The use of autologous platelet gel to treat difficult-to-heal wounds: a pilot study

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BACKGROUND: Chronic ulcers can benefit from topical treatment with growth factors (GFs). PLT gel provides tissue regeneration-inducing GFs. The aim of this study was to verify the effectiveness of autologous PLT gel in the treatment of nonhealing skin lesions.

STUDY DESIGN AND METHODS: PLT gel was produced by treating PLTs with autologous thrombin. Two groups of patients were investigated: patients with dehiscent sternal wounds and patients with necrotic skin ulcers. Patients treated with PLT gel were retrospectively compared with patients having similar lesions but undergoing conventional treatment. The clinical endpoints of the study were the healing rate, the length of hospital stay, and/or the time required to bring about adequate tissue regeneration in order to undergo reconstructive plastic surgery.

RESULTS: In patients with treated dehiscent sternal wounds the healing rate (3.5 vs. 6.0 wks, $p = 0.0002$) and hospital stay (31.5 vs. 52.5 days, $p < 0.0001$) were significantly reduced. Patients with treated necrotic skin ulcers required a notably shorter time to have surgery (median 15.0 vs. 35.5 wks, $p < 0.0001$). Neither adverse reactions nor in-situ recurrences were observed.

CONCLUSIONS: Patients with chronic unhealing wounds showed substantial improvement when treated with PLT gel lesion dressings.

Chronic ulcers are a major cost for public health and substantially impair the patients' quality of life.[1-5] Conventional treatment such as pressure relief, debridement, dressing, antibiotic therapy, surgery, and nutritional support are somewhat effective when treating nonhealing wounds. Hyperbaric oxygen, ketanserin, cultured human dermis, electrical stimulation, PLT releasate, and growth factors (GFs) are new-generation treatments providing healing acceleration, thus reducing wound-related complications;[6,7] among these, GFs seem to be particularly interesting, owing to their proliferation-eliciting activities.

Topical dressing with crude PLT releasate PLT-derived wound healing formula has been successfully used since 1986.[8-13] Later, randomized controlled trials demonstrated rHu GFs to be effective.[14-16] Finally a new blood component, PLT gel, was added to the therapeutic repertoire as an adjunctive means to accelerate tissue regeneration in a variety of clinical settings.[17-20]

PLT gel is made by treating PLT concentrates (PCs) with autologous thrombin obtained by differential centrifugation of whole blood. The concentration of PLT-derived mitogenic GFs depends on the PLT density in PCs.[21,22] In this study, we used autologous PLT gel to treat soft-tissue

ABBREVIATIONS: bGFG = basic fibroblast growth factor; GF = growth factor; PC = PLT concentrate; PDWHF = PLT-derived wound healing formula; PDGF = PLT-derived GF; VEGF = vascular endothelial growth factor.

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ulcers in two clinical settings: dehiscent sternal wounds and necrotic skin ulcers.

Our purpose was to test if dressing unhealing skin lesions with PLT gel could induce significant acceleration of tissue regeneration. Accordingly, a pilot study was carried out.

MATERIALS AND METHODS

Patients, study design, and treatment

Patients with difficult-to-treat skin and soft-tissue wounds were the target of our investigation. Patients with either dehiscent sternal wounds or necrotic skin ulcers were selected because very often these lesions heal with difficulty, owing to coexisting diseases or complications.

