Mitogenic whey extract stimulates wound repair activity in vitro and promotes healing of rat incisional wounds

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Rayner, Timothy E., Allison J. Cowin, J. Gray Robertson, Rodney D. Cooter, Richard C. Harries, Geoffrey W. Smithers, Chris Goddard, and David A. Belford. Mitogenic whey extract stimulates wound repair activity in vitro and promotes healing of rat incisional wounds. Am J Physiol Regulatory Integrative Comp Physiol 278: R1651–R1660, 2000.—The ability of single growth factors to promote healing of normal and compromised wounds has been well described, but wound healing is a process requiring the coordinated action of multiple growth factors. Only the synergistic effect on wound healing of combinations containing at most two individual growth factors has been reported. We sought to assess the ability of a novel milk-derived growth factor-enriched preparation [mitogenic bovine whey extract (MBWE)], which contains six known growth factors, to promote repair processes in organotypic in vitro models and incisional wounds in vivo. MBWE stimulated the contraction of fibroblast-populated collagen lattices in a dose-dependent fashion and promoted the closure of excisional wounds in embryonic day 17 fetal rat skin. Application of MBWE increased incisional wound strength in normal animals on days 3, 5, 7, and 10 and reversed the decrease in wound strength observed following steroid treatment. Wound histology showed increased fibroblast numbers in wounds from normal and steroid-compromised animals. These data suggest the mixture of factors present in bovine milk exerts a direct action on the cells of cutaneous wound repair to enhance both normal and compromised healing.

growth factor; wound healing; skin; fetal wound healing; fibroblast-populated collagen lattice

The topical application of several classes of growth factors has been shown to enhance all aspects of wound repair, including inflammation, matrix synthesis, wound contraction, epithelialization, and tissue remodelling (6). Studies to date have largely focussed on the effects of single recombinant growth factors, including members of the fibroblast growth factor (FGF) (17, 30), insulin-like growth factor (IGF) (22, 48), transforming growth factor (TGF)-β (34, 38, 39), platelet-derived growth factor (PDGF) (17, 18, 39), and epidermal growth factor (EGF) (19) families, the interleukins (26), growth factor (PDGF) (17, 18, 39), and colony stimulating factor (24). Importantly, these factors have been shown to reverse the healing deficit associated with diabetes (17), steroid administration (38), cytotoxic therapy (25), infection (24), radiation (9, 35), and ischemia (49). However, the activity of these factors in promoting wound repair on the experimental animal has not been reproduced in clinical studies of chronic wound healing (43). Whereas there are several possible reasons for this discrepancy, it now seems likely that the proinflammatory environment of the chronic wound adversely affects the stability and activity of the applied growth factors (29, 50). Thus the ability of any single factor to enhance the multiple aspects of wound repair to produce a clinically significant outcome may be limited.

Several studies have now clearly shown that wound repair is the result of the temporally coordinated local expression of multiple families of growth factors and their receptors (5). It would therefore seem reasonable to hypothesize that the application of mixtures of growth factors to the healing wound may be more effective than the addition of a single factor. Indeed, several studies have shown this to be the case. In particular, PDGF interacts with IGF-I (27), IGF-II (16), EGF, and TGF-β (25), insulin (18), and TGF-α (7) to promote the repair of a variety of wounds. EGF and insulin act synergistically to promote collagen synthesis in the diabetic rat (19). Similarly, coinjection of IGF-I and its binding proteins is more effective at promoting wound repair than application of IGF-I alone (22, 33).

Bovine milk is a rich source of several classes of growth factors, including PDGF (46), IGF-I and -II (11), and TGF-β (21). We have reported that cation-exchange fractionation of bovine whey results in a 100- to 200-fold enrichment of the growth factor activity present in milk (10). The resultant fraction, mitogenic bovine whey extract (MBWE), was shown to stimulate the growth of cells of mesodermal origin, although, due to its TGF-β content, was found to be inhibitory to the division of epithelial cells (3, 45). This fraction also
contains significant concentrations of IGF-I and -II, IGF binding proteins 2 and 3, PDGF, and FGF-1 and -2, although together these known growth factors account for only 50% of growth activity present (2, 44). Maximal growth of fibroblast lines in response to MBWE significantly exceeded that in the presence of several individual recombinant factors including PDGF, IGF-I, TGF-β, FGF-2, or EGF (3).

The aim of the current study was to investigate the wound repair activity of the milk-derived growth factor mixture. In vitro organotypic models initially were used to characterize the tissue repair activity of MBWE, and an in vivo model of incisional wound healing was used to assess the ability of MBWE to promote cutaneous repair in normal animals and reverse the deficit in healing associated with steroid administration.

MATERIALS AND METHODS

Materials

Human diploid skin fibroblasts (SF) were provided by the Women’s and Children’s Hospital (North Adelaide, Australia) and DMEM obtained from Flow Laboratories (Irvine, Scotland). Fetal bovine serum (FBS) was obtained from Cytosystems (Castle Hill, Australia) and [3H]inulin (1.18 Ci/mmol) and DMEM obtained from Flow Laboratories (Irvine, Scotland). Collagen was extracted from rat tail tendons in a solution of 0.1% acetic acid (ICI Australia, New South Wales, Australia). Fluothane anesthetic (Halothane) was obtained from ICI Australia (Victoria, Australia) and used in combination with oxygen and nitrous oxide (BOC Gases, South Australia, Australia). Collagen was extracted from rat tail tendons in a solution of 0.1% acetic acid according to the method of Bell et al. (4). Before use as a vehicle matrix, the collagen was diluted in sterile water to a final concentration of 1 mg/ml and brought to neutrality with phosphate-buffered saline and NaOH.

Production of MBWE

Fractionation of whey was carried out as previously described (10). Briefly, pasteurized whey obtained as a byproduct of cheese manufacture was clarified by passage through a 0.8-μm ceramic filter (Membralox, Bajet, France). The ultrafilter was adjusted to pH 6.5 and applied to a 30-cm diameter column (Moduline, Beverly, MA) packed with 5 l of S-Sepharose Fast Flow cation-exchange resin (AMRAD Pharmacia Biotech, Victoria, Australia) equilibrated with 50 mM sodium citrate buffer at pH 6.5. After washing the column with the same buffer, the absorbed material was eluted with 0.5 M NaCl. This eluate was desalted, concentrated, and freeze dried. Growth activity was confirmed using a dye-binding cell growth assay as previously described (3). TGF-β concentration was determined using an Mv1Lu cell bioassay (45). IGF-I concentration was measured by radioimmunoassay after removal of IGF binding proteins by acid gel-permeation HPLC (37). MBWE was screened for bovine diarrhea virus, infectious bovine rhinotracheitis, parainfluenza type 3 and coliforms. Endotoxin levels were also measured (Limulus Amoebocyte Lysate assay, BioWhittaker, Walkersville, MD).

Fibroblast-Populated Collagen Lattice Contraction

Human diploid SF were maintained in DMEM supplemented with penicillin (60 mg/l), streptomycin (100 mg/l), fungizone (1 mg/l), and 10% FBS. Stock cultures were maintained in 75-cm² flasks (Corning, NY) in 5% CO2-95% air at 37°C and routinely passaged after suspension in trypsin (0.125%)/EDTA (0.5 mM) in Dulbecco’s phosphate buffered saline.

A solution of rat tail collagen (2 mg/ml) was mixed with an equal volume of 2× DMEM at 4°C before addition of human SFs (200,000 cells/ml final concentration) and [3H]inulin (to 30,000–40,000 counts·min⁻¹·ml⁻¹). Five-hundred microliters of this solution were poured into each well of a 48-place tissue culture plate (Costar, Cambridge, MA) and allowed to set by incubation at 37°C for 30 min. Gels were freed from the surrounding tissue culture plastic by rimming the margin with a 25-gauge needle. Dilutions of MBWE were applied to the top of the gels, which were then maintained in a humidified atmosphere of 5%CO2-95% air at 37°C. Control wells containing either serum-free or FBS-supplemented medium were incorporated on each plate. Fibroblast-induced contraction was assessed by counting the radioactivity remaining in the gel after 24-h culture. Data were fit to a four-parameter equation with the aid of a nonlinear curve-fitting program (Graphpad Prismview, Dapple Systems, Sunnyvale, CA).

Fetal Skin Organ Culture

Wounding and culture of fetal rat skin were undertaken as previously described (1). Pregnant rats were killed by CO2 asphyxiation, the fetuses (E16–E17) removed from the uterus, weighted, and placed in Hank’s balanced salt solution. A 1 cm piece of skin was dissected from the back of each fetus and a 1-mm diameter wound created in each explant using a squared-off, sharpened 19-gauge needle. The wound margin was marked by dipping the cutting needle into Indian ink immediately before wounding. The wounded skin was then mounted on a six-pin cradle and placed in a 12-well tissue culture dish (Costar). After three washes with medium, serum-free DMEM or DMEM/MBWE (2.5 mg/ml) was added in a final volume of 2 ml. Wounds were photographed using a standard focal length (jig at the time of culture and at 24, 48, and 72 h. Wound area was calculated by tracing the wound margin onto acetate sheets which were then scanned into a computer (Apple Macintosh Onscanner connected to an Apple Macintosh LCIII) equipped with an image analysis program (Prismview, Dapple Systems, Sunnyvale, CA). For histology, cultures were fixed in methacarn for 2 h before storage in 70% alcohol and processing by graded dehydration. Skin slices were embedded vertically in wax blocks and 3-μm sections taken through the wound. Sections were stained with hematoxylin and eosin, viewed, and photographed.

Incisional Wound Healing Model

Ability to promote repair of an incisional wound was investigated as previously described (34, 38). After induction of general anesthesia by inhalation of halothane, paired 6-cm incisions were created through the panniculus carnosus either side of the midline on the dorsal surface of the rat using a standard template. MBWE was dissolved in the collagen vehicle at the indicated concentrations and a single 200-μl dose of this formulation applied to one wound. The same volume of collagen vehicle was applied to the contralateral wound. The collagen was noted to cover the wound surface
and gelled within 1–2 min. Left- and right-sided wounds were randomized with respect to treatment and were closed using five sutures (4/0 silk, Dynec, South Australia, Australia). Care was taken during suturing to evert the wound margins to ensure dermal-dermal contact along the length of the wound. All rats were housed individually after surgery and were weighed the day after surgery and then every 2 days. At various time points after wounding, rats were killed by CO₂ asphyxiation and the pelts carefully removed. A standard template was used to cut four strips of 0.5-cm width through each wound (i.e., 1 strip through each of the wound segments bordered by two sutures, excluding the top and bottom wound margins) and either peak breaking strength measured using a M1000E (Mecmesin, West Sussex, UK) tensiometer (time-course study) or a breaking profile of each strip generated using a PCM2500EL tensiometer (Mecmesin) connected to a personal computer (dose-response study). The latter allowed calculation of peak load, yield load (defined as the point at which the gradient change in load over a set displacement interval becomes <20% of the calculated start gradient), force absorbed to yield load, and force absorbed to peak load.

**Histology**

Samples of MBWE and collagen-treated wounds were collected and placed in 10% Formalin fixative immediately after harvesting and processed by graded dehydration. Three sections (3 µm) were cut through each wound, stained with hematoxylin and eosin, and scored by two independent observers (blinded). Each section was scored for mononuclear cell and fibroblast infiltration. Scores of zero to four were recorded for each section, and the average of three sections from each scorer was used to obtain an average score for the wound, which was used to calculate overall group means.

