

Original Research Article

Allogenic Lysate Platelet on Deep Second Degree Burns

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Abstract: Despite the decline in burns incidence and mortality in recent years, its management remains complex. Surface burns usually receive conservative treatment due to their re-epithelizing potential. Regenerative therapies with platelet concentrate are used as physiological reservoirs of growth factors and proteins, capable of accelerating tissue regeneration. A quasi-experimental study was conducted, with two groups of 30 patients to determine the clinical effect of Lysate Platelet on tissue regeneration in patients with deep dermal burns. Differences were identified between the use of conventional treatment with Silver Sulfadiazine and Lysate Platelet, as well as determining the final outcome of both treatments, in the Plastic Surgery and Cosmetology Service, of the General Clinical-Surgical Hospital "Dr. Juan Bruno Zayas Alfonso" of Santiago de Cuba, Cuba in the period from February 2013 to February 2014. Percentages and totals were calculated as a summary measure for qualitative variables for statistical analysis of the data. For quantitative variables, the calculation of the average and the standard deviation was performed. The female sex prevailed at ages 18 to 45, with the lower limbs highlighting as the most affected region, in both treatment groups. The application of lysate platelet favored healing and epithelialization in a shorter time than conventional treatment with silver sulfadiazine, with minimal adverse events, without complications, achieving a better quality of the final scar and excellent satisfaction level perceived by the burnt patient.

Keywords: Burns, lysate platelet, platelet concentrate, regenerative therapy.

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INTRODUCTION

Burns are one of the leading causes of injury and accident, especially at extreme ages of life. Despite the decline in both incidence and mortality in recent years, its management remains complex. Surface burns usually receive conservative treatment due to their potential re-epithelizing. However, the mixed pattern burns, deep dermal and full thickness, are those that pose an aesthetic and functional risk to the patient even the commitment to their life, if they are extensive. In general, burns bring physical disabilities, systemic disorders, and emotional and aesthetic repercussions. In these cases regional systemic and crazy treatments are critical to burn healing [1-3].

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

The persistence of the unhealed wound determines the patient's final evolution. Therefore, the

main need in the patient with lesions that cause loss of skin integrity is their recovery in the shortest time, an aspect that is guaranteed with flattering treatments or accelerators of healing, to which special attention is paid today [1].

Local application of high concentrations of growth factor through the use of lysogenic allogeneic platelet concentrate by freezing – thawing has previously been used in order to accelerate the healing process of different lesions [4-5].

The use of high concentrations of growth factor using lysate platelet (LP) is simple, safe, effective, low cost, painless, easily accessible and curative; induces minimal adverse effects. This results in the improvement and well-being of people suffering from these aggressions, so this therapeutic modality has become a fundamental pillar for the local treatment of burns [4, 5].

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The above paragraph suggests the need for simple, safe, effective and economical technique proposals that drive the acceleration of healing processes and do not require special mobilization or processing.

The purpose of this work has been to evaluate the regenerative effect of allogeneic lysate platelet on deep dermal burns.

MATERIALS AND METHODS

A longitudinal, prospective and quasi-experimental study was carried out in the Plastic Surgery and Cosmetology Service, of the General Clinical-Surgical Hospital "Dr. Juan Bruno Zayas Alfonso", in the period January 2013 to January 2014. The research was conducted on a sample of 60 patients aged between 18 and 55 who suffered recent deep AB dermal burns with boiling water, with a burnt body surface equal to or less than 5%. The patients were assigned to two groups of 30 subjects, for the application of local platelet lysate and conventional local treatment with silver sulfadiazine 1%. Patients should have an adequate physical and nutritional status, with no history of chronic non-communicable diseases and with a good humoral state according to lab test results. Those who used medications such as anticoagulants or non-steroidal anti-inflammatory drugs; pregnant and with cellular or humoral immune deficit (referred to or tested by complementary) were not included. The quality of healing was measured in both treatment modalities at 30 days after treatment using the Vancouver Scar Assessment. The Chi-square test was used to determine the association between the treatment types and each of the variables analyzed. The t-student test was used for independent samples to identify writ of significant differences between the average epithelialization times when both treatments were prescribed. Fisher's F statistical test was used to investigate whether the variance in both treatment

groups was equal or uneven. A significance level of 5% was set for all statistical tests. 95% confidence intervals were computed. The information was processed using Minitab statistical system. The results were carried on tables and graphics and also compared with international literature finding.

RESULTS

As regards the distribution of patients according to selected ages and treatment group, predominance of burn injury aged between 18 and 45 years was shown for both treatment groups, with the average age of 33.4 for the Lysate Platelet treated group and 33.0 for the group treated with Silver Sulfadiazine 1%.

For both treatment groups, the female sex predominated, which accounted for 70.0% of treated patients, which means that 73.3% and 66.7% belong to group A and Group B females respectively, for a sex ratio (H/M) of 1: 2.3.

With regard to the location of burns it was appreciated that the most frequent location of burns for both treatment groups was at the level of the lower limbs, representing 36.6% of patients, followed by localized lesions in the upper limbs, and in the anterior trunk, representing 26.7% for both regions, there were no significant differences in the location of the lesions and the type of treatment applied ($p = 1,000$).

The determination of the initial area of the burn constitutes the objective way to assess the size of the injury at the time of production and subsequently the regeneration of the tissues, more precisely that is why in our study we decided to include only lesions that covered up to 5% of the burned body surface area and decided to measure it in cm^2 in addition. The area mean for the LP-treated group was 249.8cm^2 and for those treated with silver sulfadiazine it ²²²was 253.4cm^2 .

Table 1: Patients according to burn healing time by treatment group

Healing Time	Treatment Group					
	Group A		Group B		Total	
	N°	%*	N°	%*	N°	%
7 – 13 days	20	66,7	4	13,3	24	40.0
14 – 20 days	10	33,3	19	63,3	29	48.4
21 days and more	0	0.0	7	23,4	7	11.6
Total	30	100	30	100	60	100
$\chi^2 = 20.46 p = 0.000$						
Media	12.6		18.3			
Desviación Estándar	2.33		4.10		t Student	6.702
IC (95 %)	11.7 – 13.4		16.8 – 19.9		p	0.000

* The percentage has been calculated based on the number of patients according to treatment group

Group A highlights the healing time between 7 and 13 days, in 26 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, getting faster in healing time, being statistically very significant, with an average epithelialization time of 12.6 days.

As for group B, we observed that 63.3% of patients had a healing time that oscillated between 14 and 20 days. Only 13.3% were healed between 7 to 13 days, and 23.4% healed after 21 days of treatment. Noting that the average epithelialization time with silver sulfadiazine was 18.3 days, resulting in greater than that obtained in Group A. The student t-test showed with a significance level of 5% that there are significant differences between burn healing time and the type of treatment applied ($p = 0.000$). The Chi

squared test shows, with a significance level of 5%, that there is an association between the healing time and the type of treatment ($p = 0.000$).

It is necessary to emphasize 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).



Figure 1: A: Leg Deep Second Degree Burn B: appearance of firm and confluent islands, after the second application of lysate Platelet and getting a thin and smooth-looking skin

Despite promising results with allogeneic LP in deep second degree burns, as well as the use of Sulfadiazine adverse events that prevailed in patients were itching and pain (table 2). None of these adverse events were moderate or severe in any of the patients.

In Group A (GA) itching was predominant while in Group B (GB) pain and perilesional erythema prevailed. For each treatment group, there were no significant statistical differences ($p=0.068$) between

adverse events, according to the Chi squared test ($X^2=7.14$).

When GA and GB were compared, there were no statistical differences between itching ($p= 0.981$), perilesional oedema ($p=0.061$), and pain ($p=0.279$). However, there was a significant statistical difference ($p=0.044$) between perilesional erythema, predominating in GB patients. These statistical significances were obtained with the t-Student test.

Table 2: Associated adverse events by treatment group

Adverse event	Grupo A		Grupo B		Total		p -value
	Nº	%	Nº	%	Nº	%	
Itching	12	23.1	5	9.6	17	32.7	0,981
Perilesional erythema	3	5.8	8	15.4	11	21.2	0,044
Perilesional oedema	2	3.8	6	11.5	8	15.3	0,061
Pain	7	13.5	9	17.3	16	30.8	0,279
Total	24	46.2	28	53.8	52	100	
$\chi^2 = 7,14 p = 0,068$							

Table 3 shows the complications associated with the application of silver sulfadiazine as topical therapeutics, taking into account that patients receiving LP treatment showed no complications.

Table 3: Patients for associated complications

Complications	Silver sulfadiazine	
	Nº	%*
Deepening	7	23,3
Local infection	7	23,3

*Percentage calculated based on total Group B subjects

We can appreciate that 23.3% of all patients treated with silver sulfadiazine showed clinical signs of local infection, which led to deepening of the lesions becoming hypodermic lesions, with changes in coloration, acquiring a white coloration, with abundant hemorrhagic stippling, secretions, fetid, which denoted the presence of germs, which was corroborated with microbiological studies, being found as frequent germ Klebsiella, Enterobacter, despite its wide spectrum of

activity, inhibiting the main bacteria responsible for burn infections,

Healing tissue causes changes in skin architecture that make the skin different and have characteristics of its own in terms of color, thickness, elasticity, texture and degree of shrinkage as can be seen in Figure 2.

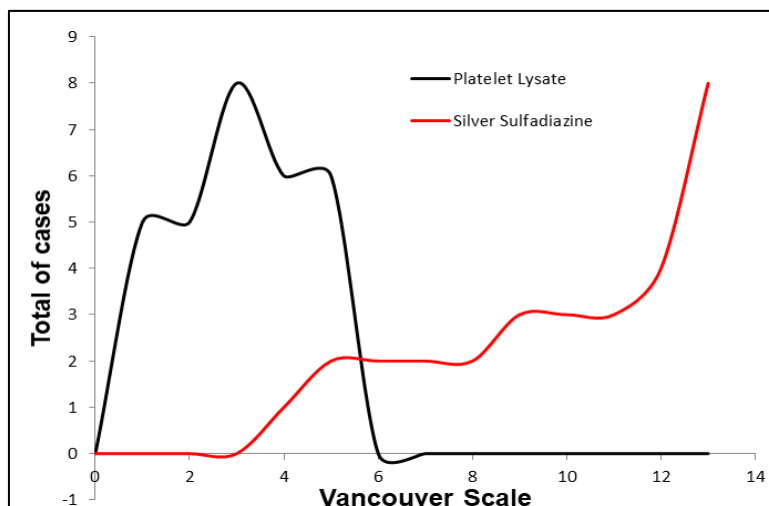


Figure 2: Vancouver scale healing assessment (VSA) for each type of treatment

By analyzing the quality of each scar quantified by the VSA, at 30 days after treatment we can see that the scores obtained are directly proportional to the appearance of the scar; i.e. the lower the better look score of the injury.

For the treatment of silver sulfadiazine, the 30 cases move in a score between 3 and 13 highlighting 8 patients, for 26.6%, in the score of 13 showing that the highest percentage of patients were found in the highest scores on the scale, with a median score of 8.5. This means that they had hyper pigmented scars, much vascularized denoting red or purple, with alterations in addition to sensitivity and flexibility and with height greater than 2mm.

However, after treatment received with LP the 30 cases move between a score of 1 and 5 with a maximum of 3 representing 26.6%, thus having that the highest percentage of patient takes scores close to normal, with a median score of 3. This results in scarring with normal pigmentation and vascularity, flexible, with adequate sensitivity and with a normal height or not exceeding 2 mm.

The evaluation of applied therapeutics, according to treatment group, was observed in Table 4. The Chi squared test shows, with a significance level of 5%, that there is an association between the type of treatment and the final evaluation of that therapeutic (p =0.000).

Table 4: Patients according therapeutic evaluation by treatment group

Evaluation	Group A		Group B		Total	
	N°	%*	N°	%*	N°	%
Success	30	100,0	12	40,0	42	70,0
Failure	0	0,0	18	60,0	18	30,0
Total	30	100,0	30	100,0	60	100,0
X ² = 25,714 p = 0,000						

*The percentage has been calculated based on the number of patients according to treatment group

DISCUSSION

The therapeutic use of platelets in regenerative medicine is based on the release, after platelet activation, at sites where a large number of growth factors and bioactive molecules are implanted with the ability to improve tissue healing. Among platelet

growth factors, platelet-derived growth factor is very important in tissue regeneration because it is attached to blood vessels, acts as a mesenchyme cell mitogen and boosts cell activity with receptors for platelet growth factors, which together helps maintain and prolong the action initiated by the rest of the growth factors [4, 6].

Platelets are considered biological pumps or bags that contain a true cocktail of biomolecules with regenerative capacity and factors that modulate angiogenesis and inflammation. They also have bacteriostatic activity against a large number of bacterial and fungal strains [4-7].

Current interest in the use of platelet concentrates in the modulation and acceleration of tissue regeneration and repair processes based on: proliferative, chemotactic, anti-inflammatory and immunomodulatory action of growth factors, chemokine and cytokines; inducing, stimulating and attractive action of some factors on stem cells (CD34+) and the hemostatic action of some factors [8, 9].

The usual form of presentation of platelets for regenerative (PPR) application can be as, concentrate of platelets obtained by apheresis (according to the techniques rated in the Blood Banks), platelet gel and LP. Some of these procedures have already been referred to in literature [10-12].

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 decreased their action, but which retain their ability to secrete plasma growth factors (FCPs) and other bioactive materials. This allows you to take advantage of the product until today discarded [4, 5].

The use of allogeneic platelets allows homogenization of PPR preparations; however, international standards, requirements and procedures for the storage of blood products provide that platelets are stored at room temperatures and discarded 5 days after they are obtained [5, 13].

Platelets retain their secreting activities for up to 21 days after being obtained and stored at room temperature, but this carries an increased risk of bacterial proliferation and accumulation of pyrogenic cytokines. Consequently, its use in wound repair is limited. As a result of these regulations, large platelet volumes listed as deciduous or inappropriate are wasted, making platelet products expensive and limitedly available. There are several strategies designed to extend the life of platelets, such as the use of PPR cushioned with protective carbohydrates such as sucrose and trehalose [14].

Cooling (80°C) and platelet cryopreservation reduce the risk of bacterial colonization and facilitate the use of platelets that have lost some of their hemostatic potential, so they are no longer suitable for use as a hemocomponent, but maintain their ability to release FCs involved in tissue repair [14].

There are other aspects documented in the literature consulted that justify the use of preserved platelets for the healing of skin lesions. It has been noted that skin keratinocytes and fibroblasts and those exposed in wounds do not express molecules from the major histocompatibility complex, allowing the use of allogeneic platelets without immune reactions. This offers certain advantages over autologous PPR, such as: the high potential for proliferation and higher concentration of FCs [5, 13, 14].

It is precisely from the allogeneic PPR that CP and LP are obtained, using internationally established methods for obtaining them. The PPR is separated from the total blood by light centrifugation (2 750 rpm × 4 to 5 min, at 22 oC) and is subsequently subjected to heavy centrifugation (3 750 rpm × 10 min, 22 oC) [14-16].

It should be noted that when using LP, not only high concentrations of Platelet Growth Factor (PGF) but also transforming growth factor are applied to increase adhesion and enhance the regenerating effect. The current interest in the use of platelet concentrates in tissue regeneration is based on the different actions they exert in the body, such as: the accumulation of a large number of platelets activated at the injured site as a natural and immediate response of the body to tissue damage; the release of the contents of alpha granules from platelets including Platelet Growth Factor (PGF), Transformation Growth Factor (TGF), Vascular Endothelial Growth Factor (VEGF) and Epidermal Growth Factor (EGF), which attract macrophages, mesenchyme cells, osteoblasts and cells responsible for removing necrotic tissue; and the secretion of presintetized proteins that occurs within the first 10 minutes of platelet activation and more than 95% are secreted within the first hour [4-6].

The main need in the burned patient is the recovery as soon as possible from the integrity of the skin, so flattering treatments or accelerators of healing become very important in the therapeutic approach. These injuries are historically related to high hospital stays, the high hospital cost and the use of large numbers of topical frontline medications.

The results achieved demonstrate the effectiveness of outpatient use of allogeneic platelet lysing in the treatment of deep dermal burns as a simple alternative to conventional treatment. The use of this treatment is benefic, because in this way the patient is kept in regular history, both family and works, contributes to raise their quality of life and also provides significant savings of resources, by decreasing the hospital stay by these traumatic injuries, also decreases the appearance of invalidating or deforming sequel in the patient who suffers them.

Deep dermal burns average a healing time of 21 days as described by numerous authors as this

process occurs from the bottoms of the sweaty and sebaceous glands as well as the hair follicle bulb that are located deep in the dermis and remain unscathed when this type of injury occurs. Under appropriate conditions of assistance, these burns usually recover for 3 to 4 weeks, although without respecting the normal architecture of the epidermis. If they reach the deep dermis, they result in hypertrophic scars, keloids and major retractions [1, 3, 14].

Rapid evolution of early epithelial healing was appreciated in all areas treated with allogeneic LP, which eventually resulted in fairly uniform skin and wound closure by 95% or more, at which point adequate revitalization was considered for the suspension of occlusive healings.

From a clinical point of view, healing was characterized by the appearance of firm and confluent epidermal islands, being faster in Group B patients. As a result, the healing time was shorter in this treated group. 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.

Alterations induced by burning tissue make it difficult to reach nutrients and regenerative factors from the vascular bed to the injured area [15]. These regenerating factors facilitate tissue repair and are stimulated when allogeneic LP is administered directly into injured tissue. The latter can be explained from the large number of FCs and bioactive molecules that are released into the lesion due to the application of the LP in line with other authors.

Similar results were found in other studies where PPR was used in burn-carrying patients. [16]. However, a previous study reports that the application of autologous PPR to second-degree burns can speed up the healing process in rats, but has no effect on third-degree burns. This is explained because the effectiveness of PPR depends on the existence of some substrate, such as the remaining epithelial cells located in the hair follicles and sweat glands that are characteristic of second-degree burns, but are missing from third-degree burns [15].

The average re-epithelialization time of burns in Group A patients was 31.2% shorter than that of Group B patients.

In addition, LP reduces the re-epithelialization time of these burns by 40 to 55% if it is assumed that these burns usually recover between 3 and 4 weeks, respectively. However, silver sulfadiazine reduces this time by 12.9% (compared to 3 weeks of recovery) and 34.6% (compared to 4 weeks of recovery).

Despite silver sulfadiazine being the most widely used of topical antimicrobials for its broad-spectrum antimicrobial effect against staph and strep, it is raised that its potential irrigative effect on tissue prolongs the time of repair of injury and revitalization [17]. Some works that have compared it to other alternatives seem to support this assumption. The delay in the healing process is due to the cytotoxic activity of this medicine in several cells, such as keratinocytes and fibroblasts [8].

Adverse events that prevailed in patients receiving LP treatment were itching and pain. None of these adverse events were moderate or severe in any of the patients.

One of the first consequences of burning is the alteration of capillary permeability in the damaged area and its neighborhoods, which brings with it the appearance of edema [1-3]. The anti-laema effect of LP is due to early angiogenesis in which a weak and permeable vascular bed is quickly replaced by an adequate vascular wall due to endothelium proliferation.

The explanation of the decrease in pain with LP has been related to the intervention of cytokines involved in the pain pathways; as an example of these, factor 4 platelets [13].

The itching present in lesions during LP treatment is considered due to the intense inflammatory reaction that causes the release of growth factors by platelets as well as the consequent release of histamine by the cells involved in this process and which is activated by platelets [18].

Our study showed less erythema in GT1 patients, because it has shown that LP is able to reduce erythema, and in daily practice, so far, no complications have been had with the cases to which they have been performed, obtaining good results and satisfaction from patients undergoing it, reason this, which would support why fewer complications were identified in treated patients.

In our daily practice, with the application of LP, we have observed the minimal onset of adverse effects, obtaining good results and satisfaction from patients undergoing it.

No infection is reported in patients treated with LP during the observation period. In addition, this study does not report adverse and urtic reactions produced by hematopoietic preparations containing a large number of platelets obtained from allogeneic plasma. Exacerbation of the inflammatory phase caused by LP on burn results in the release of growth factors and increased polymorph nuclear and macrophage leukocytes at the site of the injury. The latter results in

an increase in defense against infection in the affected area.

The non-presence of complications in patients treated with LP coincides with reports in a previous study that clinical evidence that the use of any form of PPR facilitates natural wound healing and healing processes [13, 15].

In the daily clinic, it can be generally realized that the therapeutic use of LP establishes an increase in soft tissue tissue repair processes, a decrease in infection rates in therapeutic acts; decreased pain and inflammation and eventually a decrease in blood loss through injured tissue.

The final result of the scar should not be considered as a trivial part of the type of treatment used, since the healing of ill-quality lesions can constitute an invalidating pathology and have implications, such as social exclusion, being of particular significance scar contractures that cause significant alterations of mobility.

The assessment of healing was performed using the Vancouver Scar Assessment (VSA). This objective assessment scale of the patient and the observer categorized the different valuable characteristics of the scar, such as: pigmentation, vascularity, flexibility and height/thickness, pigmentation quantitatively at 30 days after treatment.

The results of the Vancouver scar scale demonstrate better quality healing with LP application, compared to Silver Sulfadiazine, in all variables analyzed. The usefulness of the Vancouver scar scale in determining healing quality in burned patients requiring skin substitutes has been corroborated by other authors showing quality in healing donor areas. [19]. Other authors document the use of this scale for the evaluation of scar quality following the use of two topical treatments in patients with cosmetic surgery [20].

It is important to note that in any healing process a resulting scar should be obtained that does not stand out from the surrounding normal skin. It is advisable that the scar is as attenuated as possible and that it does not present any symptomatology. In addition, the patient's own perception of his wound is an important component to consider.

The successful therapeutic evaluation of Group A patients can be explained from the non-appearance of complications, obtaining the closure of the burn injury in a short period and obtaining the final scar with characteristics similar to normal skin, which means better aesthetic result and without deforming squeal in the patient.

trivial part of the type of treatment used, since the healing of ill-quality lesions can constitute an invalidating pathology and have implications, such as social exclusion, being of particular significance scar contractures that cause significant alterations of mobility.

These aspects confirm the effectiveness of LP for the treatment of deep dermal burns. However, the unfavorable evolution of 60.0% of Group B patients can be explained because 38.9% (7/18) of these patients show signs of local infection with the consequent deepening of lesions and with a healing time of more than 21 days. In addition, the healing quality of the 18 patients shows the score between 10 and 13, being noticeable for score 13, which means the scar has very different characteristics to normal, in terms of its sensitivity, coloration and height. This can lead to pathological scarring, such as hypertrophic or keloid scarring, which mostly affects the patient's self-perception. The latter negatively influences your interpersonal relationship and decreased quality of life.

The favorable evaluation and satisfaction of patients treated with allogeneic LP reported in this study are consistent with those documented in other studies in skin grafts in patients with third-degree burns [16].

The favorable evolution of patients with the application of LP demonstrated the advantages of using this treatment as an alternative to use in deep dermal burns.

All patients belonging to Group A, for 100.0% of the sample, evolved favorably, which could be expected 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 deforming squeal.

CONCLUSIONS

The population of patients with deep second-degree burns stood out for being female at intermediate ages of life, highlighting as affected regions the lower limbs, followed by the upper limbs and the previous trunk.

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

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to the research presented.

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