Group 1: sternal wounds. A minority of patients who have undergone sternotomy owing to coronary artery bypass or valve replacement may develop dehiscent nonhealing wounds. Diabetes, obesity, blood transfusions, and cigarette smoking are important factors that heighten the risk of surgical site infection and/or cause unhealing wounds. This is the case specially after cardiac surgery, particularly in patients who have had coronary artery bypass graft as the use of the internal mammary artery deprives the sternum of blood supply, all of which goes to establish the relationship between tissue perfusion, healing, and overwhelming infections. The study covered 1 year because of the limited number of such lesions. Therefore, all patients who were enrolled from January to December 2002 for treatment with PLT gel were compared with patients with similar lesions who had been treated with conventional therapy from January to December 2001. It was the latter group that constituted the control group. PLT-gel treatment consisted of wound dressing with PLT gel twice per week, followed by topical washing and cleaning. The PLT gel was then covered with Vaseline gauze and left for 48 to 72 hours, after which the wounds were treated conventionally until the next PLT-gel treatment. Conventional treatment consisted of daily topical washing and cleaning of the wounds. In the case of a single refractory patient, hyperbaric therapy was added to the conventional treatment. Patients of both groups underwent antibiotic therapy as required. The clinical endpoints for this arm of the study were the time required for complete healing starting from the beginning of the treatment (100% healing rate) and the total hospital length of stay (wks). Follow-up visits were carried out 1 and 6 months after discharge from the hospital, and patients were only asked to come back for a new visit if subsequent recurrence or complication occurred.

Group 2: severe necrotic ulcers. The patients enrolled had full thickness Stage III and IV[23-24] necrotic ulcers resulting from various primary disorders, including venous, arteriopathic, and pressure ulcers. All these patients needed reconstructive surgery because of the extension and/or deepness of lesions; both the treated and the control groups were accordingly assigned. PLT-gel treatment consisted of cleansing with sterile saline solution and a once-a-week coverage with PLT gel, followed by dressing with Vaseline gauze as previously described. Conventional treatment consisted of cleaning and dressing the wound with ialuronic acid and dressing it with synthetic collagen gauze. In only one case in the control group did conventional treatment also include the use of autologous cultured fibroblasts. The clinical endpoints for this arm of the study was the time required to have surgery (wks) starting from the beginning of the treatment leading up to surgery. Surgery was considered feasible only when the lesion kept clean, the margin and floor were occupied by healthy granulation tissue, and the surrounding tissue was trophic and free from signs of gross inflammation. The follow-up was limited to the postsurgery period, and patients were asked to come back for a new visit if subsequent recurrence or complication occurred.

A relatively small number of patients with Stage III and IV lesions was recruitable, hence randomized group allocation was not considered feasible and a pilot case series study was conducted instead. The sample size and the time required to accomplish the study were not determinable in advance. Blinding was not feasible because the physicians responsible for patient care and data evaluation were the same. Data regarding the clinical endpoints for both groups considered in the study was obtained from clinical records, thus ensuring satisfactorily objective results. The study was in compliance with the Helsinki Declaration; accordingly treated patients signed a pertinent informed-consent form.

The hypothesis under test was that, in comparison with conventional treatment, local release of GFs through PLT gel could quicken the wound-healing process, hence reducing medical care, hospital stay, and the related costs.

Autologous PLT-gel preparation

PLT gel was prepared by treating autologous PCs with autologous thrombin. PCs were produced through differential centrifugation of whole venous blood collected either in ACD-containing sterile tubes (Vacutainer, Becton Dickinson Labware, Franklin Lakes, NJ) or in transfusion CPDA1-containing blood bags (NPBI International BV, Emmer-Compascuum, The Netherlands) according to the necessary volume. PLTs were adjusted to 1.5 to 2.0 × 1012 per L. Thrombin was prepared by mixing (5 : 1, vol/vol) the patient's own PLT-poor plasma with 0.22 mol/L calcium gluconate (Ca++ 446 mEq/L). After 15 minutes incubation at 37°C and centrifugation, the thrombin-containing supernatant was collected and, when not immediately used, stored in aliquots at –80°C. PLT gel was then obtained at room temperature by mixing in sterile petri dishes (Falcon, Becton Dickinson Labware) PCs, thrombin, and

0.22 mol/L calcium gluconate (ratio 8 : 2 : 1). Manipulations were done under a Class II Type A/B3 Biohazard laminar flow cabinet (Blueair srl, Capsiolo-BS, Italy).

Statistical analysis

Data analysis included the mean and SD, or the median, according to the data distribution. Exact p values were calculated by means of statistical software (Prism 3.03, GraphPad, San Diego, CA). Demographic and clinical data were compared by a two-tailed paired t test, which calculates group difference and pairing effectiveness. Clinical results were plotted using the Kaplan-Meier method. The Mantel-Haenszel test was used to calculate the significance (95% CI). To determine the sample size to perform a randomized prospective clinical trial, software was used (Epi Info6, Version 6.04, CDC, Atlanta, GA; available free from CDC at the web site, http://www.cdc.gov/epi info/Epi6).

