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Platelet gel for healing cutaneous chronic wounds

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Abstract

Wound healing is a specific host immune response for restoration of tissue integrity. Experimental studies demonstrated an alteration of growth factors activity due to their reduced synthesis, increased degradation and inactivation. In wound healing platelets play an essential role since they are rich of α-granules growth factors (platelet derived growth factor—PDGF; transforming growth factor-β—TGF-β; vascular endothelial growth factor—VEGF). Topical use of platelet gel (PG), hemocomponent obtained from mix of activated platelets and cryoprecipitate, gives the exogenous and in situ adding of growth factors (GF). The hemocomponents are of autologous or homologous origin. We performed a technique based on: multicomponent apheretic procedure to obtain plasma rich platelet and cryoprecipitate; manual processing in an open system, in sterile environment, for gel activation. Every step of the gel synthesis was checked by a quality control programme. The therapeutic protocol consists of the once-weekly application of PG. Progressive reduction of the wound size, granulation tissue forming, wound bed detersion, regression and absence of infective processes were considered for evaluating clinical response to hemotherapy. 24 patients were enrolled. They had single or multiple cutaneous ulcers with different ethiopathogenesis. Only 3 patients could perform autologous withdrawal; in the others homologous hemocomponent were used, always considering suitability and traceability criteria for transfusional use of blood. Complete response was observed in 9 patients, 2 were subjected to cutaneous graft, 4 stopped treatment, 9 had partial response and are still receiving the treatment. In each case granulation tissue forming increased following to the first PG applications, while complete re-epithelization was obtained later. Pain was reduced in every treated patient.

Topical haemotherapy with PG may be considered as an adjuvant treatment of a multidisciplinary process, useful to enhance therapy of cutaneous ulcers.

Keywords: Wound healing; Growth factors; Platelet gel

1. Introduction

Cutaneous ulcers are characterized by tissue loss that involves the epidermis, dermis and sometimes also adipose tissue and muscular fascia;

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there is no spontaneous repair process and tissue distruption often results in a fibrotic scar [1]. The etiology of the wounds are various: peripheral vascular disease, infectious disease; secondary to trauma, to neurologic, immunologic, neoplastic, coagulotion, or metabolic disorders, and iatrogenic injury. These are all able to compromise the mechanisms of tissue repair [2–4].

Cutaneous ulceration is a rather common event with a current prevalence between 0.18% and 0.32%, an incidence of 0.78% and a clear trend to increase with the increasing median age of the population [5]. The effects on the socio-economic cost are inevitable: the European Union allocates 2% of the yearly health budget to wound treatment, while a UK clinical study concluded that a 4 month local outpatient therapy costs from 200 to 2000 pounds and that 40 millions pounds are paid yearly only for wound care [6].

Wound healing is a complex process mediated by interacting molecular signals involving mediators and cellular events; it is followed by mesenchemal cell recruitment, proliferation and extracellular matrix generation which allow scar formation [7]. The events causing ulceration are similar to a specific host immune response for restoration of tissue integrity. Wound healing is regulated by a pattern of events including coagulation, inflammation, formation of granulation tissue, epithelialization and tissue remodeling. These events are mediated and modulated by interacting molecular signals, primarily cytokines and growth factors (GF): they stimulate and modulate the main cellular activities which underscore the healing process [7,8].

Several experimental clinical studies have clearly demonstrated that chronic wounds, may in some case, lack the availability of GF to aid in the healing cascade either by way of decreased production, decreased release, trapping, excess degradation or a combination of these mechanisms [9]. Analysis of the supernatant from chronic pressure ulcers showed decreased GF compared with the values of acute wound supernatant [10]. In the chronic ulcers, on the other hand, quick GF metabolization frequently appears because of the presence of a proteinase having a bacterial or cellular origin [11]. In both venous and diabetic ulcers, GF reduction is a consequence of a sequestration mechanism by fibrin sleeves that are rolled around capillaries [12].

Growth factor deficiencies occurring in chronic ulcers suggests their therapeutic use to accelerate tissue healing processes; several researchers have evaluated the efficacy of topical GF management on animal models first and then on humans with promising results [13–15].

Studies on the restoration of tissue integrity have shown the role of the platelets in the wound healing process: during coagulation and the inflammation phase, the formation of a blood clot induces adhesion, aggregation and degranulation of circulating platelets. Platelet α-granules release numerous GF: platelet derived growth factor (PDGF) [16], transforming growth factor beta (TGF-β) [17], epidermal growth factor (EGF) [18], insulin-like growth factor-1 e 2 (IGF 1–2) [19], and vascular endothelial growth factor (VEGF) [20]. These factors play an important role in the tissue remodelling phase (re-epithelization and neovascularization) by mesenchymal cell recruitment and extra-cellular matrix synthesis [21,22].

Platelet gel (PG) is an hemocomponent obtained by associating activated hyper-concentrated platelets and cryoprecipitate: it allows an exogenuos in situ addition of GF with homeostasis restoration with tissue reparation and regeneration. In PG, the GF released from the thrombocyte α-granules is induced in "vitro" by adding gluconate calcium, thrombin or batroxobine to platelets.

Whole blood, as described in Marx's original technique [21], is the source for hyper-concentrated platelet and cryoprecipitate: the source may be autologous or homologous, if general or clinical conditions contraindicate an autologous procedure.

The methodologies for sampling and GP preparation are numerous [23]: (1) test tube, 20–60 ml sample of whole blood: it is useful for extemporaneous use and for a small size wound; (2) quadruple blood bag, 450 ml: whole blood is split into platelet concentrate (platelet rich plasma or buffy coat) and cryoprecipitate; (3) apheresis, multicomponent procedure: plasma-plateletpheresis; (4) manual preparation in an open or closed system; (5) automatic preparation devices: Cryoseal FS

Dideco, VivoStat System, SmartPrep System of Harvest, Autogel Process Cytomedix, Magellan Medtronic.

We apply a methodology based on autologous or homologous platelet multi-component collection using the Haemonetics MCS platform [24]. This procedure choice was made since we did not need to modify the normal service organization work flow, and was made in order to reduce and rationalize the work for the single procedure, and to control costs.

2. Materials and methods

2.1. PG preparation

All the hemocomponent production phases as well as the PG activation are performed in a sterile environment with a hazard cabinet.

(a) *Platelets*: an autologous or homologous collection procedure, respecting ABO compatibility between donor and patient, is done at least 48 h before the GP application. We perform a plasma-plateletpheresis (PLPLT) using the cell separator Haemonetics MCS+, LDPLPS-v.C, using an acid citrate dextrose (ACD) to blood ratio of 1:9; we preset a platelet concentration target at $\geqslant 3\times10^{11}$ and a plasma volume of 400 ml. The platelet preparation is kept for 24 h at +20 °C with continuous shaking; the unit is split into aliquotsof 20, 10, 5 and 3 ml according to the ulcer size and is then stored at −40 °C as platelet lysate; we always verify the therapeutic platelet aliquot concentration $> 1.5\times10^{6}/\mu l$) [25].

(b) *Cryoprecipitate*: the frozen plasma (400 ml) is cryopreserved for at least 24 h at −40 °C: cryo is obtained by siphoning at +4 °C. The cryoprecipitate volume is then increased to 60–80 ml by adding plasma; the final product is split into aliquots of 5 ml which are cryopreserved at −40 °C. Any connection to the satellite bags is done via a TSCD Terumo sterile connector.

(c) *Thrombin*: thrombin is obtained as follows: whole blood is sampled in a 5 ml vacutainer sodium–citrate test tube; then the tube is centrifuged at 3000 g for 15′ to separate the plasma fraction; plasma is mixed with calcium gluconate (1/0.2 rate) and then incubated at +37 °C for 15–30 min; the final supernatant, full of thrombin precursors, is recovered and split into aliquotsat −40 °C.

(d) *Platelet gel activation*: platelet lysate is thawed at room temperature and moved into a graduated sterile Falcon tube. Cryoprecipitate, thawed at +37 °C, is added in the Falcon tube with calcium gluconate in the following proportions: 5 parts of platelet lysate, 2 parts of cryoprecipitate, 2 parts of calcium gluconate. When using thrombin, the proportions to obtain PG are: 3 parts of platelet lysate, 1 part of thrombin, and 0.5 parts of calcium gluconate.

The suspension is exposed to slow shaking with the caution to complete 10–12 times a 360° tube revolution, then it is fractionated, according to the ulcer size and shape, into sterile Petri capsules (35×10 mm, Sigma) or into sterile 5/2.5 ml syringes and it is left to rest for about 15′.

In case of very large cutaneous wounds, grains of hyaluronic acid (Hyalogran Fidiapharma) are added to the platelet lysate–cryoprecipitate mix; we obtain a very malleable paste which makes PG application easier.

All these PG production phases are controlled by a product quality control program that includes: volume determination, platelet count, leukocyte contamination (residual White Blood Count, ''rWBC'' by Flow Cytometry), fibrinogen level and sterility controls for each component and for the final product.

Therapeutic protocol: patient recruitment for PG treatment is based on a preliminary screening visit (''time zero'') that consistes of a complete physical examination and of clinical data collection: general hygienic condition, life habits (smoking, alcoholism), possibility of familial support, ambulation capability, occluding arteriopathy, previous deep venous thrombosis, pain at rest/deambulation/ortho-statism/clino-statism.

The wound evaluation was based on these parameters:

- Etiology.
- Duration (month/year).
- Location, size (area measured by length × width) and depth (cm).
- Edge and peri-wound skin.

- Erythema, edema, purulence, necrotic tissue, fibrin and drainage.

Patients are excluded if there is uncontrolled infection or cellulitis at the site of target ulcer, if osteomyelitis is present or if there is vascular insufficiency in the wound area.

Photodocumentation of the target ulcer is performed at recruitment time and also periodically during the therapeutic period.

During the first screening evaluation, eligibility for autologous blood collection is also defined in respect to the suitability criteria: age, general condition, hematologic, and cardiovascular conditions, and associated pharmacological therapy. If the suitability criteria are not respected, the patient is designated for homologous component use. The traceability and suitability of the hemocomponents is assured according to the hemovigilance criteria: ABO compatibility, Rh compatibility, donor/patient crossmatch, and biological qualification. Informed written consent from the patient is always acquired.

The treatment program is based on outpatient management and consists of a weekly PG application and of preliminary simultaneous bacteriologic tests on the wound bed. Oral and/or topical antibiotic therapy starts only in case of a clear infective symptomatology: exudation, ulcer expansion, fragility and easy wound bleeding, increase in pain, lymphangitis or satellite cellulitus [26]. PG application is anticipated by ulcer cleaning based on irrigation with a isotonic salt solution; hydrogen peroxide (H_2O_2) is also used in wound cleaning to help of removal necrotic particles from the ulcer bottom. In case of Pseudomanas Aeruginosis infection, the ulcer is cleaned by a physiological solution with 1–2% acetic acid (CH_3 COOH). If needed, a surgical debridement is also carried out.

PG is applied to the ulcer bed and then covered by an occlusive dressing, with compressive or not respectively in the absence or in the presence of vascular insufficiency. The dressing is not removed for 72 h and then the patient makes daily dressing changes at home.

The response to topical hemotherapy with PG is evaluated according to the following criteria: wound area reduction, granulation tissue formation, wound bed detersion, and the absence or regression of the infective processes [27].

