Res Exp Med (1993) 193:195–205

Research in
Experimental Medicine

Original papers

Fibroblast migration and proliferation during in vitro wound healing

A quantitative comparison between various growth factors and a low molecular weight blood dialyzate used in the clinic to normalize impaired wound healing

T. Schreier[1], E. Degen[1], W. Baschong[2]

[1]Department of Biochemistry, Solco Basle, Rührbergstrasse 21, CH-4127 Birsfelden, Switzerland
[2]Maurice E. Müller Institute at the Biocenter, University of Basle, Klingelbergstrasse 70, CH-4056 Basle, Switzerland

Received: 29 September 1992 / Accepted: 2 March 1993

Abstract. During the formation of granulation tissue in a dermal wound, platelets, monocytes and other cellular blood constituents release various peptide growth factors to stimulate fibroblasts to migrate into the wound site and proliferate, in order to reconstitute the various connective tissue components. The effect on fibroblast migration and proliferation of these growth factors, and of Solcoseryl (HD), a deproteinized fraction of calf blood used to normalize wound granulation and scar tissue formation, was quantified in vitro. The presence of basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and hemodialyzate (HD) increased the number of cells in the denuded area, i.e., in the "wound space" of an artificially ruptured monolayer of LM-fibroblasts (mouse lung fibroblasts). When cell proliferation was blocked with Mitomycin C, in the first 24 h all factors, i.e., bFGF, PDGF, TGF-β and HD, promoted cell migration, whereas after 48 h it became obvious that each factor stimulated both migration and proliferation, each in a characteristic way. The effects were significant and more distinct after 48 h, following the order: PDGF (46%) ~ bFGF (87%) > HD (45%) ~ TGF-β (40%) > control (62%). The relative contributions of migration after inhibiting proliferation are given in brackets. The modulatory activity of HD was localized in its hydrophilic fraction. It was destroyed by acid hydrolysis. Furthermore, this activity could be blocked by protamine sulfate, an inhibitor blocking peptide growth factor receptor binding.

Key words: Fibroblasts – Growth factors – Drugs – Wound healing in vitro – Cell migration

Correspondence to: W. Baschong

Introduction

After wounding, peptide factors derived from blood constitutents such as fibrinopeptides A and B of the fibrinolytic pathway [20], PDGF [14, 36, 37, 42] and TGF-β from platelets and monocytes [33] and complement-derived peptides [32, 44, 47] start to direct inflammatory cells to the wound. These cells in turn produce more mediators to attract mast cells, neutrophils, fibroblasts, endothelial cells and basal cells to migrate from adjacent areas into the wound site [7]. Granulation tissue is formed and remodeled to the final scar tissue [43]. Growth factors released at sites of injury are supposed to act in concert, promoting wound repair and maintaining tissue architecture [4]. Impairment of the regulatory cascade, e.g., by insufficient blood supply or catabolism or by overshooting production of a specific growth factor, may cause poor healing, or uncontrolled fibroplasia leading to the formation of hypertrophic scars and keloids respectively [45, 46].

Theoretically, supplementing healing wounds with blood constituents should favor tissue repair. Clinical investigators have tested various blood extracts and blood preparations of human and animal origin for the capacity to act as wound healing agents, and used some of them successfully to improve the healing of ulcers and burns [1]. A deproteinized fraction of calf blood, Solcoseryl (HD), has been manufactured at an industrial level [19] and has been used ever since for the treatment of dermal and mucosal injuries [3, 6, 21]. Recent studies in vivo [16–18] and in vitro [2, 11, 12, 16] strongly indicate that HD interacts multifactorially with different steps of the wound healing cascade, improving granulation, epithelization and tissue remodeling as well as reducing the duration of the inflammatory phase [18, 19, 25, 31].

Fibroblast migration to and proliferation within the wound site are prerequisites for wound granulation. Fibroblasts then participate in the construction of the scar tissue and its remodeling. Modulation of fibroblast activity by peptide growth factors is reported as responsible for improved wound healing [23, 27, 28]. We have now quantified the influence of various blood-cell-derived peptide growth factors and of HD on fibroblast migration and proliferation in "wounded" fibroblast monolayers, i.e., in confluent monolayer cultures of fibroblasts wounded by scraping off half of the cell monolayer. Under these conditions, cells migrate into the denuded area, synthesize DNA and proliferate, in a manner comparable to that in vivo [5, 24, 26].

Materials and methods

Cell cultures

Mouse lung fibroblasts (LM cells), human skin fibroblasts (CCL 110 cells) and baby hamster kidney cells (B14F28 cells) were obtained from the American Type Culture Collection (Rockville, Md., USA). Cells were incubated at 37°C, 95% air/5% CO_2 in 75-cm^2 tissue culture flasks (Falcon, Becton Dickinson, Oxnard, Calif., USA) with 20 m*M* Hepes-buffered DMEM (LM cells and B14F28 cells), or with 10 m*M* Hepes-buffered MEM (CCL 110 cells), in the presence of 10% FCS. Cells were harvested by trypsinization (0.05% Trypsin/0.02% EDTA solution from Gibco, Grand Island, N.Y., USA).

