Reciprocal Actions of Platelet-Secreted TGF-β1 on the Production of VEGF and HGF by Human Tendon Cells

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Background: Autologous platelet-rich matrices can be an aid in surgery by promoting and accelerating tissue healing because of the release of growth factors including transforming growth factor (TGF)-β1 and platelet-derived growth factor (PDGF) from platelet α-granules.
Methods: PDGF and TGF-β1 were quantified in supernatants collected from platelet-rich matrices prepared in vitro ($n = 45$ donors) and they correlated with the number of platelets and showed a constant ratio ($p < 0.05$). Tendon cells in culture were exposed to the supernatants ($n = 4$ donors) from either platelet-rich or platelet-poor matrices, differing in their content of platelet-secreted molecules. These treatments were modified by either neutralizing or adding PDGF or TGF-β1. Effects were compared in terms of proliferation, procollagen I, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) production.
Results: PDGF was a partial contributor to cell proliferation, whereas exogenous TGF-β1 acted as a negative modulator ($p < 0.05$). The production of type I collagen was similar regardless of differences in the concentration of TGF-β1. Moreover, addition of exogenous TGF-β1 promoted a significant increase in collagen synthesis only in the absence of other platelet-released substances ($p < 0.05$). Exogenous TGF-β1 increased the synthesis of VEGF and simultaneously abolished the production of HGF. Furthermore, antibody-mediated neutralization of TGF-β1 induced a decrease in VEGF synthesis and concomitantly a substantial production of HGF ($p < 0.05$).
Conclusion: The balance between TGF-β1 and the pools of platelet-secreted molecules may have important therapeutic implications in the control of angiogenesis and fibrosis. (*Plast. Reconstr. Surg.* 119: 950, 2007.)

Tendons have low healing capabilities. Injury can cause joint instability, which may lead to deterioration of other tissues and the development of degenerative diseases. Research focused on the biology of tendon tissue and its constituent cells will help in understanding the processes by which tendons heal after injury, offering new possibilities for the development of novel therapeutic strategies that may be helpful in surgery. To address this issue, it is necessary to understand the participation of individual cell types and the role of the soluble factors that contribute to the extrinsic and intrinsic healing properties of the tissue. It has been reported that several growth factors play key roles in initiating and sustaining the different phases of tendon repair.[1] For example, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor (TGF)-β1 are up-regulated during tendon wound repair and grafting.[2–4] The presence of TGF-β receptors has been described in the tendon sheath, epitenon, and endotenon,[5] and changes in receptor numbers occur with tendon injury and subsequent repair.[2] Moreover, expression of TGF-β1 and insulin-like growth factor type I showed a characteristic temporal regulation coincident with the early inflammatory (TGF-β1, 1 week) and later regenerative (insulin-like growth factor type I, 4 weeks) phases of healing.[6] However, even though physiologic release of growth factors is fundamental to the healing process, their indi-

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vidual participation in repair is difficult to evaluate, as complex interactions almost certainly occur, possibly as a consequence of cross-talk in their signaling pathways.

In our laboratory, we are investigating whether connective tissue repair can be improved through the local administration of autologous growth factors delivered from a platelet-rich matrix at the injury site.[7,8] Platelets retained in fibrin matrices secrete significant amounts of TGF-β1, PDGF, and other growth factors from their storage pools.[9] Some growth factors would be released rapidly, whereas others may bind to the fibrin and constitute chemotactic gradients.[10] Growth factors would also depend locally on the influence of inflammatory cells present at the injured site.[11] We have previously reported that tendon cells in vitro respond to supernatants released from platelet-rich matrices by proliferation, a basic response fundamental for repair.[12] Moreover, and also crucial for repair, this treatment induced a potentially angiogenic response through the synthesis of vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) by tendon cells. Others have reported that PDGF and TGF-β1 can up-regulate VEGF expression in vascular smooth muscle cells.[13] TGF-β1 is ubiquitous; information on its indirect promotion of angiogenesis in different tissues has been described,[14,15] although its effects may be influenced by the local environment.[16]

Our current studies have been designed to further investigate the role of proteins secreted from platelets on both the proliferation of tendon cells in vitro and on their capacity to produce collagen. A known characteristic of TGF-β1 is its ability to stimulate collagen synthesis, a feature that is also linked to the development of fibrosis.[17] To do this, we have compared the effects of supernatants released from platelet-rich matrices with those of supernatants released from platelet-poor matrices that lack the bulk of the platelet-secreted molecules. In parallel, we have supplemented the supernatants released from platelet-poor matrices with recombinant TGF-β1 and PDGF to exactly match their respective concentrations in the supernatant of platelet-rich fibrin matrices. Results were also obtained from other cultures where platelet-secreted TGF-β1 or PDGF were neutralized with antibodies. Our results suggest that the balance between TGF-β1 and the pools of platelet-secreted molecules may have important therapeutic implications in the control of angiogenesis and fibrosis.

MATERIALS AND METHODS

Recombinant human (rh) TGF-β1 (active or latent) and PDGF-AB, anti-human TGF-β1, and anti-human PDGF antibodies were purchased from R&D Systems (Abingdon, United Kingdom). Quantikine enzyme-linked immunosorbent assay kits for measuring TGF-β1, PDGF-AB, VEGF, and HGF levels were also obtained from R&D Systems. The enzyme immunoassay kit for procollagen type I C-peptide was purchased from Takara Bio, Inc. (Tokyo, Japan).

