Introduction: Muscle injury is a challenging problem in traumatology and the most frequently occurring injury in sports medicine. Muscle injury is capable of healing, although slowly and with an incomplete functional recovery due to the development of fibrosis. Muscle fibers cannot regenerate efficiently across this scar tissue prohibiting a complete healing of the muscle. Recently, we have investigated the use of an anti-fibrosis substance, decorin, a human proteoglycan, as an approach to prevent muscle fibrosis. We observed that direct injection of human recombinant decorin at 2 weeks post-laceration resulted in an effective prevention of muscle fibrosis and enhancement of muscle regeneration to near complete recovery. Although the application of decorin in reducing muscle fibrosis has been very successful, it is necessary to fully understand the mechanism by which decorin blocks the scar tissue formation. In previous studies, it has been found that the formation of fibrosis is initiated by the release of the growth factor TGF-β, which acts as a signaling molecule causing myo-fibroblast cells to proliferate into the injured area and develop into scar tissue. 1 Decorin has been found to be a TGF-β inhibitor in other studies 2, therefore, we hypothesize that decorin is capable of binding to TGF-β molecules which are released at the injured site and therefore preventing TGF-β from initiating its effect on myo-fibroblasts. In order to test this hypothesis, in vitro, we conducted a series of tests to determine the effects of TGF-β and decorin on myo-fibroblasts. In vivo, we used an animal model of muscle laceration to induce fibrosis and then attempted to investigate the expression of decorin and TGF-β in the injured area.

Materials and Methods:

1. Determining the Effect of Decorin and TGF-β on Myo-fibroblasts in vitro

Myo-fibroblasts: The gastrocnemius muscles were removed from the hind limbs of 3-weeks old mice and muscle tissue was dissociated by enzymatic treatment. The pre-plate technique was used to dissociate the myo-fibroblasts from the myogenic cells in vitro. 1 The myo-fibroblasts (500,000 cells) have been incubated with TGF-β (25 µg), decorin (25 µg) or TGF-β + decorin (25 µg each). Then we have compared cell proliferation to the control (serum-free medium without TGF-β or decorin) at 24, 48 and 72 hours after incubation. The number of cells has been monitored and compared among different groups. We have also tested the cells before and after treatment with decorin for vimentin, a protein specific to fibroblasts, in order to determine whether decorin induced differentiation of myo-fibroblasts to myoblasts.

2. Determining the levels of Decorin and TGF-β in vivo

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 muscle laceration model was developed as described in previous studies. 3 At 5 and 10 days post injury, animals were sacrificed for evaluation of healing and regeneration. Regular histology was used to evaluate the development of scar tissue. The amount of decorin present in both the injured and non-injured muscle was evaluated by immunohistochemical staining, using an antibody generously donated by Dr. Larry Fisher (NIH). Immunohistochemical techniques were also used to develop the expression of TGF-β.

Results

1. In vitro, decorin was found to have an anti-proliferative effect on myo-fibroblasts.

2. In vitro, TGF-β was found to have a proliferative effect on myo-fibroblasts.

3. In vitro, decorin was capable of reducing the proliferative effect of TGF-β on myo-fibroblasts. The proliferation of the myo-fibroblasts which were cultured with both TGF-β and decorin showed a pattern of growth similar to the control (serum-free medium). (Fig. 1)

4. When exposed to decorin in culture, myo-fibroblasts did not show an increase in desmin immunoreactivity, indicating that decorin does not cause the differentiation of myo-fibroblasts to myoblasts.

5. In vivo, a high level of both decorin and TGF-β expression was found at the site of fibrosis by immunohistochemical staining. (Fig. 2)

Discussion

Our goal in this study was to elucidate the mechanism by which decorin is capable of reducing fibrosis and also promoting muscle regeneration. The major players in the process of fibrosis and muscle healing are the antagonistic cells, myoblasts and myo-fibroblasts, and the growth factors which stimulate and inhibit these cells, most notably TGF-β. The in vitro staining confirmed that both decorin and TGF-β are present at the injured site, suggesting the importance of these molecules in fibrosis. The in vitro study shows that TGF-β does in fact stimulate myo-fibroblasts, indicating that inactivating TGF-β may be the solution to reducing fibrosis. Moreover, decorin is capable of neutralizing the effect that TGF-β has on myo-fibroblasts which will reduce fibrosis after injury. Although decorin does not stimulate the differentiation of myo-fibroblasts to myoblasts, it’s affect on myo-fibroblasts and TGF-β should have beneficial effects in vivo. We will continue to study the relationship between decorin and fibrosis and apply this proteoglycan in muscle injury using other techniques such as gene therapy to deliver a higher concentration of decorin to muscle fibers.

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References


