Protective Action of Denervated Muscle Extract to the Trauma Induced Cell Death Of Motor Neuron

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Functional recovery of peripheral nerve after injury were often not so completed. One of the reasons was that peripheral nerve injury leads to the degeneration and death of a certain number of neuron. As to sensory neuron, degeneration and death can be partly prevented by a continuous local application of nerve growth factor. But to the traumatic degeneration and death of motor neurons, there is up to the present no effective protective factor available. The authors obtained an inspiration from the phenomenon that denervated muscle could induce its adjacent nerve fibers to sprout and grow, and investigated the protective effect of Denervated Muscle Extract (DME) to the degeneration and death of motor neurons after traumatic injury. Experiment was divided into two parts. In the first part, 10 Wistar rats with body weight of about 250g were selected. Under routine anesthesia both sides of their sciatic nerves were sectioned. After the surgery, samples were taken at 5 different time points, on 2, 5, 8, 12, 15 days after operation. The triceps muscles in the leg were taken, after weighting the muscles 10 times distilled water was added. The muscles were then homogenized and centrifuged (20,000g, 2hrs), clear supernatent was obtained. After ultraviolet protein quantitative determination, the above obtained clear fluid was diluted to a protein content of 1.2mg/ml, after ultra-filtration they were packed separately and stored for use under refrigeration. The second part of experiment was carried out with 5 litter (8 rats / litter) of 2 days old rats. Operation was carried out under ether anesthesia. In one litter after cutting the sciatic nerve on one side, muscle extracts collected at different time points were applied locally so as to test which muscle extracts had the strongest activity. The rest 4 litters were divided into 4 groups and each received separately: (1) bilateral sciatic axotomy (2) bilateral axotomy but with DME unilaterally; (3) unilateral axotomy and (4) unilateral axotomy with DME added to the operated site. (DME was injected locally at the wounded site, 0.5ml, once every two days). On the 15th post-operation day lumbar cords were taken, a mark was made at the ventral median fissure by puncturing the cord perpendicularly at level L6. The spinal cord was frozen sectioned longitudinally with thickness of 20μm, one in two sections were taken serially and mounted on slide. The sections were stained with thionin and examined under microscope. Using the mark as centre the number of motor neurons in the anterior horn of the cord were counted in the field (magnification 4 x 10). The results indicated that sciatic axotomy in 3 days old rats caused 92% of anterior horn motor neurons of L5 spinal cord to die; if BME was added to one side, the mortality rate could be reduced to 12% (survival rate was 88% of the normal side). The anterior horn motor neurons on the DME protected side were obviously healthier than those on the unprotected side, both in terms of the number and
morphology. This experiment, with its preliminary results, proved that in the
denervated muscle extract there is a kind of motor neuron protection factor that
can protect the motor neuron from traumatic degeneration and death. The nature
of that factor and its mechanism of action remain to be further investigated.

Investigation of Vascular Implant in Degenerated Skeletal Muscles
Bridging Lesioned Recurrent Laryngeal Nerve
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After recurrent laryngeal injury, how to reestablish the innervation of
the laryngeal muscles to improve the function of throat. Hirito etc. performed
direct suturing of the nerve and found that there was electromyographic action
in laryngeal muscles but at phonation, the movement of vocal cord was in
disorder. Analysis with laryngeal electromyogram it was shown that
regenerating axons mostly grew in wrong direction. At present most scholars
know that the erroneous growth of the regenerated axons was the main cause of
inducing vocal cord palsy after direct suture of the recurrent laryngeal nerve. In
this experiment vascular implant in autogenous degenerated muscle was used to
bridge the lesioned recurrent laryngeal nerve to correct the erroneous direction
growth problem of the regenerating axons in simple suture so as to improve
laryngeal function.

Eight domestic dogs, 10-15 kg, were selected (2 for control) for this
experiment. Sodium pentobarbital (3% 1 ml/kg) was used as intraperitoneal
anesthesia. One side of the recurrent laryngeal nerve was exposed from the
anterior tracheal oesophageal groove, then its medial and lateral branches were
exposed at the cricothyroid junction. A segment of the branches were isolated
by blunt dissection and a lesion of 1cm long was made. The adjacent
thyropharyngeal muscle was cut and made into an autogenous bridge according
to the method of Zhu Jia Kai and placed at the lesioned nerve, as diagram:

Proximal trunk of — Muscle bridge—
recurrent laryngeal nerve

Various medial branches

Lateral branch

A tunnel was made through the muscle at the space between the muscle
cascicles. Then the broken ends of the nerve were embedded into these tunnels,
the neurolemma and myolemma were suture to close up the tunnel. Adjacent
vascularized muscle bundles were first lesioned and wrapped around the muscle
bridge.

After lesion of the left recurrent laryngeal nerve, the left arytenoid of
the vocal cord was abruptly turned outward, vocal cord was obviously shifted to
the left and in a paramedian position. Barking sound of the operated dogs
became hoarse. After rearing for two months the sound of barking gradually
recovered, the frequency, loudness and sound pattern were comparable with