Short Report

MMP-2 Assessment as an Indicator of Wound Healing: A Feasibility Study

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ABSTRACT

OBJECTIVE: Evaluate the feasibility of assessment of fibroblast matrix metalloproteinase-2 (MMP-2) expression as an indicator of wound healing in wounds that change from chronically inert to actively healing.

METHODS: Phase II feasibility study of 4 patients with nonhealing wounds (duration, ≥ 3 months; surface area, ≥ 1 cm^2). Wounds were treated with a dressing impregated with oak bark extract (DerMax) and evaluated weekly; biopsies were performed every 2 weeks until wound healing.

RESULTS: Therapy-induced wound healing and immunohistochemical measurements of MMP-2 expression paralleled the clinical characteristics of wound healing.

CONCLUSION: MMP-2 expression offers a reliable indicator for clinical wound healing induced by DerMax treatment.

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Wound healing research has focused predominantly on cytokine levels found in the wound bed.[1] Cytokines are key players in wound healing, controlling many of the stress responses leading to extracellular matrix restoration following injury.[2] Cytokines, however, influence extracellular matrix production and breakdown only indirectly by regulating levels of matrix metalloproteinases (MMPs) and their natural inhibitors (tissue inhibitors of metalloproteinase, or TIMP).[3,4] Matrix metalloproteinases (endopeptidases) break down extracellular matrix components expressed by different cell types (eg, macrophages, neutrophils, fibroblasts, keratinocytes, and endothelial cells).[3,5-7] Prolonged high-MMP expression allegedly destroys local growth factors and impairs a wound's ability to heal.[8] Imbalances between MMP and TIMP activity, therefore, lead to prolonged MMP presence (atrophy and ulcers) or relatively low MMP expression (extracellular matrix hypertrophy).[7,9-12] The balance of MMPs and TIMP directly affects extracellular matrix buildup and breakdown, making assessment of MMP levels a potentially important prognostic indicator of wound healing.[13,14]

Knowledge of the effects of MMPs on wound healing is increasing, and several MMP subgroups have been identified. These subgroups are based on structure and substrate specificity: (1) collagenase, (2) gelatinase, (3) stromelysine, (4) membrane-type MMP, and (5) other MMPs (Table 1).[15] Levels of gelatinases are different during each stage of wound repair.[16] Many studies have shown basal levels of gelatinase-A (MMP-2) in noninjured skin. Prolonged periods of increased MMP-2 expression occur following injury.[17-19] Pro-MMP-2 levels detected in fibroblasts appear similar in acute and chronic dermal wounds. Activated MMP-2 protein, however, is higher in chronic wounds.[20-22] Because of the difference between MMP-2 expression levels in inert and healing wounds, specific tissue staining may effectively determine wound state and changes induced by therapeutic interventions.[23]

To evaluate the feasibility of MMP-2 assessment as an indicator of changes in wound healing, this study surveyed histologic changes in nonhealing wounds that clinically improved with dressings impregnated with oak bark extract (DerMax; Dermagenics Europe BV, Kaatsheuvel, the Netherlands).

METHODS

Four patients—1 male and 3 females (average age, 61 years)—were included in this Phase II study. All patients had nonhealing wounds that had not responded to conventional treatment (duration, ≥ 3 months; surface area, ≥ 1 cm^2). They signed an informed consent form that described the procedure, the treatment, and the potential complications and that allowed for wound biopsies every 2 weeks until wound healing (Table 2).

The wounds were covered with DerMax, which was changed daily at the patient's home following wound cleansing with

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Table 1.

MMP CLASSIFICATION

Collagenase
- Collagenase 1 (MMP-1)
- Collagenase 2 (MMP-8)
- Collagenase 3 (MMP-13)
- Collagenase 4 (MMP-18)

Gelatinase
- Gelatinase A (MMP-2) 92 kd
- Gelatinase B (MMP-9) 72 kd

Stromelysin
- Stromelysin 1 (MMP-3)
- Stromelysin 2 (MMP-10)
- Stromelysin 3 (MMP-11)

Membrane-type metalloproteinase (MT-MMP)
- MT1-MMP (MMP-14)
- MT2-MMP (MMP-15)
- Other: MMP-17, MMP-24, MMP-25

Other
- MMP-7
- MMP-12
- MMP-20

Table 2.

PATIENT CHARACTERISTICS

Sex	Age	Location	Cause	Duration (months)	Prior treatment
Male	42	Malleolus	Accident	14	Kaltostat
Female	74	Lower leg	RA/infection	>12	Duoderm, hydrogel, VAC, SDD
Female	62	Thorax	Radiotherapy	12	Kaltostat
Female	67	Achilles	Trauma	3.5	VAC

RA = rheumatoid arthritis; SDD = sterile dry dressing; VAC = vacuum-assisted closure

water. Wounds were clinically evaluated weekly at the wound center for size, granulation, epithelialization, and exudate. Punch biopsies (3 mm) were taken from the center of the wounds on day 0 and every 2 weeks thereafter until wound healing. Biopsies were fixed with Kryofix (Merck Sharp & Dohme, Haarlem, the Netherlands) for 1–3 days. Following paraffin embedding, 5-micron sections were stained with Papanicolaou or hematoxylin and eosin staining. Histologic assessment of granulation tissue spatial distribution, inflammation, and fibroblast morphology determined healing. Semi-serial sections were incubated with monoclonal MMP-2 antibodies (NeoMarkers/Immunologic; Duiven, the Netherlands) and processed according to standard immunohistochemical staining procedures. MMP-2 fibroblast expression levels were evaluated by routine indicators (eg, estimated number of positively stained fibroblasts and intensity of signal). The pathologist who examined the slides was blinded to the DerMax application protocol and the clinical course of healing.

RESULTS

Initial biopsies consistently showed broad fibroid caps with cellular debris covering the granulating wound bed in all biopsies. Histologic examination indicated quiescent fibroblasts (or fibrocytes), with slender outlines and small nuclei with dense nuclear chromatin. Immunohistochemically stained sections of these nonhealing wounds showed fibroblasts with high MMP-2 expression (Figure 1).

Clinical observations during treatment weeks 1 and 2 showed decreased wound secretions; however, histologic and immunostained sections of all biopsies consistently showed little or no change compared with initial biopsies (Figure 2). At

Figure 1.

DAY 0 OF THERAPY

A. A 74-year-old patient with rheumatoid arthritis presented with a lower leg wound of more than 1 year's duration. B. Histologic analysis (25×) of a 3-mm biopsy after Papanicolaou staining, showing (a) a broad fibrin layer with necrotic cellular debris, (b) a broad zone with breakdown of matured collagen, and (c) inflammation that declines toward bottom of the biopsy. C. After immunohistochemical staining of a semi-serial section of the same biopsy for MMP-2 expression level, the analysis (200×) appeared dark red, indicating a high expression level of MMP-2; a low expression level resulted in soft pink.

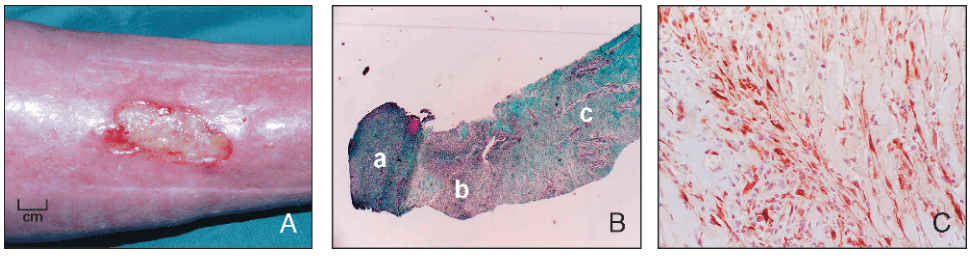
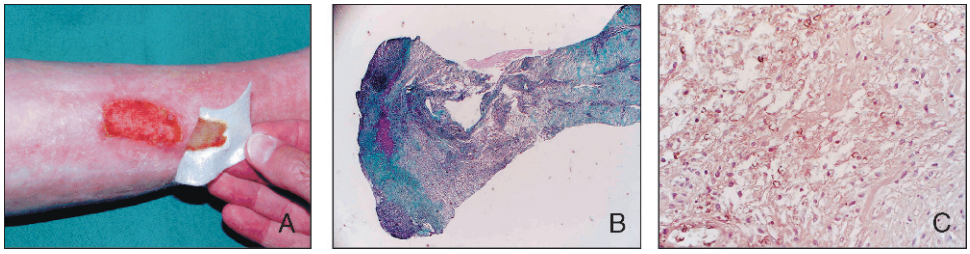


Figure 2.
WEEK 2 OF THERAPY

The wound shows little change compared with day 0. A. Clinical view. B. Histologic findings. C. Immunohistochemical findings.



week 4, however, clinical and histologic findings had clearly changed: wounds decreased in size, produced minimal secretions, and showed epithelialization and thinning fibrinoid caps. Clearly recognizable declines could be observed immunohistochemically in fibroblast MMP-2 expression in all biopsies.

