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

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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

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Introduction

After wounding, peptide factors derived from blood constituents 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% CO₂ in 75-cm² tissue culture flasks (Falcon, Becton Dickinson, Oxnard, Calif., USA) with 20 mM Heps-buffered DMEM (LM cells and B14F28 cells), or with 10 mM Heps-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-