Melatonin enhances muscular regeneration following skeletal muscle injury in rats
+1,2Stratos, I; 1Richter, N; 2Rotter, R; 2Mittlmeier, T; 1Vollmar, B
+1Institute for Experimental Surgery, University of Rostock, Rostock, Germany;
2Department of Trauma and Reconstructive Surgery, University of Rostock, Rostock, Germany;
john.stratos@gmail.com

ABSTRACT INTRODUCTION:
Melatonin is a well known pleiotropic cytokine with anti-oxidative properties and cell regenerative capacity in liver, heart and brain. Although the beneficial effects of melatonin can be traced back to anti-oxidative properties, improved microcirculation and amplified tissue protective effects, little is known about the action of melatonin after muscle injury. In the current study we tested for the first time the hypothesis that application of melatonin could accelerate muscular regeneration after skeletal muscle injury in rats.

METHODS:
For this purpose we anesthetized 56 male Wistar rats (275-300 g body weight) with pentobarbital sodium and induced a blunt injury of the left soleus muscle by using an instrumented clamp as described before [1]. All animals received a daily dose of either melatonin 10 mg/kg body weight i.p. (melatonin; n=28 animals) or equivalent volumes of 4.5% ethanol as vehicle i.p. (control; n=28 animals). Subsequent observations were performed at day 1, 4, 7 and 14 after injury induction. After bilateral stimulation of the sciatic nerve fast twitch and tetanic forces of the soleus muscles were analyzed and given in percent of the forces of the non-injured muscle. By using histology and immunohistochemistry we analyzed leukocyte infiltration (CAE-analysis), satellite cell number (Pax-7 immunohistochemistry), muscle cell proliferation (BrdU immunohistochemistry) and the muscle cell apoptosis (cleaved caspase 3 immunohistochemistry). All data is given as means ± standard error of the mean (SEM). Differences between groups were assessed using a t-test. Statistical significance was set at p<0.05.

RESULTS SECTION:
Muscle injury caused a massive reduction of muscle force. From day 1 up to day 14 after injury the muscle tissue in the control group showed a continuous improvement of strength (twitch force: 62 % and tetanic force: 50 % of the contralateral muscle at day 14). Daily application of melatonin caused a significant improvement of muscle force throughout the entire experimental period. At the end of the experiment we could show a 1.3-fold rise of the twitch force and a 1.2-fold improvement of the tetanic force in the melatonin group compared to the control group (Fig. 1a and b).

Staining for satellite cells was performed by Pax-7 immunohistochemistry and revealed in the melatonin group significantly higher numbers of satellite cells at day 1 when compared to vehicle treated animals (Fig. 2a). At later time points, no difference between the control group and the melatonin group was noted. Incorporation of BrdU served as an indicator of cellular DNA synthesis and was found highest at day 1 and day 4 upon injury in both groups. At day 4 after injury, muscle tissue of melatonin treated animals revealed slightly higher mean values of BrdU-positive cells when compared to the corresponding values of the control group (Fig. 2b).

DISCUSSION:
The present study demonstrates that chronic treatment with melatonin enhances the biomechanical characteristics of the injured muscle by improving the twitch and the tetanic force after injury. Furthermore melatonin attenuates leukocyte infiltration immediately after injury. Finally we could show that the functional restoration of the muscle is based on an increased cellular turnover, i.e. increased proliferation and decreased apoptosis, and enhanced number of satellite cells. Thus we like to state that melatonin might represent an attractive therapeutic strategy to optimize the postraumatic course of muscle tissue healing.

REFERENCES: