Therapeutic possibilities of brachial plexus avulsion injuries: clinical and experimental approaches

Ph.D. Thesis

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Original papers related to this thesis

I. Simonka JA, Nagy E, Vörös E, Császár J, Pintér S: A felső végtag fedett érsérüléseinek diagnosztikája és kezelése. Magyar Traumatológia, Ortopédia, Kézsebészet, Plasztikai Sebészet 1995;4:321-327

II. Nagy E, Morvay Z, Pintér S: A plexus brachialis MRI vizsgálata
Magyar Radiológia 1997;1:11-15.

III. S Pintér, L Mendler, L Dux: Neural impacts on the regeneration of skeletal muscles Acta Biochimica Polonica 2003;50:1229-1237 IF. 1,863

IV. A Nógrádi, A Szabó , S Pintér and G Vrbová: Delayed riluzole treatment is able to rescue injured rat spinal motoneurons Neuroscience 2007;144:431-438 IF. 3,41

V. Pintér S, Janka Cs, Kószó B, Czifra A, Simonka JA: Könyök flexió helyreállítása musculus gracilis funkcionális szabadizom transzplantációval C5C6 (C7) szülési plexus brachialis sérülés után 16 évvel. Magyar Traumatológia Ortopédia Kézsebészet Plasztikai Sebészet 2007;50:63-66.

Abstracts related to the thesis

I. Nagy E., Morvay Z., Pintér S.: MRI scanning of brachial plexus. XVIII. Magyar Radiológus Kongresszus, Tihany, 1996. május 8-11. Magyar Radiológia (Suppl) 1996. 1. kötet.

II. J. A. Simonka, S. Pinter, J. Császár: Combined Brachial Plexus Surgery and Closed Arterial Injuries of the Upper Extremity. Abstract in Swiss Surgery (Suppl) 2/1996.

III. Pintér Sándor, Horváth Attila, Kincses László: Plexus brachialis sérülés utáni késői rekonstructio felnőttkorban. Magyar Traumatológia Ortopédia Kézsebészet Plasztikai Sebészet. XLII.évf. (Suppl) 1999.

IV. S. Pintér, L. Mendler, E. Zádor, L. Dux: Skeletal muscle adaptation capacities after denervation/reinnervation Neuromuscular Disorders Vol. 13 Nos. 7-8 September 2003 (abstract) IF 3,34.

V. Simonka J.A..Kincses L.,Pintér S. Császár J. Vörös E.: Advantages of the new computer analysed myelo-CT method for evaluation and treatment of avulsion injuries of brachial plexus J.Hand Surg. 2005;30B:S1 71 (abstract) IF 0,759.

VI. L. Mendler, S. Pintér, M. Kiricsi, L. Dux
Changes in fiber-type composition of re-innervated rat soleus muscle regenerating from notexin-induced necrosis Neuromuscular Disorders Vol 16 (Issues 9-10 Page 693) October 2006 (abstract) IF 3,34.

List of abbreviations in the text

AchE: acetylcholinesterase
ABPI: adult brachial plexus injury
ALS: amyotrophic lateral sclerosis
BMRC: British Medical Research Council
ChAT: cholinacetyltransferase
CNS: central nervous system
EM: electron microscopy
GH: glenohumeral
HE: haematoxilin-eosin
mRNA: messenger ribonucleic acid
NMDA: N-methyl-D-aspatare
OBPI: obstetrical brachial plexus injury
R L: retrograde labelling
SERCA: Sarco/Endoplasmic Reticulum Ca^{2+} -ATPase
TOS: thoracic outlet syndrome
MRF myogenic regulatory factor

1. Introduction:

Brachial plexus injuries usually occur at birth (Obstetrical Brachial Plexus Injury; OBPI) or in traffic accidents (Adult Brachial Plexus Injury; ABPI). They are usually induced by a high energy trauma but partial injuries can develop even by a blunt trauma to the shoulder as a result of a complicated clavicular fracture or shoulder dislocation. Direct and open injuries of the brachial plexus are rare.

Clinical symptoms vary according to the type and the degree of the injury. Particularly serious is the avulsion injury where spinal roots are torn out from the spinal cord. In these cases, conventional peripheral nerve reconstruction methods can not be applied.

The incidence of brachial plexus injuries in newborns is 1-2 per 1000 live births (Gilbert, 1993). The most frequent etiological causes are macrosomic fetus, shoulder dystocia at delivery and prolonged labor, but it can occur in the case of premature birth and caesarean delivery as well (Slooff, 1995; Dyachenko *et al.*, 2006). In severe cases it is accompanied by other injuries such as clavicular or vertebral fractures, epiphyseolysis of the humerus and Horner's syndrome (Alfonso *et al.*, 2006). In the majority of cases traction with scar formation develops. In 15% of all cases truncal disruption or avulsion occurs, which requires surgical repair (Bahm, 2003). According to clinical symptoms, upper (Erb-Duchenne), lower (Klumpke) or total paresis are distinguished (Geutjens *et al.*, 1996; Jennett *et al.*, 2002). The diagnosis and surgical indication are essentially based on clinical examinations and the Tassin criteria (Gilbert *et al.*, 1987).

Post-traumatic brachial plexus paralysis is usually caused by high-velocity and high impact motor vehicle accidents that occur in the young productive population (Alnot, 1995). Avulsion injuries of the brachial plexus result in the most devastating palsies of the affected upper extremity. The prognosis is grave and the functional results are of limited degree. These injuries are usually associated with polytrauma and as a result the diagnosis is often late. Previously we gained experience in MRI mapping of the supra-ganglionic region (Nagy *et al.*, 1997), (it is the intravertebral course of spinal root from the spinal cord to the spinal ganglion). Modern devices give the chance of reliable investigation on the infraganglionic

part of the brachial plexus, which courses from the spinal ganglion to the level of clavicule (Rankine, 2004). On the basis of our experience, recently we preferred and used myeloCT in the diagnostics of avulsion injuries (Hems *et al.*, 1999; Simonka *et al.*, 2005). We do not use MRI examination to follow the changes of denervated muscle in the daily practice (West *et al.*, 1994).

Exploration of the brachial plexus is performed from the frontal supraclavicular approach, which can be extended to include the infraclavicular part with or without a clavicular osteotomy. This latter method can be useful in cases with neurolysis and grafting or with associated injury of the subclavian or axillary artery (Millesi, 1977). The suggested surgery in the early nerve reconstructive period can be neurolysis (removing the scar or reactive tissue from a peripheral nerve or nerve root), or in cases of truncal injuries nerve suture or grafting (bridging the nerve gap with autologous segment of peripheral nerve). In serious avulsion injury these interventions can not be applied as the injury occurs more proximally. Depending on the type of injury, root avulsion may affect the anterior horn and/or the posterior horn of the spinal cord (Nagano, 1998; Terzis & Kostas, 2006). Avulsion injury causing a true sensory lesion alone does not occur typically (Vekris & Soucacos, 2001).

Currently in C5-C6-C7 avulsion injuries we can apply neurotization to restore the most important functions such as the upper arm abduction and elbow flexion. In case of neurotization, reinnervation of the injured muscle is performed with the peripheral part of a functioning motor pathway (phrenic nerve, accessory spinal nerve, Th3-Th6 intercostal nerve, motor branch of ulnar nerve), or with a peripheral nerve graft (usually the sural nerve). In our experience restoration of abduction can be most successfully achieved by connecting the distal part of the accessory spinal nerve to the suprascapular nerve. To regain the biceps function we performed mostly an intercostal nerve to musculocutaneous nerve anastomosis (Bahm *et al.*, 2005).

When functional muscle reinnervation was made possible by anatomical reinnervation, after the process of nerve recovery first the muscle tone develops. Then motion triggered by the original innervation stimulus recovers. Finally, voluntary motion appears (Kanamaru *et al.*, 1999).

Additionally a large group of secondary surgical interventions can be performed for brachial plexus injuries, mostly done as a palliative procedure, several years after the initial trauma to

restore at least some of the lost functions (Berger & Brenner, 1995; Mohammed, 1998; Ruhmann *et al.*, 1999).

After a brachial plexus injury reconstruction corresponding to the original intact state is not possible, especially if complicated with avulsion of one of the cervical roots. Surgical intervention usually comprises a series of surgeries, first to stabilize the shoulder and to reconstruct abduction in the shoulder and flexion in the elbow function. In addition to this, a distal tendon transfer and arthrodesis may be necessary (Ochiai *et al.*, 1995).
Although brachial plexus injuries divided into the same categories, individual results vary due to the differences in accompanied injuries, postoperative physiotherapeutic possibilities and the motivation of the patient.
Efficiency of the surgical intervention can be assessed by the degree of stability or reinnervation. On the whole, results can be assessed by a grading score which indicates the state of the limb: BMRC scale M0-M5, Seddon score, Constant score, Mallet score (Bae *et al.*, 2003).