3. Results

PG components were collected in 56 multicomponent plasma-plateletpheresis procedures, 6 of them autologous and 50 homologous (normal volunteer donors). Quality control assays were carried out in accordance with defined criteria: (1) the mean platelet concentration was 4.4×10^{11} % ±1.1 (SD) in PLPLT and $1.9\times10^{6}/\mu l$ % ±0.40 (SD) in PG aliquotes; (2) the mean fibrinogen level in the plasma was 248 mg% ±57 (SD) and in the cryoprecipitate was 1141 mg% ±350 (SD); (3) the rWBC 0.99 cells % ±1.7 (SD); (4) all sterility controls were negative.

Since March 2002, 24 patients with cutaneous ulcers have been recruited (Table 1): 22 of them had not responsed to conventional therapy and 2 had recent wound onset. The male/female ratio is 9/15 and the median age is 73 year (range 46–88 year).

Three patients were eligible for autologous platelet gel treatment, in all the other cases the source was homologous. One patient, a Jehovah's witness, refused therapy.

Ulcer etiologies were varied: diabetic ulcer, 9 cases, vascular insufficiency, 9 cases (3 arterial, 6 venous), post-traumatic, 3 cases, neuropathic, 2 cases, vasculitis, 1 case. The median initial wound size was 67.4 cm^2 (range 0.5–560 cm^2) with a median depth of 0.69 cm (range 0.2–3 cm). Ulcer duration prior to PG use was very wide: the most recent was one month old, the oldest, 30 year old. In all patients the inferior limbs were affected; 7 patients showed multiple ulcers. In 2 cases, preliminary bacteriology was positivity for Staphylococcus Aureus and Pseudomonas Aeruginosis was found; oral antibiotic therapy was started with resolution of local infection.

In 9 cases complete wound healing was achieved after a mean of 10 applications (range 1–33); in eight of them the wounds remained healed, while in one patient the ulcer reopened during 4 month of follow up. Nine patients are now on treatment:

Table 1
Wound etiology, patient demographics and ulcer response to PG therapy

Patient	M/F	Age (year)	Location of ulcer	Size (cm)	Duration of ulcer prior to platelet gel use	Cause of ulcer	No. of application of platelet gel	Duration of platelet gel use	Response to platelet gel
1	F	64	Right and left lower leg[b]	7×7	6 month	Diabetes	57	14 month	Healed
2[a]	M	73	Left lower leg[b]	7×6	4 year	Trauma	44	11 month	Recovery > 50%
3	F	80	Left malleolar leg	4×3	5 month	Diabetes	24	6 month	Healed
4	F	70	Left heel	9×9	2 year	Neuropathy	13	3 month	Stopped
5	F	76	Left lower leg	6×4	2 year	Venous insufficiency	19	5 month	Healed, reopened
6	F	66	Left heel	10×8	1 month	Diabetes	21	5 month	Recovery > 50%, skin graft
7	M	52	Right and left lower leg[b]	15×4	30 year	Venous insufficiency	20	5 month	Recovery < 50%
8	F	81	Right lower leg	12×30	13 year	Venous insufficiency	14	4 month	Recovery < 50%, skin graft
9	M	72	Thigh stump	1×1	3 month	Arterious insufficiency	8	2 month	Healed
10	F	81	Right malleolar leg	5.5×1	3 year	Diabetes	14	4 month	Healed
11[a]	F	46	Right foot plantar	2×6	2 month	Diabetes	7	2 month	Stopped
12	M	83	Right and left lower leg[b]	8×4	1 year	Venous insufficiency	12	3 month	Recovery > 50%
13	F	70	Left lower leg	3×1.5	7 month	Secondary to trauma	10	3 month	Healed
14	M	71	Right and left lower leg[b]	3×3	7 month	Venous insufficiency	15	4 month	Recovery > 50%
15	M	66	Left Foot plantar	1×1	1 month	Diabetes	1	1 week	Healed
16	F	80	Left lower leg	7×18	16 month	Vasculitis	5	5 week	Stopped
17	F	61	Right heel	3×3	1 month	Neuropathy	2	2 week	Healed
18	F	88	Right and left lower leg[b]	5×3	3 year	Diabetes	6	6 week	Recovery > 50%
19	F	83	Right and left lower leg[b]	5×15	2 year	Venous insufficiency	4	1 month	Recovery > 50%
20	F	77	Left lower leg	16×35	5 year	Diabetes	6	6 week	Recovery > 50%
21	M	68	Right lower leg with exposed tendon and heel	5×5	4 month	Arterious insufficiency	4	4 week	Recovery > 50%
22	M	62	Right lower leg	5×5	1 month	Arterious insufficiency	7	7 week	Stopped
23	F	65	Right foot third digital ulcer	1×0.5	1 month	Secondary to trauma	6	6 week	Healed
24[b]	M	66	Right foot, third and forth digital ulcer	6×1.5	1 year	Diabetes	4	4 week	Recovery < 50%

[a] Autologous procedure.
[b] Multiple ulcers.

7 of them have a decrease of wound area (cm^2) > 50% and 2 patients < 50% from the start of PG use. In 2 patients, the platelet gel allowed are treatment with a skin, graft, that had not previously been before because of the ulcer size. Finally, in 4 cases the treatment was stopped: behind patients' request (Table 1: patient nos. 4 and 16), onset of osteomyelitis (Table 1: patient no. 11) and cellulits in another area (Table 1: patient no. 22).

In total, 323 platelet gel applications were carried out. During treatment an infection was observed in 2 cases, both with positive bacteriological tests for Staphylococcus Aureus: infections were cured with specific oral antibiotic therapy.

No adverse effects have been occurred so far. In all cases we have observed that the promotion of granulation tissues is faster after the first applications and that complete re-epithelization needs a longer time,due to the different size and duration of the ulcers.

In all cases the planned gel application schedule was respected. Patients' compliance was optimum and all the patients agreedthat the pain was decreased during treatment.

4. Discussion

Wound healing is a complex process. The first injury damages blood vessels, triggers coagulation and provokes an acute local inflammatory response. Because of circulating platelet aggregation and degranulation, several cytokines are released: TGF-β 1 induces chemotaxis of neutrophils and monocytes in the wound site; PDGF leads to fibroblast recruitment and proliferation and to matrix remodelling; VEGF is a vascular permeability factor that influences extravasation of plasma proteins to create a support for epithelial and endothelial cells. Restoration of tissue integrity involves a cascade of overlapping events including inflammation, epithelialzation, angiogenesis and matrix deposition.

Platelet gel is a tool for bringing to the wound area growth factors by a platelet lysate and matrix proteins by cryoprecipitate; it is useful to promote the functional recovery of physiological tissue reparation [22].

Platelet gel application is an adjuvant treatment within a multidisciplinary therapeutic programe for chronic cutaneous ulcers. In the protocol proposed, the continuous relationship between the vascular surgeon, dermatologist, infectious disease and transfusion medicine specialists has proved to be effective.

Our experience concerning topicals PG use is based on treatment of chronic nonresponsive and severe ulcers: we have achieved encouraging results. Most of the patients of our Service Center are affected by chronic ulcers that need long term treatment. This aspect, together with the noneligibility for an autologous program induced us to use homologous blood components. The biological safety of the homologous hemocomponents is high and comparable to that of autologous ones. Apheresis collection allows us to obtain each PG component in a single session and to achieve platelet efficiency; the hemocomponent separation allows us to contain the number of donors required to complete a patient therapy cycle; finally the use of homologous blood components has no impact on the overall costs or to routine activities.

In conclusion, topical PG hemo-therapy, an extension of hemocomponent use, allows us to increase and improve the therapeutic approach to the cutaneous wound.

