Introducing A New Model Of Combined Nerve And Muscle Injury In Rats

Ioannis Stratos, M.D., Oliver Karle, Thomas Mittlmeier, Brigitte Vollmar, Prof.
University of Rostock, Rostock, Germany.

Disclosures:
I. Stratos: None. O. Karle: None. T. Mittlmeier: None. B. Vollmar: None.

Introduction: Major trauma of the lower extremities, like fractures or compartment syndromes, are often associated with secondary lesions of peripheral nerves and muscles. This phenomenon is usually a product of increased pressure on muscles and nerves at the site of injury by posttraumatic hematoma formation or by displaced bone fragments. Patients that experience complex extremity injuries are often dealing with impeded muscle regeneration of the injured region, loss of muscle strength, chronic pain and a prolonged rehabilitation period. Goal of our study was to evaluate the changes that occur in the peripheral skeletal muscle and nerve, after a combined muscle and nerve injury.

Methods: For this purpose we anesthetized 24 male Wistar rats (275-325 g body weight) with pentobarbital sodium (60 mg/kg body weight i.p.) and induced a chronic constriction injury of the left sciatic nerve (CCI) or a sham CCI (sCCI) [1]. At day 4 after the CCI and the sCCI all animals underwent a closed soft tissue injury (CSTI) on the left lower limb by means of a standardized impact device or a sham CSTI (sCSTI) [2]. Subsequent observations were performed at day 8 in all 4 groups (CCI/CSTI; sCCI/CSTI; CCI/sCSTI; sCCI/sCSTI; n=6 animals per group). Analysis of tactile and thermal allodynia served as indicators for pain. In addition, motor nerve conduction velocity as well as nerve fiber density (HE histology of the sciatic nerve; n=3 animals per group) allowed assessing the posttraumatic nerve lesion. The muscle regeneration of the hind limb was evaluated by analyzing in the soleus muscle, the fast twitch (sciatic nerve stimulation with 9 mA/75 Hz, 5 times for 0.1 s in 5 s intervals) and tetanic force (sciatic nerve stimulation with 9 mA/75 Hz, 5 times for 3 s in 5 s intervals). Muscle force data are given in Newton (N). The dry to wet soleus muscle weight ratio was also quantified and expressed in %. By using immunohistochemistry and histology we analyzed in the soleus muscle the cell proliferation (BrdU immunohistochemistry) and the apoptosis (TUNEL histology). Data are given as means ± standard error of the mean (SEM). Differences between groups were assessed using a one-way-ANOVA, * p<0.05 vs sCCI/sCSTI, # p<0.05 vs sCSTI/CSTI, + p<0.05 vs CCI/sCSTI.

Results: Planimetric analysis of the sciatic nerve showed a significant difference in the nerve fiber coverage between CCI and sCCI groups whereas the CSTI did not influence the nerve fiber density (nerve fiber coverage in % per observation field: CCI/CSTI: 78±6%; sCCI/sCSTI: 74±7%; sCCI/CSTI: 89±3; sCCI/sCSTI: 91±1). Tactile allodynia of the non-injured (right) hind limb did not show any major differences between all groups (Figure a). On the contrary quantitative examination of thermal and tactile allodynia in the injured (left) hind limb revealed a significant difference between CCI and sCCI groups (Figure b and c). The CSTI alone did not induce any allodynic symptoms in the injured hind limb (Figure b and c). The nerve conduction velocity could not be deduced in the CCI/CSTI and CCI/sCSTI groups whereas sCCI/CSTI and sCCI/sCSTI animals showed a response time of 0.33±0.04 and 0.28±0.04 ms respectively which was almost equal to the response time of the contralateral non-injured limb. Evaluation of the muscle force in the non-injured soleus muscle showed almost equal values between all groups regarding tetanic force and fast twitch. Muscle force analysis of the left soleus muscle showed a massive decrease of fast twitch and tetanic force after CCI (see Table). CSTI after sCCI caused a significant decrease of the tetanic force and a moderate reduction of the fast twitch (see Table). Similar results were observed for muscle cell apoptosis (see Table) indicated by the huge increased muscle cell apoptosis after CCI and relative low increase of muscle cell death after CSTI. Muscle cell proliferation and dry to wet muscle weight did not show any difference between groups (see Table).

Discussion: The present study demonstrates that the CCI massively reduces the muscle force of peripheral muscles, is associated with allodynia, reduction of nerve fiber density and an impaired nerve conduction velocity. On the contrary CSTI is associated with a moderate reduction of muscle force and is not accompanied by allodynia or a compromised nerve conduction velocity. Apoptotic events in the peripheral muscle were majorly observed after CCI and also after the CSTI but in a lesser extent. Changes in the proliferation were not observed in the end of our experimental setup.

Significance: Current study highlighted some changes that occur during closed soft tissue injury and chronic nerve injury. This experimental setup can be used as a model for further studies that evaluate pathophysiological changes or innovative therapies after a combined nerve and muscle lesions.

Acknowledgments: The authors kindly thank Berit Blendow, Eva Lorbeer-Rehfeld, Dorothea Frenz, Maren Nerowski (Institute for Experimental Surgery) for excellent technical assistance.
