Randomized trial and local biological effect of autologous platelets used as adjuvant therapy for chronic venous leg ulcers

Patricia Senet, MD,[a,d,e] François-Xavier Bon, PhD,[a] Marc Benbunan, MD,[b] Annette Bussel, MD,[b] Richard Traineau, MD,[c] Fabien Calvo, MD, PhD,[d] Louis Dubertret, MD, PhD,[a] and **Christine Dosquet, MD, PhD,[b]** *Paris and Ivry-sur-Seine, France*

Objectives: **Platelet products have been proposed as adjuvant therapy for wound healing. We undertook this study to determine the healing effect of topically applied frozen autologous platelets (FAP) on chronic venous ulcers, compared with effect of placebo, and whether use of topical FAP modifies local expression of vascular endothelial growth factor (VEGF), keratinocyte growth factor (KGF), interleukin 8 (IL-8), and tissue inhibitor of metalloproteinase-1 (TIMP-1) in wound fluid.**

Methods: **This randomized, placebo-controlled, double-blind trial was carried out in institutional practice, with ambulatory patients with proved chronic venous leg ulcers. In all patients, whole venous blood was drawn for preparation of FAP. FAP or normal saline solution was applied three times per week for up to 12 weeks, together with hydrocolloids and standardized compression bandages. Leg ulcer surface was assessed with numerical pictures. IL-8, VEGF, KGF, and TIMP-1 levels were determined (enzyme-linked immunosorbent assay) in wound fluid after each 4 weeks of treatment.**

Results: **Fifteen patients were randomized into two groups with comparable leg ulcer characteristics. Mean percent reduction in ulcer area was 26.2% in the FAP group versus 15.2% in the placebo group ($P = .94$). One ulcer in each group was completely healed at study end. Levels of TIMP-1 increased significantly during FAP treatment. IL-8 concentration was significantly lower in wound fluid of healing ulcers than in the fluid of nonhealing ulcers, in both FAP and placebo groups. Growth factor levels were not modified with FAP treatment.**

Conclusion: **Topical autologous platelets have no significant adjuvant effect on healing of chronic venous leg ulcers and increased wound fluid TIMP-1 concentration. Ulcer healing is associated with a decrease in wound fluid IL-8. (J Vasc Surg 2003;38:1342-8.)**

Venous ulcers are chronic wounds associated with long-standing venous hypertension. The main goals of venous ulcer treatment are to counteract or eliminate transmission of increased venous pressure to the skin and to improve local wound care. Despite recent improvements in dressings, no drug or local adjuvant wound treatment is widely accepted as standard therapy because the reasons for nonhealing of chronic venous ulcers are poorly understood.[1] The prognosis for healing of large, long-standing venous ulcers is poor, and these ulcers could particularly benefit from alternative therapies to speed the healing process.[2] Cutaneous wound repair is the result of a complex set of interactions among inflammatory and resident cells, soluble mediators, and extracellular matrix.[3] Blood platelets have a major role in initiation of cutaneous wound healing. They adhere, aggregate, and release numerous growth factors, adhesive molecules, and lipids that regulate the migration, proliferation, and functions of keratinocytes, fibroblasts, and endothelial cells.[4-8] It was demonstrated in a porcine model of a cutaneous wound that platelets and fibroblasts are the main inducers of granulation tissue formation.[9] Implication of growth factors in tissue repair has led to abundant animal and clinical research on local application of platelet products or recombinant growth factor as adjuvant therapy for healing of a variety of wound tissues, such as skin, bone, and nervous tissue.[10-12] Supernatants of thrombin-activated platelets—that is, platelet-derived wound healing factors—have been widely used since 1990 in wound care centers in the United States as topical adjuvant therapy for cutaneous wound healing.[13]