RESULTS

A total of 59 patients entered the pilot study. Six refused treatment. Of the remaining 53 patients, 22 had dehiscent sternal wounds (10 treated and 12 controls), and 31 had skin necrotic ulcers (17 treated and 14 controls). There were no drop-outs in the study. Demographic and clinical characteristics of the enrolled patients are shown in Table 1. Treated and control subgroups were comparable (Group 1, p = 0.903; Group 2, p = 0.887).

The clinical endpoints of the study are summarized in Fig. 1.

Compared with patients under conventional treatment, patients with dehiscent sternal wounds (Group 1) treated with PLT gel achieved 100-percent healing in nearly half the time (median, 3.5 vs. 6.0 wks; p = 0.0002). The difference in hospital stay between treated and control groups was substantial (median, 31.5 vs. 52.5 days; p < 0.0001). During the follow-up, neither recurrence nor complication occurred.

In patients with necrotic skin ulcers (Group 2) treated with PLT gel, the time required to have surgery was significantly shorter (median, 15.0 vs. 35.5 wks; p < 0.0001). Local recurrence did not occur in patients after surgery.

All significance tests were carried out censoring from the analysis two patients from the control group with preexisting variables that could bias the statistical results: one patient from Group 1 who had received additional hyperbaric therapy and one patient from Group 2 who had received cultured fibroblasts. However, this data elimination was noninfluential for the final p value.

Most of the patients in both groups had positive microbiological cultures. Infections were treated with specific antibiotic therapy. Figure 2 illustrates progressive clinical results obtained using PLT gel to treat a Stage IV refractory necrotic ulcer.

TABLE 1. Demographic and clinical characteristics

	Treated	Control
Group 1: Dehiscent sternal wounds		
Number	10	12
Mean age ± SD, years	64 ± 8	66 ± 5
Male/female ratio	6/4	8/4
Primary disease, number (%)		
ischemic disease	8 (80)	9 (75)
Valvular defect	2(20)	3 (25)
Coexisting disease, number (%)		
Diabetes	5 (50)	7 (58)
Endocarditis	1 (10)	NA
Mediastinitis	1 (10)	NA
None	3 (30)	5 (41)
Group 2: Skin necrotic ulcers		
Number	17	14
Mean age ± SD, years	61 ± 18	63 ± 16
Male/female ratio	8/9	5/9
Primary disease, number (%)		
Venous ulcers	3 (17)	5 (35)
Postthraumatic ulcers	5 (29)	3 (21)
Paraplegic ulcers	3 (17)	2 (14)
Arteriopathic ulcers	1 (6)	2 (14)
Leg-foot pressure ulcers	5 (29)	2 (14)
Coexisting disease, number (%)		
Diabetes	2 (11)	2 (14)
Lymphoma	1 (6)	NA
Haemolytic anemia	1 (6)	NA
Multiplemyeloma	NA	1 (7)
None	10 (59)	9 (64)

DISCUSSION

In this pilot study, we have described how dressing unhealing wounds with PLT gel is clinically effective. Compared with lesions of patients under conventional treatment, the sternal dehiscent wounds and skin necrotic ulcers treated with PLT gel recovered more quickly; complete recovery of sternal wounds took half the time and Stage III and IV necrotic ulcers improved even more promptly, strikingly reducing the time to have surgery. All this led to a dramatic reduction of hospital stay and of the related costs. Neither adverse reactions nor in-situ recurrences were observed when treating patients with PLT gel. It is worth mentioning that almost all patients treated with PLT gel reported significant pain relief, thus bettering their quality of life. The analysis of this variable was not planned in advance in the study. We have mentioned it here to underline that the relevance of prospective evaluation of pain by means of objective pain scales should be further studied.

