Matrix metalloproteinase-1 therapy improves muscle healing

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Muscle undergoes time-dependent phases of healing after injury, which ultimately results in residual fibrosis in the injured area. The use of exogenous matrix metalloproteinases (MMPs) may improve recovery after muscle injury by promoting the digestion of existing fibrous tissue and releasing local growth factors. In the current experiment, bilateral gastrocnemius (GM) lacerations were created in severe combined immunodeficient mice. Twenty-five days after injury (peak postruminal fibrosis), C2C12 cells (myoblasts) transduced with the LacZ reporter gene were injected with exogenous MMP-1 into the right GMs at the site of injury; the cells were also injected along with PBS (control) at the site of injury in the left GMs. The muscle tissues were examined histologically via X-gal, hematoxylin and eosin, and Masson’s trichrome staining. The MMP-treated limbs contained more regenerating myofibers than did the control limbs (MMP 170 ± 96 fibers, control 62 ± 51 fibers; P < 0.001). Less fibrous tissue was observed within MMP-treated muscles (MMP: 24 ± 11%, control: 35 ± 15%; P < 0.01). These results suggest that the direct injection of MMP-1 into the zone of injury during fibrosis can enhance muscle regeneration by increasing the number of myofibers and decreasing the amount of fibrous tissue.

Muscle injury; fibrosis; regeneration

SKELETAL MUSCLE PAIN, the majority of which is related to muscle strain injuries, is among the most common ailments treated by physicians in a general medical practice according to ICD-9 code billing data. (http://www.aafp.org/pfm/20050900/491cd9.html). The natural process of muscle injury repair follows a highly coordinated sequence of steps with the goal of restoring normal architecture and function. Unfortunately, the regenerative capacity of injured skeletal muscle is limited; the growth of fibrotic tissue commonly predisposes the muscle to diminished function and possible reinjury (13). Clinical experience reveals a high recurrence rate of skeletal muscle strain injuries among athletes, approaching 12% in professional soccer players and up to 30% in Australian footballers (20, 25). The majority of these recurrent injuries appears following early return to play. This vulnerable clinical time period correlates histologically with the presence of enhanced type III collagen formation and decreased myofiber regeneration (3). Experimental work has elucidated key events in the formation of fibrous tissue following muscle injury and the apparent central role of transforming growth factor-β1 (TGF-β1) in orchestrating this process (16, 17). Studies demonstrated the successful use of TGF-β1 blockade in restoring near normal architecture and function (5, 8, 9, 18). Although known therapeutic interventions may prevent the onset of fibrous changes after injury, a more likely clinical scenario would be the presentation of a patient after the onset of fibrotic remodeling with associated pain and dysfunction. This presents a challenge to the treating physician as conventional therapies of rest (12), ice (26), and anti-inflammatory medications (1) are of limited efficacy in preventing reinjury.

A better approach for treating fibrosis following muscle injury may rest with a family of proteolytic enzymes, the matrix metalloproteinases (MMPs). MMPs are widely found in plants and animals and are intimately involved in the maintenance of the extracellular matrix (ECM) and cell migration; each member of this proteolytic family has a specific affinity toward certain elements of the ECM (4). MMP collagenases, including MMP-1, -8, -13, and -18, have an ability to cleave interstitial collagen types I, II, and III, whereas MMP gelatinases (MMP-2 and -9) degrade denatured collagen (24).

After an injury, satellite cells, muscle cell precursors known to express MMP, become activated; an elevated level of MMP production is thought to result in cell migration into injured areas with subsequent differentiation and fusion of satellite cells into myofibers (7, 14, 15). While this process of regeneration proceeds for 10–14 days after injury, its abrupt end is heralded by the onset of fibrous changes within the muscle (13). This dense organization of the ECM prohibits further cell migration and regeneration, thus resulting in a terminal residual fibrotic state within the muscle.

We hypothesize that the introduction of exogenous MMP-1 into the zone of injury following skeletal muscle laceration will decrease the amount of residual fibrosis and, in turn, result in more regenerating myofibers in the area of injury.

