Losartan Improve The Muscle Regeneration Potential Of Muscle Derived Stem Cell

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Introduction: Muscle contusions are one of the most common muscle injuries seen by sports medicine physicians. Although these injuries are capable of healing, incomplete functional recovery often occurs. We have previously reported that losartan, one of the FDA approved Angiotensin II Receptor Blockers (ARBs), can promote muscle healing and reduce the formation of fibrosis in injured skeletal muscle [1]. It is also known that rapid revascularization after muscle injury is very important for early muscle healing [2], and we have previously reported that better functional recovery of injured skeletal muscle can be achieved by transplanting muscle with muscle derived stem cells (MDSCs). The enhancement in muscle regeneration was shown to be the result of an increase in angiogenesis and a reduction in fibrosis formation [3]. However, it has also been shown that the MDSCs can differentiate toward a fibrotic lineage [4] [5] and in an attempt to limit this potential, and due to the fact that the transplantation of the MDSCs could not completely inhibit the formation of fibrosis [3], the current study combined the transplantation of MDSCs with the administration of losartan to see if we could further reduce the formation of scar and increase muscle regeneration.

Methods: The contusion injury model was created in the tibialis anterior (TA) muscle of C57BL/6 wild-type mice [6]. MDSCs were isolated from 3-week-old wild-type mice (C57BL/6J) using a modified preplate technique as previously described [7]. Concentrations of losartan(125mg) in 1 liter of drinking water were administered beginning 3 days post-injury and continued until the endpoint - doses were calculated based on the average fluid intake of the mice as 10mg/kg/day. At 4 days post-injury, 3 × 10^5 MDSCs were transplanted directly into the injured TA muscle region (n = 15 mice for each group). Mice were divided into 3 groups, 1) MDSC/losartan group, 2) MDSC group, and 3) injured control group with PBS injection. At 1, 2, and 4 weeks post injury, a modified in situ force physiological testing was performed [8]. After the testing, animals were euthanized and the TA muscles were harvested for histological evaluations (Fig. 1). Statistical analysis was performed with ANOVA and Scheffe’s F test as post hoc test. Statistical significance was defined as p < 0.05.

Results: MDSC Transplantation Enhanced Muscle Regeneration in Injured Muscle: Hematoxlin and eosin staining revealed centronucleated regenerating myofibers in the injured muscle which were counted and compared among the groups at 2 weeks post-injury. The MDSC treated groups (MDSC and MDSC/losartan groups) showed a significantly higher number of regenerating myofibers (MDSC group, 131.8±32.1; MDSC/losartan group, 132.4±28.5/hpf) when compared with the control group (40.7±36.2/hpf) (Fig. 2A). Moreover, the diameters of the regenerating myofibers in the MDSC/losartan group (400.6±75.9μm) were significantly larger when compared with the other groups (MDSC group, 167.3±38.3μm; control group, 92.7±46.0μm) (Fig. 2B).
Administration of Losartan Decreased Fibrosis Formation in Injured Muscle: After Masson’s trichrome staining; the area of fibrotic scar tissue was evaluated and compared among the groups at 4 weeks post-injury. The MDSC/losartan group had significantly less fibrotic area (3.74±1.41%) when compared with the control and MDSC groups (48.24±22.99 and 19.39±4.12%, respectively) (Fig. 2C).

MDSC Transplanted Muscle Showed Rapid Improvement of Muscle Torque: At 2 weeks post-injury, the MDSC treated groups (MDSC and MDSC/losartan groups) showed significantly greater specific peak twitch and tetanic torques (twitch; 44.1±10.3 and 47.2±13.7, tetanic; 67.2±16.2 and 69.4±13.0g/cm², respectively) when compared with the control group (twitch; 24.9±5.3, tetanic; 46.8±11.6g/cm²). Moreover, there was no significant difference between the MDSC treated groups and the normal group (twitch; 36.0±11.1, tetanic; 64.9±18.8g/cm²) (Fig. 3). At 4 weeks post-injury, the control group showed significantly less specific twitch and tetanic torques (twitch; 24.9±2.0, tetanic; 46.8±5.9g/cm²) when compared with the other groups (MDSC and MDSC/losartan groups) (twitch; 37.3±3.1 and 40.4±2.1, tetanic; 68.9±7.4 and 85.8±7.9g/cm², respectively). Interestingly, at 4 weeks post-injury the MDSC/losartan group showed a significantly greater specific peak tetanic torque when compared with the MDSC group (Fig. 3).

MDSC Transplantation after Losartan Treatment Enhanced Follistatin, Smad7 and MyoD Expressions in Injured Muscle: Follistatin (FSTN) positive areas in the injured TA muscles were measured and compared among the groups at 1 week post-injury. The MDSC/losartan group (0.60±0.22%) showed significantly greater FSTN expression when compared with the MDSC and control groups (0.29±0.19 and 0.00±0.00 %, respectively) (Fig. 4A). Smad7 expression in the injured TA muscles were measured and compared among the groups at 2 weeks post-injury. The MDSC/losartan group showed significantly greater Smad7 expression (28.77±26.37%) when compared with control and MDSC groups (2.15±1.86 and 8.25±7.64%, respectively) (Fig. 4B). MyoD expression in the injured TA muscles were also measured and compared among the groups at 2 weeks post-injury. The MDSC treated groups (MDSC and MDSC/losartan groups) showed significantly greater MyoD expression (9.40±3.78 and 30.75±9.85%, respectively) when compared with the untreated control group (0.80±0.52%). Moreover, the MDSC/losartan group showed significantly greater MyoD expression when compared with the MDSC group (Fig. 4C).

Discussion: The combinatorial therapy of intramuscular transplantation of MDSCs and administration of losartan following a contusion injury improved overall skeletal muscle healing when compared to the individual treatment. Losartan enhanced MDSC differentiation towards myogenic cells and inhibited fibrosis formation via an increase in Smad7 and MyoD expression. Furthermore, losartan enhanced the recovery of muscle torque 4 weeks post-injury. These results suggest a possible mechanism for the beneficial effects imparted by losartan when administered to MDSC transplanted skeletal muscle following injury. These findings could contribute to the development of biological treatments to accelerate muscle healing after muscle injury.

Significance: Losartan could effectively enhance the beneficial effect of transplanted MDSC in the injured skeletal muscle.
Figure 1.

Muscle contusion injury → Physiological test → Histological evaluation → RT-PCR analysis → Physiological test → Histological evaluation

1wk → 2wks → 3wks → 4wks

MDSC group
Transplantation of MDSCs at Day 4

MDSC/losartan group
Administration of losartan from Day 3
Transplantation of MDSCs at Day 4

Figure 2

A

Number of regenerative myofibers/gram

B

Diameter of regenerative myofibers/gram

C

Fibrosis area [%]

PBS  MDSC  MDSC/losartan
PBS  MDSC  MDSC/losartan
PBS  MDSC  MDSC/losartan

* indicates significant difference (p < 0.05)