MICE DEFICIENT IN PLASMINOGEN ACTIVATOR INHIBITOR-1 HAVE IMPROVED SKELETAL MUSCLE REGENERATION

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ABSTRACT

Skeletal muscle possesses a remarkable capacity for regeneration. Although the regulation of this process at the molecular level remains largely undefined, the plasminogen system appears to play a critical role. Specifically, mice deficient in either urokinase-type plasminogen activator (uPA) or plasminogen demonstrate markedly impaired muscle regeneration following injury. In the present study, we tested the hypothesis that loss of the primary inhibitor of uPA, plasminogen activator inhibitor-1 (PAI-1), would improve muscle regeneration. Repair of the extensor digitorum longus muscle was assessed following cardiotoxin injury in wild-type, uPA deficient (uPA-/-) and PAI-1 deficient (PAI-1-/-) mice. As expected, there was no uPA activity in the injured muscles of uPA-/- mice, and muscles from these transgenic animals demonstrated impaired regeneration. On the other hand, uPA activity was increased in injured muscle from PAI-1-/- mice to a greater extent than wild-type controls. Furthermore, PAI-1-/- mice demonstrated increased expression of MyoD and developmental myosin following injury as well as accelerated recovery of muscle morphology, protein levels and muscle force compared to wild-type animals. The injured muscles of PAI-1 null mice also demonstrated increased macrophage accumulation, contrasting with impaired macrophage accumulation in uPA deficient mice. The extent of macrophage accumulation correlated with both the clearance of protein following injury and the efficiency of regeneration. Taken together, these results indicate that PAI-1 deficiency promotes muscle regeneration, and this protease inhibitor represents a therapeutic target for enhancing muscle regeneration.

Keywords: Muscle injury, muscle repair, urokinase-type plasminogen activator, muscle inflammation, macrophage
INTRODUCTION

Skeletal muscle possesses an extraordinary capacity for regeneration following injury caused by disease, trauma or intense exercise. The process of regeneration can be separated into three overlapping phases. The first phase is marked by the accumulation of inflammatory cells that remove tissue debris and may play further critical roles in regeneration [10; 18; 24; 40]. The second phase involves the activation and proliferation of quiescent muscle precursor cells called satellite cells, which migrate and fuse to replace damaged fibers [7; 16; 32]. The third phase involves the growth of newly formed fibers and remodeling of damaged fibers, a process highlighted by synthesis of proteins to form new sarcomeres and associated structures. Although much has been learned about the events involved in muscle regeneration, the molecular mechanisms that regulate this process remain largely undefined.

Many studies have demonstrated that the plasminogen system plays an important role in the repair of skeletal muscle as well as other tissues [19; 31; 33-35; 43]. The plasminogen system is a serine protease cascade that includes the urokinase-type plasminogen activator (uPA). The proteolytic activity of uPA is tightly regulated by its primary inhibitor, plasminogen activator inhibitor-1 (PAI-1). The classic function of uPA is to activate plasminogen to plasmin, and plasmin possesses broad-spectrum protease activity against a variety of extracellular matrix molecules including fibrin, fibronectin and proteoglycans [37]. Plasmin activity may be required to remove fibrin clots following injury, and clearing a path to allow migration of different cells to the site of injury. uPA and PAI-1 may also contribute to tissue repair through pathways that do not involve plasminogen, including the regulation of growth factor activity and cell migration [6; 27; 29; 36; 41].
Recent studies support the idea that uPA and PAI-1 play critical roles in muscle regeneration. *In vitro*, uPA appears to stimulate satellite cell proliferation, migration and fusion [2; 12; 13; 25]. *In vivo*, muscle injury results in increased expression of uPA [11; 19], and mice with a targeted deletion of uPA or plasminogen show impaired expression of myogenic factors and markedly deficient muscle regeneration [19; 38]. Muscle injury also results in increased expression of PAI-1 [9; 11; 44] which, through the inhibition of uPA activity, could limit the efficiency of muscle regeneration. Thus, the main hypothesis of this study is that PAI-1 deficiency will result in increased uPA activity and improved muscle regeneration. On the other hand, antibodies that block PAI-1’s interaction with uPA were found to impair satellite cell migration and fusion *in vitro* [2; 8]. Based on this result, one could speculate that the presence of PAI-1 in the injured muscle is required for efficient *in vivo* regeneration. Thus, the alternative hypothesis for this study is that PAI-1 deficiency will result in impaired muscle regeneration. The available data make it difficult to predict whether PAI-1 deficiency would promote or impair muscle regeneration. To explore the effect of PAI-1 on muscle repair, we injured the extensor digitorum longus muscle in wild-type, uPA deficient (uPA-/-), and PAI-1 deficient (PAI-1-/-) mice, and assessed the efficiency of repair with measures of muscle function, morphology, and protein levels. We also assessed inflammatory cell accumulation to determine whether PAI-1 regulates the inflammatory response following injury.

