Antifibrotic effects of suramin in injured skeletal muscle after laceration

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Chan, Yi-Sheng, Yong Li, William Foster, Takashi Horaguchi, George Somogyi, Freddie H. Fu, and Johnny Huard. Antifibrotic effects of suramin in injured skeletal muscle after laceration. J Appl Physiol 95: 771–780, 2003. First published May 2, 2003; 10.1152/japplphysiol.00915.2002.—Muscle injuries are very common in traumatology and sports medicine. Although muscle tissue can regenerate postinjury, the healing process is slow and often incomplete; complete recovery after skeletal muscle injury is hindered by fibrosis. Our studies have shown that decreased fibrosis could improve muscle healing. Suramin has been found to inhibit transforming growth factor (TGF)-β1 expression by competitively binding to the growth factor receptor. We conducted a series of tests to determine the antifibrotic effects of suramin on muscle laceration injuries. Our results demonstrate that suramin (50 μg/ml) can effectively decrease fibroblast proliferation and fibroblast-protein expression (α-smooth muscle actin) in vitro. In vivo, direct injection of suramin (2.5 mg) into injured murine muscle resulted in effective inhibition of muscle fibrosis and enhanced muscle regeneration, which led to efficient functional muscle recovery. These results support our hypothesis that prevention of fibrosis could enhance muscle regeneration, thereby facilitating more efficient muscle healing. This study could significantly contribute to the development of strategies to promote efficient muscle healing and functional recovery.

Muscle injury; fibrosis; transforming growth factor-β1; suramin; muscle regeneration

Muscle injuries, some of the most frequently occurring injuries in sports medicine, pose challenging problems in traumatology. Muscle injury can occur via a variety of mechanisms, ranging from direct mechanical deformation (such as muscle laceration, strain, or contusion) to indirect damage related to ischemia and neurological dysfunction (12, 33, 39, 40, 49, 62). Injured muscle usually undergoes a healing process of degeneration and regeneration comprising three phases (25, 28, 44). The destruction phase is characterized by formation of a hematoma, necrosis of muscle tissue, degeneration, and inflammatory-cell response. The repair phase includes phagocytosis of the damaged tissue, regeneration of the striated muscle, production of connective-tissue scar, and capillary in-growth (1, 7, 11, 12, 24). In the final remodeling phase, the regenerated muscle matures and contracts with reorganization of the scar tissue (42, 49). Although muscle tissue retains its ability to regenerate after injury, the healing process tends to be slow and often incomplete (21, 28, 44, 49). Complete skeletal muscle recovery is hindered by the development of fibrosis, which typically appears during the second week after muscle injury and increases over time (21, 38, 39, 42, 47–49, 53). More importantly, the scar tissue that often replaces damaged myofibers may be a contributing factor in the tendency of strains to recur (28, 38, 42, 44, 48, 62).

We have established various animal models for muscle injuries and have observed a regeneration process in injured muscle that is consistent with the literature on muscle healing (20, 21, 38–40, 47, 49). The long-term goal of our study is to develop biological interventions to improve muscle healing after injuries (28, 39, 44, 48). To achieve this goal, our research team is investigating two major procedures: the enhancement of muscle regeneration and the prevention of muscle fibrosis. We have characterized the effects of several growth factors on the enhancement of myoblast proliferation and fusion in vitro. This led to the identification of three promising growth factors (nerve growth factor, basic fibroblast growth factor, and, particularly, insulin-like growth factor (IGF)-1) that improve muscle regeneration (38–40, 48). However, the full recovery of the injured muscle continues to be limited by the development of fibrosis (38–40, 47, 49).

Therefore, we have focused our recent efforts on the development of biological approaches to prevent muscle fibrosis after muscle injuries. The overproduction of transforming growth factor (TGF)-β in response to injury and disease is a major cause of tissue fibrosis in humans and other animals (5, 6, 15, 25, 28, 44, 45, 52, 64, 65). In skeletal muscle, TGF-β1 is expressed at high levels and is associated with the massive muscle fibro-
sis present in the skeletal muscle of Duchenne muscular dystrophy patients (6). Similarly, during muscle injury, an inflammatory reaction that takes place at the injury site leads to the focal release of TGF-β, which triggers fibrosis via the activation of the extra-cellular matrix and the proliferation of connective tissue (15). We have reported that muscle-derived cells can differentiate into fibroctic cells on laceration injury in skeletal muscle (47). We also have reported that TGF-β is a major factor in triggering the fibrotic cascade within injured skeletal muscle (45, 47). By blocking the fibrotic effects of TGF-β via injection of the bioactive molecule decorin, a direct antagonist of TGF-β (51), we found that both the structure and function of the lacerated muscle reached near-complete recovery (21). However, a large amount of decorin was required to efficiently improve the healing of a small mouse muscle. Decorin also is not Food and Drug Administration-approved for clinical use (66). Hence, we have focused our efforts on the identification of a biological agent that could be used clinically and displays antifibrotic effects comparable to those of decorin.

Suramin (Sigma Chemical, St. Louis, MO) was originally designed as an antiparasitic drug and has been found to inhibit TGF-β expression by competitively binding to the growth factor receptor (60, 66). With this in mind, we hypothesized that suramin also could bind to TGF-β receptors commonly found in cells present at the site of muscle injuries and thereby prevent TGF-β1 from initiating its effect on myofibroblasts. To investigate this hypothesis, we conducted a series of tests in vitro to determine the effects of suramin on fibroblasts. In vivo, we chose a laceration model to confirm the antifibrotic effect of suramin and to evaluate its influence on muscle healing.

**MATERIALS AND METHODS**

**Effect of Suramin on Fibroblasts In Vitro**

*Cell cultures.* National Institutes of Health (NIH) 3T3 cells, a well-known fibroblast cell line, were cultured with Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum, 10% horse serum, 0.5% chicken embryo extract, and 1% penicillin/streptomycin. All of the cells were grown at 37°C in 5% CO2 for 3 days with regular medium changes.

*Growth experiments.* NIH 3T3 cells (2,000 cells/well) were seeded into 12-well plates. After a 12-h incubation period, the medium was completely removed, and fresh growth medium (serum free) was added with suramin (50 μg/ml) and lysed with a lysis buffer [ratio of (β-mercaptoethanol to sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol) = 1:20]. After they were boiled, the whole cell lysates were centrifuged at 3,000 g for 5 min, and the supernatant was collected and stored at 4°C. The samples were analyzed by using 12% SDS-polyacrylamide gel electrophoresis. Protein concentrations were determined by using the Bradford methodology (9). Four micrograms of protein from each sample were loaded and separated with 55 V for 1 h and 75 V for 3 h. We then transferred the total protein to a nitrocellulose membrane (Bio-Rad, Hercules, CA) by using 60 V overnight. Membranes were washed in PBS for 5 min and blocked with 1% nonfat dry milk and 2% horse serum in Triton-PBS (T-PBS; 0.01%) for 1 h at room temperature. Nonfat dry milk (50 cycles/min). Afterward, the membranes were washed, they were developed by using enhanced chemiluminescence (Supersignal West Pico Chemilluminence Substrate, Pierce), and the positive bands were identified by using X-ray film. To assess vimentin expression, the same protocol was performed by using different antibodies. A primary antibody of anti-vimentin (1:1,000, Sigma Chemical) and a secondary antibody of anti-goat IgG conjugated with horseradish peroxidase (1:8,000, Pierce, Rockford, IL) was applied for 1 h, at which time the blots again were washed 4 times in T-PBS (15 min/wash). A secondary antibody of anti-mouse IgG conjugated with horseradish peroxidase (1:8,000, Pierce) were used. The X-ray films from three independent Western blots for each antibody were scanned and evaluated with Northern Eclipse software (version 6.0, Empix Imaging, North Tonawanda, NY) by calculating the optical density of each protein band. The image analysis of the films was performed in black and white, and the signals of the images on the linear portion were expressed in gray values ranging from 0 to 256 pixels.

**Determination of the Biological and Physiological Effects of Suramin on Muscle Healing After Laceration**

*Animal model.* A muscle laceration model was developed in mice (C57BL10J/+; Jackson Laboratory, Bar Harbor, ME) based on previously described studies (20, 21, 47, 49). Forty-four mice, with an average age of 8 wk and approximate weight of 18–22 g, were used in this experiment. 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. The Animal Research and Care Committee of the authors’ institution approved the research protocols used for these experiments (protocol no. 5/01). The mice were anesthetized by using a low dose (80 mg/kg of animal body wt) of pentobarbital sodium (Anpro Pharmaceuticals, Arcadia, CA) delivered by intraperitoneal injection. The muscles were CA treated with a surgical blade (no. 11) at the largest diameter, through the lateral 50% of their width and 100% of their thickness (23, 47–49). Polydioxanone suture material (PDSII 5-0, Ethicon, Somerville, NJ), 4 mm in length, was placed at the medial edge of the lacerated side of each leg as a marker for the
lacerated site. After the laceration was made, the skin was closed with 4-0 black silk. The suramin was injected along the polydioxanone suture material with a microsyringe. The mice were divided into three groups on the basis of different time points of injection after laceration (0, 7, and 14 days). At each time point, four different concentrations of suramin (0, 0.25, 1, and 2.5 mg in 20 μl of PBS) were used. In the sham-injected group (the negative control), the muscle was lacerated as in the experimental groups but was injected only with PBS. Both gastrocnemius muscles of the mice were lacerated. The mice were then killed for evaluation of muscle healing and regeneration 2 wk postinjection. The gastrocnemius muscles were isolated and frozen in 2-methylbutane precooled in liquid nitrogen. Regular histological and immunohistochemical techniques to assess the expression of vimentin were utilized to evaluate the development of scar tissue postinjury.

Evaluation of muscle fibrosis after suramin therapy. Twenty-four mice were used for this experiment. Both gastrocnemius muscles of the mice were lacerated. The mice were then divided into 12 groups with 2 mice/group (4 muscles/group). Various concentrations of suramin (0, 0.25, 1, and 2.5 mg in 20 μl of PBS) were injected at each time point (0, 7, and 14 days postlaceration). Two weeks after injection, all animals were killed for evaluation of muscle healing and regeneration. Sixteen muscles per time point (4 muscles/concentration group) were used for regular histological and immunohistochemical staining. Total collagen staining and vimentin staining were performed to detect fibrosis in the injured muscle as previously described (8, 10, 20, 21, 39, 47, 49). The intermediate filament, vimentin, was used as a marker of scar tissue in this study because it is expressed by connective tissue. Vimentin is expressed by myofibers early in development and shortly after injury (<2 wk); however, mature myofibers do not express this protein (8). In brief, for collagen staining, sections were fixed in 10% formalin for 10 min. After the sections were washed in deionized water, Masson modified trichrome staining was performed according to the manufacturer's protocol (IMEB, San Marcos, CA). This histological technique stained muscle red and collagen blue. Vimentin immunohistochemistry was performed by fixing the sections as described above. After 45 min of blocking in 5% horse serum (Vector, Burlingame, CA), these slides were incubated with anti-vimentin conjugated with Cy3 (1:500, Sigma Chemical) (8, 45, 47, 49). The surface area of muscle fibrosis in the different groups was measured by using a previously described technique (20, 21, 39, 40, 45, 47, 49). These measurements were obtained by an blinded independent investigator who was unaware of which groups were treated or used as controls, thereby ensuring the objectivity of the results. To measure the total vimentin- or collagen-positive area under the fluorescence microscope, 10 random fields were selected for each sample. The images were collected by using an Olympus Provis epifluorescence microscope (Olympus Optical, Tokyo, Japan) and a Sony 970 3 chip charge-coupled device camera (Sony, Tokyo, Japan). After they were digitized by using a Coreco frame grabber board (Coreco Imaging, St. Laurent, Quebec, Canada) and rendered to monochrome, features were extracted, and the absolute area of vimentin-positive staining was measured in each image field.

Evaluation of muscle regeneration after suramin therapy. Hematoxylin and eosin was used to monitor the number of regenerating myofibers within the injured sites treated with suramin (0, 0.25, 1, and 2.5 mg in 20 μl of PBS), and the results were compared among the different groups. Centronucleated cells were considered to be regenerating myofibers (7, 30). Nuclei with no discernible surrounding cytoplasm were discarded. A blinded observer performed the counting. The total number of regenerating myofibers within the injured site was quantitated by using five random fields selected from each sample in accordance with a previously described protocol (20, 39, 40, 49).