Platelet-Rich and Platelet-Poor Matrices and Their Supernatants

These were obtained as previously described[12] and are illustrated in Figure 1. Briefly, blood was collected into 3.8% (wt/vol) sodium citrate from healthy adult donors. Samples were centrifuged either at 4500 *g* for 12 minutes at 4°C to separate platelet-poor plasma or at 460 *g* for 8 minutes to obtain platelet-rich plasma. Care was taken to avoid collecting the buffy coat. Platelet-poor and platelet-rich fibrin matrices were formed by adding calcium chloride at a final concentration of 22.8 mM to samples in glass tubes and incubating them at 37°C. Matrices were allowed to retract for 1 hour and, after centrifugation at 4500 *g* for 10 minutes at 4°C, the supernatants containing the released materials were collected by aspiration.

Determination of TGF-β1 and PDGF-AB Levels

Forty-five healthy volunteers, aged 20 to 69 years (11 women and 34 men; mean age, 42 ± 13 years) gave informed consent and constituted the study group. Samples (10 ml) of whole blood were drawn from each subject. The platelet count was determined in the platelet-rich plasma before clotting. PDGF-AB and TGF-β1 were quantified in the supernatants released from platelet-rich matrices using the Quantikine enzyme-linked immunosorbent assay according to the manufacturer's instructions and as described previously by us.[12]

Cell Culture Experiments

Blood collected from four representative donors of this group, three middle-aged men and one woman, was used for the cell culture experiments. For these subjects, additional measurements of the concentration of growth factors in unclotted plasma were determined in addition to the analysis of the supernatants of platelet-poor and platelet-rich matrices.

Culture, Cells, and Media

Human tendon were obtained during surgical reconstruction of the anterior cruciate ligament

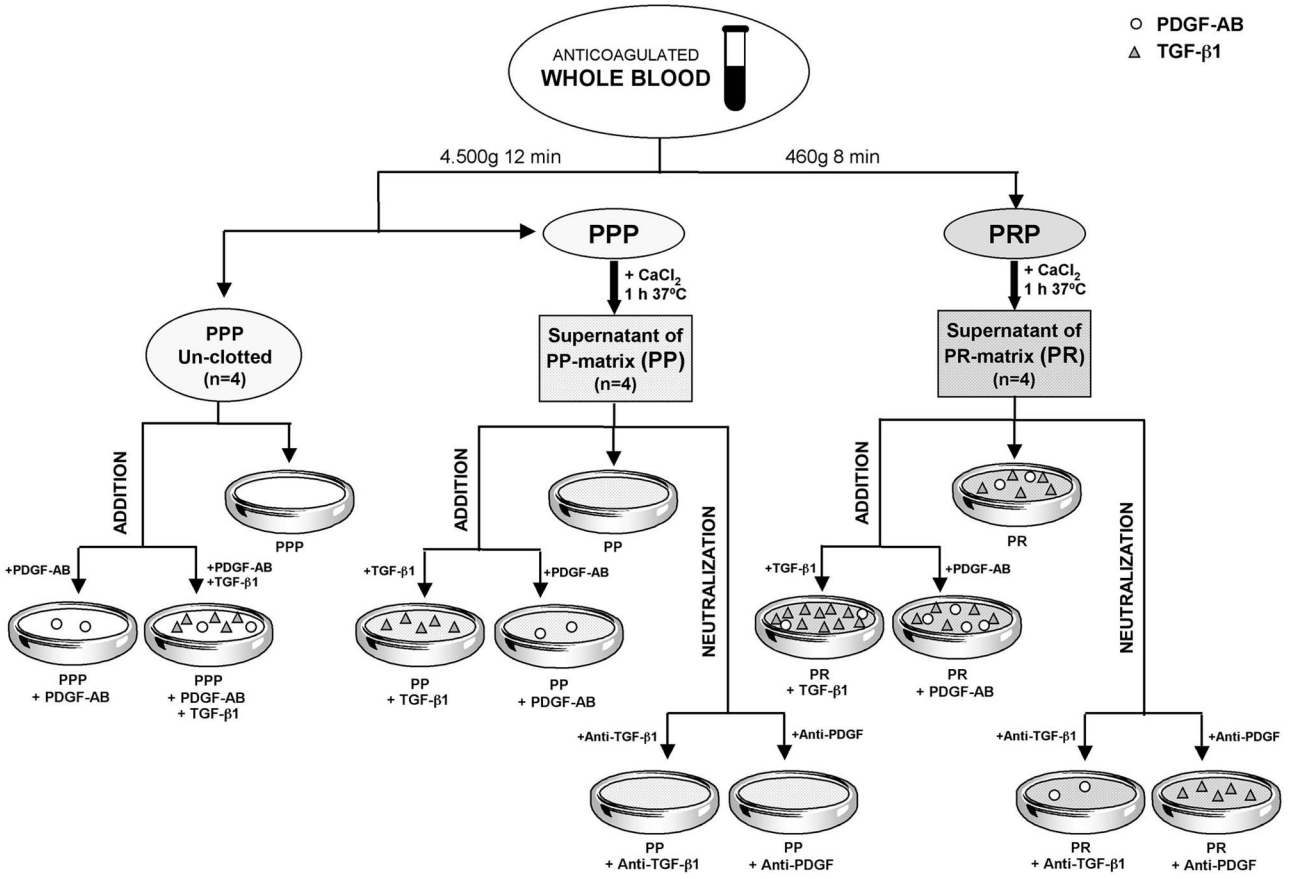

Fig. 1. Diagram of the preparations of the different treatments that were assayed in human tendon cells in culture. Culture media were supplemented with the supernatants released from either platelet-poor or platelet-rich matrices or their modifications based on parallel additions and neutralizations of PDGF or TGF-β1.

with semitendinosus tendon autograft from an otherwise healthy young donor after informed consent and project approval from the local ethics committee. The tendon samples were cleaned of surrounding adipose tissue. Tendon fragments collected in phosphate-buffered saline supplemented with antibiotics (penicillin-streptomycin solution and amphotericin B; Sigma Chemical Co., St. Louis, Mo.) were minced and treated with 0.3% collagenase II (GIBCO Life Technologies, Gaithersburg, Md.) at 37°C for 2 hours. The resulting cell suspension was filtered and centrifuged at 460 *g* for 10 minutes. Cells were seeded into culture flasks and maintained with Dulbecco's Modified Eagle Medium/F12 (vol/vol) (GIBCO) culture medium supplemented with 10% fetal bovine serum (Biochrom AG, Berlin, Germany), 2 mM L-glutamine (Sigma), antibiotics, and amphotericin B in a humidified atmosphere at 37°C with 5% carbon dioxide. All experiments were performed on cells obtained between the third and fifth passages. The cells were characterized immunocytochemically by staining for collagen type I (BIODESIGN International, Saco, Maine).

Culture Experiments

Culture medium was replaced by serum-free medium supplemented with either (1) 20% (vol/vol) supernatant released from a platelet-rich matrix, (2) 20% (vol/vol) supernatant released from a platelet-poor matrix, or (3) 20% (vol/vol) unclotted platelet-poor plasma. Parallel cultures analyzed the effects of TGF-β1 and PDGF in supernatants of platelet-rich matrices; these were identical to the above except that they were incubated at 37°C with either PDGF neutralizing or TGF-β1 neutralizing antibodies (2000×) for 1 hour. Additional experiments analyzed the effects of exogenous TGF-β1 and PDGF. In these cultures, platelet-poor plasma and supernatants released from platelet-poor and platelet-rich matrices were supplemented with either rh-TGF-β1 or latent rh-TGF-β1. In addition, rh-PDGF-AB was added to the three treatments in other parallel cultures. When these factors were added to plate-

let-poor plasma or supernatants of platelet-poor matrices, we matched exactly the levels found in the supernatants of platelet-rich matrices. In this way, we could analyze the effect of TGF-β1 and PDGF in a similar milieu but without other proteins released from platelets; at the same time, we doubled the natural levels when they were added to the supernatants of platelet-rich matrices. Experiments were run simultaneously for four donors. Control cultures received equivalent volumes of medium, with no addition of platelet-poor plasma or supernatant. The culture medium was not changed during the study periods. Additional control cultures were set up to evaluate the neutralization efficiency of the antibodies against recombinant growth factors under our experimental conditions. The experimental design for cell culture experiments is shown in Figure 1.

Cell Proliferation Assay

Cells were plated at a density of 800 cells per well in 48-well plates and maintained with serum-free medium for 24 hours. Cell proliferation was evaluated using the tetrazolium salt WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)] colorimetric assay (Roche, Basel, Switzerland). Control wells with the same treatments, but without cells, were used for background correction. All experiments designed to study proliferation were repeated in triplicate on three different occasions and gave essentially the same results. The mean data of repeated analyses are shown for each experiment. As an index of cell number, calibration curves, ranging from 800 to 80,000 cells per well, were established using the WST cell counting kit.

Determination of Collagen Production

Collagens are synthesized as precursor molecules called procollagens; these contain additional peptide sequences that are cleaved off from the collagen triple helix during its secretion. Thus, the amount of free propeptides reflects stoichiometrically the amount of collagen molecules synthesized. Human procollagen type I C-peptide was measured in the media conditioned by tendon cells after 6 days of treatment using an in vitro solid-phase enzyme immunoassay kit according to the manufacturer's instructions. The results were normalized for cell number and expressed as micrograms per 10^6 cells.

Measurements of HGF and VEGF in Conditioned Medium

Cells were plated at a density of 5000 cells per well in 48-well plates and maintained with serum-free medium for 24 hours and then exposed to the described treatments for 4 days. WST-1 was used to assess cell number. Culture media were collected on day 4 of treatment, centrifuged for 5 minutes at 460 *g*, and stored at −80°C until assayed. VEGF and HGF were quantified in the culture medium conditioned by tendon cells. Control wells without cells were used for background correction. Growth factor concentrations were adjusted for cell number and expressed as nanograms per 10^6 cells.

Statistical Analysis

Scatter plots and Pearson correlations were used to examine the relationship between platelet counts in the platelet-rich plasma used for the platelet-rich matrix and secreted PDGF-AB and TGF-β1 concentrations. Results of proliferation or of type I collagen, VEGF, and HGF production are expressed as mean ± SD. Significant differences among defined groups were evaluated using the nonparametric Kruskal-Wallis procedure, and differences were detected graphically using notched box plots. The medians were compared box to box; boxes for which notches do not overlap indicate that the medians of the two groups differ at the 5 percent significance level. In addition, the magnitude of changes, obtained by modifying platelet-poor and platelet-rich treatments by either adding or neutralizing TGF-β1 or PDGF, were expressed as percentages of variation (mean ± SD) relative to platelet-poor or platelet-rich treatments, respectively. Here, differences were assessed using the Wilcoxon test. A difference of $p < 0.05$ was considered to be statistically significant (Statgraphics Plus, Manugistics Inc., Rockville, Md).

RESULTS

Levels of TGF-β1 and PDGF-AB in the Supernatants Released from Platelet-Rich Matrices

The mean platelet count in the platelet-rich plasma (506 ± 124 × 10^6 platelets/ml) and the levels of secreted TGF-β1 (48.69 ± 13.68 ng/ml) and PDGF-AB (21.45 ± 9.21 ng/ml) were determined for a group of 45 healthy subjects (Fig. 2). Both PDGF-AB and TGF-β1 showed a significant correlation with the platelet count ($r = 0.6849$ and $r = 0.7796$, respectively; $n = 45$; $p < 0.01$). Interestingly, the TGF-β1/PDGF-AB ratio of 2.463 ± 0.644 remained fairly constant.

Activities of PDGF and TGF-β1 in Supernatants Released from Platelet-Rich Matrices

We aimed to ascertain whether the platelet-secreted factors TGF-β1 and PDGF from platelet-rich matrices were the regulators of cell proliferation and synthesis of VEGF and HGF by tendon

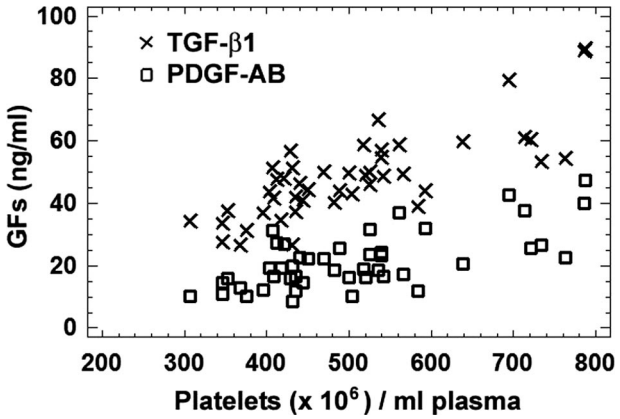

Fig. 2. Relationship between platelet counts and growth factor levels in the supernatants released from platelet-rich matrices prepared in vitro for a group of 45 healthy subjects. Scatterplot of TGF-β1 and PDGF-AB versus platelet count in the platelet-rich plasma revealed significant correlations (TGF-β1, $r = 0.7796$; PDGF-AB, $r = 0.6668$; $n = 45$; $p < 0.01$).

cells. The concentrations of TGF-β1 and PDGF-AB assessed in unclotted platelet-poor plasma and in the supernatants released from platelet-poor and platelet-rich matrices for the four donors are shown in Table 1. Supernatants of platelet-rich matrices contained high amounts of PDGF-AB (8.5 to 20.7 ng/ml) and TGF-β1 (26.7 to 59.8 ng/ml) when compared with platelet-poor matrices (PDGF-AB, 0.3 to 1.0 ng/ml; TGF-β1, 1.6 to 5.3 ng/ml) or unclotted platelet-poor plasma. To modify the composition of these latter preparations, rh-PDGF-AB or rh-TGF-β1 was added in amounts that matched exactly the concentration present in the supernatant of platelet-rich matrix of each donor. This was designed as a strategy to examine the effect of TGF-β1 or PDGF in a similar milieu but without other proteins released from platelets. It was also possible to hypothesize that the effect of platelet-secreted TGF-β1 in our experimental conditions could be regulated by its activation from the latent form.[16] After verification that latent TGF-β1 decreased tendon cell proliferation and regulated the synthesis of angiogenic factors in a manner identical to that of active rh-TGF-β1, the latter was used throughout the study.

Cell Proliferation

We have previously reported that the supernatant released from platelet-rich matrices considerably enhanced the proliferation of tendon cells compared with the supernatant of platelet-poor matrices.[12] As shown in Figure 3, *above*, addition of rh-TGF-β1 significantly decreased cell proliferation, as seen with both supernatants, even though the decrease in proliferation was significantly less with platelet-rich supernatant–treated cells ($-35.7 \pm 8.7\%$) than with platelet-poor cells ($-81 \pm 3.6\%$) ($p < 0.05$). Results are expressed as percentages of variation for each donor relative to their platelet-poor or platelet-rich treatments, respectively. Supporting these results, addition of neutralizing antibody against platelet-secreted TGF-β1 in supernatants of platelet-rich-matrices increased tendon cell proliferation by $22.5 \pm 14.8\%$ ($p < 0.05$) (Fig. 3, *above*). When tendon cells were incubated with unclotted platelet-poor plasma, only a weak proliferation was induced (Fig. 3, *center*), and little effect was seen on adding recombinant TGF-β1 (result not shown). However, when exogenous PDGF-AB was added, proliferation was markedly enhanced, and this effect was strongly counterbalanced by adding recombinant TGF-β1 in combination ($p < 0.05$) (Fig. 3, *center*). In fact, incubation of tendon cell cultures with either unclotted platelet-poor plasma or su-