It is well established that avulsion injury in the experimental animal models induce cell death of the affected motoneurons (Chan *et al.*, 2001; Chan *et al.*, 2002; Gu *et al.*, 2004). It has been postulated that this motoneuron loss can be prevented by blocking the NMDA receptors and other cell membrane channels contributing to the cell damage following an excitotoxic injury. It has been recently shown that the NMDA receptor antagonist riluzole is able to rescue injured motoneurons destined to death in adults (Nógrádi & Vrbová, 2001). However, it was not clear, how long the avulsed motoneurons could be left untreated without losing their capacity to reinnervate peripheral targets.
Not all of the motoneurons die instantaneously after an avulsion, some of them can be rescued if the ventral root is reinserted into the spinal cord (Nógrádi & Vrbová, 1996; Hems *et al.*, 1999; Chai *et al.*, 2000; Nógrádi & Vrbová, 2001). This reimplantation represents an interesting surgical strategy for the treatment of brachial plexus avulsion injuries but is not always possible because of the physical distance between the spinal cord and the avulsed rootlets. Some experiments have shown that this gap can be bridged by the use of a synthetic tube (Liu *et al.*, 1997; Liu *et al.*, 1998; Kassar-Duchossoy *et al.*, 2001). Other data have

shown that implantation of peripheral nerve grafts after spinal root avulsion greatly enhances the motoneuron survival (Bertelli & Mira, 1994; Gu *et al.*, 2004).

After successful reimplantation or proximal nerve reconstruction, the muscle remains denervated for some time. This period of time depends on the time of axon regeneration and the course of reinnervation process. It is well-known that the innervation is essential for the normal development, and for the regeneration of skeletal muscles, also (Pette & Staron, 1990; Pette & Vrbová, 1992). Denervated muscles show impaired regeneration capacities (Sesodia & Cullen, 1991) which is most pronounced in the second part of regeneration, *i. e.* the newly formed primitive fibres are not able to differentiate to reach their original size.

Clinically, nerve-reconstruction surgery (reinnervation) could help in the recovery of skeletal muscles after a nerve injury, at least partially (Kirjavainen *et al.*, 2007). However, muscle function does not full returning after the high level injury of the mixed type peripheral nerve, even if the microsurgical nerve reconstruction was successful. This is probably caused by the loss of axon adaptation after microsurgery and by the limited regeneration of the reinnervated muscles. However, the regeneration capacities of denervated/reinnervated muscles have not been investigated at the morphological/molecular level yet.

The snake venom notexin causes complete necrosis to skeletal muscle followed by a regeneration process. This regeneration process has been thoroughly characterized in the last decade in normal and dystrophic skeletal muscles (Sewry *et al.*, 1992; Dux *et al.*, 1993; Wilson *et al.*, 1994a; Wilson *et al.*, 1994b; Zádor *et al.*, 1996; Zádor *et al.*, 1998; Mendler *et al.*, 1998a; Mendler *et al.*, 1998b; Zádor *et al.*, 1999; Mendler *et al.*, 2000; Zádor *et al.*, 2001). The many aspects of this model has been already described allows to make comparison between the regeneration process of normally innervated muscles and that of denervated/reinnervated ones (Pintér et *al.*, 2003)

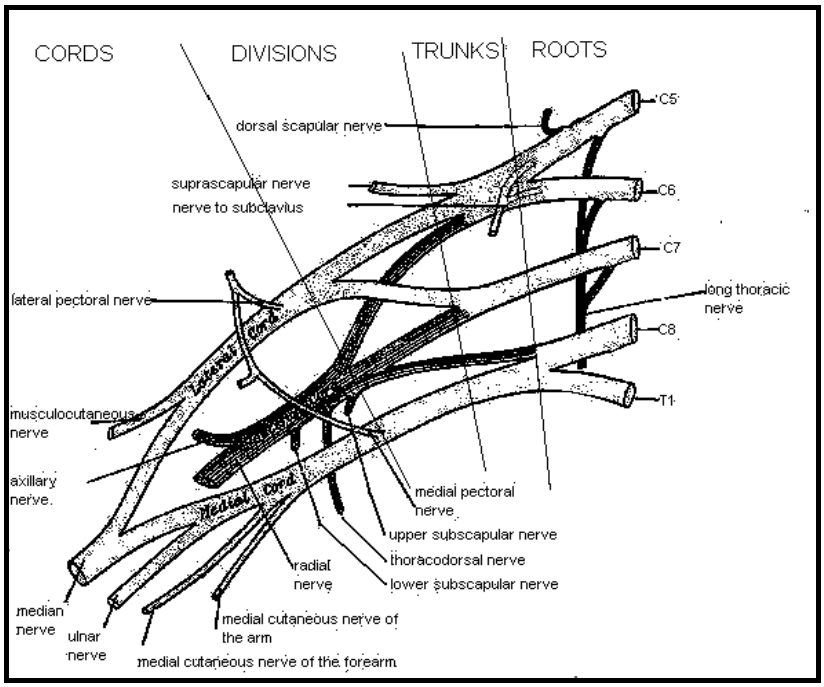

Figure 1.Schematic drawing of the right brachial plexus; modified figure taken from Alnot & Narakas.A., (1996).