Growth factors and hemodialyzate

We obtained bFGF and PDGF from Boehringer Mannheim, Germany. TGF-β and EGF were from Sigma (Sigma Chemicals, St. Louis, Mo., USA). Stock solutions were prepared accord-

ing to manufacturer's instructions and kept in aliquots at −20°C. Protamine sulfate was from Sigma. The hemodialyzate Solcoseryl (HD) (42.5 mg/ml dry weight from Solco, Basle, Switzerland) was further separated by $CHCl_3$/methanol extraction (2/1, V/V) according to Folch et al. [13] into a lipophilic (HD-lip) and a hydrophilic (HD-hyd) fraction. HD-lip was stored as an ethanolic stock solution (approx. 100 × concentrated) and diluted to its original volume with double-distilled water prior to use. For acid hydrolysis, 1 ml of HD (or HD-hyd) was evaporated to dryness and hydrolyzed with 1 ml of 6 *N* HCl (Pierce, Rockford, Ill. USA) in a sealed glass tube at 110°C for 20 h.

Migration assay

We seeded 50,000 cells into "Easy grip" culture dishes of 35 mm diameter (21 cm^2, Falcon) and let them grow to confluence. Fibroblast monolayers were then "wounded" by removing half of the layer with a razor blade: the blade was pressed down in the middle of the dish, thus cutting the cell layer and concomitantly marking the "wound boundary" on the underlying plastic. Then the blade was gently slid unidirectionally to remove half of the confluent cell layer. The "wounded monolayer" was washed twice with phosphate-buffered saline pH 7.4 (PBS) and then incubated with serum-deprived medium containing the various test agents at 37°C for 24 h or for 48 h respectively. In the respective assays, protamine sulfate was added to confluent monolayers immediately prior to wounding. Cell monolayers were then wounded and rinsed with medium containing the respective concentrations of protamine sulfate, and incubated accordingly.

For evaluation, cultures were rinsed twice with PBS, fixed by absolute methanol, stained according to Giemsa, and examined by a light microscope equipped with a calibrated ocular at a magnification of × 190. The number of cells having migrated at least 180 µm (day 1) or 300 µm (day 2) from the "wound boundary" were counted within six areas (600 µm by 600 µm) randomly chosen adjacent to the migration lines. For each test agent, three cultures were prepared and evaluated. Since the experimental errors were markedly lower than the systematic errors, each of the 3 × 6 counts was treated as an independent random sample. The values, expressed as average number of cells per field ($\pm SD_{n-1}$), were analyzed for significance by Student's *t*-test. Significant differences versus the respective controls are indicated by * for $P < 0.05$ and by ** for $P < 0.01$.

DNA labeling

Wounded monolayer cultures (see above) were cultivated in the presence of various factors for 2 days. Then, 5 h prior to evaluation, 0.5 µCi/ml [^{3}H]-thymidine (Amersham, 5 Ci/mmol) was added to the medium, and the cells were incubated for a further 5 h. The medium was then removed, the cultures rinsed with PBS, and fixed by PBS/methanol/acetic acid: 4/3/1 (V/V) for 15 min. The solution was then changed to methanol/acetic acid (3/1; V/V) and the cell cultures were fixed for another 10 min. The fixative was removed and the cultures were left to dry at ambient temperature. In the dark, the dried dishes were coated by a thin layer of NTB2 emulsion (Kodak, Rochester, N.Y., USA) in water (1/1). Dishes were left to dry for 1 h at ambient temperature and then stored at 4°C under exclusion of light. After 6 days the NTB2 emulsion was developed using D-19 developer (Kodak) and Kodak Ektaflo for fixation. Developed culture dishes were left to dry. The dishes were then rinsed with methanol, and the cells stained according to Giemsa. In the autoradiographed cultures, labeled and unlabeled nuclei were counted within six areas (0.36 mm^2) adjacent to the 300-µm migration line, and within the confluent monolayer, so as to give the relative contribution of labeled cells to the total number of cells.

Results

Evaluation of the cell line

Several fibroblast cell lines were stimulated in the "migration assay" with PDGF in order to select the one most convenient for quantification. Figure 1a shows

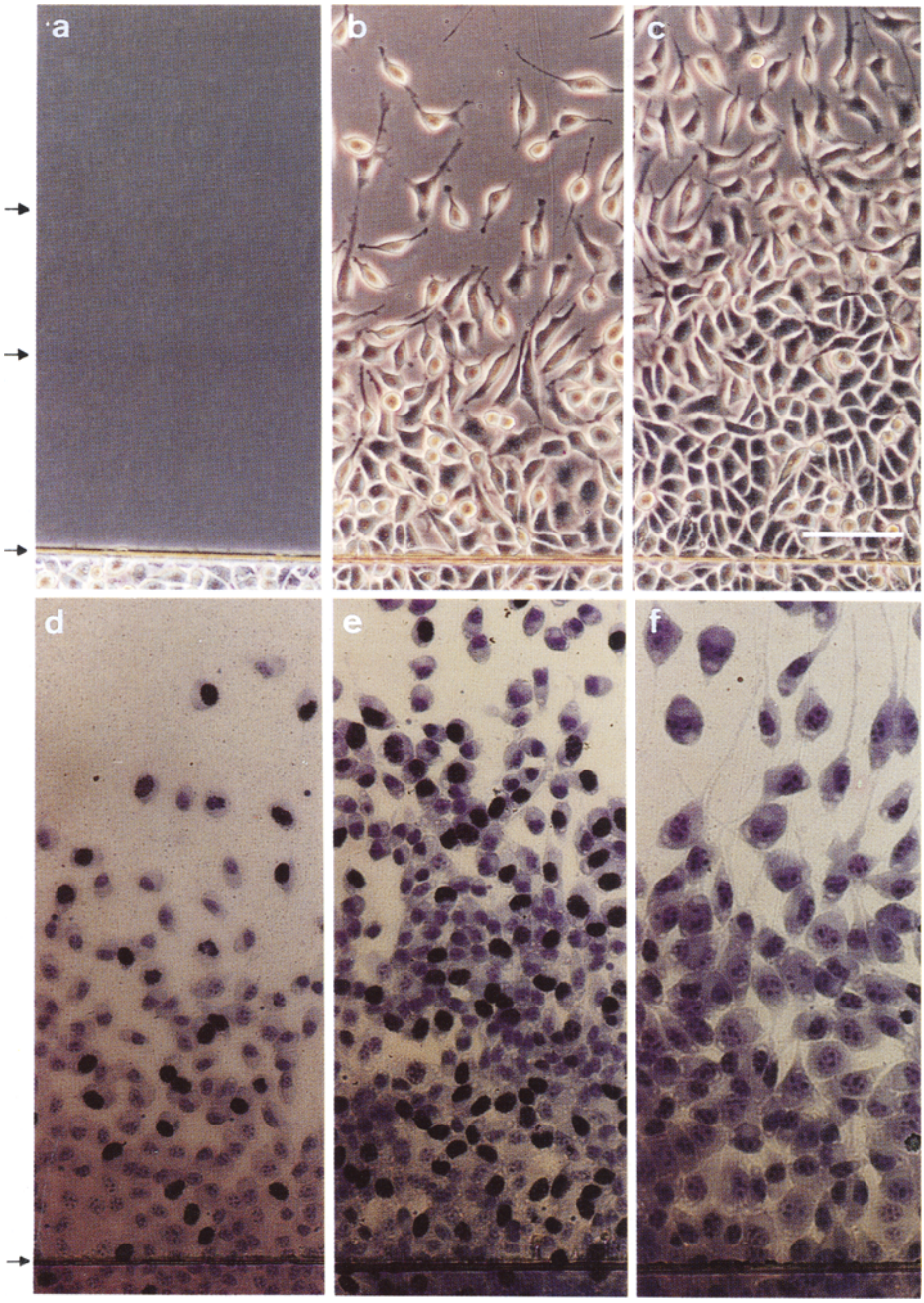

Fig. 1a–f. Scratch assay. A confluent monolayer of LM-fibroblasts was "wounded" by scraping off half of the cell layer. **a** Immediately after wounding. *Arrows* indicate the wound edge, and the 180-μm and the 300-μm migration lines. **b–f** After 48 h; **b, d** controls (no factors added); **c, e** in presence of 8 ng/ml PDGF; and **f** in presence of 8 ng/ml PDGF and 5 μg/ml Mitomycin C. **d–f** Proliferating cells visualized by in situ autoradiography of incorporated [^{3}H]-thymidine. *Bar*, 100 μm

Table 1. Chemokinetic activities of various factors: dose response relationship. Values represent mean number of cells ± SD_{n-1} ($n = 18$) per field. Differences versus controls: $^{*}P < 0.05$; $^{**}P < 0.01$