Physiological Evaluation of Muscle Contractile Properties after Suramin Therapy

Twenty-four mice were utilized for the physiological test, which was based on a previously described protocol (20, 21, 38, 40, 48, 49). The gastrocnemius muscle of the left leg in each mouse was lacerated as previously described and injected with suramin 14 days postinjury. The gastrocnemius muscle of the right leg in each mouse was simply exposed and kept intact. Six mice in each group (injected with 0, 0.25, 1, or 2.5 mg of suramin in 20 μl of PBS) were examined by physiological testing for functional recovery 14 days after injection. The mice were anesthetized by using a low dose (80 mg/kg of animal body wt) of pentobarbital sodium delivered by intraperitoneal injection. Both gastrocnemius muscles were removed and mounted in a double-jacketed organ bath of 5 ml at 36°C in Krebs solution (in mM: 113 NaCl, 4.7 KCl, 1.2 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1.2 KH2PO4, 11.5 glucose) and constantly agitated via bubbling with a mixture of 95% oxygen-5% carbon dioxide. The time between the extraction of the muscle and the initiation of force measurements was <1 min for all evaluated muscles. The initial tension was set at 20 mN; isometric contractions were measured with strain-gauge transducers coupled with a TBM4 strain gauge amplifier (World Precision Instruments, Sarasota, FL) and recorded on a computer using a data acquisition program (Windaq, DATAQ Instruments, Akron, OH). The sampling rate per channel was set at 500 Hz. The amplitude of the stimulation-evoked contractions was computed by using a calculation program (WindaqEx, DATAQ). After 20 min of equilibration, electrical field stimuli were applied through two platinum wire electrodes positioned on top and bottom of the organ bath (separated by 4 cm). The muscles were stimulated with square-wave pulses of 0.25 ms duration with maximal voltage (50 V). First, a 1-Hz stimulation was applied, and the muscle twitches were recorded. Six tetanic stimulations then were applied with 0.5-s train duration at 100 Hz every 10 s. Finally, each muscle was weighed by using a microbalance (Mettler Toledo, Greifensee, Switzerland). The strength measurements were recorded and expressed in milli-Newtons per gram.

Statistical Analyses

Differences in cell proliferation between the 3T3 control group and the experimental groups treated with suramin (50 μg/ml) were analyzed by using a two-tailed t-test. Statistical comparison of the fibrotic area, number of regenerating myofibers, and twitch and tetanus strength in the various groups were performed by ANOVA. Differences among the groups were analyzed by using Bonferroni multiple comparisons (post hoc test). Statistical significance was defined as $P < 0.05$.

RESULTS

Effect of Suramin on Fibroblasts In Vitro

The anti-proliferative effect of suramin on the proliferation of NIH 3T3 fibroblasts is described in Fig. 1. After incubation ranging from 72 h up to 144 h, the experimental group treated with suramin (50 μg/ml)
had a significant decrease in the number of fibroblasts compared with the control group (a serum-free medium without suramin).

Effect of suramin on intracellular α-SMA and vimentin: Western blot analysis. To evaluate whether suramin influences α-SMA and vimentin protein production, we investigated the effect of suramin on intracellular α-SMA and vimentin protein content in the treated cells. To make sure that the cells were responding to suramin, the growth rate was determined in parallel. After 72 h of incubation with suramin (50 μg/ml), one set of cells was counted to confirm that the growth of NIH 3T3 fibroblasts was inhibited significantly. Western blot analysis revealed a decrease in the intracellular α-SMA and vimentin protein expression at a suramin concentration of 50 μg/ml. Densitometric evaluation revealed a 100% decrease in α-SMA and vimentin proteins in cells incubated with 50 μg/ml suramin, a 73 and 81% decrease in the respective proteins at 10 μg/ml suramin, and a 53 and 45% decrease in the respective proteins at 1 μg/ml suramin compared with the control (0 μg/ml suramin) (Tables 1 and 2).

Determination of the Biological and Physiological Effects of Suramin on Muscle Healing After Laceration

Evaluation of muscle fibrosis after suramin injection. Histological assessment in combination with quantitation of collagen and vimentin immunofluorescence at the injured site suggested that the injection of high concentrations of suramin (2.5 mg in 20 μl of PBS) resulted in better prevention of fibrosis than did injection of lower concentrations of suramin (1, 0.25, and 0 mg; Figs. 2–5). Significantly less scar tissue formed in the experimental group treated with 2.5 mg of suramin than in the control group (P < 0.05). The inhibitory effect of suramin on the development of muscle fibrosis seemed to be dose dependent; however, although there was a significant reduction of fibrosis observed with doses between 1.0 (indicated by vimentin) and 2.5 mg of suramin (indicated by vimentin and collagen), no significant differences in outcome were detected between the 0.25- and 1-mg or the 1- and 2.5-mg doses. The percentage of the muscle containing scar tissue 4 wk postlaceration ranged from ~12.8% of the total area in the control (0 mg suramin) to 7.9% in the 0.25-mg group, 4.7% in the 1-mg group, and 2.6% in the 2.5-mg group.

Evaluation of muscle regeneration after suramin injection. The suramin-injected muscles displayed numerous regenerating myofibers at the laceration site. These regenerating myofibers were located uniformly throughout the injured region in the superficial as well as the deeper parts of the muscle (Fig. 2). The control muscles also contained regenerating myofibers, but they were predominantly located in the deeper regions of the lacerated areas. In the superficial region of the control muscles, there was an infiltration of mononucleated cells with only a few regenerating myofibers; the majority of this area was occupied by fibrotic tissue (Fig. 2). Figure 6 shows the mean number of regenerating myofibers.

Table 1. Effects of suramin on levels of α-smooth muscle actin on CT cells

<table>
<thead>
<tr>
<th>Densitometric Evaluation</th>
<th>Control Culture</th>
<th>Suramin-Treated Culture</th>
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<tr>
<td>Unit: gray value</td>
<td></td>
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<tr>
<td>151 ± 30</td>
<td>71 ± 37</td>
<td>42 ± 18</td>
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<tr>
<td>Inhibition, %</td>
<td>0</td>
<td>53</td>
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Values are means ± SD. CT cells, C2C12 cells expressing transforming growth factor β1.

Table 2. Effects of suramin on levels of vimentin on CT cells

<table>
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<th>Densitometric Evaluation</th>
<th>Control Culture</th>
<th>Suramin-Treated Culture</th>
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<tbody>
<tr>
<td>Unit: gray value</td>
<td></td>
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</tr>
<tr>
<td>144 ± 38</td>
<td>80 ± 30</td>
<td>27 ± 14</td>
</tr>
<tr>
<td>Inhibition, %</td>
<td>0</td>
<td>45</td>
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Values are means ± SD.
present in the injured muscle were counted and compared among the groups. We observed an increased number of regenerating myofibers in all the suramin-treated groups when compared with the controls. However, only a high dose of suramin (2.5 mg) led to a significantly higher number of regenerating myofibers compared with the control animals injected only with PBS ($P < 0.01$; Fig. 6).

Physiological Evaluation of Muscle Contractile Properties After Suramin Injection

The tetanus and twitch strength of the lacerated muscles was observed by physiological testing and compared with results from animals in the control group (PBS injection). The twitch muscle strength was $217.2 \pm 26.3$ mN/g in the normal muscle, $119.6 \pm 32.0$
mN/g in the control group, 148.2 ± 36.7 mN/g in the group treated with 0.25 mg of suramin, 164.8 ± 51.2 mN/g in the group treated with 1.0 mg of suramin, and 184.5 ± 20.4 mN/g in the group treated with 2.5 mg of suramin. There was no significant difference between the normal muscle and muscle from the group treated with 2.5 mg of suramin (Fig. 7). However, there was a significant difference between the control group (injured + PBS) and the group treated with 2.5 mg of suramin (Fig. 7). To minimize variation between animals, data also were normalized with respect to untreated controls, i.e., the strength in the experimental muscle was divided by that in the control muscle on the contralateral side and multiplied by 100 to determine the percent change. For twitch strength, the control group produced 55%, the 0.25 mg of suramin group produced 63%, the 1 mg of suramin group produced 69%, and the 2.5 mg of suramin group produced 82% of the twitch strength measured in the intact muscles 4 wk after injury (Fig. 8). The difference between the control group and the group treated with 2.5 mg of suramin was found to be significant (P < 0.05). Similarly, the tetanus muscle strength was 54% in the control group, 59% in the 0.25 mg of suramin group, 72% in the 1 mg of suramin group, and 87% in the 2.5 mg of suramin group of the tetanus strength measured in the intact
muscles. Again there was a significant difference between the tetanus strength of the control group (injured + PBS) and that measured in the group treated with 2.5 mg of suramin.