**MATERIALS AND METHODS**

**Mice.** uPA and PAI-1 null mice were originally generated by Dr. Peter Carmeliet [4; 5]. Both knockout strains had been bred into a C57BL/6 background for a minimum of 8 generations. C57BL/6 mice (wild-type) were obtained from Harlan Biosciences. Experiments were
performed on mice aged 10-12 weeks. All experimental procedures were approved by the University Animal Care and Use Committee of the University of Michigan and the Animal Care Committee at the University of Illinois at Chicago.

**Cardiotoxin injection and muscle function assay.** Extensor digitorum longus (EDL) muscle force measurements and cardiotoxin injuries were performed using standard procedures [17; 44]. Briefly, mice were anesthetized with an intraperitoneal injection of tribromoethanol (avertin; 400 mg/kg), and fixed on a heated Plexiglas platform (37°C). Pre-injury *in situ* maximal isometric force was measured as described [17] and then 10 µl cardiotoxin (10 µM; Calbiochem) was injected into the EDL muscle in 3 locations to ensure distribution of cardiotoxin throughout the muscle. After injection, the skin incision was closed with 7-0 nylon suture, and procedures repeated on the contralateral muscle. Mice were allowed to recover and then subjected to a post-injury muscle function test using the same muscle testing procedures at 1 to 20 days post-injury.

**Sample preparation.** Following the terminal muscle function assay, EDL muscles were dissected, blotted dry, weighed, and processed for histological or biochemical analyses. For histological assays, muscles were mounted in tissue freezing media, and frozen in isopentane chilled with dry ice. For biochemical assays, muscles were homogenized in buffer A (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 5 mM EDTA) supplemented with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 µM leupeptin and 0.3 µM aprotinin). Samples were centrifuged at 15,000 g for 10 minutes at 4°C, the soluble fraction collected, and the insoluble fraction resuspended in buffer A.

**Histology.** Cross-sections were cut at 10 µm from the mid-belly of each EDL muscle and either stained with haematoxylin and eosin for morphological analysis or processed for
immunohistochemistry for inflammatory cell analysis. For morphological analysis, images of two fields using a 20x objective were captured for each muscle section (Nikon Labphot-2 with Spot Diagnostics imaging). For each field, fibers were classified as normal, damaged or regenerating, and the number and area of these fibers were recorded. Normal fibers were identified as those demonstrating no clear evidence of damage, damaged fibers identified as those demonstrating overt damage (e.g. infiltration of inflammatory cells, pale and/or discontinuous staining of the cytoplasm, or a substantially swollen appearance), and regenerating fibers were identified as those containing centrally located nuclei without evidence of damage. Damaged area for each muscle section was estimated by subtracting the summed area of normal and regenerating fibers from the total area of each field.

Analysis of inflammatory cells was performed using immunohistochemical methods as described [28]. Neutrophils were labeled with a Ly6G antibody (1:100; Pharmingen) and macrophages were labeled with an F4/80 antibody (1:100; Serotec), followed by incubation with biotinylated mouse adsorbed anti-rat IgG (1:200; Vector Laboratories) and then avidin D horseradish peroxidase (1:1000). Sections were then developed with the AEC kit (Vector Laboratories). The number of positive cells were counted in two entire sections for each muscle with the aid of an eyepiece grid and normalized to the volume of muscle sampled (area of section x section thickness; 10 µm).

Zymography. uPA activity in soluble fractions of EDL muscle homogenates was assessed using zymography as described [34]. Briefly, protein content of the soluble fraction was determined [23] and 10 µg of each sample was then separated on 10% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) containing α-casein (4 mg/ml) and human Glu-plasminogen (20 µg/ml). Gels were then washed in 1% Tween-20 in PBS for 1 hour at 37C and
then incubated in 0.1% Tween-20 in PBS overnight at room temperature. Finally, gels were stained with Coomassie blue dye, then destained. Murine uPA activity was identified by the position of its lytic band (45 kD) relative to that of a recombinant human uPA standard (55 kD).

**Fibrinolysis assay.** uPA activity was also measured in EDL muscle homogenates quantitatively using a microplate fibrinolysis assay. Samples (10 µg protein) were mixed with plasminogen (0.5 µM final concentration) and fibrinogen (8 µM final concentration). Thrombin was added to a final concentration of 0.4 U/ml to initiate fibrin clot formation. Clot formation was followed by the increase in absorbance at 405 nm and fibrinolysis resulted in the subsequent decrease in OD405. The time required for the OD405 to fall to one-half its maximal value was taken as a measure of uPA activity. Controls were run without addition of thrombin and without addition of sample.

**Protein levels.** Aliquots of both soluble and insoluble fraction of EDL muscle homogenates were mixed with concentrated reducing sample buffer, boiled, and protein concentrations and total muscle protein content determined [23]. Equal amounts of protein (1 µg for myosin heavy chain-fast isoform; 10 µg for developmental myosin heavy chain) were separated on 8% SDS-PAGE gels and transferred to nitrocellulose membranes. After transfer, membranes were stained with Ponceau-S to confirm equal loading and then blocked in 5% milk overnight. Membranes were incubated with primary antibodies against MyoD (1:500; Novocastra), developmental myosin heavy chain (1:500; Novocastra) or fast isoform myosin heavy chain (1:5000; Sigma), washed and then incubated with secondary antibody conjugated to horseradish peroxidase (1:25,000; Pierce). Following another wash, protein bands were detected using enhanced chemiluminescence (Amersham) and band densities were determined by image
analysis (BioRad FluorS). Individual band densities were normalized to the average of the control lanes for wild-type mice and multiplied by 100%.

**Data analysis.** Values reported in the text and Figures are given as means ± standard errors. Data were compared over different mouse strains and time points using two-way analysis of variance. Post-hoc tests were performed using the Student-Neumann-Keuls test. The 0.05 level was taken to indicate statistical significance.

**RESULTS**

**uPA activity.** Casein zymograms were used to assess the time course of uPA activity in injured muscle (Figure 1). Although uPA was barely detectable in non-injured muscle, proteolytic activity was markedly increased on days 1, 3 and 5 post-injury in both wild-type and PAI-1 null mice. Thereafter, uPA activity decreased to near control levels by day 20 post-injury. As expected, muscle homogenates from uPA null mice showed no uPA activity at any time point. Of note, muscle injury also resulted in an increase in activity of tissue-type plasminogen activator (tPA) in all mouse types, with a time course that lagged behind uPA. A microplate fibrinolysis assay was performed to quantify uPA activity in muscles from wild-type and PAI-1 null mice at day 1 post-injury. Injured muscle from PAI-1 null mice showed greater fibrinolytic activity (fibrin clot dissolution \( t_{1/2} = 211\pm15 \) minutes) than muscle from wild-type mice (\( t_{1/2} = 374\pm54 \) minutes). Increased fibrinolytic activity in injured muscle of PAI-1 null mice is consistent with loss of uPA inhibition.

**Muscle function.** Muscle force production was measured following injury to assess recovery of muscle function (Figure 2). Prior to injury, specific tension (muscle force divided by physiological cross sectional area) was not different between muscles of wild-type, uPA null,
and PAI-1 null mice (~22 N/cm^2), indicating that muscle function did not differ between genotypes prior to injury. On days 1 and 3 post-injury, maximal isometric muscle force for each mouse strain was reduced to ~20% of control values, indicating that cardiotoxin-induced injury to the EDL muscle was independent of genotype. On day 5 post-injury, only muscles from PAI-1 null mice showed a significant recovery of force. By days 10 and 20 post-injury, wild-type mice showed substantial improvement, and PAI-1 null mice continued to exhibit significantly greater recovery of force than wild-type. On the other hand, uPA null mice showed little, if any, recovery over the time course of the experiment.

**Muscle morphology.** Haematoxylin and eosin stained cryosections were used to assess changes in muscle morphology following injury (Figure 3). In non-injured muscle, muscle fiber morphology showed no overt differences between wild-type, uPA null and PAI-1 null mice. Furthermore, fiber area was not different between genotypes (923 ± 36 μm), suggesting no differences in muscle fiber development. On day 3 following cardiotoxin injection, cross-sections of EDL muscles from each mouse type showed substantial fiber damage and edema. The amount of damage (~90% of total area in cryosections) was indistinguishable between the three genotypes. On day 5 post-injury, wild-type mice demonstrated a significant reduction in damaged area. At the same time point, PAI-1 null mice showed a significantly greater reduction in damaged area compared to wild-type mice, consistent with greater force production. By days 10 and 20 post-injury, damaged area in both wild-type and PAI-1 null mice approached zero. In contrast, uPA null mice showed little, if any, reduction in damaged area up to 20 days post-injury.

**Protein levels.** Levels of total protein and of the fast isoform of myosin heavy chain were measured to assess muscle protein turnover following muscle injury (Figure 4). In muscles from
both wild-type and PAI-1 null mice, total protein and fast myosin levels decreased rapidly following injury, reaching a nadir on day 3. Muscles from uPA null mice demonstrated little change in protein levels during this time period, suggesting impaired clearance of damaged tissue. During the subsequent phase of regeneration, muscle of wild-type mice demonstrated increases in total protein and fast myosin to near normal levels by day 20 post-injury. PAI-1 null mice showed accelerated recovery of muscle protein levels compared to their wild-type counterparts, consistent with accelerated recovery of function and morphology. In contrast, uPA null mice exhibited a gradual loss of muscle protein to 20 days. Together, these data show that protein turnover was impaired in injured muscle of uPA null mice, and that protein accretion was accelerated during the regenerative phase in PAI-1 null mice.

Myogenesis. Myogenesis following injury was assessed by measuring expression of the myogenic transcription factor MyoD and the developmental isoform of myosin heavy chain using Western blots, and by counting numbers of central nucleated fibers in muscle sections (Figure 5). On day 1 post-injury, protein expression of MyoD was significantly greater in injured muscle of PAI-1 null mice compared to wild-type mice. Similarly, the expression of developmental myosin was significantly greater in injured muscle of PAI-1 null mice than in wild-type mice on day 5 post-injury. In contrast, developmental myosin protein expression was significantly lower in injured muscle of uPA null mice than in muscle of wild-type mice. Also on day 5 post-injury, muscle sections from wild-type and PAI-1 null mice showed substantial numbers of central nucleated fibers. Mean values for PAI-1 null mice were greater than those for wild-type mice, although the difference did not reach statistical significance (p = 0.08). On the other hand, muscle sections from uPA null mice showed significantly fewer central nucleated fibers following injury than wild-type mice.
Inflammatory cells. Immunohistochemical methods were used to quantify inflammatory cell accumulation in injured muscle (Figure 6). Neutrophil accumulation peaked on day 1 post-injury in each mouse strain, and the number of neutrophils did not differ significantly between genotypes. In muscle of wild-type mice, macrophage accumulation peaked on day 3 post-injury and then declined by day 5. In muscles of PAI-1 null mice, there was a trend towards increased macrophage accumulation on day 3 (p = 0.08) and there was significantly accumulation at day 5, compared to wild-type mice. In contrast, the accumulation of macrophages was significantly impaired in injured muscles of uPA null mice compared to wild-type mice; the number of macrophages was less than 5% of wild-type values on day 3 post-injury.

DISCUSSION

Although skeletal muscle regeneration is typically efficient, we have found that manipulation of the plasminogen system can further enhance muscle repair. Specifically, mice deficient in PAI-1 demonstrated increased MyoD and developmental myosin expression following injury, and accelerated recovery of muscle morphology, protein levels and function compared to wild-type mice. The more rapid muscle regeneration in PAI-1 null mice was also associated with an increased accumulation of macrophages following injury. As anticipated from previous studies [19; 38], we also found that mice deficient in uPA have a nearly complete abrogation of muscle fiber regeneration and macrophage accumulation following injury. Taken together, our data indicate that PAI-1 deficiency accentuates plasminogen activator activity and promotes muscle regeneration.

The balance of uPA and PAI-1 may influence muscle regeneration through many pathways. Previous in vitro studies have demonstrated that uPA and PAI-1 can directly
influence myogenesis. Exogenous uPA stimulated proliferation of human satellite cells, and antibodies that prevented interaction between uPA and its receptor uPAR blocked this increase in proliferation [12]. These same antibodies also blocked an fibroblast growth factor (FGF)-stimulated increase in satellite cell proliferation, indicating that the uPA system may play a role in growth factor-mediated proliferation [13]. In addition, exogenous uPA stimulated migration of human satellite cells through a matrix composed of basement membrane components (Matrigel), and antibodies that blocked binding of uPA to uPAR inhibited both exogenous uPA-dependent and exogenous FGF-dependent migration [12; 13]. Finally, uPA activity may be required for satellite cell fusion, as \textit{in vitro} studies have shown that uPA activity is increased during this process, and antibodies that block the catalytic activity of uPA or block the interaction of uPA with uPAR inhibited fusion [2; 25]. Taken together, these \textit{in vitro} results demonstrate that uPA is capable of modulating satellite cell proliferation, migration and fusion. Furthermore, these processes appear to depend on both the proteolytic (e.g. plasmin formation, growth factor activation) and the non-proteolytic (e.g. uPA receptor binding, matrix binding) functions of uPA. In the present study, injured muscle of PAI-1-/- mice demonstrated increased uPA activity and increased expression of developmental muscle proteins compared to wild-type mice. Whether the enhanced expression of these proteins resulted from a direct effect of uPA on muscle cells, and whether it required proteolytic or non-proteolytic uPA functions, remain to be determined.

In addition to influencing myogenesis directly, uPA and PAI-1 may modulate regeneration by regulating the inflammatory response. uPA, PAI-1 and the uPA receptor have been reported to be important in the regulation of inflammatory cell activation and infiltration into injured tissues, through both proteolytic and non-proteolytic mechanisms [1; 6; 14; 15]. In
the present study, macrophage accumulation was largely abrogated in uPA-/- mice, whereas PAI-1 deficiency augmented this process. Impaired macrophage accumulation in damaged muscle has been previously reported in uPA-/- and plasminogen-/- mice [19; 38]. The mechanism by which macrophage accumulation is altered in these transgenic animals is unclear, but differences in the level of activation, the ability to migrate and/or the levels of chemotactic factors could be important. Following muscle injury, macrophages appear to be critical for both clearance of damaged tissue and for promoting muscle regeneration [10; 18; 24; 40]. In support of the latter role, isolated macrophages, presumably through the release of soluble factors, have been found to stimulate satellite cell proliferation and migration in vitro [3; 22; 30].

uPA and PAI-1 may also modulate muscle regeneration through the regulation of extracellular matrix turnover. Through the activation of plasminogen, uPA promotes the degradation of several extracellular matrix proteins, including fibrin, fibronectin, and proteoglycans [37]. Furthermore, plasmin can also activate a subset of matrix metalloproteinases, which can degrade other matrix components, including collagen, elastin and laminin [6; 26]. Previous investigators [19; 38] have reported the persistence of intramuscular fibrin deposition following injury in both uPA-/- and plasminogen-/- mice. Furthermore, systemic defibrinogenation with ancrad limited fibrin deposition and, in turn, improved muscle regeneration in these transgenic mice. The removal of extracellular matrix barriers may be necessary for efficient macrophage and satellite cell migration during muscle regeneration. Whether or not impaired fibrin removal is the primary cause of delayed regeneration in uPA-/- mice requires further study.

In addition to matrix turnover, uPA and PAI-1 may influence muscle repair by regulating the bioactivity of a variety of growth factors. For example, plasmin can activate latent
transforming growth factor-β (TGF-β) and can enhance the bioactivity of FGF-2 by releasing it from its extracellular matrix reservoir [20; 29]. uPA is also capable of activating growth factors directly, without involvement of plasminogen. Specifically, uPA can cleave inactive single chain hepatocyte growth factor (HGF) in order to generate the active two chain form [21; 27]. Since HGF and FGF are thought to play key roles in regulating satellite cell activity [39; 42], regulation of their activity could be an important mechanism by which uPA and PAI-1 influence muscle repair.

Tissue-type plasminogen activator (t-PA) activity was also increased following cardiotoxin injection in wild-type mice with a time course that lagged behind the increase in uPA activity. Based on this finding, it is possible that tPA also participates in the regenerative response following muscle injury. However, a previous study [19] demonstrated that muscle repair is not qualitatively different in tPA deficient mice compared to wild-type controls, demonstrating that tPA is not required for efficient regeneration.

Efficient muscle regeneration helps to restore muscle function following injury, but repair is inadequate to prevent progressive degeneration in muscle diseases such as Duchenne Muscular Dystrophy. Furthermore, the efficiency of muscle repair declines with age. Our results suggest that PAI-1 may be an attractive therapeutic target in patients with insufficient regenerative capacity in their skeletal muscles. The mechanism by which PAI-1 deficiency accelerates muscle regeneration remains unclear and requires further study. Because macrophages appear to be critical in this process, we speculate that PAI-1 deficiency may promote muscle repair, at least in part, by modulating the inflammatory response following injury. This hypothesis is currently being addressed in ongoing experiments. A further definition of the mechanism(s) by which uPA and PAI-1 influence muscle regeneration may provide insight into improving regeneration
in muscle diseases and in the aging population. Furthermore, since uPA and PAI-1 have been implicated in the repair of other tissues, elucidating the mechanisms of action of this system in muscle regeneration may provide insights into improving the repair of other tissues that do not efficiently regenerate after injury (e.g. heart).
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REFERENCES


23. Minamide LS and Bamburg JR. A filter paper dye-binding assay for quantitative
determination of protein without interference from reducing agents or detergents. *Anal Biochem*

24. Mitchell CA, McGeachie JK and Grounds MD. Cellular differences in the regeneration of
murine skeletal muscle: a quantitative histological study in SJL/J and BALB/c mice. *Cell Tissue*

plasminogen activator (uPA) abrogates myogenesis in vitro. *Thromb Haemost* 77: 526-534,
1997.


Y, Tsubouchi H, Blasi F and Comoglio PM. Extracellular proteolytic cleavage by urokinase is

28. Pizza FX, Koh TJ, McGregor SJ and Brooks SV. Muscle inflammatory cells after passive
stretches, isometric contractions, and lengthening contractions. *J Appl Physiol* 92: 1873-1878,
2002.

29. Rifkin DB, Mazzieri R, Munger JS, Noguera I and Sung J. Proteolytic control of growth

30. Robertson TA, Maley MA, Grounds MD and Papadimitriou JM. The role of
macrophages in skeletal muscle regeneration with particular reference to chemotaxis. *Exp Cell*


FIGURE CAPTIONS

1. uPA activity is upregulated following muscle injury. Top: Gel zymography analysis of uPA and tPA activities in muscle of wild-type (WT), uPA null (uPA-/-) and PAI-1 null (PAI1-/-) mice at different time points following cardiotoxin injury. PA activity forms clearing bands in zymogram gels. Note the increase in uPA activity following muscle injury in wild-type and PAI1-/- mice and the lack of clearing bands for uPA-/- mice. Note also the increase in tPA activity that lags the increase in uPA. The identity of uPA and tPA bands was determined using protein standards (not shown).