Agent	Migrated cells	
	> 180 µm/24 h	> 300 µm/48 h
bFGF		
Control	25 ± 4	45 ± 6
0.1 ng/ml	35 ± 6**	49 ± 8*
1.0 ng/ml	43 ± 9**	55 ± 9**
10 ng/ml	76 ± 16**	95 ± 22**
100 ng/ml	81 ± 15**	150 ± 18**
EGF		
Control	21 ± 4	42 ± 6
0.1 ng/ml	26 ± 5*	50 ± 8*
1 ng/ml	29 ± 7*	73 ± 12**
10 ng/ml	30 ± 6**	59 ± 15**
100 ng/ml	21 ± 5	63 ± 14**
PDGF		
Control	24 ± 3	48 ± 9
0.5 ng/ml	48 ± 6**	67 ± 15**
2 ng/ml	63 ± 9**	140 ± 22**
8 ng/ml	67 ± 12**	153 ± 29**
16 ng/ml	76 ± 10**	158 ± 31**
TGF-β		
Control	24 ± 4	46 ± 4
0.1 ng/ml	44 ± 7**	75 ± 15**
0.5 ng/ml	40 ± 5**	81 ± 11**
1 ng/ml	45 ± 9**	74 ± 13**
5 ng/ml	58 ± 5**	56 ± 8**
10 ng/ml	57 ± 9**	57 ± 13**
Solcoseryl		
Control	24 ± 4	46 ± 4
0.2% (V/V)	26 ± 4	58 ± 7**
1% (V/V)	35 ± 4**	90 ± 7**
3% (V/V)	38 ± 4**	100 ± 8**
5% (V/V)	45 ± 5**	88 ± 10**
7% (V/V)	35 ± 6**	62 ± 10**

the "wound" produced by removing half of a confluent fibroblast monolayer with a razor blade.

PDGF significantly increased the number of human skin fibroblasts (CCL 110) in the denuded space. However, these cells migrated as cell clusters rather than as single cells. B14F28 cells, which are very small in diameter (10–15 µm), were not able to cross the incision mark at the wound boundary (data not shown). In

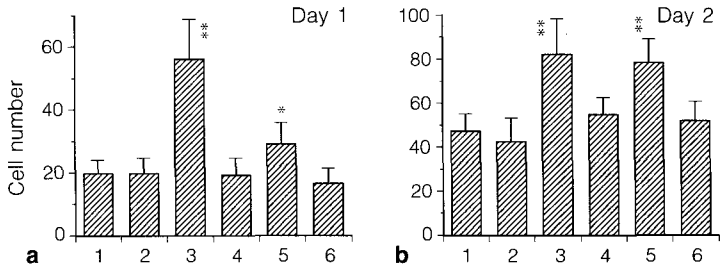

Fig. 2a, b. Influence of autocrine factors. Inhibition of peptide growth factor receptor-binding by protamine sulfate. Cells immigrated within 24 h (**a**) and within 48 h (**b**) expressed as cells/0.36 mm^2 beyond the 180-μm or 300-μm migration line. *1*, Control; *2*, 5 μg/ml protamine sulfate; *3*, 10 ng/ml bFGF; *4*, 10 ng/ml bFGF + 5 μg/ml protamine sulfate; *5*, 3% (V/V) HD; *6*, 3% (V/V) HD + 5 μg/ml protamine sulfate

contrast, LM cells (average size: 30–40 μm) were large enough to cross the incision mark and migrated as single cells (Fig. 1b, c). In the presence of PDGF, significantly more LM cells were found in the denuded area (Fig. 1c) compared to the control (Fig. 1b).

Dose-finding studies

PDGF, bFGF, TGF-β, EGF and HD increased the number of cells in the denuded area specifically and dose-dependently. Highest responses were measured with 16 ng/ml PDGF (0.5 ng/ml–16 ng/ml) and with 100 ng/ml bFGF (0.1 ng/ml–100 ng/ml). HD at 3% v/v (0.2%–7%) stimulated in a manner comparable to 0.5 ng/ml TGF-β (0.1 ng/ml–10 ng/ml) and significantly better than 1 ng/ml EGF ($P < 0.05$) (0.1 ng/ml–100 ng/ml) or the control ($P < 0.005$). Dose-ranges covered are given in brackets. Stimulatory activities after 24 h and 48 h were PDGF ~ bFGF > HD ~ TGF-β > EGF > control. The stimulatory effects became more distinct after 48 h (Table 1). When a mixture of PDGF, bFGF and TGF-β was used, stimulatory effects were additive (data not shown).

Absence of endogenous growth factors

Human skin fibroblasts and NIH3T3 fibroblasts have been observed to release endogenous bFGF [39]. Protamine sulfate, a non-specific inhibitor binding to various peptide growth factor receptors (e.g., PDGF and bFGF) [10, 39] was used to block the activity of autocrine bFGF or other growth factors eventually released by LM-fibroblasts. LM-fibroblast migration, stimulated by bFGF, was inhibited by about 80% in the presence of 5 μg/ml protamine sulfate. However, protamine sulfate did not alter the migratory behavior of unstimulated controls (Fig. 2a, b), indicating the absence of autocrine stimulatory peptide growth factors.

Differentiation between migration and proliferation

The number of cells in the denuded area increases either due to immigration of cells from the wound edge, or by mitosis of the migrated cells. The contribution of cell migration to the increase of the cell population in the denuded space was determined after inhibiting cell division with the antimitotic agent Mitomycin C.

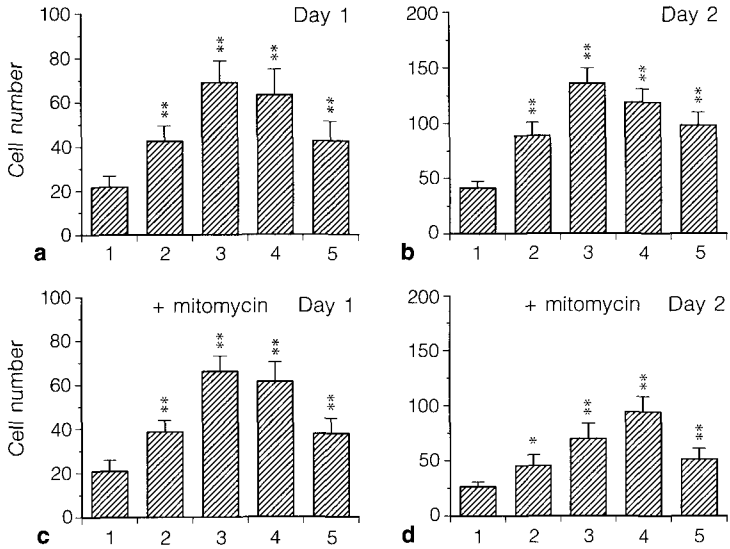

Fig. 3a–d. Migratory and proliferatory activities. Cells immigrated after 24 h (**a, c**) and 48 h (**b, d**) expressed as cells/0.36 mm^2 beyond the 180-μm or 300-μm migration line in the absence (**a, b**) or presence (**c, d**) of 5 μg/ml of the antimitotic Mitomycin C. *1*, Control (no factor added); *2*, 0.5 ng/ml TGF-β; *3*, 16 ng/ml PDGF; *4*, 100 ng/ml bFGF; *5*, 3% (V/V) HD

The lowest effective dose of Mitomycin C inhibiting [^{3}H]-thymidine uptake was found to be 5 μg/ml. This concentration completely blocked cell division, but was not cytotoxic (Fig. 1f).

Wounded monolayer cultures were incubated with the various factors at optimal dose (see above) for 24 h (Fig. 3a, c) and 48 h (Fig. 3b, d) respectively, in absence (Fig. 3a, b) and presence (Fig. 3c, d) of Mitomycin C. The number of cells counted in the denuded space after 24 h was not influenced by the presence of Mitomycin C, implying cell division to be marginal within the 24 h after "wounding" (Fig. 3a, c). After 48 h in the absence of Mitomycin C (Fig. 3b) there were significantly ($P < 0.005$) more cells in the "wounded" area than in the presence of the antimitotic agent (Fig. 3d). The overall stimulatory effects in the absence of Mitomycin C, i.e., migration + proliferation, followed the order PDGF ~ bFGF > HD ~ TGF-β > control, as observed in the dose-finding studies (Table 1). The relative migrations were calculated as the ratio between the effects measured in the presence (migration) and in the absence (migration + proliferation) of Mitomycin C. The contribution of migration was highest with bFGF (87%; $P < 0.01$). The relative migration in the control was 62%, whereas the other growth factors or HD stimulated proliferation more than migration (PDGF: 46%; HD: 45%; TGF-β: 40%), the relative migrations corresponding to bFGF » control > PDGF ~ HD ~ TGF-β. Because of its weak overall response (cf. Table 1), the influence of EGF was not further investigated.

The extent of fibroblast mitosis was estimated by in situ autoradiography via [^{3}H]-thymidine incorporation into single cells (Fig. 1d–f). In the presence of the various factors or in the control, about 3% of the cells were found to proliferate within the confluent area. Within the wounded area proliferation was significantly ($P < 0.005$ vs the confluent area) higher: HD: 30 ± 6%; PDGF: 25 ± 6%;

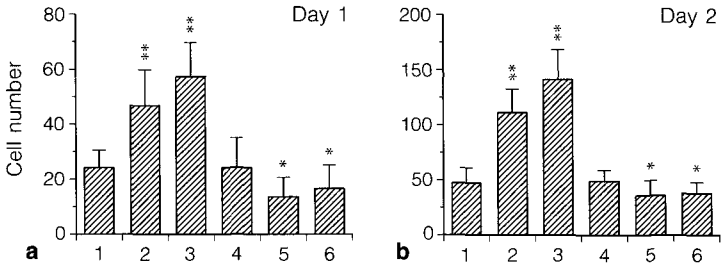

Fig. 4a, b. Characterization of the activity in the hemodialyzate HD. Cells immigrated within 24 h (**a**) and within 48 h (**b**) expressed as cells/0.36 mm^2 beyond the 180-μm or 300-μm migration line. *1*, Control; *2*, 3% (V/V) HD; *3*, 3% (V/V) hydrophilic fraction HD-hyd; *4*, 3% (V/V) lipophilic fraction HD-lip; *5*, 3% (V/V) acid hydrolyzate of HD; *6*, 3% (V/V) acid hydrolyzate of HD-hyd

control 25 ± 5%; EGF: 24 ± 6%; TGF-β: 21 ± 12%; bFGF: 19 ± 6%. The latter differences did not reach statistical significance (data not shown).

