**GAMMA INTERFERON AS AN ANTIFIBROSIS AGENT IN SKELETAL MUSCLE**

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**Introduction:** Muscle injuries are commonly encountered in sports medicine. A major problem with the process of muscle healing is the formation of scar tissue. Shortly after injury there is a dramatic increase in muscle regeneration, but by two weeks post injury there is a substantial increase in fibrosis. The addition of growth factors (such as b-FGF, IGF-1, NGF) was attempted to further increase muscle regeneration, but it did not lead to a complete functional recovery. Growth factors increased the number of myoblasts in the injured muscle, but the muscle regeneration was limited by the accumulation of fibrosis. If the area of fibrosis is reduced, myoblasts should contribute to efficient muscle regeneration beyond two weeks, leading to a better recovery of the injured muscle. In other tissues, such as those of the kidney and lung, Interferon has been shown to decrease the amount of fibrosis. In lung fibroblasts, Interferon has been shown to block production of collagen I, which were induced by transforming growth factor-β (TGFβ1). In muscle, Interferon should alleviate fibrosis, allowing for a better healing of the injured muscle.

**Methods:** Isolation of muscle derived cells: Muscle derived fibroblasts were isolated from the gastrocnemius muscle of mice using a protocol previously described.

1. (Cell proliferation) pp1 cells (mostly fibroblasts) were plated into four different groups with equal numbers of cells in each group. After 24 hours of incubation, cells were treated with four different concentrations of γ-Interferon (0, 100, 500, and 1000 units of activity/ml (U/ml)). After three days, the number of cells was counted using a hemocytometer. The Students t-test was used for statistical comparison of the treated group and the control group.

2. (RT-PCR) NIH/3T3 cells were plated into four groups: the control group was cultured in normal media, the γ-Interferon group received 1000 U/ml γ-Interferon, the TGFβ1 group received 1 ng/ml of TGFβ1 and the dual treatment group received both 1000 U/ml γ-Interferon and 1 ng/ml TGFβ1. The mRNA was isolated at 48 hours and was amplified via RT-PCR with α-smooth muscle actin (αSMA) primers.

**In Vivo** Mice were lacerated at 50% of the width and 100% of the height of both gastrocnemius muscles. The left gastrocnemius muscles were injected at either 1 or 2 weeks post laceration with 250 units of γ-Interferon, and the right gastrocnemius was left as the control. They were later sacrificed at 4 weeks post injury.

3. (Physiological testing) Four weeks after the injury the gastrocnemius was removed from the hindlimb. The origin was tied to a fixed point, and the tendon was attached to a force transducer. A 0.5s train duration of 100Hz every 10s was applied to stimulate tetanic contractions. The force was then measured by Windaq software, and the Kruskall Wallis H-test and Student Newman Keuls test were used to determine statistical differences between groups (p< 0.05).

4. (Histology) Four weeks after injury, the muscle was removed: Cryosections were made and histologically stained for hematoxylin and eosin.

**Results:** Cell proliferation A dose dependent inhibition of muscle derived fibroblast (pp1) growth upon γ-Interferon treatment was observed in vitro. A significant decrease in proliferation was found when the cells were treated with 1000 U/ml of γ-Interferon. (Figure 1)

(RT-PCR) NIH/3T3 cells did not express the myofibroblast marker αSMA without treatment (Fig. 2, Lane 1) or with γ-Interferon (Fig. 2, Lane 4) treatment, but when incubated with TGFβ-1 (Fig. 2, Lane 2), αSMA mRNA was detected. When NIH/3T3 cells were incubated with both TGFβ-1 and γ-Interferon (Fig. 2, Lane 3) simultaneously, there was no detectable αSMA mRNA. (Figure 2)

(Physiological testing) When the muscle was injected with γ-Interferon at two weeks, it showed a statistically significant increase in tetanuss strength when compared to the control muscle, while the muscle injected at one week did not exhibit a significant difference when compared to the control. (Figure 3)

(Histology) Cryosections stained for hematoxylin and eosin showed that the muscle injected with γ-Interferon had a smaller area of fibrosis than did the control muscle. (Figure 4)

**Discussion:** Based on our experiment γ-Interferon can be used as an anti-fibrosis agent in skeletal muscle. We have shown that it slows down the growth of muscle derived fibroblasts, which are the cells mainly involved in scar tissue formation. This decreases the availability of fibroblasts to form large areas of fibrosis. Not only were we able to decrease fibroblast growth, but we were also able to alter their phenotype. αSmooth muscle actin (αSMA) has been implicated in the pathology of fibrosis in the kidney and lung. This marker was not expressed by the untreated fibroblast cell line NIH/3T3. When the cells were treated with TGFβ-1, they began to express αSMA, but the addition of γ-Interferon to TGFβ-1 blocked the αSMA expression of the cells. In vivo, the fibrotic area of the muscle was smaller after γ-Interferon treatment, and the strength of the injured muscle was increased. We found that when we injected the mice at two weeks, the strength was greater, but when it was injected at one week after injury no difference was seen when compared to the control. This demonstrates that γ-Interferon can increase the strength after injury, but the timing of the injection is very important for the functional recovery of the muscle. These results demonstrate that blocking muscle fibrosis is enough to improve the functional recovery (i.e. strength) of muscle, but this research needs to be further investigated to understand the mechanisms by which γ-Interferon blocks muscle fibrosis and improves muscle healing following injury.

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**References:**