Table 1. Platelet Counts and PDGF-AB and TGF-β1 Levels in the Supernatants Obtained from Either Platelet-Poor or Platelet-Rich Matrices or Unclotted Platelet-Poor Plasma from Four Donors*

			Concentration (ng/ml)	
Treatment	**Donor**	**Platelets (×106/ml)**	**PDGF-AB**	**TGF-β1**
Supernatant of platelet-rich matrix	1	366	10.43	33.75
	2	642	20.70	59.75
	3	431	8.46	26.70
	4	402	13.73	31.15
Supernatant of platelet-poor matrix	1	3	0.41	2.21
	2	11	1.03	5.28
	3	3	0.62	1.58
	4	4	1.04	3.04
Platelet-poor plasma	1	3	0.37	1.41
	2	11	0.16	1.10
	3	3	0.41	1.72
	4	4	0.89	1.25

*Culture media used to treat tendon cells were supplemented with 20 percent of these preparations.

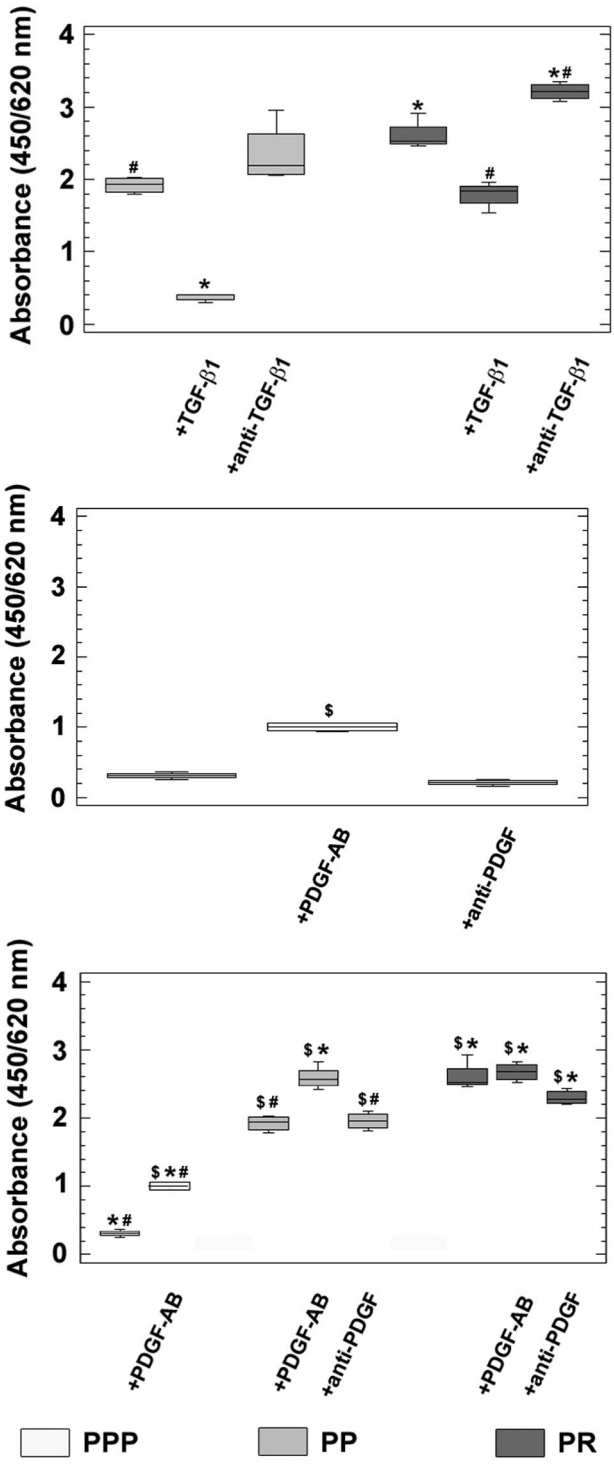

Fig. 3. Effect of TGF-β1 and PDGF on proliferation of tendon cells. The cells were seeded at a density of 800 cells per well and treated for 6 days with 20 percent of the following supplements: (*above*) supernatants of either platelet-poor or platelet-rich matrices and either supplemented with TGF-β1 (26 to 60 ng/ml) or treated with neutralizing anti–TGF-β1 antibody as described in the text; (*center*) unclotted platelet-poor plasma, supplemented either with PDGF-AB or a PDGF-AB plus TGF-β1 mixture; and (*below*) supernatants of platelet-poor matrices supplemented with PDGF-AB (8 to 20.5 ng/ml) or treated with neutralizing anti-PDGF antibody or supernatants of platelet-rich plasma treated with neutralizing anti-PDGF antibody as described in the text. Box plot representation based on the median (line across the box): the box limits contain 50 percent of the values (including 25th and 75th percentiles). The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. Data summarize the combined values obtained for four different donors in three independent experiments (*$p < 0.05$ as compared with platelet-poor plasma; #$p < 0.05$ as compared with platelet-rich plasma; \$$p < 0.05$ as compared with platelet-poor plasma).

pernatant of platelet-poor matrix supplemented with PDGF-AB enhanced proliferation significantly ($p < 0.05$) (Fig. 3, *below*). This confirms the hypothesis that PDGF in the platelet releasate was indeed stimulating proliferation. When PDGF was depleted by using PDGF-specific neutralizing antibody, a weak decrease in proliferation was observed. Overall, these results suggest that PDGF contributes partially to the proliferative effects induced by the supernatant of platelet-rich matrices (Fig. 3, *below*).

Production of Type I Collagen by Tendon Cells

Tendon cells treated with supernatants of either platelet-poor or platelet-rich matrices synthesized type I procollagen in readily detectable amounts, although these were higher with platelet-rich matrices (platelet-poor, 4.58 ± 1.03 μg/10^6 cells; platelet-rich, 6.67 ± 2.45 μg/10^6 cells). When supernatants of either platelet-poor or platelet-rich matrices were supplemented with TGF-β1 (26 to 60 ng/ml, according to the donor), we found an increase in the synthesis of type I collagen by the tendon cells. However, the magnitude of this increase was much higher in platelet-poor (520 ± 125) than in platelet-rich supernatant–treated cells (84 ± 40; $p < 0.05$), thus confirming the importance of TGF-β1. It was noteworthy that the platelet-poor supernatant of donor 2 that had the highest concentration of TGF-β1 showed the highest boost in collagen synthesis in response to TGF-β1 addition (Fig. 4).

Angiogenic Factors in the Culture Medium: Reciprocal Actions of TGF-β1 in the Production of VEGF and HGF by Tendon Cells

The addition of TGF-β1 (26 to 60 ng/ml) to supernatants of platelet-rich and platelet-poor matrices resulted in an increase in the production of VEGF by tendon cells; however, this increase was significantly higher in platelet-poor–treated cells

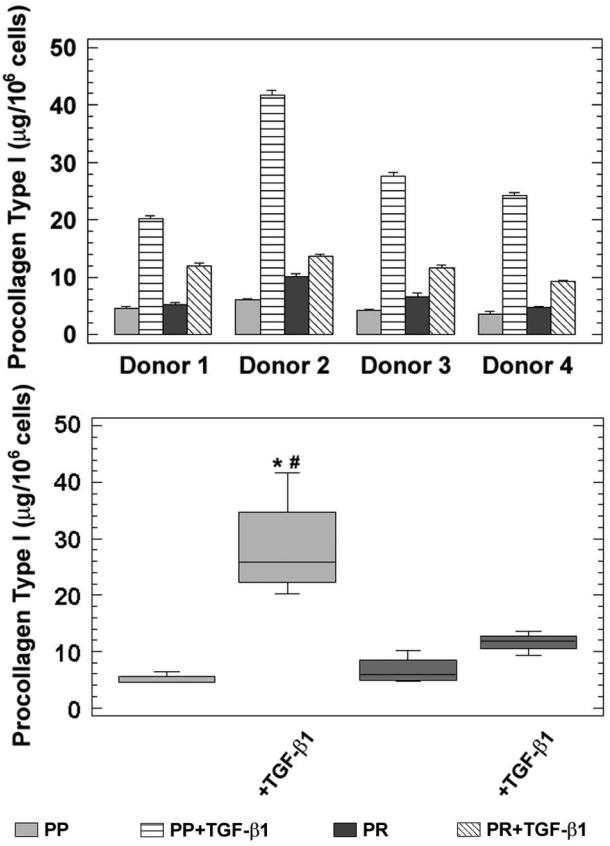

Fig. 4. Synthesis of procollagen type I. Cells were seeded at a density of 800 cells per well and (*above*) treated for 6 days with supernatants released from either platelet-poor (*PP*) or platelet-rich matrices (*PR*) supplemented with TGF-β1 (26 to 60 ng/ml). For each donor, the amount of TGF-β1 added was equal to that present in their platelet-rich supernatant. Results are expressed as mean ± SD. (*Below*) Summary of all individual data expressed as box plots showing the median and 25th and 75th percentiles (*$p < 0.05$ as compared with platelet-poor matrices; #$p < 0.05$ as compared with platelet-rich matrices).

(167 ± 32%) than in platelet-rich–treated cells (84 ± 33%; $p < 0.05$). Neutralization of platelet-secreted TGF-β1 in platelet-rich supernatants also elicited a significant decrease in the production of VEGF (42 ± 12%). Data are expressed as percentages of variation for each donor relative to their platelet-poor or platelet-rich treatments, respectively. These results considered together confirm the role of TGF-β1 in promoting VEGF synthesis and highlight the fact that the observed difference in VEGF synthesis between both treatments can be attributed to platelet-secreted TGF-β1 (Fig. 5). The synthesis of VEGF remained unchanged from the second day of treatment; nevertheless, HGF production increased over longer periods of treatment (2 to 5 days; data not shown). However, after addition of TGF-β1 to platelet-poor or to platelet-rich supernatants, HGF could not be detected, indicating an inhibition of its synthesis by TGF-β1; this was corroborated by the neutralization of platelet-secreted TGF-β1, which in turn induced a substantial increase in the production of HGF (83 ± 46 ng/10^6 tendon cells) (Fig. 5).