References

[1] Champion RH, Burton JL, Burns AD, et al., editors. Textbook of Dermatology. 6th ed. Oxford: Blackwell; 1998.
[2] Eaglstein WH, Falanga V. Chronic wounds. Surg Clin North Am 1997;77:689–97.
[3] Stadelmann WK, Digenis VD, et al. Impediments to wound healing. Am J Surg 1998;176:S39–47.
[4] Knighton DR, Fiegel VD, et al. Classification and treatment of chronic nonhealing wounds. Ann Surg 1986;204:322–30.
[5] Ippolito E. Epidemiologia e implicanze socio-economiche delle ulcere degli arti inferiori. In: Monti M, editor. L'ulcera cutanea: approccio multidisciplinare alla diagnosi ed al trattamento. Milano: Springer-Verlag Italia; 2000. p. 171–7.
[6] Ruckley CV. Socioeconomic impact of chronic venous insufficiency and leg ulcers. Angiology 1997;48:67–9.
[7] Goss JR. Regeneration versus repair. In: Cohen IK, Diegelmann RF, Lindblad WJ, editors. Wound healing,

biochemical and clinical aspects. Philadelphia: WB Saunders; 1992. p. 40–62.
[8] Rothe M, Falanga V. Growth factors. Arch Dermatol 1989;125:1390–8.
[9] Stadelmann WK, Digenis AG, Tobin GR. Physiology and healing dynamics of chronic cutaneous wounds. Am J Surg 1998;176(suppl 2A):26S–38S.
[10] Cooper DM, Yu EZ, Hennessey P, et al. Determination of endogenous cytokines in chronic wounds. Ann Surg 1994;219:688–92.
[11] Robson MC. The role of growth factors in the healing of chronic wounds. Wound Repair Regen 1997;5:12–7.
[12] Greenhalgh DG. Wound healing and diabetes mellitus. Clin Plast Surg 2003;30(1):37–45.
[13] Ksander GA, Chu GH, McMullin H, et al. TGFs-beta 1 and beta 2 enhance connective tissue formation in animal models of dermal wound healing by secondary intent. Ann NY Acad Sci 1990;593:135–47.
[14] Uhl E, Barker JH, Bondar I, et al. Basic fibroblast growth factor accelerates wound healing in chronically ischaemic tissue. Br J Surg 1993;80(8):977–80.
[15] Xia YP, Zhao Y, Marcus J, et al. Effects of keratinocyte growth factor—on wound healing in a ischaemia-impaired rabbit ear model and on scar formation. J Pathol 1999;188:431–8.
[16] Ross R. Platelet-derived growth factor. Ann Rev Med 1987;38:71–9.
[17] O'Kane S, Ferguson MW. Trasforming growth factor betas and wound healing. Int J Biochem Cell Biol 1997;29(1):63–78.
[18] Cohen S, Carpenter G. Human epidermal growth factor: isolation and chemical and biological properties. Proc Natl Acd Sci USA 1975;72:1317–21.
[19] Bhora RY, Dunkin BJ, et al. Effect of growth factors on cell proliferation and epitheliazation in human skin. J Surg Res 1995;59:n236–244.
[20] Dvorak HF, Brown LF, Detmor M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability and angiogenesis. Am J Pathol 1995;146:1029–39.
[21] Marx RE et al. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Maxillofac Surg 1998;85:6.
[22] Adam J et al. Cutaneous wound healing. N Engl J Med 1999;341:738–46.
[23] Zimmemann R et al. Different preparation methods to obtain platelet component as a source of growth factors for local application. Transfusion 2001;41:1217–24.
[24] O'Neill EM, Zalewski WM, Eaton LJ, et al. Autologous platelet-rich plasma isolated using the Haemonetics Cell Saver 5 and Haemonetics MCS+ for the preparation of platelet gel. Vox Sanguinis 2001;81(3):172–5.
[25] Gollehon TJ, King DE, Craig FE. Does Hyperconcentration result in platelet activation? Vox sanguinis 1998;75:124–7.
[26] Monti M, Motta S. L'uso degli antisettici nel trattamento delle ulcere. In: Monti M, editor. L'ulcera cutanea: approccio multidisciplinare alla diagnosi ed al trattamento. Milano: Springer-Verlag Italia; 2000. p. 407–15.
[27] Embil JM, Papp K, Sibbald G, et al. Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. Wound Rep Reg 2000;8:162–8.