2. Aims:

1, To establish the clinically relevant methods, suitable for the functional recovery of upper limb following an avulsion injury.

2, To determine the number of surviving and reinnervating motoneurons following avulsion injury when the excitotoxic environment of motoneurons has been considerably reduced.

3, To determine the possible maximum delay at which the NMDA receptor antagonist riluzole is still effective following ventral root avulsion injury.

4, To establish the degree of regenerative capacity of the reinnervated muscle after a necrosis induced by injection notexin.

3. Materials and methods

Clinical studies

Since 1994, we have been treating patiens with brachial plexus injury at Department of Traumatology, Albert Szent-Györgyi Medical and Pharmaceutical Centre Faculty of Medicine, University of Szeged, including surgical interventions of brachial plexus injuries in newborns and adults, primary nerve and secondary reconstruction surgery, as well as surgery of the shoulder girdle tunnel syndromes and Thoracic Outlet Syndrome (TOS).
The present thesis work is based on the 287 operations I performed within this period. (Fig.2)

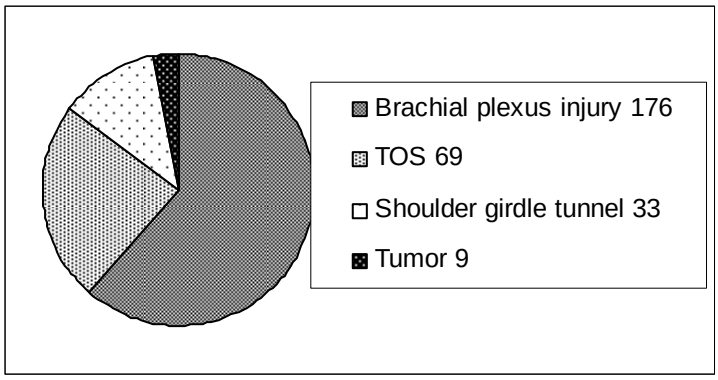

Figure 2. Cases of brachial plexus surgery performed between 1994 and 2007

Supraclavicular lesions which account for 81% of all cases in our experience may be grouped as follow (Table 1):

Types of palsies	OBPI %	ABPI %
C5C6 or C5C6C7	56	22
C8Th1	8-10	2-3
Total	34	74-76

Table 1. Types of palsies in our experience

Experimental models

Ethical issues

The experiments were carried out with the approval of the Committee for Animal Experiments, University of Szeged regarding the care and use of animals for experimental procedures. All the procedures were carried out according to the Helsinki Declaration on Animal Rights. Adequate care was taken to minimize pain and discomfort.

Surgery

All the operations were carried out under deep chloralhydrate anaesthesia (4%, 1ml/100 g body weight). The operations were performed under aseptic circumstances.

Avulsion model

Sprague-Dawley rats (200-250 gr) were used in the avulsion study groups. In the first experimental setup the left L4 ventral root was avulsed from the cord and then the L4 ventral root were reimplanted dorsolaterally into the spinal cord. Four of these animals remained untreated while the other groups of animals were treated with riluzole for 3 weeks. The intact animals were used for counting the L4 motoneuron pool.

In the other groups of animals the right C6 ventral root was avulsed from the spinal cord. The animals were divided into five groups. Group I.: the untreated controls served only to determine the number of motoneurons in the C6 spinal segment, Group II.: avulsion only, Group III.:the avulsed root was reimplanted dorsolaterally into the spinal cord, Group IV.: after avulsion, peripheral nerve grafts were implanted to bridge the spinal cord and the surrounding muscle, Group V.: the avulsed roots were reimplanted dorsolaterally into the spinal cord and treated immediately with riluzole (Fig.3).