DISCUSSION

In the present work, we have investigated whether the mitogenic and angiogenic activities in supernatants released from platelet-rich and platelet-poor matrices are mediated by PDGF and TGF-β1. These proteins are abundant in the α-granules of platelets, contrasting with the lower levels of other growth factors such as VEGF, EGF, and bFGF. On assessing the amounts of PDGF and TGF-β1 in supernatants of a group of healthy subjects, we noted constancy in their relative concentrations and a good correlation with the number of platelets present in the matrices. Previously reported differences in TGF-β1 and PDGF-AB ratios in platelet-rich plasma from different donors may reflect differences in methodology, including a high leukocyte count that could modify the platelet contribution.[18,19]

As in all tissue repair, cell proliferation in the injured area is a necessary step in tendon healing. Many studies have evaluated the mitogenic effects of individual growth factors on cells in culture. However, care must be taken not to oversimplify the clinical situation in which multiple growth factors are present, some acting as positive and other as negative regulators of proliferation. PDGF has mitogenic activities on healing tendons and ligaments.[20] Moreover, PDGF is described in the literature as a powerful mitogen for mesenchyme-derived cells.[21] In our work, rh-PDGF increased cell proliferation when added to platelet-poor plasma or the supernatant of platelet-poor matrix, even though its effect was modest when added to the supernatant of platelet-rich matrix. However, in the latter situation, considerable amounts of PDGF were already present. The relatively modest inhibition achieved with the anti-PDGF antibody suggests that other released proteins or metabolites may contribute to the total proliferation seen in these experiments.

TGF-β1 is also involved in cell proliferation, although its effects are controversial and have been reported to be dose dependent.[16] Our data are in accordance with other studies that report inhibition of cell proliferation as central to the TGF-β1 response in epithelial, endothelial, hematopoietic, neural, and certain types of mesen-