MATERIALS AND METHODS

In Vitro

MMP-1 activity assay. To investigate the inherent activity of the MMP-1 supplied by the manufacturer (M1802, Sigma, St. Louis, MO) as a combination of active and proenzyme, an MMP activity assay was performed. Initially, latent MMP-1 was activated via incubation with trypsin (0–20 ng trypsin/ng proMMP-1) in buffer (50 mM HEPES, 10 mM calcium chloride, pH 7.5) for 1 h at 37°C. The activation reaction was terminated by the addition of soybean trypsin inhibitor (Sigma) at a minimum of a 2:1 mass ratio of inhibitor to enzyme (4). MMP collagenases, including MMP-1, -8, -13, and -18, have an affinity toward certain elements of the ECM (4). MMP collagenases, including MMP-1, -8, -13, and -18, have an ability to cleave interstitial collagen types I, II, and III, whereas MMP gelatinases (MMP-2 and -9) degrade denatured collagen (24).

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measuring the rate of hydrolysis of the peptide substrate acetyl-Pro-Leu-Gly-[2-mercapto-4-methyl-pentanoyl]-Leu-Gly-OC\textsubscript{H\textsubscript{5}} (P, 125, BIOMOL, Plymouth, PA). Free sulfhydryls formed by cleavage of the peptide reacted with DTNB (5,5'-dithiobis 2-nitrobenzoic acid) to produce a colorimetric product. Trypsin-activated MMP-1 (0–0.35 ng/\mu{l}) was added to buffer (50 mM HEPES, 10 mM calcium chloride, pH 7.5) containing substrate (100 \mu{M}) and DTNB (1 mM) (Sigma) in 96-well polystyrene microplate. The total volume of the reaction solution was 100 \mu{L}. Enzyme activity expressed as change in (optical density) per second was measured by monitoring substrate hydrolysis at 412 nm for 5 min at 37°C using a SpectraMax 340PC\textsuperscript{384} microplate spectrophotometer (Sunnyvale, CA).

Matrix-assisted laser desorption/ionization mass spectrometry analysis of the MMP-1. Matrix-assisted laser desorption/ionization (MALDI) analysis of the untreated MMP-1 preparation was carried out using an Applied Biosystems PerSeptive STR Mass Spectrometer (Foster City, CA). A saturated sinapinic acid matrix solution (0.4 ml water, 0.3 ml acetonitrile, and 1 \mu{l} trifluoroacetic acid) was mixed with the enzyme in a 1:1 volumetric ratio. The mixture was spotted onto a MALDI sample plate and incubated at RT until dry. After evaporation of the solvent, the protein spectrum was recorded. The instrument was operated in a linear mode using a 2.5 kV accelerating voltage.

Cell culture and proliferation assays. National Institutes of Health (NIH) 3T3 cells (fibroblast cell line) and C2C12 myoblasts, purchased from American Type Culture Collection (ATCC, Rockville, MD) were chosen as a well established, standardized, purified, and homogenous cell line population that represents muscle fibroblast and myoblasts. These cells were cultured with DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS, 10% horse serum, 0.5% chicken embryo extract, and 1% penicillin/streptomycin. All cells were cultured at 37°C in 5% CO\textsubscript{2}, NIH 3T3 and C2C12 cells were separately seeded at 1000 cells per well in 150 \mu{l} of DMEM supplemented with 10% FBS in a 96-well microplate. The plate was transferred to a cell culture incubator maintained at 37°C, 5% CO\textsubscript{2} for 24 h to allow cell attachment. Two groups of the plate were used for cell proliferation assay. The first group was used for cell attachment assay, and the second group was used for CellTiter 96 AQ\textsubscript{1} proliferation assay (Promega, Madison, WI) according to the manufacturer’s specifications.