**Data Analysis**

Five percent of strips in the steroid-treated group showed evidence of hemorrhage or dehiscence before dissection and were removed from the wound strength data set. Similarly, spontaneous separation of the dermal margins of the wound during processing for tensiometry (i.e., once they had been removed from the support of the sutures) was recorded as dehiscence, and the strip was excluded from wound-strength data analysis. All results are presented as means ± SE with wound-strength data analyzed using Student's t-test (time-course study) or paired t-test (time-course study). Histology scores were analyzed by Kruskall-Wallis one-way analysis of variance on ranks with multiple comparisons versus the control group (combined data) performed using Dunn's method. P < 0.05 was considered significant.

**RESULTS**

**Activity of MBWE**

Cell growth activity of MBWE was confirmed using Balb/c 3T3 fibroblasts and SFs as previously described (3). Growth activity corresponding to that observed in 10% FBS for Balb/c 3T3 fibroblasts and SFs was achieved with 150 and 610 µg/ml MBWE, respectively. The batch of MBWE used in the current study contained 1.6 ng/mg active TGF-β and 61 ng/mg total TGF-β bioactivity as determined by Mv1Lu cell bioassay before and after acid activation, respectively. The batch of MBWE also contained 32 ng/mg IGF-I. The concentrations of other growth factors known to be present in MBWE, including PDGF and FGF, were not routinely determined. In addition, MBWE was shown to be free of all microbiological contaminants tested and contained <1 ng/ml endotoxin.

**Effect of MBWE on Fibroblast-Induced Gel Contraction**

The ability of MBWE to stimulate growth of several fibroblast cell lines (3) led us to further examine its repair activity using organotypic models of wound healing. MBWE markedly stimulated fibroblast-induced gel contraction in a dose-responsive manner when applied to fibroblast-populated collagen lattices (FPCL; Fig. 1). The dose that exerts half-maximal effect above baseline in this assay over a series of similar experiments was 195 ± 44 µg/ml (mean ± SE; n = 3).

**Effect of MBWE on Wound Closure in Organ Cultured Fetal Rat Skin**

The effect of MBWE on wound repair was further examined using a fetal skin organ culture model. Skin harvested from E16–17 rat embryos retains the ability to heal an excisional wound in serum-supplemented suspension culture (1) and allows the effect of agents with potential wound-healing activity to be evaluated in an organ culture model isolated from blood-borne or environmental factors. Wounded E16–17 rat skin cultures were established as described and cultured in DMEM alone or DMEM plus MBWE (2.5 mg/ml). In DMEM alone, wounds contracted to 35–40% of the original wounded area over 72 h, although they never completely closed (Fig. 2A, a and c) (1). Addition of MBWE to the medium promoted complete closure over
the 72-h culture period (Fig. 2A, b and d). The contraction of wounds in skin explants treated with MBWE was greatly accelerated compared with skin incubated in DMEM alone (Fig. 2B). The wound areas of MBWE-treated explants at 24 and 48 h were 20 ± 10% and 2.8 ± 2.2% their original area, respectively (Fig. 2B, means ± SE of triplicate cultures). In agreement with a previous study (1), histology through the wound after 48 h in DMEM alone showed no evidence of epithelial or mesenchymal movement into the wound defect (Fig. 3A). In contrast, histology through healed wounds after 48 h in the presence of MBWE showed that final wound closure had been achieved by the movement of the epidermal layer over the wound edge to form a “plug” of epithelium between the dermal margins (Fig. 3B).

Time Course of Response to MBWE

To examine the ability of MBWE to stimulate wound healing in normal rats and determine the time course of this response, MBWE was formulated at 2.5 mg/ml, corresponding to a total dose of 0.5 mg/wound, and applied to linear incisions on the dorsum of adult rats. This concentration of MBWE has previously been shown to stimulate maximal growth of human SFs in vitro (3), although concentrations up to 5 mg/ml were tolerated by these cells without a significant decrease in the maximal growth response. Wound strength was assessed at days 3, 5, 7, 10, 14, 21, and 42 after wounding.

The peak load tolerated by MBWE- and vehicle-treated wounds is shown in Table 1. Highly significant increases in wound strength in response to topical MBWE were seen on days 5, 7, and 10, corresponding to average increases of 44%, 28%, and 20% over control wounds, respectively. The strength of MBWE-treated wounds on days 3 and 21 were increased an average of 21% and 10% over control values, respectively. Break-

Fig. 2. Fetal excisional wound closure. Fetal skin was dissected from dorsum of E17 rats and wounded using a squared off 19-gauge needle. Wound margin was marked by dipping cutting needle into Indian ink immediately before wounding. A: wounds are shown at time of culture (a and b) and after 72 h in serum-free DMEM (c) or DMEM + 2.5 mg/ml MBWE (d). B: contraction curves from triplicate experiments after measurement of wound areas at times shown for wounded skin incubated in serum-free DMEM (○) or DMEM + 2.5 mg/ml MBWE (●).

Fig. 3. Histology of excisional wounds in fetal rat skin after 48 h culture in MBWE. Experimental details are given in Fig. 2 legend. At 48 h culture, skin explants were fixed in methacarn, processed for wax embedding, and 3-µm sections were taken through wound that was identified by concentration of ink particles. Sections were stained with hematoxylin and eosin. A: wound margin after 48 h in serum-free DMEM. B: healed wound after 48 h in DMEM + 2.5 mg/ml MBWE.
ing strengths on days 14 and 42 were not significantly different from vehicle-treated controls.

**Dose Response to MBWE**

A series of studies aimed at determining the optimal dose and assessing the histological picture of the MBWE-treated wounds was undertaken using both normal and steroid-compromised animals. Steroid-treated rats received an intramuscular injection of methylprednisolone (15 mg/kg) immediately before surgery. MBWE was formulated at 10, 2.5, and 1.25 mg/ml, each wound therefore receiving a total of 2, 0.5, and 0.25 mg of MBWE, respectively. As the maximal response to MBWE in normal animals occurred 5 days after wounding and application of the extract, all wounds were harvested at this time point.

Normal animals. The force absorbed by each strip harvested from normal animals 5 days after wounding is shown in Fig. 4A. Both force absorbed to yield load (not shown) and force absorbed to peak load were significantly increased in wounds treated with 0.5 mg/wound MBWE compared with vehicle-treated wounds. In addition, the yield load and peak load in the MBWE-treated wounds were increased an average of 62% and 25% over control wounds, respectively (data not shown). Application of either 0.25 or 2.0 mg/wound MBWE did not significantly alter wound strength.

Steroid-treated animals. Administration of methylprednisolone on the day of wounding induced an average 15% weight loss compared with an 8% weight gain in the normal group over the course of the experiment and significantly reduced all indices of wound strength. Yield load, peak load, force absorbed to yield load, and force absorbed to peak load of vehicle-treated wounds were decreased an average of 44%, 39%, 42%, and 39%, respectively, in the steroid animals (P < 0.0001 for all). Whereas only ~1–2% of all strips taken through wounds on normal animals showed any signs of dehiscence, 28% of strips cut through vehicle-treated wounds on steroid-treated rats disrupted before placement in the tensiometer. This degree of wound breakdown in the steroid-treated group was reduced to 13% in wounds treated with 0.5 mg/wound MBWE, whereas the dehiscence rate in wounds treated with 0.25 and 2.0 mg/wound MBWE was consistent at 21%. Wounds treated with the 0.5 mg/wound dose of MBWE also exhibited a significant increase in both the force absorbed to yield load (not shown) and force absorbed to peak load compared with vehicle-treated wounds (Fig. 4B). Indeed, 0.5 mg/wound MBWE increased the wound strength to levels recorded from normal control wounds.

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<th>Day</th>
<th>Collagen</th>
<th>MBWE</th>
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<td>5</td>
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<td>169.8 ± 10.8</td>
<td>203.5 ± 10.6</td>
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<td>14</td>
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<td>297.4 ± 13.8</td>
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<td>42</td>
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*Values are means ± SE for 16 wounds (64 strips) pooled from 2 separate experiments. Paired linear incisions were created on the dorsal surface of adult male rats as described in MATERIALS AND METHODS. Mitogenic bovine whey extract (MBWE; 2.5 mg/ml of collagen) was applied to one wound and collagen vehicle was only applied to the contralateral wound. On the indicated days after wounding 1 group was killed and the maximal load (grams) tolerated by 4 strips of 0.5-cm width were taken through each wound determined using a tensiometer. Data were analyzed by paired t-test.*

Fig. 4. Wound strength in normal (A) and steroid-treated animals (B) in response to MBWE. Paired linear incisions were created on the dorsal surface of adult male rats. MBWE was applied to one wound at indicated doses and collagen vehicle was only applied to the contralateral wound. Animals in the steroid group were given an intramuscular injection of 15 mg/kg methylprednisolone at time of surgery. Animals were killed 5 days after wounding and 4 strips of 0.5-cm width were taken through each wound and placed in a tensiometer. Force absorbed to peak load was calculated from load-displacement curves. Bars represent means ± SE of indicated number of strips (in parentheses) pooled from 2 experiments for both MBWE (filled bars) and matched vehicle-treated (open bars) wounds. Different number of strips analyzed reflects incidence of wound strip dehiscence in respective treatment groups. *P < 0.05 vs. vehicle-treated wounds.
Furthermore, the values for both the yield load and peak load were increased an average of 60% above their vehicle-treated controls (data not shown). As observed with wounds from normal animals, application of either 0.25 or 2.0 mg of MBWE did not significantly affect the force absorbed by the wound compared with their vehicle-treated controls (Fig. 4B).

Wound histology. Representative sections taken through wound samples from normal and steroid rats are shown in Fig. 5 with semiquantitative histology scores of sections from all treatment groups presented in Fig. 6. Comparison of Fig. 5, A and C, shows the difference in wound histology expected between normal and steroid-treated rats, which is characterized by the marked absence of cellular infiltrate and granulation tissue. Administration of methylprednisolone significantly reduced the scores, reflecting fibroblast and inflammatory cell infiltration by 46% and 34%, respectively (Fig. 6, A and B). Treatment with MBWE increased the cellular infiltrate in wounds on both normal and steroid-treated rats (Fig. 5, B and D). This response to MBWE was most noticeable in sections from wounds

![Fig. 5. Wound histology. Photomicrographs show cross sections through day 5 incisional wounds from normal (A and B) and steroid-treated (C and D) rats that received MBWE (B and D) or collagen vehicle only (A and C) at time of wounding. Sections were stained with Masson's trichrome and photographed at ×250 magnification.](image-url)
with either 0.25 or 0.5 mg/wound MBWE showed a significant increase in fibroblast score. MBWE had no significant effect on the inflammatory score in wounds on both normal and steroid-treated animals (Fig. 6B).

**DISCUSSION**

We previously showed that cation-exchange chromatography results in a 100- to 200-fold enrichment of the growth factor component of bovine milk. Although consisting of only 0.5% of whey protein, it contains the bulk of growth factor activity present in bovine milk (10). Importantly, this fraction contains several growth factors and their binding proteins that have been shown by others to act in synergistic fashion in the wound, including IGF-I and PDGF (27), IGF-II and PDGF (16), and IGF-I and IGF binding protein 3 (33). In addition, MBWE contains significant concentrations of members of the TGF-β family, with TGF-β1, a factor known to significantly enhance the healing strength of an incisional wound (34). Approximately 85% of the TGF-β activity in MBWE is accounted for by TGF-β2, which exists as an acid labile 80-kDa species consistent with the small latent complex of TGF-β (45). In this regard, it is interesting to note that some 30–40% of platelet-derived TGF-β is retained in the clot as the small latent complex, where it may act as a slow release form of the molecule during the wound repair process (15).

The activity of the growth factor-enriched milk-derived fraction in promoting different aspects of tissue repair was investigated in the first instance using in vitro organotypic models. The ability of fibroblasts incorporated in a collagen gel to reorganize the collagen fibers and cause gel contraction is considered the in vitro counterpart of wound contraction in the adult mammal (4, 32). This phenomenon has been found to be dependent on the trophic activity of FBS (14). However, in the present study, MBWE was able to substitute for serum in stimulating fibroblast-mediated collagen lattice contraction (Fig. 1). MBWE-induced contraction was dose dependent, with the maximal effect over 24 h found to be at least equivalent to that observed in serum-supplemented culture (not shown). Given that the individual growth factors EGF, PDGF, and basic FGF (bFGF) failed to stimulate FPCL contraction (32), it would appear that the novel mixture of growth factors contained within MBWE may account for the response observed in this assay, with IGF-I or TGF-β likely to be at least partly responsible for the activity evident in MBWE (13, 41).

The assertion that a mixture of individual growth factors such as that present in MBWE may be effective at stimulating tissue repair is further supported by the ability of MBWE to promote wound closure in organ-cultured fetal skin. Skin harvested from the E16–17 embryo is able to heal a wound in vitro by a combined process of mesenchymal contraction and substrate-independent movement of the epithelium over the dermal margins of the wound to effect final closure (1). This repair response is dependent on the presence of serum but is not promoted by the addition of single...
recombinant factors such as IGF-I, EGF, bFGF, TGF-β, or PDGF. Although wound closure is likely to result from the coordinated action of epithelial cells to mobilize contractile proteins and close the wound by a purse-string mechanism (28), the trophic requirement for this form of epithelial repair is unknown. MBWE was able to substitute for serum in promoting wound closure, histology clearly revealing the presence of epithelium spanning the dermal margins of the wound (Fig. 3). Nevertheless, the dermal margins were consistently more approximated after culture in MBWE than in serum-supplemented cultures compared with previously published results (1), possibly reflecting the action of the factors in MBWE on the mesenchymal layer to enhance wound contraction. Thus the mixture of factors contained in MBWE has the capacity to induce novel effects on fetal wound closure that are not observed after application of individual factors, suggesting that appropriate combinations of growth factors may act in a coordinated fashion to stimulate the sequence of events required for successful healing. We cannot, however, rule out the possibility that an as yet unidentified molecule(s) may be present in MBWE that has unique and singular effects on the repair process.

All surface wounds heal by a process of wound contraction, matrix synthesis and remodelling, and epithelialization. Apposition of the wound margins allows healing by primary intention, which is mainly achieved by matrix synthesis. Thus the ability of a topical agent to enhance the strength of an incisional wound reflects a direct or indirect ability to enhance wound fibroblast division, migration into the wound, or biosynthetic capacity. The ability of MBWE to promote wound repair was most evident in steroid-treated animals, suggesting a direct action of the growth factors in MBWE on the cells of wound repair. Steroid administration profoundly inhibits both the inflammatory response to wounding and the expression of procollagen genes by SFs (12). A single dose of methylprednisolone at or before wounding inhibits wound contraction (23), synthesis of granulation tissue, and angiogenesis and decreases wound strength (38). In the current study, all indices of wound strength were reduced by ~40% of control values 5 days after administration of methylprednisolone. The most effective dose of MBWE (0.5 mg/wound) returned the force absorbed by wounds on steroid-treated animals to levels observed in normal animals and resulted in a histological picture similar to that seen on sections from control wounds on normal animals.

The ability of MBWE to promote wound repair was concentration dependent. Higher concentrations of MBWE promoted fibroblast influx but did not increase the strength of incisional wounds on either the normal or steroid-treated animals. Whereas we cannot rule out the presence of some inhibitory activity in MBWE, it is possible this phenomenon is due to the bimodal action of some individual growth factors in MBWE. Both TGF-β and bFGF have been shown to have apparent concentration-dependent bimodal effects on the strength of incisional wounds in vivo (36, 39). Similarly, a bimodal effect of TGF-β and bFGF on the volume of new granulation tissue detected in rabbit ear excisional wounds has also been reported (40).