DISCUSSION

Muscle injuries are frequently encountered in professional and recreational sports, although the optimal treatment has not been clearly defined. Furthermore, significant morbidity, including early functional and structural deficits, reinjury, muscle atrophy, contracture, and pain, often occurs after muscle injury (3, 11, 16, 27, 31, 32, 55). Skeletal muscle is capable of extensive regeneration after injuries (12, 22, 23, 29, 39–41, 49, 62) via satellite cell activation, proliferation, and fusion into mature multinucleated myofibers (7, 30, 57, 58). However, scar tissue formation occurs concurrently and competes with muscle regeneration during the muscle healing process (20, 39, 40, 48).

Various growth factors can stimulate growth of and protein secretion by many musculoskeletal cells. During muscle regeneration, growth factors and cytokines released by the injured muscle are believed to activate the satellite cells (1, 7, 30, 37, 47). Preliminary data also have suggested that growth factors play additional roles during muscle regeneration (2, 4, 14, 17, 19, 24, 34–36, 41). Of particular interest, IGF-1 and -2 have been found to be mitogenic for myoblasts (19, 54). IGF-1 is critical in mediating the growth of muscle and other tissues (18). We have characterized the efficiency of muscle recovery after different models of muscle injury. In these models, we observed an active muscle regeneration process, which occurred in the early stages of healing, that eventually became impaired by the development of scar tissue in the injured muscle (21, 47, 49). In addition, we identified specific growth factors (IGF-1, basic fibroblast growth factor, and nerve growth factor) that are capable of enhancing myoblast proliferation and differentiation in vitro, and we investigated the delivery of these growth factors into the injured muscle to improve muscle healing in vivo (38–40, 48). Although the direct injection of the recombinant basic fibroblast growth factor and nerve growth factor proteins conveys some beneficial effects on muscle healing after injuries, IGF-1 has been found capable of mediating the highest improvement of muscle healing for all muscle injuries tested (i.e., laceration, contusion, and strain) (38–40, 48). These growth factors can enhance muscle regeneration but are unable to prevent the formation of scar tissue that ultimately impairs the healing process (38–40, 48).

Large amounts of scar tissue that appear in injured muscle 3–4 wk postinjury suggest that the natural healing of contused, lacerated, and strained muscles is incomplete. Fibrosis generally develops during the second week after muscle injury and increases over time (21, 39, 40, 45, 47, 49). We recently attempted to develop biological approaches to directly block muscle fibrosis. Our laboratory’s previous report (49) showed that immobilization of the lacerated muscle had no significant effect on the development of fibrosis, whereas suture was capable of limiting fibrosis deep in the injured muscle but not superficial muscle fibrosis. We recently have determined that myogenic cells un-
under the influence of TGF-β1, can differentiate into fibroblastic cells on muscle laceration injury (45, 47). On the basis of this finding, decorin, an anti-fibrotic proteoglycan that inhibits the effect of TGF-β (51), was used to prevent fibrosis and thereby improve muscle healing after injury in murine muscle (21). We observed that direct injection of decorin was able to efficiently prevent fibrosis and enhance muscle regeneration in the lacerated muscle (21).

Suramin, a polysulphonated naphthylurea, was originally synthesized and designed as an antiparasitic drug (26); because of its inhibitory effect on reverse transcriptase, it has recently been introduced into clinical trials for AIDS (43), selected malignancies, and metastatic diseases, including prostate, adrenal cortex, lymphoma, breast, and colon cancer (60). Because suramin is a heparin analog, it binds to heparin-binding proteins. The substance blocks the effects of growth factors on tumor cells in vitro and interferes with the action of growth factors by competitively binding to growth factor receptors (60, 66). Growth factors inhibited by suramin include TGF-β1, -2, and -3; PDGF A and B; and EGF. Of the many growth factors known, TGF-β1 and -2 and PDGF A and B have the strongest influence on fibroblasts (61, 63). Growth factors inhibited by suramin include TGF-β1, -2, and -3; PDGF A and B; and EGF. Of the many growth factors known, TGF-β1 and -2 and PDGF A and B have the strongest influence on fibroblasts (61, 63). Growth factors inhibited by suramin include TGF-β1, -2, and -3; PDGF A and B; and EGF. Of the many growth factors known, TGF-β1 and -2 and PDGF A and B have the strongest influence on fibroblasts (61, 63).

In an animal model, we found that direct injection of suramin into a lacerated muscle decreases the amount of fibrosis within the injured area. Histological examination and quantification of the vimentin and collagen at the injured site revealed that the injection of a high concentration of suramin (2.5 mg in 20 μl of PBS) appeared to prevent fibrosis better than injection of lower suramin concentrations (1, 0.25, or 0 mg). The ability of suramin to impede muscle fibrosis appears to be dose dependent. We also evaluated the effects of suramin injection on muscle regeneration. There were many regenerated myofibers within the lacerated sites in the experimental groups treated with suramin, whereas the lacerated site was usually filled with fibrous scar tissue in the non-suramin-treated groups.

Our goal in the present study was to investigate the antifibrotic effect of suramin in skeletal muscle. In vitro, we determined the effects of suramin on fibroblast proliferation and the expression of fibrotic-related proteins. In vivo, we investigated the antifibrotic effects of suramin in injured muscle by direct injection of various concentrations of suramin at different time points postlaceration. Our results indicate that suramin has a strong antiproliferative effect on NIH 3T3 cells. Similar results have been reported in various cells, including tumor and nontumor cells (56, 60, 66).

α-SMA and vimentin have been implicated in the pathology of fibrosis and are marker proteins for the myofibroblastic phenotype (8, 10, 59). We treated CT cells (C2C12 cells transfected with a plasmid encoding TGF-β1 and neomycin) (45, 46), which display high expression of α-SMA and vimentin, with suramin. Quantitative Western blot analysis revealed a 100% decrease in α-SMA and vimentin proteins for cells incubated with 50 μg/ml suramin, a 73 and 81% reduction in the respective proteins after treatment with 10 μg/ml and a 53 and 45% decrease in these proteins with 1 μg/ml compared with the control. We demonstrated that suramin abolishes the stimulating effects of TGF-β1 on fibroblast protein production in CT clone cells. Our results also support the theory that suramin reduces the biological activity of extracellular growth factors in a dose-dependent manner by competitively binding to the growth factor receptors and thus inactivating them.
creased in the suramin-treated group. This finding suggests that direct injection of suramin can prevent fibrootic scar tissue formation and consequently muscle regeneration.

The results of physiological testing paralleled the histological findings. The tetanus and twitch strength evaluations indicated no major difference between the noninjured group and the injured group treated with 2.5 mg of suramin. However, there was a significant difference between the group treated with 2.5 mg of suramin and the control group (lacerated muscle injected only with PBS). To minimize interanimal variation, the data were normalized with respect to untreated controls. The control group produced 55% and the group treated with 2.5 mg of suramin produced 82% of the twitch strength measured in intact muscles at 4 wk after injury. Similar results were found with the tetanus muscle strength assessment. These findings strongly suggest that injection of 2.5 mg of suramin at 2 wk postlaceration can effectively improve the functional recovery of lacerated muscles.

The results from this study demonstrate that suramin (50 μg/ml) can inhibit the proliferation of fibroblasts and the expression of fibrotic proteins (α-SMA and vimentin) effectively in vitro. In vivo, injection of suramin (2.5 mg) at 2 wk postlaceration efficiently prevented muscle fibrosis and enhanced muscle regeneration, leading to efficient functional muscle recovery. In the future, we need to study the effect of suramin on other muscle injuries, particularly muscle strain, the most common type of muscle injury in sports medicine. The healing process of muscle after strain injury is similar to that after laceration injury, i.e., the development of significant fibrosis seems to hamper efficient, complete muscle regeneration (38, 39, 44, 48). Therefore, we should apply similar techniques of suramin injection in future studies. The main advantage of suramin treatment for fibrosis prevention is its clinical availability (26, 43, 60). However, the safety of the use of suramin as an antifibrotic agent must be determined before these results can be applied clinically to treat muscle injuries.

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DISCLOSURES

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REFERENCES


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