In Vivo

Safety testing. Seven mice (C57BL/6J Pkrdc; Jackson Laboratory, Bar Harbor, ME) were allocated for safety dose testing of MMP-1. These 4- to 6-wk-old mice were injected with 10, 100, or 400 ng of MMP-1 in 10 \mu{l} volume PBS in the mid-gastrocnemius muscle. The solutions contained fluorescence beads (1 \mu{g}/ml, Molecular Probes, Eugene, OR) and were prepared by diluting stock with PBS. Control limbs were injected with 10 \mu{l} of PBS only. Three mice were killed 6 days after injection, and an additional four were killed 21 days after injection. The muscle was isolated and snap frozen as described below. Muscle sections were analyzed by both hematoxylin and eosin (H&E) staining as well as by immunofluorescence with anti-laminin (Chemicon, Temecula, CA) and anti-\alpha-smooth muscle actin (SMA; Sigma) antibodies to assess the integrity of the basal lamina.

MMP in injured mouse skeletal muscle. Twenty-one SCID mice (C57BL/6J Pkrdc; Jackson Laboratory) were used for histological analysis to prevent an immunogenic reaction following the intramuscular delivery of the localizing C2C12 (Lac-Z) cells. Seventeen immunocompetent mice (C57Bl/6j; Jackson Laboratory) were used for physiological testing in this experiment as no C2C12 cells were injected in this group. The histological testing group had an average age of 82 ± 33 days, average weight of 28 ± 6 g, and contained 8 females and 13 males. The physiological testing group had an average age of 48 ± 1 days, ranged in weight from 20.7–27.0 g, and consisted of males only to eliminate any variability in inherent strength due to differences in sex. The animals were housed in cages and fed with commercial pellets and water ad libitum. The policies and procedures of the animal laboratory are in accordance with those detailed by the US Department of Health and Human Services, and the Animal Research Care Committee (ARCC) of the authors’ institution approved the research protocols for these experiments (ARCC protocol #25-03). A previously reported muscle laceration injury model was employed for these experiments. However, the differences in the laceration protocol between the histological group [50% width and 100% thickness of gastrocnemius muscle (GM)] and the physiological testing group [100% width and 100% thickness of the tibialis anterior (TA) muscle] were due to constraints of the experimental apparatus and the fact that preliminary experiments had been performed with 50% width injury in the TA muscle with the healing process being significantly faster than the healing process for similarly injured GM muscle. Specifically, the healing time for 100% injured TA muscle most closely corresponded with 50% width injury (histological group) healing time for the GM muscle. Therefore, we performed a 100% width injury in the TA muscle to mimic the healing process in the 50% width injured GM.

The healing process for this model is histologically similar to strain injury models, an injury type that accounts for the most common clinically reported pattern of injury (5, 9, 10, 13, 17). The mice were anesthetized with 0.016 ml of ketamine and 0.008 ml of xylazine in 0.025 ml of PBS by intraperitoneal injection. For the histological analysis group (the SCID group of mice), lacerations were performed bilaterally on the GM through 50% of its width and 100% of its thickness at 60% of its length from its insertion. In the physiological testing group, lacerations were performed bilaterally through 100% of the width and thickness of the TA at 60% of its length from insertion to ensure a detectable difference. We used the group of 17 immunocompetent mice in accordance with prior physiological testing protocols. All laceration sites were tagged with a single 4-0 silk suture, and the skin was then closed with a 4-0 silk suture. After an average time period of 21 ± 3 days (range 21–28 days), to allow for the formation of muscle fibrosis, 100 ng of human MMP-1 (Sigma) in 10 \mu{l} PBS was injected at the tagged laceration sites of the right GMs of the SCID mice; 10 \mu{l} PBS was injected at the tagged laceration sites of the left GMs of the SCID mice. C2C12 myoblasts (5 × 10^6 cells) transduced with the Lac-Z reporter gene were injected at each site to serve as confirmation of delivery of the MMP-1 solution into the appropriate site following subsequent histological evaluation. Immunocompetent mice had the same MMP-1 therapy delivered into the TAs (without including the injection of transduced C2C12 cells). Fourteen days later, all the SCID mice were killed. The injured muscles (GMs) were isolated, mounted, and snap frozen in liquid-nitrogen-cooled 2-methylbutane. Samples were serially sectioned at 10 \mu{m} with a cryostat for histological analysis; the entire area of injury identified by the suture tag was sectioned. Immunocompetent mice were killed at 6 days (7 mice) and 21 days (10 mice), and the isolated muscles (TAs) were prepared for physiological testing.

Evaluation of MMP-1 delivery. Muscle sections from all SCID mice were fixed in 2% glutaraldehyde for 1 min and rinsed with PBS. Sections were then stained with X-gal for sufficient time to identify Lac-Z-expressing cells (13, 14); all sections were then counterstained with eosin to confirm delivery of the therapeutic intervention to the appropriate site. Only those animals with Lac-Z-positive cells in the area of injury were included for additional histological analysis.

Muscle fibrosis. Sections from each limb of each animal were washed in deionized water and stained with a modified Masson’s trichrome staining kit (IMEB, San Marcos, CA) according to the manufacturer’s specifications. This particular technique stains nuclei black, muscle red, and collagen blue (3, 14) and was previously validated through immunohistochemistry as an accurate technique for evaluating fibrous tissue within skeletal muscle (8). Five randomly selected high-powered image fields within the injured area for each limb were obtained using a Nikon Eclipse 800 fitted with a Spot camera (Diagnostic Instruments). Images were analyzed using the Northern Eclipse image analysis software (Empix, Ontario, Canada).
to measure the percent area of collagen (blue staining tissue) within the injury zone. Color threshold levels within the software program were set to isolate the blue staining regions and calculate the area of that region within the zone of injury, corresponding to the area of fibrosis. This value was expressed as a percentage of the entire cross-sectional area of the muscle. A blinded observer performed all analyses.

Muscle regeneration. Muscle sections were stained with hematoxylin and eosin. Muscle regeneration was assessed by counting the number of centronucleated myofibers (3, 6, 7, and 15). Five high-powered fields from the injury zone were analyzed for each muscle, and the average number of regenerating myofibers per field was compared with other groups. A blinded observer performed all analyses.

Physiology testing. Technical and financial constraints excluded the use of immunodeficient animals for physiological testing. Therefore, 17 immunocompetent mice were used for muscle strength testing; 7 mice were tested 6 days after MMP-1 treatment, and 10 mice were tested 21 days after MMP-1 treatment. The mice were anesthetized by using 70 mg/kg of pentobarbital sodium via intraperitoneal injection. After the mice were killed, TA muscles were removed with their bony origins and insertions intact and mounted in a vertical chamber that was constantly perfused with mammalian Ringer solution aerated with 95% O2-5% CO2 and maintained at 25°C. The origin was mounted to a glass tissue support rod, and the insertion was connected to a force transducer and length servo system (Aurora Scientific, ON, Canada). The length at which the peak force (single burst stimulation) for each muscle was generated determined the optimal testing length of each muscle. The muscle was allowed to rest and reach an equilibrium state for 5 min after the determination of the optimal testing length to reduce the effects of fatigue. The tetanic force for each muscle was then measured by stimulating the tissue with monophasic rectangular pulses of current (1 ms in duration) delivered through a platinum plate electrode 1 cm apart. Current was increased to 50% over the necessary current to obtain the peak twitch force (250–300 mA) so as to ensure the greatest stimulation. Maximal tetanic force was assessed with a stimulation frequency of 75 Hz delivered in a 500-ms-duration train. Each muscle was then weighed after tendon and bone attachments were removed. The Close formula was used to correct force values for cross-sectional area to normalize for variability in muscle size, which are expressed as specific peak force and specific tetanic force in Newtons per cross-sectional area.

Statistical analysis. Comparisons in cell proliferation between 3T3 and C2C12 cells, muscle fibrotic area, number of regenerating myofibers, and physiological testing were compared with ANOVA and two-tailed Student’s t-tests.

RESULTS

Intrinsic Activity of MMP-1 Preparation

MMP-1, like all MMPs, is expressed as a zymogen (proMMP-1) and must, therefore, be activated by disrupting the interaction between a cysteine (Cys92) molecule in the enzyme’s pro-peptide domain and a zinc molecule situated in the active site. Latent MMP-1 can be activated in vitro by incubation with organomercurial compounds and other chemical agents that modify sulphydryl groups. Nonspecific proteases such as trypsin, which cleave the pro-peptide domain, can also be used to activate latent MMP-1 (6, 24). In vivo, latent MMPs are activated by the proteolytic activity of plasmin as well as other MMPs (4).

As a first approximation to in vivo systems, the MMP-1 was not activated prior to use in all experiments described in this paper. According to the supplier, the protein preparation consisted of a mixture of zymogen and active forms of the enzyme. The level of enzymatic activity in the preparation was assayed using a colorimetric peptide substrate. Results indicated that the activity of the enzyme relative to the enzyme that had been activated with 20 ng trypsin/ng latent MMP-1 (considered to be fully active) was 12.5% (Fig. 1A). The fraction of active enzyme in the MMP-1 preparation was also approximated by MALDI mass analysis (Fig. 1B). In the MALDI spectra of the protein, peaks at 44,451 and 51,939 Da are consistent with the molecular weights of active and latent MMP-1, respectively (6, 19, 23, 27). Results of the MALDI analysis confirm, based on quantification of the relative ratio of peak heights corresponding to active and latent MMP-1, that <26% of the protein preparation was active.

Influence of MMP-1 on Cell Proliferation

To determine the effect of MMP-1 on cell proliferation, fibroblasts (NIH 3T3 cell line) and myoblasts (C2C12 cell line) were cultured with varied concentrations of MMP-1 for a period of 1–5 days. MMP-1 appeared to have no deleterious effect on NIH 3T3 or C2C12 proliferation in vitro compared...
with controls (see supplementary data Figs. S1 and S2 in the online version of this manuscript).

**MMP-1 Use is Safe in Normal Muscle in Vivo**

A large dose range of MMP-1 was injected into the GM of otherwise healthy mice with the highest dose being near the stock concentration of the MMP-1 enzyme. When the muscles were isolated 6 or 21 days after injection, the muscles showed no signs of injury or increased fibrosis at the site of MMP-1 injection as determined by the location of fluorescence beads and compared with control limbs on H&E sections. Immunofluorescent anti-laminin staining was also used to assess the integrity of the basal lamina (Fig. 2). There were no defects observed in any of the sections.

**Evaluation of MMP-1 Delivery**

Twenty-one mice had bilateral GMs injected with MMP-1/C2C12-Lac-Z cells (right side) and PBS/C2C12-Lac-Z cells (left side). Lac-Z-positive staining cells were recovered from 16 right GMs (MMP-1 therapy group) and 17 left GMs (control group). Fourteen animals had Lac-Z-positive cells within GMs injected bilaterally. The percutaneous injection of the MMP-1 into the desired target was observed >75% of the time. Three weeks after cell transplantation, the injured muscles demonstrated more regenerating myofibers in the MMP-1-treated fibrotic areas than in the control fibrotic areas (Fig. 3, A–C, \( P < 0.01 \)). The transplanted Lac-Z-positive myoblasts were unable to fuse and form myofibers in the control fibrotic areas (Fig. 3A, arrowheads), whereas transplanted Lac-Z-positive myoblasts in the MMP1-treated fibrotic areas did fuse and form myofibers (Fig. 3B, arrows). Furthermore, more Lac-Z-positive myofibers were observed in the treated group than in the control group (Fig. 3C).

**Effect of MMP-1 on Skeletal Muscle Fibrosis After Injury**

Sixteen GM sections were analyzed from the MMP-1 group and 17 GM sections from the control group to determine the percent area of fibrosis within the zone of injury. The MMP-1 therapy group had significantly less fibrosis (24 ± 11% fibrosis) compared with the control group (35 ± 15% fibrosis; \( P < 0.01 \)) as demonstrated in Fig. 4.

**Muscle Regeneration with MMP-1 Therapy**

Sixteen MMP-1-treated muscles and 17 control muscles were examined for the number of centronucleated myofibers within the zone of injury that represented the number of regenerating myofibers. The MMP-1 therapy group demonstrated enhanced numbers of regenerating (centronucleated) myofibers per high-powered field after injury; the MMP-1 therapy group had 170 ± 96 myofibers whereas the control groups (\( P < 0.01 \)) had 62 ± 51 myofibers as demonstrated in Fig. 5.

**Muscle Physiology**

The cross-sectional area specific peak force for MMP-1 and the control groups were 5.2 ± 1.7 and 5.4 ± 0.7 N/cm², respectively, at 6 days after MMP-1 therapy and 5.8 ± 0.8 and 6.0 ± 0.8 N/cm² at 21 days after therapy. The cross-sectional area specific tetanic force for the MMP-1-treated group and the control group were 16 ± 4 and 18 ± 2 N/cm² at 6 days and 19 ± 4 and 19 ± 4 N/cm² at 21 days, respectively (Fig. 6).

**DISCUSSION**

The role of MMP-1 in the treatment of fibrotic diseases has shown much success in other organ systems, most notably experimentally induced hepatic fibrosis, where MMP-1 has been shown to reverse fibrosis and begin the restoration of...
Fig. 3. Evaluation of MMP-1 delivery. Our preliminary results showed that the transplanted Lac-Z-positive myoblasts were unable to fuse and form myofibers in the control scarred areas (A, arrowheads). Whereas, the transplanted Lac-Z-positive myoblasts in the MMP-1-treated scarred areas fused and formed myofibers (B, arrows). Furthermore, we observed more Lac-Z-positive myofibers in the MMP-1-treated group rather than in the nontreated muscle tissues (C), $P < 0.01$.

Fig. 4. Effect of MMP-1 on skeletal muscle fibrosis after injury. Two weeks after therapy, the MMP-1 treatment group had significantly less fibrosis (24 ± 11% fibrosis; A) compared with the control group (35 ± 15% fibrosis; $P < 0.01$; B). A single injection of MMP-1 directly into the site of fibrous tissue formation is able to digest overgrown elements of the ECM (C).
sition of connective tissue that supports these myofibers. After skeletal muscle injury, the deposition of connective tissue predominates while the regeneration of myofibers is limited. The predominant components of the ECM connective and fibrous tissue are collagen types I and III (2). MMP-2 and -9 have minimal effect on these types of collagen. The introduction of MMP-1 (collagenase), which specifically targets collagen I and III, may aid regeneration by removing this blockade. We postulated that the removal of this barrier will reestablish an environment conducive to muscle regeneration. Myoblasts require MMPs for migration, and it is known that satellite cells express gelatinases to cross the basement membrane (7, 15). Since satellite cells are capable of producing MMPs that are relatively ineffective against scar tissue, it would be reasonable to hypothesize that these precursors would be prevented from migrating, fusing, and contributing to the reparative process. Thus it is possible that the elimination of this barrier would aid in repair through either the digestion of scar tissues or the activation of muscle cell migration and invasion.

The first portion of this study was to assess the affect of MMP-1 delivery on normal muscle tissue by injecting a broad range of doses, ranging from 10 to 400 ng, into normal skeletal muscle. Even in the highest concentrations, MMP-1 did not show any negative effect to either muscle or to the structural integrity of the ECM that surrounds normal muscle fibers. These data are corroborated by the lack of effect demonstrated by MMP-1 on the proliferation of myoblasts and fibroblasts in culture.

The technique of intramuscular injection of MMP-1 directly into the zone of injury as performed in this model is technically challenging. The simultaneous intramuscular injection of C2C12 myoblasts transduced with the Lac-Z reporter gene and the MMP-1 or the injection of our placebo (MMP-1 and PBS control) into each limb served as a biologically observable confirmation that the MMP-1 reached its intended target. Only those samples containing cells that stained positively for Lac-Z in the zone of injury (nearly 80%) were analyzed. This eliminated the inclusion of samples in which the MMP-1 may have been erroneously targeted.

