TROPHIC EFFECTS OF DENERVATED SKELETAL MUSCLE EXTRACTS

*+Fujiwara, H., Takimoto, Y., Okajima, S., Hitomi, S., Tamai, K., Hirasawa, Y. *+Department of Orthopaedic Surgery, Kyoto Prefectural University of Medicine, Kyoto 602-8041, Japan, PHONE 81-75-251-5540, FAX 81-75-251-5841

Introduction
Skeletal muscle, which is the target organ for motor neurons, influences the survival, growth, and differentiation of nerve cells. Protein factors derived from skeletal muscle promote neurite elongation and acetylcholine synthesis in vitro. Especially, extracts from denervated skeletal muscle are reported to increase the neurite elongation, however, the trophic effects of denervated skeletal muscle in vivo are not clearly understood. The aim of this study is to examine the effects of denervated muscle extracts on promoting rat’s peripheral nerve regeneration.

Materials and Methods
To prepare denervated muscle extracts, the limb skeletal muscles of Wister rats that was innervated by sciatic nerve were removed 1 week after denervation. Then they were homogenized, centrifuged, and dialyzed through cellulose membrane for 24hrs at 4 degrees. In same process, intact muscle extracts were prepared. 32 male wister rats weighing about 200g were used. T-shaped silicone tube was implanted between 15mm gap of the left sciatic nerve. The animals were divided into four groups. Group A was administrated with 0.2 mg/ml denervated muscle extracts. Group B was administrated with 0.2 mg/ml intact muscle extracts. Group C was control group administrated with saline. Group D was normal group without tube. After 3 months, 1,1’-dioctadecyl-3,3,3’,3’,-tetramethylindocarbocyanine perchlorate (DiI) was injected to the left anterior tibial muscle of all rats. Then 3 weeks later, all animals were perfused with 4% paraformaldehyde. The spinal cord and the dorsal root ganglion (DRG) were excised, frozen, and sectioned 20µm thick on a cryostat. We examined and counted the labeled cells by a fluorescent microscope.

Results
The motor neurons in segments L4-L5 of anterior horn and DRG cells in part of L4,5 root were labeled with DiI retrogradely in Group A, B, and D (Fig. 1 and 2). The number of labeled motor neurons were about 170 in Group A, about 30 in Group B, 0 in Group C, and about 520 in Group D. The number of labeled DRG cells in four Groups were counted about 130, 120, 0, and 1000 respectively (Fig. 3). There were significant differences in the numbers of labeled anterior horn cells between Group A and Group B (t-test, P<0.001), but no significant difference in labeled DRG cells.

Discussion
We previously indicated that muscle extracts prepared from new born rats have the neurotrophic effect on promoting not only motor but also sensory nerve regeneration. The results of this study demonstrated that the denervated skeletal muscle extracts of adult rats have the specific effects on the regeneration of motor nerve in vivo.

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.
☐ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.