Partial characterization of the chemokinetic activity in the hemodialyzate

The migratory/proliferatory activity of the HD (Fig. 4) was found to be localized in the hydrophilic fraction (cells in the "wounded" area: HD-hyd 24 h: 57 ± 12, 48 h: 142 ± 24; HD-lip 24 h: 24 ± 10, 48 h: 48 ± 8; HD 24 h: 47 ± 12, 48 h: 112 ± 18; control: 24 h: 24 ±6, 48 h: 48 ± 12). Hydrochloric acid (6 *N* HCl, 20 h at 110°C) destroyed the activities in the HD (24 h: 14 ± 6, 48 h: 36 ± 12) and in HD-hyd (24 h: 17 ± 8, 48 h: 38 ± 8). Protamine sulfate suppressed stimulation by HD to the level of the control (Fig. 2).

Discussion

Fibroblast migration and proliferation within a dermal wound are prerequisites for the formation of granulation tissue, and in turn, for successful wound closure [36]. In vivo, within their extracellular matrix, dermal fibroblasts limit their proliferative activity to that required to retain their steady-state number [38]. However, upon wounding of the dermal matrix, fibroblasts, attracted by growth factors released during fibrin clot formation, concentrate at the edge of the ruptured matrix to synthesize a first set of matrix proteins and to concomitantly migrate and proliferate along these proteins until the dermal gap has been filled with granulation tissue [7]. Comparably, in vitro, in a confluent monolayer, contact inhibition reduces cell proliferation to that required for maintaining the steady state number. Upon wounding of the confluent monolayer, however, cells start to migrate and proliferate at the edge of the rupture [5], proliferation increasing by about tenfold as shown by Lipton et al. [26] and our own observations (see below).

We assayed fibroblast migration and proliferation after "in vitro wounding" using the monolayer scratch assay [5, 24, 26]. This test compares well to the Boyden chamber assay [29, 30] and at the same time makes it possible to differentiate between migration and proliferation [24]. For routine assays, LM-fibroblasts proved the most convenient with respect to migration behavior and

evaluability. These cells were then used to establish optimal dose-response for each factor 24 h and 48 h after wounding.

We quantified the migratory and proliferatory potential of bFGF, PDGF and TGF-β – all factors that are released during fibrin clot formation – and compared these with Solcoseryl (HD), a product from calf blood used to improve granulation and epithelization of poorly healing dermal wounds [3, 6, 21]. The peptide growth factors as well as HD characteristically and significantly increased the fibroblasts in the denuded space. We then tested for the presence of autocrine peptide growth factors, since the migrations of human skin fibroblasts, of BHK-21 and of NIH 3T3 cells [39], and of aortic endothelial [40] and smooth muscle cells [41] have been reported to be influenced by endogenous bFGF, as revealed after neutralizing bFGF with anti-bFGF antibodies, or by blocking its receptor by protamine sulfate [39]. Protamine sulfate is a small basic protein that competitively inhibits cell-surface binding of various peptide growth factors [8, 10, 39]. We found no inhibition of spontanous migration of LM-fibroblasts by protamine sulfate, implying the absence of autocrine bFGF or other protamine sulfate-sensitive autocrine peptide growth factors.

In vitro wound repair starts with the spreading of individual cells at the wound edge and the synthesis of matrix fibrils (e.g., fibronectin), followed by cell migration (translocation) along the fibronectin bundles and by cell proliferation [9, 15, 26, 34, 35]. Blocking mitosis with Mitomycin C allowed us to discriminate between stimulation of translocation and proliferation. Mitomycin C did not interfere during the 24 h, implying that all factors, i.e., bFGF, PDGF, HD and TGF-β, exclusively stimulated migration. However, after 48 h Mitomycin C significantly reduced the stimulatory behavior of various factors, revealing the strong proliferatory effects of PDGF, HD and TGF-β. PDGF, HD and TGF-β may well exert multiple activities, stimulating – apart from migration and proliferation – the recovery and spreading of resting fibroblasts after loss of contact inhibition, and the synthesis of fibronectin for migratory guidance. An increase of fibronectin around the cells migrating into the "wound spaces" in the presence of HD has been detected using immunofluorescence labeling (our unpublished results). In contrast, bFGF seems merely to enhance migration. Fibroblast mitosis during migration as visualized via [^{3}H]-thymidine incorporation and in situ autoradiography (Fig. 1d–f) [24], though statistically not significant, was in line with the measurements in absence and presence of Mitomycin C (see above). Cell labeling within the confluent part of the cell monolayers, i.e., steady state proliferation, was about 3%, whereas in the wound space mitosis was found to have increased by up to tenfold.

The modulatory activity of HD was confined to the hydrophilic fraction of HD and was labile to acid hydrolysis. Moreover, this activity could be blocked by protamine sulfate. As a protein-free dialyzate/ultrafiltrate (nominal cut-off 5000 Da) of hemolyzed calf blood, HD contains oligopeptides as well as autolytic fragments originating from higher molecular weight proteins. In that respect, the presence of minute amounts of EGF in HD (0.4 ng/ml, [22]) has been reported, but not in sufficient concentration to account for the growth-factor-like effect observed in in vivo ulcer healing [22]. No relevant quantities of TGF-α, EGF or Somatostatin C [12] have been observed in HD. A lipidic compound of HD not yet structurally identified, was found to stimulate S6-kinase activity [12]. This investigation shows that a hydrophilic moiety of HD influences fibroblast migration and proliferation in a growth-factor-like manner.

References

1. Allgöwer M, Pomerat CM, Blocker TG (1952) Influence of normal serum, its derivatives, and of "wound healing agents" on human epidermis in vitro. Arch Surg 135:923–937
2. Alsbjörn BF, Jensen MG, Sörensen B (1989) Effect of Solcoseryl on cadaveric split-skin oxygen consumption during 4°C storage and in frozen biopsies. Cryobiology 26:119
3. Biland L, Hürlimann F, Gloor W, Körner WF, Kündig A, Madar G, Widmer LK, Ziegler WJ (1985) Treatment of venous ulcers – a multi-center randomized double-blind study. VASA (Bern) 14:383–389
4. Brown GL, Nanney LB, Griffen J, Cramer AB, Yancey JM, Curtsinger LJ, Holtzin L, Schultz GS, Jurkiewicz MJ, Lynch JB (1989) Enhancement of wound healing by topical treatment with epidermal growth factor. N Engl J Med 321:76–79
5. Bürk RR (1973) A factor from a transformed cell line that affects cell migration. Proc Natl Acad Sci USA 70:369–372
6. Charlesworth D, Harris PL, Palmer MK (1975) Intra-arterial infusion of Solcoseryl: a clinical trial of a method of treatment for pre-gangrene of the lower limb. Br J Surg 62:337–339
7. Clark RAF (1988) Overview and general consideration of wound repair. In: RAF Clark, PM Hensen (eds) The molecular and cellular biology of wound repair. Plenum Press, New York, pp 3–33
8. Coffey RJ Jr, Leof EB, Shipley GD, Moses HL (1987) Suramin inhibition of growth factor receptor binding and mitogenicity in AKR-2B cell. J Cell Physiol 132:143–148
9. Coomber BL, Gotlieb AI (1990) In vitro endothelial wound repair. Arteriosclerosis 10: 215–222
10. Deuel TF, Senior RM, Huang JS, Griffin GL (1982) Chemotaxis for monocytes and neutrophils to platelet-derived growth factor. J Clin Invest 69:1046–1049
11. Dri P, Cramer R, Mittenzwei H, Patriarca P (1989) Dual effect of a protein-free hemodialyzate on the oxygen uptake of phagocytosing human polymorphonuclear leucocytes. Drug Res 39:1565
12. Fabbro D, Imber R, Huggel R, Baschong W (1992) Growth promoting effect of a protein-free hemodialyzate used in situtations of hypoxya and for tissue repair as measured via stimulation of S6-Kinase. Drug Res 42:917–920
13. Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 226:497–509
14. Grotendorst GR, Chang T, Seppä HEJ, Kleinman HK, Martin GR (1982) Platelet-derived growth factor is a chemoattractant for vascular smooth muscle cells. J Cell Physiol 113: 261–266
15. Halfter W, Liverani D, Vigny M, Monard D (1990) Deposition of extracellular matrix along pathways of migrating fibroblasts. Cell Tissue Res 262:467–481
16. Hartung T, Leist M, Tiegs G, Baschong W, Wendel A (1991) Solcoseryl prevents inflammatory and hypoxic but not toxic live damage in rodents. Inflammopharmacology 1:49–60
17. Isler H, Bauen A, Hubler M, Oberholzer M (1991) Morphometric assessment of wound healing in rats treated with a protein-free haemodialyzate. Burns 17:99–103
18. Isler H, Bauen A, Baschong W (1991) Topical treatment of standardized burns with a protein-free haemodialyzate. Burns 17:93–98
19. Jaeger KH, Leybold K, Mittenzwei H, Staudinger HJ, Waldstätten L von (1965) Die Förderung der Zellatmung durch einen Blutextrakt. Drug Res 15:750–754
20. Kay AB, Pepper DS, McKenzie R (1974) The identification of fibrinopeptide B as a chemotactic agent derived form human fibrinogen. Br J Haematol 27:669–677
21. Knudsen L, Solvhoj L, Christensen B (1982) The use of a haemodialyzate in the treatment of decubital ulcer: a double-blind randomized clinical study. Curr Ther Res 32:498–504
22. Konturek SJ, Drozdowicz D, Patko-Polonczyk J, Brzozowoski T, Bielanski W (1991) Solcoseryl in prevention of stress-induced gastric lesion and healing of chronic ulcers. J Phys Pharmacol 42:43–84
23. Ksander GA, Sawamura SJ, Ogawa Y, Sundsmo J, McPherson JM (1990) The effects of platelet relesate on wound healing in animal models. J Am Acad Dermatol 22:781–791
24. Levi-Schaffer F, Kupietzky A (1990) Mast cells enhance migration and proliferation of fibroblasts into an in vitro wound. Exp Cell Res 188:42–49

25. Liebich HG, Hamm D, Jöchle W (1988) Histologic evaluation of wound healing in horses treated with the protein-free haemodialyzate Solcoseryl and its hexosylceramid fraction. J Vet Med 35:84–95
26. Lipton A, Klinger I, Paul D, Holley RW (1971) Migration of mouse 3T3 fibroblasts in response to a serum factor. Proc Natl Acad Sci USA 68:2799–2801
27. Lynch SE, Nixon JC, Colvin RB (1987) Role of platelet-derived growth factor in wound healing: synergistic effects with other growth factors. Proc Natl Acad Sci USA 84:7696–7700
28. Lynch SE, Colvin RB, Antoniades HN (1989) Growth factors in wound healing. Single and synergistic effects on partial thickness porcine skin wounds. J Chir Invest 84:640
29. Mawatari M, Okamura K, Matsuda T, Hamanaka R, Mizoguchi H, Higashio K, Kohno K, Kuwano M (1991) Tumor necrosis factor and epidermal growth factor modulate migration of human microvascular endothelial cells and production of tissue-type plaminogen activator and its inhibitor. Exp Cell Res 192:574–580
30. Morimoto A, Okamura K, Hamanaka R, Sato Y, Shima N, Higashio K, Kuwano M (1991) Hepatocyte growth factor modulates migration and proliferation of human microvascular endothelial cells in culture. Biochem Biophys Res Commun 2:1042–1049
31. Niinikoski J, Renvall S, Laato M, Tschannen R, Fraefel W (1986) Effect of a hexosylceramide fraction of the haemodialyzate Solcoseryl on experimental granulation tissue. Eur Surg Res 18:58–64
32. Postlethwaite AE, Seyer JM, Kang AH (1979) Generation of a fibroblast chemotactic factor in serum by activation of complement. J Clin Invest 64:1379–1385
33. Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH (1987) Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor β. J Exp Med 165:251–256
34. Raff EC, Houck JC (1989) Migration and proliferation of diploid human fibroblasts following „wounding" of confluent monolayers. J Cell Physiol 74:235–244
35. Rosen EM, Goldberg ID (1989) Protein factors with regulate cell motility. In Vitro Cell Dev Biol 25:1079–1087
36. Ross R (1968) The fibroblast and wound repair. Biol Rev 43:51–96
37. Ross R, Vogel A (1978) The platelet-derived growth factor. Rev Cell 14:203–210
38. Sarber R, Hull B, Merrill C, Sorranno T, Bell E (1981) Regulation of proliferation of fibroblasts of low and high population doubling levels grown in collagen lattices. Mech Ageing Dev 17:107–117
39. Sato Y, Rifkin DB (1988) Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J Cell Biol 107:1199–1205
40. Sato Y, Abe M, Takaki R (1990) Platelet factor 4 blocks the binding of basic fibroblast growth factor to the receptor and inhibits the spontaneous migration of vascular endothelial cells. Biochem Biophys Res Commun 172:595–600
41. Sato Y, Hamanaka R, Ono J, Kuwano M, Rifkin DB, Takaki R (1991) The stimulatory effect of PDGF on vascular smooth muscle cell migration is mediated by the induction of endogenous basic FGF. Biochem Biophys Res Commun 174:1260–1266
42. Seppä H, Grotendorst G, Seppä S, Schiffmann E, Martin GR (1982) Platelet derived growth factor is chemotactic for fibroblasts. J Cell Biol 92:584–588
43. Skalli O, Gabbiani G (1988) The biology of the myofibroblast. Relationship to wound contraction and fibrocontractive diseases. In: Clark RAF, Henson PM (eds) The molecular and cellular biology of wound repair. Plenum Press, New York, pp 373–402
44. Snyderman R, Philips J, Mergenhagen SE (1970) Polymorphonuclear leukocyte chemotactic activity in rabbit serum and guinea pig serum treated with immune complexes: evidence for C5a as the major chemotactic factor. Infect Immunol 1:521–525
45. Uitto J, Santa Cruz DJ, Eisen AZ (1980) Connective tissue nevi of the skin: the clinical, genetic and histopathological classification of hamartomas of the collagen, elastin and proteoglycan type. J Am Acad Dermatol 3:441–461
46. Uitto J, Rhyhänen L, Tan EML, Oikarinen AI, Zarazoga EJ (1984) Pharmacological inhibition of excessive collagen deposition in fibrotic diseases. Fed Proc 43:2815–2820
47. Ward PA, Cochrane CG, Muller-Eberhard HJ (1965) The role of serum complement in chemotaxis of leukocytes in vitro. J Exp Med 122:327–346