In testing the main hypothesis of this study, we demonstrated that the use of a single dose of 100 ng of MMP-1 delivered directly into the fibrous tissue produced a significant...
reduction of residual collagen in the zone of injury. This, in turn, appears to have led to a significant improvement in the number of regenerating myofibers. Thus MMP-1 delivered into the area of fibrosis that develops following skeletal muscle injury results in the dual therapeutic effect of decreasing residual fibrous tissue while simultaneously enhancing the process of repair.

While the presumed biological half-life of MMP-1 in vivo is no longer than a few hours, our results indicated a remarkable outcome after a relatively short exposure to MMP-1. This may be related to the dose of MMP-1, the timing of therapy, or the timing of exposure. Similarly, it may also be related to the choice of reagent of the MMP-1 mixture rather than fully activated MMP-1. The activity of the MMP-1 preparation, supplied and administered as a mixture of active and pro-enzyme, was determined to be a ratio of ~1:3. While the true enzyme kinetics of our dose delivery was not specifically measured, one may speculate that the activated portion may have initiated the granulation tissue remodeling cascade, whereas the bulk of the enzyme, in its latent form, may have allowed for a slow and sustained release in vivo through the plasminogen/urokinase system (4).

The injection of MMP-1 3 wk after a laceration, while empirically selected, represents the time point at which the regenerative phase has declined substantially and the deposition of fibrous tissue appears to be at its peak. This may represent the time of highest potential for improved regeneration and the formation and degradation of the different elements of the ECM as they are in a dynamic state. Various biological factors such as growth factors and satellite cells are still active; the tissue is still poised for regeneration and its plasticity can be manipulated more easily. Although beyond the scope of this study, the release of intrinsic growth factors within the ECM might aid initially in the regenerative process. Other authors observed the relationship between MMP and the local release of various growth factors (21).

The histological response to MMP-1 injection into fibrous skeletal muscle demonstrates promising results; however, the goal of this therapeutic intervention is to also restore the functional status of the tissue. There was no difference demonstrated in regard to peak forces generated by control and experimental groups at either time point. As for the measured tetcatic force produced by the muscles, we observed no change in the control group between 6 and 21 days after PBS injection. This suggests that there is little improvement in the functional status of untreated injured muscle between 4 and 6 wk after a laceration injury. One might speculate that any functional recovery regained after injury is obtained by 4 wk after healing has begun and will remain unchanged, mirroring the end point of the regenerative phase observed during histology. Although there were no statistically significant differences between the control and MMP-1-treated groups, interesting trends were observed. Six days following MMP-1 therapy, there was a reduction in the tetcatic force produced in the treated group compared with the control group. This may be a result of the active degradation and subsequent remodeling initiated by MMP-1. At this early time point, the tissue has most likely lost tensile properties in the area of previous fibrous tissue, which is mirrored as reduced force production. Three weeks after treatment, as the tissue matures and the zone of injury is populated by regenerating myofibers (as observed histologically), the force produced in the MMP-1-treated group improves from that measured at 1 wk and returns to the level of the control group. This improvement most likely represents a delay in the correlation of histological findings and functional findings, a phenomenon commonly seen in skeletal muscle injury studies. As the regenerating muscle continues to regenerate and mature, the force produced may eventually exceed that of the control group; however, this is speculative and requires future study.

Skeletal muscle fibrosis following an injury represents a difficult therapeutic dilemma. Traditional therapies have proven ineffective and experimental therapies thus far have focused on prophylactic treatment of injuries to prevent fibrosis. Our work with MMP therapy might represent an initial stage in addressing this problem. This study demonstrated that a single dose of MMP-1 delivered intramuscularly into the site of fibrosis effectively reduces residual fibrosis and enhances muscle regeneration. Continued work on this topic may yield more strategies to optimize dose, delivery, and timing, which might eventually lead to an improvement in functional status.

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