The possible complex interactions among the known growth factors in MBWE render any discussion of its mechanism of action in the wound somewhat speculative. Furthermore, it is impossible to rule out effects on wound healing by components of MBWE that are not growth factors. Moreover, although the activity of MBWE in steroid-treated rats would suggest a direct action on the wound, we cannot exclude indirect effects of the components of MBWE on the cells of wound repair. Nevertheless, comparative studies using single growth factors may provide some clues. PDGF, TGF-β, and FGF all stimulate granulation tissue deposition. TGF-β1 in particular has been shown to act directly on the cells of cutaneous healing to induce the fibrotic and angiogenic components of repair in vivo (42). Thus TGF-β1 is effective in promoting repair of wounds on animals rendered monocytopenic by steroid administration (38) or total body irradiation (9), although it is ineffective in rats whose dermal fibroblasts have been damaged by surface irradiation (9). In contrast, PDGF does not directly stimulate collagen synthesis (42) and cannot reverse the wound healing deficit associated with circulating and wound monocytopenia as a result of steroid treatment (38), although it was able to improve wound repair in animals whose dermal fibroblasts had been damaged by surface irradiation by recruiting macrophages to the wound (35).

The degree of improvement in wound strength observed in response to MBWE 5 days after wounding steroid-treated rats is greater than that reported following administration of recombinant TGF-β1 in a comparable study (38), suggesting the potential synergistic action of factors present in MBWE. In this regard, IGF-I and -II are also known to promote fibroblast division (8), protein, collagen, and fibronectin synthesis (20), and local administration of IGF-I has been shown to reverse the effects of steroids on inhibition of granulation tissue ingrowth to a subcutaneous chamber (48). Coadministration of IGF-I with its binding proteins increases wound strength (22). Similarly, both acidic and basic FGF have been shown to increase wound strength (30, 31). Indeed, Lynch et al. (27) found a combination of PDGF-2 and IGF-I to be the most potent inducer for healing partial-thickness porcine wounds compared with various other individual growth factors or combinations tested.

Although some studies have shown the maximum wound strength achieved by the application of single growth factors (principally TGF-β) to be greater than that observed in our experiments, it should be noted that comparative studies have generally added microgram quantities of single recombinant growth factors to wounds (27, 34, 36, 39, 40). The amount of individual growth factors applied to wounds treated with MBWE was in the low nanogram range. It is possible further purification of MBWE designed to concentrate specific growth factor components of the extract may enhance the wound healing activity of these fractions.
The current report represents the first to describe the ability of a mitogenic extract containing the complement of growth factors present in milk to stimulate a healing response in organotypic wound models and promote healing of the incisional wound in vivo. Together with in vitro data showing significant activity in assays of cell growth (3, 10), our results would suggest the unique mix of agents in MBWE may confer an ability to promote wound repair over and above that obtained with single growth factors and that is particularly relevant to rectifying the healing deficits associated with compromised conditions resulting in chronic cutaneous wounds (47).

Perspectives

Wound healing is a complex series of events aimed at restoring the integrity of damaged tissue. Included in these events are the actions of growth factors, which play an essential role in directing wound repair. The action of such mediators, however, is disturbed in tissue compromised by lack of blood flow or systemic diseases such as diabetes, leading to failure of the cellular processes of healing. Whereas growth factors have been shown to enhance all aspects of wound repair in experimental models, the effectiveness of individual growth factors has generally been difficult to replicate when applied to clinical studies in chronic wounds. As wound repair results from the temporally coordinated local expression of multiple families of growth factors and their receptors, it would seem reasonable that the application of mixtures of growth factors to the healing wound may be more effective than a single factor. Bovine milk is a rich source of several classes of growth factors, and cation-exchange fractionation of bovine whey results in a mitogenic extract that contains a 100-200-fold enrichment of the growth factor activity present in milk. Our results demonstrate that this mitogenic extract derived from milk has considerable ability to promote wound repair over and above that do not account for the cell growth activity present in bovine milk. J Endocrinol 154: 45–55, 1997.