2. Recovery of muscle function following injury is accelerated in PAI-1 null mice and impaired in uPA null mice. Maximal isometric force was measured before, and at different time points after, cardiotoxin injury of the EDL muscle. Muscle force expressed as percentage of pre-injury value. Data are means with standard error bars (n = 4-10 per time point). Two-way analysis of variance showed significant interaction effect of mouse strain x day after injury. Mean value significantly greater than that for 3 days (within strain), mean value significantly greater than that for wild-type (within day), mean value significantly smaller than that for wild-type (within day).

3. Recovery of normal morphology following injury is accelerated in muscle of PAI-1 null mice and impaired in muscle of uPA null mice. Top: Hematoxylin and eosin stained cryosections from uninjured control muscle (C) and from muscle at different time points after injury in wild-type (WT), uPA null (uPA-/-) and PAI-1 null (PAI1-/-) mice. Note the robust regenerative response in muscle of wild-type and PAI-1 null mice, and the absence of regeneration in muscle of uPA null mice. Bottom: Damaged area estimated by subtracting area of normal and regenerating fibers from total area of 2 fields per section. Damaged area
expressed as percentage of total area. Data are means with standard error bars (n = 4-8 per time point). Two-way analysis of variance showed significant interaction effect of mouse strain x day after injury. Mean value significantly greater than that for 3 days (within strain), mean value significantly greater than that for wild-type (within day), mean value significantly smaller than that for wild-type (within day).

4. Recovery of total protein and adult myosin levels following injury is accelerated in muscle of PAI-1 null mice and impaired in muscle of uPA null mice. Left: Total protein levels in injured muscle of wild-type (WT), uPA null (uPA-/-) and PAI-1 null (PAI1-/-) mice at different time points after injury were normalized to protein level in non-injured control muscle. Right: Fast myosin heavy chain (MHCf) protein levels measured by densitometry of western blots (top) and expressed relative to levels in non-injured control muscle. Data are means with standard error bars (n = 4-8 per time point). Two-way analysis of variance showed significant interaction effect of mouse strain x day after injury for both total protein and fast myosin levels. Mean value significantly different from control (within strain), mean value significantly greater than that for wild-type (within day), mean value significantly smaller than that for wild-type (within day).

5. Expression of MyoD and developmental myosin in muscle of wild-type (WT), uPA null (uPA-/-) and PAI-1 null (PAI1-/-) mice. Top: MyoD protein levels at 1 day post-injury were measured by densitometry of Western blots and expressed relative to levels in muscle of wild-type mice. Middle: Developmental myosin heavy chain (MHCd) protein levels at 5 days post-injury measured by densitometry of Western blots and expressed relative to levels in muscle of wild-type mice. Bottom: Number of central nucleated fibers counted per cryosection for muscles of WT, uPA-/- and PAI1-/- mice at 5 days post-injury, and normalized to total area of two fields.
per section. Central nucleated fibers were considered to be regenerating fibers. Data are means with standard error bars (n = 4-8 per time point). One-way analysis of variance showed effect of mouse strain for both central nucleated fibers and developmental myosin expression. 1 Mean value significantly smaller than that for wild-type, 2 mean value significantly greater than that for wild-type.

6. Macrophage accumulation is impaired in injured muscle of uPA null mice and increased in injured muscle of PAI-1 null mice. Top: Neutrophil accumulation assessed at different time points following muscle injury in wild-type (WT), uPA null (uPA-/-) and PAI-1 null (PAI1-/-) mice by immunostaining for the neutrophil specific Ly6G antigen, counting number of Ly6G+ cells per cryosection, and normalizing to cryosection volume. Bottom: Macrophage accumulation assessed by immunostaining for the macrophage specific F4/80 antigen, counting number of F4/80+ cells per cryosection, and normalizing to cryosection volume. Data are means with standard error bars (n = 4-8 per time point). Two-way analysis of variance showed significant effect of day for neutrophils (all time points significantly greater than controls), and significant interaction effect of mouse strain x day after injury for macrophages. 1 Mean value significantly different from control (within strain), 2 mean value significantly greater than that for wild-type (within day), 3 mean value significantly smaller than that for wild-type (within day).
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