

Matrix metalloproteinase-1 treatment of muscle fibrosis

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Abstract

The onset of scarring after injury may impede the regeneration and functional recovery of skeletal muscle. Matrix metalloproteinase-1 (MMP-1) hydrolyzes type I collagen and thus may improve muscle regeneration by resolving fibrotic tissue. We examined the effect of recombinant human MMP-1 on fibrosis in the lacerated gastrocnemius muscle of NOD/scid mice, allowing treatment potential to be ascertained in isolation from immune response. The efficacy of proMMP-1 and active MMP-1 were compared with or without poly(ethylene glycol) (PEG) modification, which was intended to increase the enzyme's stability. Active MMP-1 was most effective in reducing fibrosis, although treatment with proMMP-1 was also beneficial relative to controls. PEG-modified MMP-1 had minimal activity *in vivo*, despite retaining activity towards a thioester substrate. Moreover, the modified enzyme was inactivated by trypsin and subtilisin at rates comparable to that of native MMP-1. These results and those of computational structural studies suggest that modification occurs at the C-terminal hemopexin domain of MMP-1, which plays a critical role in collagen turnover. Site-specific modifications that spares catalytic and substrate binding sites while protecting susceptible proteolytic digestion sites may be beneficial. We conclude that active MMP-1 can effectively reduce muscle scarring and that its activity is related to the ability of the enzyme to digest collagen, thereby facilitating remodeling of the injured muscle.

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1. Introduction

Formation of collagen, which is the result of the body's natural wound-healing process, is a critical barrier to the regeneration of injured skeletal muscle [1–3]. The fibrotic tissue, which consists of predominately type III collagen in early stages of healing and type I collagen in later stages, is produced by fibroblasts that differentiate in response to injury [4,5]. This phase of healing is referred to as the fibro-

sis phase and occurs after the initial clearing of damaged tissue and the onset of muscle repair [6,7]. The collagen matrix blocks progenitor cells from infiltrating the site of injury where they can differentiate into new muscle tissue. Incomplete muscle regeneration due to scarring ultimately compromises the structural integrity of the tissue, prevents full recovery of function and increases the likelihood of re-injury, thus having long-lasting effects [2,6,8].

Several approaches aimed at improving muscle regeneration and functional recovery have been investigated. The growth factors insulin-like growth factor-1, basic fibroblast growth factor and nerve growth factor improve regeneration when delivered to injured muscle by promoting myoblast proliferation and fusion [9]. Leukemia inhibitory

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factor, which induces the proliferation and subsequent differentiation of progenitor muscle cells, has also been shown to enhance muscle healing in a mouse model of Duchenne muscular dystrophy [10,11]. Moreover, drugs that inhibit or down-regulate expression of key fibrosis activators, such as transforming growth factor- β 1, can enhance muscle regeneration. Suramin, decorin and γ -interferon have been reported to reduce the extent of fibrosis in injured skeletal muscle in vivo, thus leading to improved regeneration [12–15]. Although these approaches have met with clinical success, related treatments are only effective if administered prior to scar formation [16].

Because muscle injuries are often left untreated prior to scarring, a therapy that degrades scar tissue during or after completion of the normal healing process would be beneficial. The key to developing such a therapy may be an enzyme that targets and digests the fibrous scar tissue. Delivery of the exogenous enzyme to injured tissue could potentially degrade pre-existing scar tissue, thereby mimicking natural tissue remodeling processes.

Tissue remodeling is driven by matrix metalloproteinases, a family of endopeptidases that catalyze the hydrolysis of native and denatured extracellular matrix proteins [17–19]. The first member of this family, matrix metalloproteinase-1 (MMP-1), otherwise known as interstitial collagenase, was discovered in amphibian tissues, where its activity facilitates tissue resorption during metamorphosis [20]. Later, the same enzyme was found to be intimately involved in the remodeling of human tissues [21]. MMP-1 is expressed as a zymogen (proMMP-1), containing an N-terminal propeptide that must be cleaved for the enzyme to be catalytically active. Naturally, because of its specificity towards type I collagen, a major component of mature scar, MMP-1 may be effective in resolving scar in injured muscle tissue.

The potential utility of MMP-1 in the treatment of fibrotic conditions was established by Iimuro et al. [22], who employed recombinant adenovirus expressing proMMP-1 to treat liver fibrosis in a rat model. More recently, we showed that direct injection of proMMP-1 into fibrotic skeletal muscle in mice resulted in the reduction of collagen content without adversely affecting the basal lamina of uninjured muscle [23]. However, by using the pro-form of MMP-1, it is difficult to determine the amount of enzyme that is activated in the scarred tissue prior to it being enzymatically degraded or cleared via diffusion from the tissue. A more efficient approach to using MMP-1 may be to deliver the pre-activated enzyme, which would allow for direct control over the activity of MMP-1 administered. Moreover, the required treatment time may be reduced by administering active MMP-1 since the enzyme would begin to work immediately.

In this study, we have compared the efficacy of three different forms of recombinant human MMP-1 in resolving muscle fibrosis in a laceration model in NOD/scid mice including proMMP-1 and active MMP-1 (rhMMP-1). The active form of the enzyme (43 kDa) was produced by

expressing cDNA that encodes for residues 101–469 of the full-length enzyme. Additionally, a poly(ethylene glycol) (PEG)-modified form of rhMMP-1 (PEG-rhMMP-1), which was prepared by reacting the enzyme with an amine-reactive PEG (PEG-SPA; 5000 M_w), was also employed in the in vivo model. The covalent attachment of PEG may improve the enzyme's in vivo stability and thus effectiveness in degrading interfibrillar collagen.

2. Materials and methods

2.1. Materials

Recombinant MMP-1 was produced and purified by Aldevron, LLC (Fargo, ND) using cDNA from human fibroblasts from the American Type Culture Collection (Manassas, VA). Full-length human proMMP-1 was from Sigma (St. Louis, MO). PEG-succinimidyl propionate (PEG-SPA; 5000 M_w) was from Nektar Therapeutics (San Carlos, CA). Electrophoresis reagents were from Bio-Rad (Hercules, CA) and Pierce Biotechnology (Rockford, IL). The thioester substrate Acetyl-Pro-Leu-Gly-[2-mercapto-4-methyl-pentanoyl]-Leu-Gly-OC₂H₅ was from Biomol International (Plymouth Meeting, PA). Type I acid-soluble, lyophilized collagen from calf skin was from Elastin Products (Owensville, MO). All other reagents were from Sigma.

2.2. Methods

2.2.1. PEGylation of rhMMP-1

PEG-SPA was added to native rhMMP-1 in 50 mM HEPES, 5 mM calcium chloride, pH 7.5, at a PEG-to-protein molar ratio of 1000:1. The reaction was mixed at room temperature for 1 h after which residual free PEG was removed via centrifugal filtration (Millipore, Billerica, MA) with a molecular weight limit of 10 kDa. Modification of rhMMP-1 was characterized by SDS-PAGE on a 10% polyacrylamide gel. After separation, the gel was stained using the SilverSNAP Stain Kit II (Pierce Biotechnology).

2.3. Muscle laceration model

The lacerated muscle model used NOD.CB17-Prkdcscid/J mice (Jackson Laboratories, Bar Harbor, ME) as described by Menetrey et al. [24]. The mice were cared for and used in accordance with protocols approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Initially, mice were anesthetized via isoflurane inhalation. A posterior longitudinal incision was made in the skin to expose the right and left gastrocnemius muscles (GMs). The GMs were lacerated bilaterally such that the injury spanned 50% of the width and 100% of the thickness of the muscle. Following the laceration, the skin at the wound site was sutured closed and the mice were allowed

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