To our knowledge, local production of mediators such as growth factor, cytokines, and metalloproteinases and their inhibitors in wounds treated with platelet products has not been reported. Therefore we analyzed the therapeutic effect of a platelet product and its local biological effect on chronic venous ulcers. The primary purpose of the study was to determine, in a randomized, double-blind, placebo-controlled trial, the healing effect of topically applied frozen autologous platelets (FAP) on chronic venous ulcers. Our secondary goal was to determine the level of growth factor (keratinocyte growth factor [KGF], vascular

From the Institut de Recherche sur la Peau,[a] Paris, Unité de Thérapie Cellulaire et de Clinique Transfusionnelle,[b] Paris, Etablissement Français du Sang,[c] Paris, Centre d'Investigations Cliniques Hôpital Saint-Louis[d] Paris, and Service de Gérontologie V, Hôpital Charles Foix[e] Ivry-sur-Seine.

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Reprint requests: Christine Dosquet, MD, PhD, Unité de Thérapie Cellulaire, 1 Ave Claude Vellefaux, 75 475 Paris Cedex 10, France (e-mail: dosquet@chu-stlouis.fr).

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endothelial growth factor [VEGF]) of an inflammatory cytokine (interleukin 8 [IL-8]) and of a tissue inhibitor of metalloproteinase-1 (TIMP-1) in wound fluid from chronic venous ulcers treated with FAP or placebo.

METHODS

Study design. The study was conducted at Saint-Louis Hospital (Paris, France) between January 1998 and December 2000, in accordance with the Declaration of Helsinki. Informed written consent was obtained from each patient before entry to the study. The protocol was approved by the local ethics committee. The study was a one-center, randomized, double-blind, placebo-controlled, parallel group clinical trial. Its purpose was to compare the effect of topically applied FAP suspension in saline solution versus placebo (normal saline solution) for up to 12 weeks on healing of venous leg ulcers (rate and time to complete healing) in patients who were already receiving standard topical treatment and compression therapy.

Patients. Patients with one or more venous leg ulcers were enrolled in the study and assessed with the CEAP classification.[14] Patients who complied with entry and exclusion criteria were randomized to one of two treatment groups. All patients received standardized conservative treatment of leg ulcers. Concomitant medications for other illnesses were continued.

Inclusion criteria included age 18 years or older; presence of at least one venous ulcer; ulcer duration at least 2 months; reference ulcer surface 3 to 50 cm^2; no tendency for healing in the past 2 months; clinical findings consistent with established venous disease (skin hyperpigmentation, varicose veins, lipodermatosclerosis) and confirmed at venous duplex ultrasound (US) scanning during the preceding 6 months; absence of significant arterial insufficiency assessed at clinical examination (intermittent claudication or resting pain, necrotic or distal wound on the foot) and by the presence of either systolic homolateral ankle-brachial index greater than 0.8 or peripheral pulses; ability to give informed consent and to follow the treatment procedure; and platelet count greater than 150,000/mm^3, hemoglobin greater than 11 g/dL, and albumin concentration greater than 35 g/L. Exclusion criteria included pregnancy; allergy to hydrocolloid dressings; uncontrolled or evolving systemic disease (cardiac or renal failure, hepatic insufficiency, malignant disease, diabetes mellitus, rheumatoid arthritis, other connective tissue disorder); serum creatinine concentration greater than 180 μmol/L; systemic treatment with corticosteroid agents or cytotoxic drugs; limited physical capacity or total immobility; ulcers with exposed tendons or bones; infected ulcer requiring systemic antibiotic treatment; history of poor compliance with compression therapy; positive viral serologic finding of human immunodeficiency virus, hepatitis C, or human T lymphocyte virus I or II; presence of antibodies against hepatitis B surface antigen or hepatitis B core antigen; serologic test results positive for syphilis; or abnormal results of liver function test. Patients with diabetes could be included if glucose concentration was less than 2 g/L with appropriate treatment.

