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Matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with different types of scars and keloids

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Summary *Background:* Hypertrophic scars and keloids are fibroproliferative skin disorders characterised by progressive deposition of collagen. Our study is designed to investigate the expression and concentration of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in different types of scars and keloids.
Methods: Total RNA from 19 proliferative hypertrophic scar samples of patients with extended burns (total body surface area (TBSA): 21 ± 12%), 18 mature hypertrophic scar samples from patients after elective surgery, 14 keloid samples and 18 normotrophic scar samples was, respectively, extracted, and then mRNA was isolated. Besides, biopsies were obtained from non-scarred skin of the patients and extraction of total RNA performed. Relative mRNA expression of MMP 2, MMP 9, TIMP 1 and TIMP 2 was measured with reverse transcriptase polymerase chain reaction (RT-PCR). Serum concentrations of MMP-1, -2, -9, TIMP-1, and -2 were determined using an enzyme-linked immunosorbent assay (ELISA).
Results: Patients with extended hypertrophic scars after burn trauma presented a significantly higher TIMP-1 concentration ($p < 0.05$) in their sera than the other patients. The relative expression of MMP 2 was significantly higher in samples of proliferative hypertrophic scars after burn injury. The relative expression of TIMP 1 and TIMP 2 was significantly higher in scar tissue of patients with proliferative and mature hypertrophic scars and keloids than in their regular skin and in scar samples of patients with normotrophic scars. The expression of TIMP 1 was significantly higher in samples of patients with keloids than in patients with hypertrophic scars.

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Conclusions: The concentration of TIMP-1 in sera of patients varies depending on the size of the involved fibrotic scar tissue. A decrease in MMP-to-TIMP expression in scar tissue may contribute to increased synthesis and deposition of collagen, leading to a severe fibrotic reaction with pathologic scar formation. The results implicate non-operative therapy options in these patients that not only down-regulate TIMPs but also increase the activity of MMPs.

The process of wound repair and restructuring is complicated. Ideally, wounds heal without the development of atrophic or hypertrophic scars (normotrophic scars).[1] Tissue remodelling of the extracellular matrix (ECM) is an essential and dynamic process in wound healing associated with physiological and pathological responses.[2] Remodelling of the ECM involves both the degradation and clearance of its components, as well as the production and deposition of newly synthesised ones.[2,3] The balance of these processes results in either preservation or alteration of the structure and functions of the supported tissue.[4] Resorption of the ECM is mediated predominantly by matrix metalloproteinases (MMPs).[5,6] To date, 24 different vertebrate MMPs have been identified, of which 23 are found in humans.[7] The key feature of collagenases (MMP-1, -8, -13) is their ability to cleave interstitial collagens I, II and III at a specific site three-fourths from the N-terminus.[8] Gelatinases (MMP-2, -9) digest denatured collagens, called gelatins.[6] Furthermore, MMP-2 digests type I, II and III collagens. The activity of MMPs is carefully regulated by controlling their conversion from proenzymes to the catalytic form and by the presence of a family of specific inhibitors, called tissue inhibitors of metalloproteinases (TIMPs).[7] Four TIMPs (TIMP-1, -2, -3, -4) have been identified that bind MMPs in a 1:1 stoichiometry.[8]

Disturbance in the balance between ECM production and degradation due to an uncontrolled activity of MMPs leads to formation of chronic ulcers.[9] In contrast, a decrease in the ratio of MMPs to TIMPs can cause fibrotic diseases by excessive accumulation of ECM components (e.g., liver cirrhosis and lung fibrosis).[10,11] Keloids and hypertrophic scars are other examples of fibroproliferative disorders that result from excessive collagen deposition.[12] They are two forms of excessive dermal fibrosis and cutaneous scarring thought to be caused by some kind of disorder in the regulation of cellularity increase and decrease during the wound-healing process.[13] Their pathophysiology is not completely understood but appears to result from an excessive healing response in the wound.[14] Proliferative hypertrophic scars are typically raised, erythematous and stiffer than the surrounding skin. Most hypertrophic scars mature, subside and pale over time.[12,13] Several morphological and immunohistochemical differences between hypertrophic scars and keloids were found that support the suggestion that different mechanisms are responsible for their development.[15,16] Abnormalities in cell migration and proliferation, inflammation, synthesis and secretion of extracellular matrix proteins and cytokines, as well as remodelling of the wound matrix have been described. Increased activity of fibrogenic cytokines (e.g., transforming growth factor β1, insulin-like growth factor 1 and interleukin 1) and exaggerated responses have also been noted.[17–19]

In 1999, Neely et al. determined levels of MMP-2 and -9 in tissue from hypertrophic scars, keloids and donor skin.[20] MMP-2 activity was significantly elevated in hypertrophic scars and keloids versus donor skin. In contrast, little MMP-9 activity was present in keloids and hypertrophic scars. There is evidence that the local behaviour of MMPs and TIMPs in tissue can also be detected in biological body fluids such as blood and urine.[21] In patients with hypertrophic scars after major burn injury, we were able to show a decrease in the ratio of MMPs to TIMPs systemically due to an increase of TIMP-1 after operative therapy that correlated with the burn scar index.[22]

It is still unknown if patients with smaller hypertrophic scars after non-burn injury and keloids after operative treatment present a pathological concentration of MMPs and TIMPs in their sera that might point to their behaviour in the involved tissue. In the present study, concentration and expression of different MMPs and TIMPs were determined for the first time in different types of scars, keloids and non-scarred tissue samples. Serum concentrations of MMP-1, MMP-2, MMP-9, TIMP-1 and TIMP-2 were analysed in sera of patients with proliferative hypertrophic scars after major burn injury and mature hypertrophic scars after elective plastic surgery, as well as in patients with keloids and normotrophic scars after elective surgery. Relative mRNA expression of MMP-2, MMP-9, TIMP-1 and TIMP 2 was measured in tissue samples of patients with hypertrophic scars, keloids and regular scar formation using a quantitative reverse-transcription polymerase chain reaction (PCR) methodology.

Material and methods

Patients

Patients with proliferative hypertrophic scars after major burn injury

The study included 19 patients (8 female, 11 male; average age: 48.2 ± 19 years) with proliferative hypertrophic scars and wound contraction after major burn injury with involving dermal or full-thickness burn wounds >5% total body surface area (TBSA, 21 ± 12 percent) and a burn scar index of 10.4 ± 3.7 points.[20] The burn trauma had been occurred more than 4 months earlier (7 ± 2 months). Steroid injections that might affect protease and TIMP activity had been performed finally more than 2 months earlier. The patients underwent correction of scar contractures.

Patients with mature hypertrophic scars after elective plastic surgery

Eighteen patients (12 female, 6 male; average age: 39.6 ± 13 years) with hypertrophic scars after reduction