Immediate Local Transplantation of Mesenchymal Stem Cells into a Severely Injured Skeletal Muscle in Rats Improves the Functional Outcome Comparable to Delayed Transplantation

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INTRODUCTION:
Skeletal muscle trauma often leads to severe functional deficits due to insufficient regeneration of the musculature itself, but on the other hand also due to impaired bone healing. (1) Mesenchymal stem cell (MSC) therapy is a promising but still experimental tool in the regeneration of muscle function after severe trauma. (2) One of the most important questions, which has to be answered prior to a possible future clinical application is the ideal time of transplantation. Due to the initial inflammatory environment we hypothesized that a local injection of the cells immediately after injury would result in an inferior functional outcome compared to a delayed transplantation.

MATERIAL AND METHODS:
Twenty-seven female Sprague Dawley rats weighing 140 – 160 g (Charles River, Germany) were used for this study. All animal experiments were carried out according to the policies and principles established by the Animal Welfare Act, the NIH Guide for Care and Use of Laboratory Animals and the national animal welfare guidelines. The study was approved by the local legal representative. (Landesamt für Arbeitsschutz, Gesundheitschutz und technische Sicherheit, Berlin) Bone marrow was aspirated from both tibiae of each animal and autologous MSC cultures obtained from the material. Two weeks later, the animals were separated into three groups (each n=9) and the left soleus muscles of the animals of group 2 were transplanted with the same number of MSCs. Group 1 and 3 received a sham treatment with the application of saline solution in an identical manner. In vivo functional muscle testing was performed three weeks after transplantation to quantify muscle regeneration. The tendon of the soleus muscle was connected to the force transducer of a muscle force measuring device (Experimetria, Hungary). Subsequently the sciatic nerve was stimulated bipolarly in two different modes. In the fast twitch mode 6 pulses at 9 mA / 75 Hz with a duration of 0.1 s and 5 s intervals were used. After this the maximum muscle strength was evaluated via sciatic stimulation periods of 3 s at 9 mA / 75 Hz for 6 times with 5 s intervals, reaching tetany in all cases. Statistical significance analysis was performed using the non-parametric Wilcoxon test for dependent samples when comparing measurands intraindividually. The non-parametric Mann-Whitney-U test for independent samples was used for comparisons between the treatment and the control group. The level of significance was set to 0.05.

RESULTS:
Fast twitch stimulation of the healthy right soleus muscles of all animals resulted in an average contraction force of 0.52 ± 0.14 N. Tetanic contraction forces amounted to 0.98 ± 0.27 N in the uninjured soleus muscles, Four weeks after trauma the ratio of fast twitch and tetanic forces, which represents the contraction reserve of the muscles, showed a significant difference between traumatized and healthy muscles with a mean value of 0.80 ± 0.12 (left) and 0.54 ± 0.15 (right, p < 0.001). No difference could be observed between the twitch/tetanus ratios of the treatment and the control groups.

Maximum contraction forces after twitch stimulation decreased to 39 ± 18 % of the non injured right control side after crush trauma of the soleus muscles as measured in group 3. (Fig. 1) Tetanic stimulation showed a reduction of the maximum contraction capacity of 72 ± 12 % of the value obtained from intact internal control muscles. The transplantation of 2 x 10^6 MSCs one week after trauma improved the functional regeneration of the injured muscles as displayed by significantly higher contraction forces in group 2 (twitch: p = 0.014, tetany: p = 0.018). Local transplantation of the same number of MSCs immediately after crush injury was able to enhance the regeneration process to a similar extent with an increase of maximum twitch contraction forces by 73.3 % (p = 0.006) and of maximum tetanic contraction forces by 49.6 % (p = 0.037) compared to the control group. A comparison of the contraction forces between muscles treated by immediate and by delayed transplantation showed no significant difference between groups with p-values of 0.93 (fast twitch) and 0.73 (tetany).

DISCUSSION:
The presented results underline the effectivity of MSC transplantation in the treatment of severe skeletal muscle injuries. The most surprising finding was that despite of the fundamental differences of the local environment into which MSCs had been transplanted, similar results could be obtained in respect to functional skeletal muscle regeneration. Although a certain extent of inflammation is necessary to support transplanted stem cells in a host environment, we hypothesized that the initial processes of necrotic tissue removal would rather reduce the functional effect of the cells. We assume that the effect of the MSC after immediate injection can partly be explained by their known immunomodulatory competences. (3) The data of our study provide evidence for a large time window of MSC transplantation after muscle trauma.

REFERENCES: