous work [23].

In this study, the intense vascularization observed after treatment with LP demonstrates the acceleration of the angiogenesis process with the marked proliferation of newly formed vessels and fibroblasts. Platelets act as a biological support on which the endothelial cells of the new blood vessels are deposited. In addition, they secrete a variety of biochemical factors, including pro and antiangiogenic growth factors that promote tissue reaction to inflammation and the recruitment of progenitor cells from connective tissue [23].

This finding demonstrates the efficacy of the different growth factors on soft tissue wounds and their performance as a polymerized fibrin matrix rich in platelets and cytokines that promote fibroblast proliferation, as shown in other studies[24].The latter are responsible for maintaining the integrity of the tissues and participate in the composition of the granulation tissue. As a result, it can be stated that angiogenesis is the key link in the process of tissue healing, revascularization and regeneration, and LP is essential to promote angiogenesis.

Histopathological findings revealed the early deposition of collagen fibers due to the abundance of fibroblasts, which also have an effect on the proliferation and differentiation of keratinocytes, essential in re-epithelialization. In addition, the presence of newly formed epithelium was found due to the stratum corneum present in the samples. These findings are reported in preclinical studies [24].

The above mentioned may suggest that the success of LP in the rapid improvement and quality of healing of deep second degree burns, after three applications, is based on the direct action that the FCs released by platelets have in the process. of regeneration, in agreement with other authors[10-12, 16].

The promising results with LP, reported in this research and in the literature, may turn it into a potential by-product for burns of any extent, depth and etiology, a way that is consistent with a previous study.

CONCLUSIONS

The application of LP favored the healing of deep dermal burns and was an effective tool within the therapeutic arsenal to be used given by the completion of epithelialization in less time than conventional treatment, pruritus being the most prominent adverse effect, with less pain, and without complications and obtaining as a final result a cutaneous architecture very similar to the patient's skin and with the excellent level of satisfaction perceived by the patients studied.

Histopathological findings showed that LP acts directly in the healing phases, which stimulates tissue regeneration early.

Conflict of interest

The authors declare that they have no conflict of interest in relation to the research presented

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