Collection and preparation of FAP. Whole venous blood was drawn at the hospital blood bank at a maximal volume of 7 mL/kg into a sterile bag (Baxter SA, La Châtre, France) containing citrate-phosphate-dextrose after standard blood donation procedures. After 2 hours of storage at room temperature (22°C ± 2°C), first-step centrifugation at 5000*g* for 7 minutes was performed, to separate platelet-containing buffy coat from red blood cells. Red blood cells were transfused the same day into the patient. The buffy coat was stored overnight at room temperature (22°C ± 2°C), then centrifuged at 300*g* for 5 minutes to separate platelet-rich plasma from white blood cells and residual red blood cells, which were discarded. Platelet-rich plasma was transferred into sterile tubes after counting (Sysmex), then centrifuged at 1500*g* for 10 minutes at 20°C, and the platelet-poor plasma was decanted. Platelets were gently resuspended in normal saline solution at a final concentration of 5×10^8/mL, and the platelet suspension was divided into 1 mL aliquots (minimum of 36 aliquots per patient) and cryopreserved at −80°C. For each patient, aliquots of 1 mL of normal saline solution (placebo) were also prepared (36 aliquots) and cryopreserved before randomization.

Treatment procedure. At baseline, a general medical history was obtained and physical examination performed. When several ulcers were present, the largest ulcer was designated the reference ulcer. The surface was estimated on the first day by measurement of wound length and width, to determine the appropriate volume of either FAP or placebo to apply topically.[15] Platelet dosage was 10^7 platelets/cm^2 of initial wound surface. The volume of applied placebo or platelets was maintained throughout the study for each patient. Immediately after collection and preparation of platelets, patients were randomized to receive either placebo or platelets. Thereafter patients attended the Clinical Investigation Center for ulcer treatment, three times a week, until either complete healing (full epithelialization) or 12 weeks of treatment. Dressings were changed three times per week, but only at the center, not at home. After the wound was cleansed with normal saline solution, the appropriate volume of either FAP or placebo was applied to the wound surface with a syringe. FAP and placebo appeared identical. Standardized dressing and compression bandages were replaced after each application. In both groups, dressings used were hydrocolloid (Comfeel Plus Opaque; Coloplast, Fontenay-Sous-Bois, France). All patients received standard graded compression, with cotton bandages (Nylex; Laboratoires URGO, Chenove, France) and elastic bandages (Biflex Plus Forte; Laboratoires Thuasne, Levallois-Perret, France). If the treatment was interrupted more than once or a patient could not continue treatment for any reason, related or not to the study, that patient was withdrawn and data were included in the analysis as treatment failure.

Wound assessment. Ulcer evaluation was performed at entry and at 4-week intervals until either 4 weeks after

complete healing or 16 weeks after enrollment. Surface evaluation was assessed by measuring wound width and length and with four successive numerical pictures at each visit. Pictures were obtained with a Kodak DC 120 camera (1280 × 960 pixels, 16.7 million colors) and standardized for light and distance from the wound. Focal distance was set at 30 cm throughout the study. The optical axis always formed a right angle with the wound plane. The dimensions and form of a semirigid frame with colorimetric (white and black) and metric scales were determined before the study, and this standardized frame was used for all patients. Once placed on the wound, it entirely framed the picture when the camera was placed at 90 degrees and 30 cm from the ulcer. The camera was turned so that the optical axis remained the same. Reflections were eliminated, because light came from the left for the first two pictures and from the right for the last two pictures. The metric scale of the frame was used to resize the picture on the computer. Reflections were eliminated by superimposition of the resized pictures on the computer. Wound outlines were manually determined on the computer by one of us (P.S.) on the resized and recorded pictures. Each picture was subsequently computer-processed, and the final wound area and perimeter were taken as the mean of the measurements calculated from the four pictures. Inasmuch as change in area is independently influenced by initial area and perimeter, we estimated wound healing rate by calculating the linear healing of the wound edge[16]:

$$D = \Delta A/P,$$

where D is linear healing, ΔA is change in area, and P is mean perimeter.

To test intraobserver reproducibility of this wound-outlining method before the study, 23 chronic leg ulcers were measured three times by the same clinician (P.S.) with this method, at 1-month intervals. The three repeated measurements were compared with analysis of variance, and did not show any significant difference (data not shown). Interobserver reproducibility of the method was not assessed, because it has been always reported as excellent in the literature.[17]

Wound fluid sample collection. Samples of wound fluid were obtained at entry to the study and every 4 weeks during treatment (12 weeks). Wound fluid was collected with a standardized method, as described.[18] In brief, after cleansing the wound with sterile saline solution, the leg ulcer was covered with polyurethane film (Opsite; Smith & Nephew, LeMans, France). The patient rested in the study center, and after 6 hours the fluid was aspirated with a syringe from beneath the dressing. The fluid was transferred to collection tubes and centrifuged at 2000*g* for 10 minutes to remove any debris. Supernatant was separated in aliquots and stored immediately at −80°C until assay.

Growth factors and TIMP-1 assay in wound fluid and platelet products. Commercial quantitative sandwich enzyme-linked immunoassay techniques were used to determine concentrations of IL-8, VEGF, KGF, and TIMP-1 in wound fluid, and platelet-derived growth factor (PDGF)–AB, and transforming growth factor beta$_1$ (TGFβ_1) levels in platelet products. The IL-8 assay system was obtained from Immunotech (Marseille, France), and used two monoclonal antibodies. Other assay systems were obtained from R&D Systems (Minneapolis, Minn), and used a monoclonal capture antibody and a second polyclonal antibody for quantification. Assay reagents and working standards were provided with the kits, and were prepared according to the manufacturer's instructions. Samples and standards were diluted with the diluents appropriate for serum sample dilution provided with the kits. Standard curves were established with serial dilutions of the corresponding recombinant protein. All samples were analyzed in duplicate. Interassay and intra-assay coefficients of variation were all less than 10%. Sensitivity of each assay was 8 pg/mL for IL-8, 5 pg/mL for VEGF, 15 pg/mL for KGF, 1.25 ng/mL for TIMP-1, 8.4 pg/mL for PDGF, and 7 pg/mL for TGFβ_1. The VEGF assay recognizes the different isoforms of VEGF-A. Each cytokine level in wound fluid was related to the corresponding total protein concentration (Roche Diagnostic, Meylan, France). PDGF and total TGFβ_1 levels in FAP were determined after five cycles of freezing and thawing to disrupt platelet membranes. Results are expressed for 10^7 platelets.

Statistical analysis. The data were analyzed on an intent-to-treat basis, and represent mean and standard deviation (SD), except where indicated. The Fisher exact test was used to compare categorical data between groups. The Mann-Whitney *U* test for unpaired populations was used to compare numerical data between groups. The Mann-Whitney *U* test for paired populations was used to compare numeric data on two different days in the same group. All tests were two-sided, and $P = .05$ was considered significant. Analysis of variance was used to analyze ulcer size and biological parameter evolution in each treatment group. Statistical analysis was performed with Statview software (ABACUS Concepts; Berkeley, Calif).

RESULTS

Epidemiologic data. Fifteen patients were randomly assigned, eight in the FAP group and seven in the placebo group. Patients were categorized according to the CEAP system (Table I). The groups were comparable after randomization. Two patients (one in each group) were withdrawn from the final clinical analysis; one discontinued participation in the study because of personal reasons, and the other did not have a photographic assessment that could be evaluated. Data for these two patients were included in the analysis as failure to heal. Characteristics of the two groups are summarized in Table I. No significant difference was found between groups for ulcer size and duration, type of venous insufficiency, or other characteristics. Overall mean ulcer diameter in enrolled patients was 12.4 ± 7.9 cm^2.

Characterization of FAP. Concentration of PDGF-AB and TGFβ_1 in frozen autologous platelet sus-

Table I. Characteristics of treatment groups

Patient characteristics	*FAP*	*Placebo*	P
Demographic data			
Age (y)	72.3 [45–88]	72.3 [50–83]	NS
Sex (F/M)	3/4	3/3	NS
Body mass index (cm/kg^2)	29.1 [18–42.3]	29 [23.9–35.6]	NS
Diabetes	1/7	1/6	NS
Reference ulcer			
No. of episodes of complete healing	0.29 [0–1]	0.3 [0–1]	NS
Duration of reference ulcer (mo)	50.6 [4–240]	70 [24–120]	NS
Mean surface area at baseline	13.7 [4.8–27.25]	10.85 [3.7–26.5]	NS
Venous disease			
Deep venous insufficiency	4/7	4/6	NS
Superficial venous insufficiency	4/7	3/6	NS
Popliteal reflux	3/7	4/6	NS
Postthrombotic syndrome	3/7	2/6	NS
Homolateral venous stripping	2/7	4/6	NS
CEAP classification			
Clinical C6	7/7	6/6	
Etiology			
Ep	4/7	1/6	
Es	2/7	3/6	
Ec	0/7	3/6	
Anatomy			
As	4/7	3/6	
Ad	4/7	4/6	
Pathophysiology			
Pr	5/7	4/6	
Pr+o	2/7	2/6	

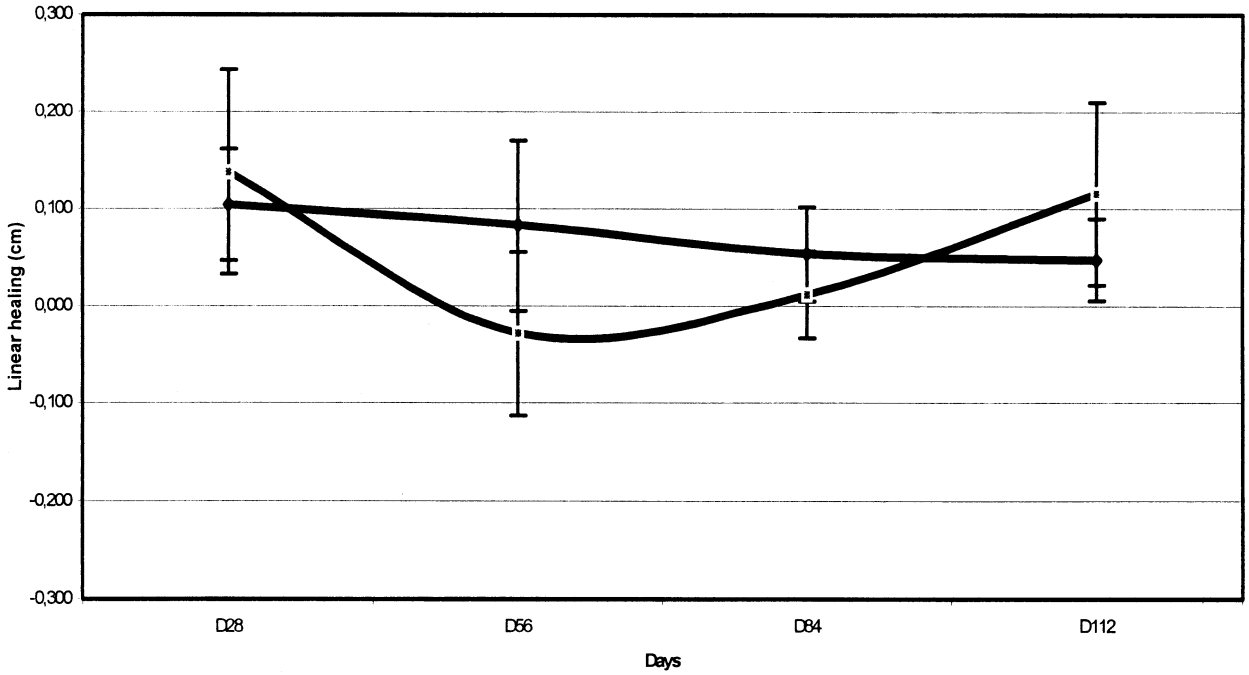
Values in parentheses are range.
FAP, Frozen autologous platelets; *NS,* not significant.

pensions was measured in prepared samples from 14 of 15 patients. PDGF-AB concentration was 0.50 ± 0.18 ng/10^7 platelets, and TGFβ_1 concentration was 1.40 ± 0.46 ng/10^7 platelets.