We retain as very plausible the fact that PLT-derived GFs locally released by PLT gel play a pivotal role inducing the results we have described. Several in-vitro and in-vivo models show that all kinds of cells involved in tissue regeneration are sensitive to GFs. Fibroblasts are strongly reactive to basic fibroblast GF (bFGF), PLT-derived GF AB

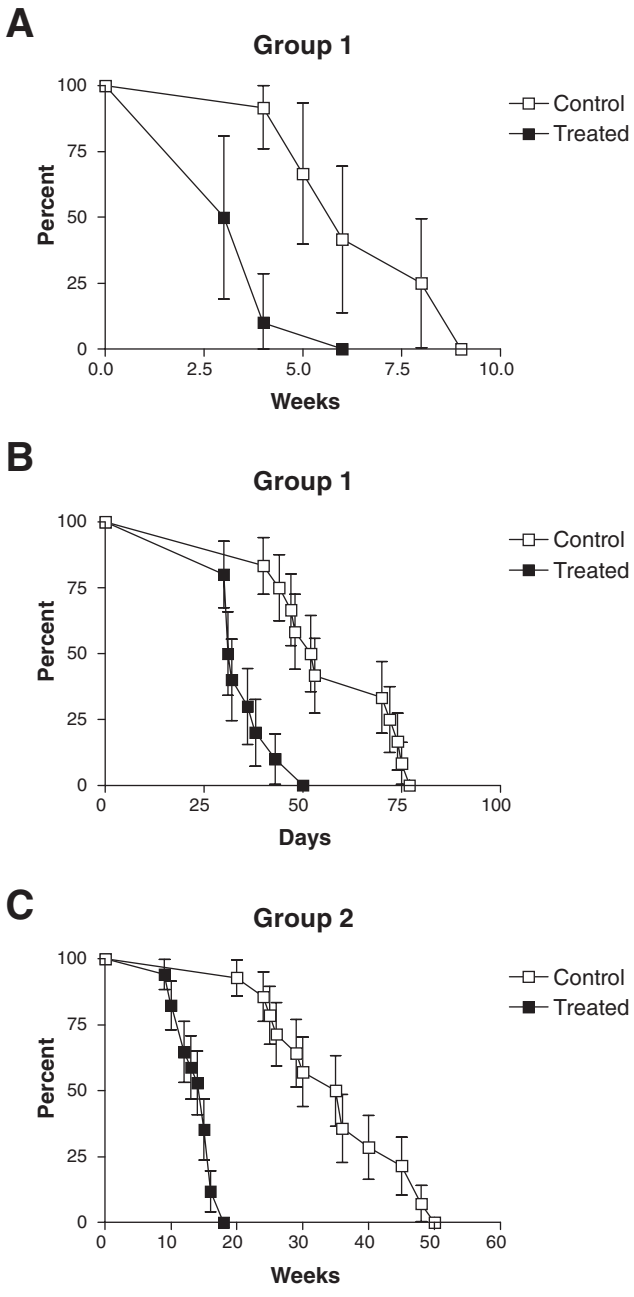

Fig. 1. Clinical endpoints: treated versus control group. Kaplan-Meier plots. (A) Group 1 (dehiscent sternal wounds): complete (100%) healing time (wks). (B) Group 1 (dehiscent sternal wounds): hospitalization time required to achieve complete healing (days). (C) Group 2 (Necrotic skin ulcers): time required to have surgery.

(PDGF-AB), insulin-like GF, and epidermal GF.[25] Endothelial cells are sensitive to bFGF and vascular endothelial GF (VEFG).[26] Human mesenchimal cells, which are recruitable during the tissue-regeneration process, are up-regulated by PLT-derived GFs as well.[27] Angiogenesis is particularly stimulated by VEGF, PDGF, and bFGF.[28] Angioneogenesis is also helped by pericytes, which are in turn dependent on PDGF and VEGF availability.[29] All the above-mentioned GFs are released by PLTs. In addition, factors such as VEGF, PDGF, and bFGF are thermo-resistant,[28] hence, in in-vivo treatment, their local availability for sustaining angiogenesis should be guaranteed by well-timed applications of PLT gel. Besides soft-tissue cells, also dense-tissue cells such as chondrocytes, osteoblastic, and periosteal cell growth is up-regulated by PLT-derived factors such as PDGF and bFGF.[21,30,31] For some time now PLT gel has been proved to be effective in human oral pathology care.[17]