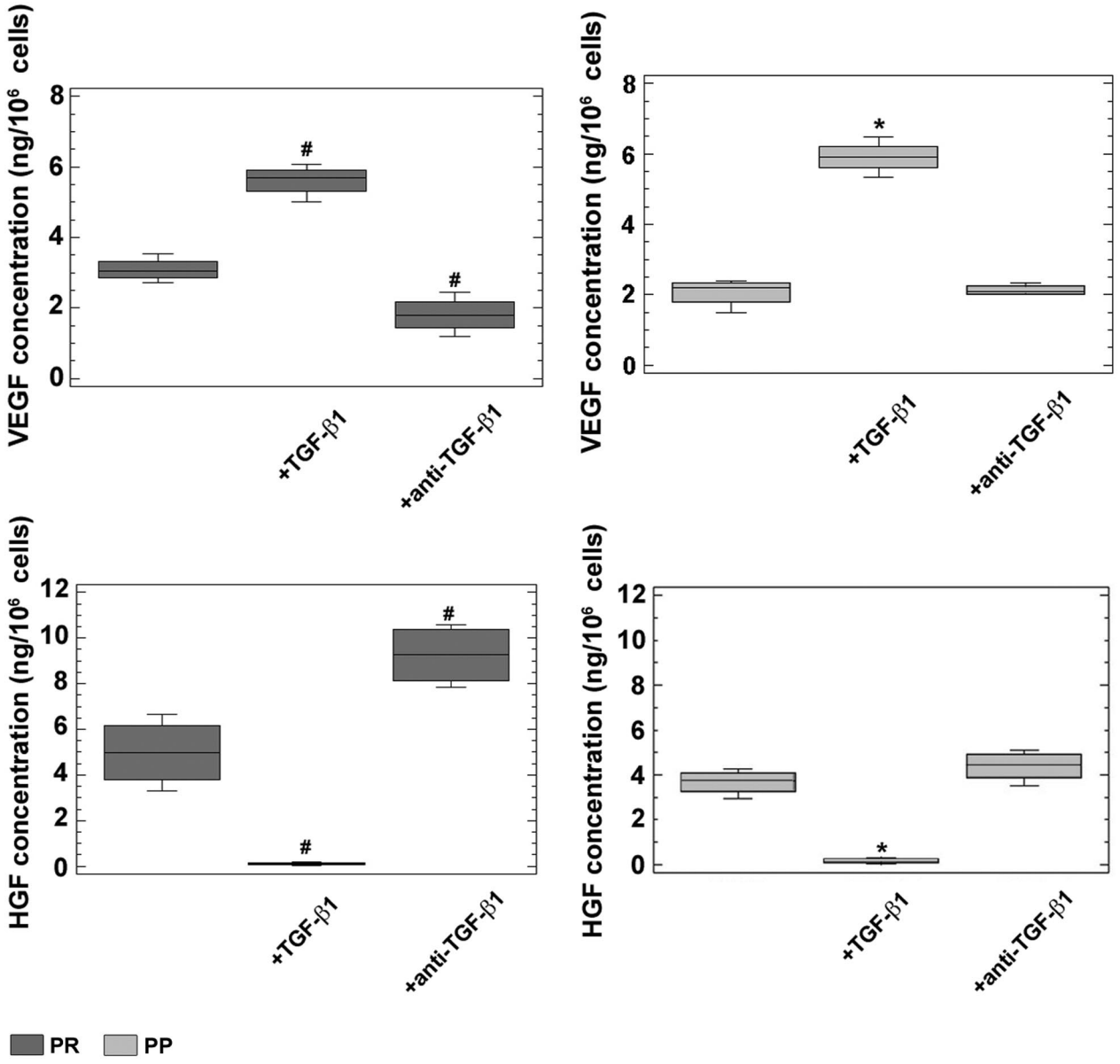

Fig. 5. Synthesis of VEGF and HGF, expressed as nanograms per 10^6 tendon cells. Cells were seeded at a density of 5000 cells per well; media were harvested after 4 days of treatment with supernatants released from platelet-rich (*left*) or platelet-poor matrices (*right*), either supplemented with TGF-β1 (26 to 60 ng/ml, according to the donor) or incubated with anti–TGF-β1 antibody as described in the text. Results are combined values obtained for four different donors in three independent experiments. Boxes show the median and 25th and 75th percentiles; differences of the end of the whiskers indicate sample range (#$p < 0.05$ as compared with platelet-rich matrices; *$p < 0.05$ as compared with platelet-poor matrices).

chymal cells.[22,23] However, addition of TGF-β1 to supernatant of platelet-rich matrix suppressed proliferation only partially. Seemingly, in platelet-rich supernatant–treated cells, the final effect is a combination of both positive and negative regulators of proliferation. It is also possible to speculate that the presence of platelets could provide homeostatic properties to platelet-rich matrices. Their released substances could weaken the effects induced by growth factors released from other cells present at the injured site. In situ, application of plasma matrices will ensure an initial release of growth factors such as TGF-β1. This has a wide range of functions, including fibroblast and macrophage recruitment, which may further amplify TGF-β1 levels at the injury site. Our results highlight the complexity of platelet-rich matrices in which the functional changes induced by the

addition of rh-TGF-β1 are reduced in intensity in contrast to platelet-poor matrices.

Our previous studies established that, in the context of tendon repair, applied platelet-rich matrices release signaling molecules that stimulate the production of VEGF and HGF by tendon cells at the site of injury and which could also promote therapeutic angiogenesis.[12] Experimental studies involving other tissues suggest a more effective therapeutically induced angiogenesis by combining HGF and VEGF.[13,24] In contrast, in vitro assays have shown different properties of both growth factors in vascular development.[25,26] Interestingly, in the present study, we observed VEGF production after merely 2 days of treatment with the supernatants. Synthesis of HGF was not detectable at the beginning of the treatment but increased throughout the period of culture. Nevertheless, we could not establish a relationship between VEGF concentration and the delayed synthesis of HGF.

An early and faster vascularization, and an enhancing healing, would lessen the risk of fibrosis. Our results lead us to speculate that, at least under the culture conditions used in the present study, platelet-rich treatments exhibit buffer capacities that soften the effects induced by the addition of rh-TGF-β1. In terms of the influence of TGF-β1 on the synthesis of type I collagen by tendon cells, the neutralizing properties of platelet-rich matrices could lessen the risk of fibrosis. Lactate has been shown to increase the synthesis of TGF-β1 in tendon wound healing.[27] The effect of platelet-rich matrices on lactate production by tendon tissue merits investigation. In the present study, we report the suppression of HGF synthesis on addition of TGF-β1 to the supernatants. HGF has been reported to act as an antifibrotic agent in several tissues[28,29] by increasing collagenase expression and decreasing the expression of TIMP-1, resulting in metalloprotease-dependent matrix degradation.[30] In contrast, TGF-β1 is known to be profibrotic and involved in scar formation because it triggers the production of type I collagen by local cells. Our results show that this action of TGF-β1 is reduced in the presence of platelet-secreted molecules. The potential buffering role of platelet-rich matrices relative to TGF-β1 alone merits further investigation and has to be confirmed experimentally.

Research toward the control of fibrosis during tendon healing has been focused on using anti–TGF-β1 antibodies to block the effect of this growth factor.[3] In accordance with this in vivo study, our data have demonstrated that neutralization of TGF-β1 would be beneficial toward hindering fibrosis by increasing local production of HGF, an antifibrotic agent. In contrast, additional results of our work showed that TGF-β1 neutralization would attenuate VEGF synthesis, as administration of VEGF has been shown to improve tensile strength early in tendon healing.[31] Considered together, our results suggest that blockade of TGF-β1 activities would have reciprocal effects. Because TGF-β1, VEGF, and HGF are being secreted or synthesized in the same local environment, the question arises regarding which molecules may orchestrate the cellular response and tilt the balance to determine whether the tissue will repair or become fibrotic.

CONCLUSIONS

Treatments that stimulate tissue-containing cells to synthesize signaling molecules targeting endothelial cells such as VEGF and HGF constitute new potential angiogenic therapies. The reciprocal actions of TGF-β1 that we report in this study suggest a potential ability to control scar formation and fibrosis by using platelet-rich matrix therapies. Additional in vitro and in vivo studies are necessary to provide further understanding as to how the regulation of the growth factor network controlling fibrosis and tissue repair mechanisms is regulated and, in particular, the role of PDGF in collagen synthesis and HGF production. This knowledge would have considerable impact in many situations requiring surgery and tissue repair.

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DISCLOSURE

None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article.

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