Clinical safety and efficacy of FAP on wound healing. FAP had no significant influence on venous ulcer healing (Fig). There was no statistically significant difference in mean baseline ulcer diameter between the FAP and placebo groups (respectively, 13.7 ± 7.9 cm^2 and 10.9 ± 8.4 cm^2; $P = .9$). One ulcer in the FAP group and one in the placebo group healed completely by study end ($P > .05$), after 12 and 4 weeks of treatment, respectively. No relapse occurred during the 4 weeks after the end of the treatment. Mean linear healing rate per day during the study was .0033 ± .0061 cm/d in the FAP group and .0021 ± .0058 in the placebo group ($P = .47$). Linear healing of wound edge was not statistically different between the two groups at any time during the study. Mean percent reduction in ulcer area was 26.2% in the FAP group versus 15.2% in the placebo group ($P = .94$). Decrease in ulcer area tended to be greater in the FAP group than in the placebo group, but this difference did not reach significance. No statistically significant difference was detected between the two groups with respect to incidence of adverse events. Adverse events included one wound infection in the FAP group, which required treatment with oral synergistin for 10 days, compared with no wound infection in the placebo group; thrombophlebitis during blood sampling in one patient in the FAP group, compared with none in the placebo group; and irritative dermatitis around the wound in two patients in each group. There was no evidence of adverse effect specifically related to use of FAP.

Induction of local production of growth factors in leg ulcers treated with FAP. Growth factor and TIMP-1 levels were assessed to determine whether platelet treatment influenced local secretion of these mediators. In a few cases wound fluid volume was not sufficient to perform all of the assays. Levels of the various factors in wound fluid at different times during the study are presented in Table II. Concentrations of growth factors and TIMP-1 in wound fluid did not differ significantly between platelet and placebo groups at inclusion (day 0) or during the study. Concentrations of IL-8, KGF, and VEGF were not significantly modified during the study in either group. TIMP-1 levels in wound fluid increased significantly during the study in the FAP treatment group ($P = .04$).

When considering ulcer outcome at the end of the study, in either group, no significant difference was found between healing (n = 7) and nonhealing (n = 8) ulcers for VEGF, TIMP-1, or KGF levels. IL-8 levels in wound fluid were not significantly different between healing and nonhealing ulcers at the beginning of the study, but at the end of the study IL-8 level was significantly lower in the healing group (29.8 ± 10.4 ng/mg) compared with the nonhealing group (69.7 ± 35.7 ng/mg; $P = .04$).



Linear healing of wound edge (mean ± SEM) calculated at each visit for platelet group *(line with diamonds)* and placebo group *(line with squares)*. $D = \Delta A/P$, where D is linear healing, ΔA is change in area, and P is mean perimeter).

Table II. IL-8, VEGF, TIMP-1, and KGF concentrations in wound fluid

	Day 0	*Day 28*	*Day 56*	*Day 84*
IL-8 (ng/mg)				
FAP	97.2 ± 60.1 (n = 7)	67.1 ± 33.0 (n = 8)	74.9 ± 91.4 (n = 7)	52.3 ± 26.8 (n = 7)
Placebo	57.0 ± 48.4 (n = 6)	38.7 ± 18.3 (n = 5)	24.5 ± 0.1 (n = 2)	59.7 ± 50.1 (n = 4)
VEGF (pg/mg)				
FAP	369 ± 123 (n = 7)	507 ± 408 (n = 8)	520 ± 630 (n = 8)	373 ± 250 (n = 7)
Placebo	379 ± 95 (n = 6)	351 ± 221 (n = 5)	256 ± 20 (n = 2)	579 ± 365 (n = 4)
TIMP-1 (ng/mg)				
FAP	7.4 ± 2.9 (n = 7)	14.2 ± 7.3 (n = 8)	13.7 ± 6.3 (n = 7)	15.1 ± 7.9 (n = 7)
Placebo	13.9 ± 7.2 (n = 6)	20.5 ± 11.9 (n = 5)	20.0 ± 0.8 (n = 2)	18.1 ± 4.6 (n = 3)
KGF (pg/mg)				
FAP	41 ± 37 (n = 6)	43 ± 18 (n = 5)	81 ± 55 (n = 7)	69 ± 26 (n = 4)
Placebo	57 ± 53 (n = 3)	46 ± 32 (n = 4)	61 ± 18 (n = 2)	78 ± 38 (n = 2)

Values per milligram of total protein content.
IL-8, Interleukin-8; *VEGF,* vascular endothelial growth factor; *TIMP-1* tissue inhibitor of metalloproteinase-1; *KGF,* keratinocyte growth factor.

DISCUSSION

This study evaluated in a randomized double-blind manner the clinical and biological effects of autologous platelets as adjuvant treatment for venous ulcers. Compared with placebo, FAP resulted in modest improvement in venous ulcer healing, as measured by the rate of wound area reduction, but the difference was not significant. During FAP treatment, local secretion of VEGF and KGF was not modified in chronic venous ulcer wound fluid, but TIMP-1 concentrations increased significantly. Moreover, IL-8 levels in wound fluid were significantly lower at the end of the study in healing venous ulcers in both groups.

Large ulcer diameter (mean, >12 cm^2) and long duration of ulceration (>50 months) are predictors of poor healing and long time to healing.[2] Indeed, only large and long-standing ulcers that are slow to heal may benefit from alternative therapies such as topically applied platelet products.[2] The severity of inclusion criteria in our study could account for the homogeneity of the enrolled patients and also for their small number. The 12-week treatment period

could be too short to observe substantial benefit in patients with venous ulcers with poor prognosis. However, recently, in a randomized trial versus placebo conducted in 86 patients with chronic venous ulcers, a platelet product had no adjuvant effect, even when the treatment was continued for 9 months.[19]

Various types of platelet products have been used for tissue repair adjuvant therapy. Platelet releasates (platelet-derived wound healing factors) are supernatants of autologous platelets that have been activated in vitro with thrombin.[20] Platelet lysates are autologous platelets disrupted by sonication and centrifuged to remove platelet fragments from the solution.[19] Our group has used autologous suspensions of functional platelets for treatment of macular holes.[11] In the present study, we prepared platelet suspensions according to the method described for treatment of macular holes.[11,21] For use during 12-week treatment of chronic venous ulcers, platelet suspensions were prepared, aliquoted, and frozen. Platelet suspensions prepared for macular hole treatment were adherent, aggregated, and mitogenic for glial cells (maximum, approximately 3×10^7 platelets/mL), and contained significant amounts of PDGF, epithelial growth factor (EGF), and TGFβ_1.[11,22] In the present study, the same type of platelet suspension was frozen until use, and therefore the platelets applied to the wounds were no longer functional. We observed in vitro a dose-dependent stimulatory effect of FAP on IL-8, VEGF, KGF, and TIMP-1 production by adult dermal fibroblasts, with a maximal effect at 10^8 platelets/mL (P. Senet, 1999). The methods of blood collection (drawing of venous blood or apheresis) and platelet preparation (with or without activation with thrombin) modify the final platelet product composition.[23] Mean content of growth factor in FAP was 50 ng/10^9 platelets for PDGF and 140 ng/10^9 platelets for TGFβ_1. The quantity of FAP applied was adjusted according to wound area, and mean quantity of growth factor applied on the ulcer three times a week was 7 ng for PDGF and 19 ng for TGFβ_1. Growth factor platelet content could account for the adjuvant effect of growth factor for tissue repair. However, after activation, platelets also release adhesive proteins, mainly thrombospondin-1, and lipid mediators, which have a role in angiogenesis regulation and wound healing.[6,24] It is noteworthy that it is impossible to reproduce the effect of functional platelets observed on glial cells in culture with recombinant growth factors.[22] These findings concerning platelet products used for wound healing adjuvant therapy justify conducting trials in homogeneous groups of patients treated with standardized and well-characterized platelet products.[23]

For chronic cutaneous wounds, it has been suggested in two recent retrospective studies and one randomized trial that platelet releasates may improve the chance for healing of diabetic foot ulcers and decrease the risk for amputation.[10,13,25] Platelet releasates have been tested also in two small studies in patients with leg ulcers from different causes, with contradictory results.[20,26] In a randomized controlled trial, platelet lysates failed to improve healing of chronic venous ulceration.[19] Similarly, results of clinical trials of topical recombinant growth factor on venous leg ulcers have been disappointing.[1,3] Thus neither platelet products nor recombinant growth factors appear to have any clear adjuvant effect on chronic venous ulcer healing.

The biological and cellular mechanisms of impaired healing in venous ulcers are not well known, because of lack of an animal model for this disease process. Studies have focused on analysis of wound fluid, and also on biopsied tissues, but these specimens are difficult to obtain, for practical and ethical reasons. Several concepts have recently emerged from the molecular and cellular analysis of the chronic wound environment. Chronic venous ulcers are associated with a chronic inflammatory state that is not present in acute surgical wounds. Chronic venous ulcers exhibit a long-standing inflammatory cellular infiltrate in tissues, and elevated levels of proinflammatory cytokines in wound fluid.[27,28] Higher levels of IL-1, IL-6, and tissue necrosis factor-α, and similar levels of various growth factors have been reported in nonhealing ulcers compared with healing ulcers.[27-30] Of interest, in our study IL-8, which is implicated in recruitment of inflammatory cells to the wound site, was present in a significantly lower concentration in wound fluid of healing compared with nonhealing ulcers. Concentrations of VEGF and KGF did not differ between healing and nonhealing ulcers, as reported for PDGF, EGF, basic fibroblast growth factor (bFGF), and TGF.[27,29] Therefore the results of our study and of others in the literature suggest that the cellular environment of chronic venous ulcers becomes less inflammatory as the wound heals. In this study we also observed an increase in wound fluid TIMP-1 concentrations during treatment with FAP. The angiogenic effect of platelets is normally required for induction of granulation tissue formation in acute wounds.[9] It was recently reported that fluid exudates from long-standing venous ulcers inhibit experimental angiogenesis.[31] This inhibiting effect could be related to presence of antiangiogenic agents such as TIMP-1 in wound fluid, which counteract the proangiogenic effect of growth factors contained in topical platelet products.[32]

Fibroblasts isolated from venous ulcers are more senescent compared with fibroblasts isolated from healthy skin, and have decreased proliferative ability, dependent on ulcer age.[33-35] Indeed, chronic wound fibroblasts generally exhibit a decreased mitogenic response to PDGF-AA, PDGF-BB, bFGF, TGFβ_1, and EGF, compared with normal or acute wound fibroblasts.[34,36] This may explain the poor healing response to topical recombinant growth factors[34] and to platelet products of long-standing venous ulcers, as in our study. The absence of healing effect of the platelet product on venous ulcers could also be due to destruction of its components by wound proteases. High levels of proteases are found in wound fluid from chronic venous ulcers, impairing the healing process by degradation of exogenous added growth factors such as EGF.[18,37] This raises the question of the possible delivery of platelet products together with protease inhibitors, by means of a polymeric device to control release of the growth factors and prolong the biological effect of the various components.[38]

In summary, we did not find any adjuvant therapeutic effect of FAP for treatment of chronic venous ulcers. With both FAP and placebo, ulcer healing was associated with a decrease in local production of the inflammatory cytokine IL-8. Thus it appears that local application of a platelet product alone, in association with standard treatment, does not improve healing of chronic venous ulcers.

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