PLT gel has been described to be effective in combined soft and bony tissue reconstruction in facial plastic surgery as well.[20] A few years ago, nerve GF was said to be effective in treating chronic vasculitis ulcers.[32] Most recently, nerve GF has been shown to be effective in treating pressure ulcers in a randomized, double-blind, placebo-controlled trial,[33] thus demonstrating that the healing process is a multifactorial, complex event and that GFs, chemokines, and cells interact with each other in a very complex fashion.

In Italy, only blood transfusion centers belonging to the National Health Service are allowed to prepare PLT gel, as well as any other labile blood products, to provide patients with GMP-certified and quality-control-assured products. In other countries, this process may be regulated differently. In our country, almost all public hospitals have their own blood transfusion centers, all processing blood donations and labile blood products; blood components are also supplied to private hospitals by the nearest public blood transfusion center. This organization, which can hardly be considered cost-effective overall, does, however, allow prompt interaction between the blood transfusion center and the consumer, thus avoiding long-distance transport of blood products. PLT gel must be used within 30 minutes of preparation. However the separate components (e.g., PLT suspension [freshly prepared, stored at room temperature for up to 5 days, or cryopreserved], thrombin, calcium gluconate, and sterile petri dishes) can be transported long distance and used to prepare PLT gel within a few hours.

PLT gel is easy to prepare and is relatively low cost. We maintain that PLT gel is clinically and cost effective and that it can be accessible to most physicians in metropolitan areas and in those areas with hospital facilities. Even though PLT gel is very effective, it should not be considered a panacea, but it must be used as an adjunctive treatment, combining it with the other typical treatments known to quicken tissue repair and regeneration.

We have stated in the Materials and Methods section that the sample size and the time required to accomplish the study were not determinable in advance. This together with some other difficulties encountered in carrying out a more proper prospective randomized trial convinced us

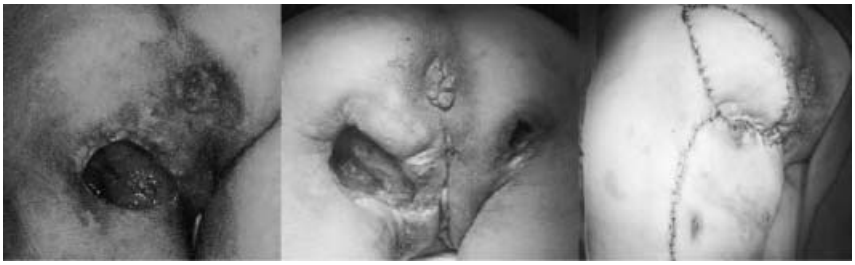

Fig. 2. Ischiatic left pressure sore, Grade IV NPUAP staging classification (National Pressure Ulcer Advisory Panel, 1989). (A) Pretreatment at presentation. Necrotic pressure ulcer for more than 200 months. This patient reported refractoriness to conventional treatment, including autologous cultured fibroblasts graft. (B) After-treatment with PLT gel (16 wks, 31 dressings overall), considered satisfactory for operation (see Materials and Methods section). (C) Postsurgery result after use of myocutaneous island flap from *gluteus maximus* and from posterior side of the thigh.

to opt for a pilot study. At the end of this study, we got substantial information to support reasonable assumptions, which enabled us to predict the necessary sample size to run a prospective randomized clinical trial, using Info Epi Info6 software. In the case of an unmatched case-control study with a control-treated ratio of 1 : 1 and with the following variables: power, 80 percent; exposure to treatment, 0.75; OR (treated vs. untreated recovery rate), 1.5; the predictable sample size turned out to be 13 treated and 13 controls (90% CI), 16 treated and 16 controls (95 CI confidence), 24 treated and 24 controls (99% CI). A prospective randomized clinical trial could then be run using such sample sizes.

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