Introduction:
Muscle injuries are a challenging problem in traumatology, and they represent some of the most frequently occurring injuries in sports medicine. Even though muscle tissue retains its ability to regenerate after injury, the healing process involved has been found to be slow and often incomplete. We have shown that muscle regeneration occurs after injury, but the development of scar tissue greatly limits the healing process.1,2,3 Our previous studies have shown that blocking TGF-ß1 with decorin decreases fibrosis and improves muscle healing.3 Suramin was originally designed as an antiparasitic drug and has been found to be a TGF-ß1 inhibitor by competitively binding to the growth factor receptor.1,3 With this in mind, we hypothesized that suramin is also capable of binding to TGF-ß1 molecules, which are released at the injured site, and preventing these molecules from initiating their effect on myofibroblasts. Previously, we found that suramin could improve the healing of lacerated muscle. Now we have investigated whether suramin can improve the healing in the strain model, the most common muscle sports-related injury.4 To determine this, we first conducted a series of tests in vitro to assess the effects of suramin on fibroblast proliferation. In vivo, we observed that the direct injection of suramin at 1 and 2 weeks after strain resulted in an effective prevention of muscle fibrosis and improved muscle regeneration.

Materials and Methods:
Cell proliferation: Myofibroblasts were isolated from mice and were incubated with either suramin (50 µg/ml), TGF-ß1 (5 ng/ml), or suramin and TGF-ß1. The growth curve for each of these groups was then measured and compared among the groups.

Suramin for the prevention of muscle fibrosis in strained muscle: 32 mice (C57BL10J+/4) were used in this experiment. 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’ institutions approved the research protocols used for these experiments. A strain model was developed in mice based on previously described studies.1,2,3,4 The injection of suramin was performed at the site of strain. The mice were divided into two different time points (7 and 14 days) of injection after strain. At each time point, 4 different concentrations (0, 1, 5, 10 mg in 25 µl of PBS) of suramin were used. The control group was injected with PBS. All animals were sacrificed for evaluation of healing and regeneration at 2 weeks after the injection. Regular histological and immunohistochemical techniques for the expression of vimentin were performed to evaluate the development of scar tissue formation after injury. We have quantitated the surface area of muscle fibrosis among the different groups to measure the total vimentin positive area under the fluorescence microscope. For physiological testing, we tied the origin to a fixed point and the tendon to a force transducer. A 0.5s train duration of 100Hz every 10s was applied to stimulate contractions, and fast twitch and tetanic strength were measured. The average and standard deviations of all data were compared among the different groups using a Student t-test for statistical analysis. Statistical significance was defined as p<0.05.

Results:
Suramin inhibits myofibroblast cell proliferation: Suramin (50 µg/ml) had an anti-proliferative effect on myofibroblasts (p<0.05 and inhibited the stimulating effect of TGF-ß1 on myofibroblasts after incubation for 72 hours (p<0.05, Figure 1).

Suramin decreases the fibrosis area of strained muscle: From histological analysis (Figure 2.3), the injection of a high concentration of suramin (5 mg) at 2 weeks after strain appeared to result in better prevention of fibrosis than a lower concentration of suramin. By quantifying the vimentin immunofluorescence at the injured site, scar tissue formation was found to be significantly decreased in the groups treated with 5 mg of suramin at 2 weeks after strain when compared to the control group (p<0.05).

Discussion:
Suramin could interfere with the action of growth factors by competitively binding to growth factor receptors. Growth factors inhibited include TGF-ß1, 2 and PDGF A, B. These growth factors have a strong influence on fibroblasts during fibrosis. Our study has shown that suramin can decrease the proliferation of fibroblasts, as well as inactivate the stimulating effect of TGF-ß1 on myofibroblasts. We have also observed that the injection of suramin at 2 weeks following muscle strain injury resulted in an effective prevention of muscle fibrosis in vivo. More importantly, we found that suramin could improve the function of the strain-injured muscle. These results agreed with previous findings, where suramin improved muscle healing in a laceration model. Now we have shown that suramin can not only inhibit the fibrosis formation in strained muscle but that it also improves the function. Although the role of suramin in muscle regeneration was not evaluated in this study, our further studies will monitor the number and diameter of regenerating myofibers of the injured muscle treated with suramin.

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References: