THE IMPROVEMENT OF INJURED MUSCLE HEALING BY RELAXIN THERAPY

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Introduction:

Muscle injuries are very common in traumatology and sports medicine. Although injured muscle can usually regenerate itself, the healing process is very slow and incomplete[1]. After injury, the regenerating muscle is hindered by the development of scar tissue formation[1,2]. The current treatment is intended to minimize bleeding in the injured site. This will prevent the formation of a large hematoma, which has a direct impact on the size of the scar tissue at the end of the regeneration process. In general, after the initial treatment with RICE principle and medication, mobilization will follow, sometimes combined with these therapeutic treatments. However, because relapses are frequently seen, athletes are unable to return to their sports, and players' lives can fall into crisis. Therefore, it is important to try to obtain better regeneration of the muscle and control the fibrosis when considering the treatment of the muscle injury[1,2]. The polypeptide cytokine/growth factor relaxin is elevated in the serum during pregnancy. It is now accepted as a member of the growing family of insulin-like growth factors[3]. Relaxin is thought to play a role in the structural remodeling of the interpubic ligament and cervix in preparation for partuition[3]. The most interesting aspect is that it is already clinically available. The purpose of this study is to characterize the effect of relaxin as an antifibrotic agent to decrease the development of scar tissue formation in the injured muscle and improve muscle healing after injury.

Materials and Methods:

***In vitro,* Cell proliferation:** C2C12 cells (mouse skeletal muscle myoblast cell line) were plated into four different groups with equal numbers of cells in each group. These cells were cultured in DMEM containing 10% fetal bovine serum, 10% horse serum, and 0.5% chicken embryo extract. After 6 hours incubation, the medium was removed and 2% horse serum was added with four different concentrations of relaxin (0, 1, 10, and 100 ng/ml). The numbers of cells were counted and compared to the control at different time points post-culturing. CT cells (TGF-ß1 transfected C2C12 cells) were thought to be myofibroblasts, as they express myofibroblastic markers: a-smooth muscle actin (α-SMA) and vimentin. These CT cells were co-cultured with four different concentrations of relaxin. Cell proliferation was monitored and compared to the control through one to six days as described above.

Western blot: The same number of CT cells was plated into four different T-25 flasks. These cells were incubated with relaxin at different concentrations for 48 hours. The same amount of protein was separated and analyzed for expression of a-SMA.

In vivo, Twenty mice (C57BL6J, aged 6 weeks) were used for this experiment. The gastrocnemius muscles (GMs) were lacerated as described before[4]. These mice were divided into two groups with different time points (1 and 2 weeks) of injection after laceration. The injection of relaxin was performed at the site of laceration with a microsyringe. Phosphate buffer saline solution (PBS) was injected for the control. The mice were sacrificed at 4 weeks after laceration, and then their GMs were analyzed by regular histological and immunohistochemical techniques. The expression of vimentin was monitored to evaluate the development of scar tissue formation within the injured muscle after laceration.

Results:

In vitro, *1.* Relaxin was found to have proliferative effects on C2C12. A dose dependent increase of the myoblast growth with relaxin treatment was observed. The group treated with relaxin had a significant increase in the number of myoblasts compared to the control group after incubation for 4 and 5 days (Fig. 1). *2.* Relaxin inhibits the number of myofibroblast cells. After incubation for 5 and 6 days, there was a significant decrease in cell number with the 10, 100, and 1000 ng/ml relaxin doses (Fig. 2). *3.* a-SMA protein, a myofibroblast marker, decreased with relaxin in a dose dependent manner and was not detected with the 100 and 1000 ng/ml doses. This finding suggests that relaxin blocks the expression of the myofibroblast marker a-SMA (Fig. 3).

In vivo, Histological and immunohistochemical cryosections showed that the muscle injected with a high concentration of relaxin had a smaller area of fibrosis than the control. The scar formation was found to be smaller with injection of relaxin at 2 weeks after laceration when compared to 1 week (Fig. 4).

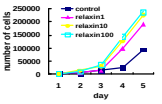
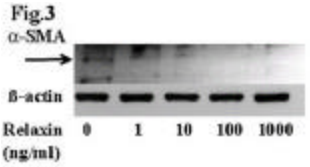


Fig.1



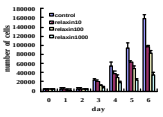
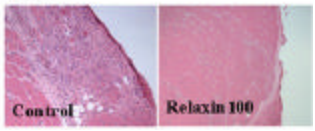


Fig.2



Discussion:

In the treatment of muscle injuries, the release of growth factors at the injured site is an important step in the healing process. Previously, we have shown that specific growth factors (IGF-1, b-FGF, and NGF) are capable of enhancing myoblast proliferation and differentiation in vitro, and we have investigated the delivery of these growth factors into the injured muscle to improve muscle healing in vivo[4]. These growth factors enhanced muscle regeneration, but were unable to prevent scar tissue formation[4]. Our previous research has shown the antifibrotic agent decorin can inhibit scar tissue formation and improve the healing of injured muscle[5]. However, decorin is not a good candidate clinically because it is not FDA approved. Relaxin has already been used in clinical practice to inhibit collagen deposition[6]. In our experiment, we found that relaxin could decrease the proliferation of myofibroblasts and decrease the expression of a fibrotic protein (a-SMA) in a dose dependent manner. Moreover, relaxin could stimulate the proliferation of myoblasts. Therefore, relaxin can be helpful in muscle healing. The injection of relaxin at 2 weeks compared to 1 week after muscle injury was more effective in preventing the development of scar formation in vivo. This shows that the timing of therapy is very important for muscle healing. Our findings will help to clinically aid muscle healing to functional recovery. To understand the function of relaxin in injured skeletal muscle, our further investigations will include longer-term analysis and physiological tests.

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