At 6 weeks, the wounds showed clinically healthy wound beds, with signs of epithelial outgrowth and decreased inflammation. Histologic changes were obvious in all biopsies, with only small rims of fibrinoid material on top of broadened granulation tissue and low expression of MMP-2 in the fibroblasts. Compared with the first series of biopsies, upper layer fibroblasts had a stellate morphologic appearance, with expanded cytoplasm; larger, more openly structured nuclei; and recognizable nucleoli associated with increased cellular production (Figure 3).

DISCUSSION

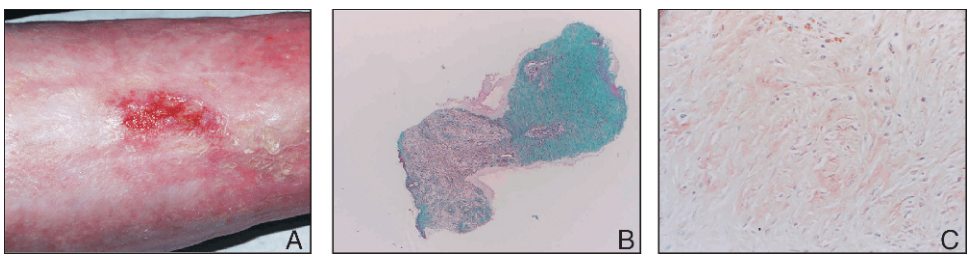
Assessment of new therapies for chronic wounds are typically based on clinical observation. Microscopic biopsy examination has not played an important role because cell morphology does not fully reflect function and, hence, routinely stained sections lack prognostic indicators. Therefore, a single biopsy, or even a short series of biopsies, is inadequate to predict final outcomes. In addition, practitioners are often hesitant to order biopsies because of the related patient stress caused by an invasive procedure. These issues can only be overcome with greatly enhanced histologic results.

The wounds included in this study showed similar clinical and histologic evolutions. Clinically, little wound healing was seen after the first 2 weeks of treatment with DerMax. Correlation of clinical data with histologic observation revealed that the clinical lag-phase paralleled an immunohistochemical lag-phase. After this phase, the authors observed loss of the fibronecrotic cap covering the wound bed, change from quiescent to active fibroblasts, and significantly decreased fibroblast MMP-2 expression that paralleled clinical wound improvement.

Feasibility of fibroblast MMP-2 expression assessment, therefore, was validated as an indicator of wound healing changes when DerMax therapy was used. Levels of MMPs in wound biopsies correlated with the severity of impaired wound healing as a result of imbalanced extracellular matrix processes. Unlike conventionally used parameters, MMP-2 measurement objectively assesses healing and minimizes intraobserver bias.

Figure 3.
WEEK 6 OF THERAPY

A. The lower leg wound has diminished, and it is now showing epithelialization and formation of a neodermis. B. Histologic view: There are fewer signs of inflammation. C. Immunohistochemical expression level of MMP-2 in the surrounding tissue has dramatically decreased.



Rather than relying on serial observations and empirical wound treatment, MMP-2 levels assess both wound state and changes induced by therapeutic interventions.

Immunohistochemical measurements paralleled clinical observations to such an extent that, in hind sight, biopsies seemed superfluous after 2 weeks of treatment. Similar outcomes may prove true with different therapeutic interventions or wound circumstances, although clinical lag-phases may differ from the immunohistochemical lag-phases. In these cases, fibroblast MMP-2 expression assessment may prove more valuable because it may guide clinical treatment or termination. Additional study in this area is warranted.

CONCLUSION

Study results show that assessment of fibroblast MMP-2 expression reliably indicates clinical wound healing when using DerMax treatment. Determination of MMP-2 assessment effectiveness with other therapeutics and different wound circumstances still must be established. ●

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