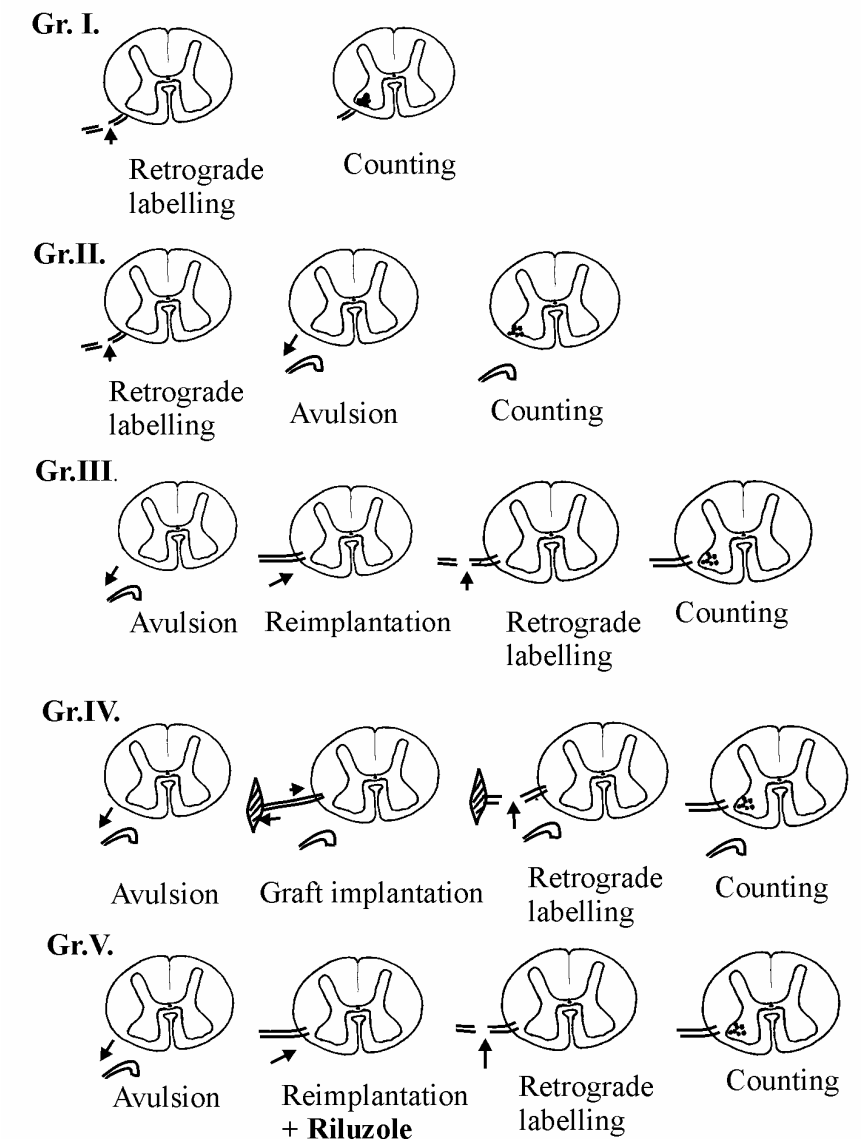

Figure 3. Schematic draw of experimental model at the C6 level.

Lumbar model

Laminectomy was performed at the level of T13-L1 vertebrae, the dura was opened and the left L4 ventral root was pulled out leaving the dorsal roots intact. Then the cut end of the ventral root was gently inserted into the dorsolateral part of the spinal cord (Nógrádi & Vrbová, 1996; Nógrádi & Vrbová, 2001). The spinal cord was covered with the remaining dura, the wound was closed and the animals were allowed to recover.

Cervical model

Laminectomy was performed at the level of C4-C6 vertebrae, the dura mater was opened, the right C6 dorsal root was cut, the ventral root was avulsed by traction with a fine hook controlled under a surgical microscope. The avulsed ventral root was gently inserted into the dorsolateral part of the spinal cord. The ipsilateral common peroneal nerve was dissected and approx. 2 cm part of it was removed. This peripheral nerve graft was used in group IV. The end of the graft was inserted into the dorsolateral part of the spinal cord, the other end inserted into the rectus capitis posterior major muscle. The spinal cord was covered with the remaining dura mater, the wound was closed and the animals were allowed to recover.

Riluzole treatment

Animals were treated with riluzole (-2-amino 6-trifluoromethoxy-benzothiazole-), (kind gift from Tocris Cookson Ltd, Langford, UK) 4mg/kg./for 3 weeks. Riluzole treatment started either immediately on the day of surgery or 5, 10, 14 and 16 days following surgery (n=5 in each group). The drug was injected intraperitoneally daily for 1 week and every second day for the next 2 weeks. Four animals remained untreated. This treatment protocol was based on the successful riluzole treatment described earlier (Nógrádi and Vrbová, 2001).

Retrograde labelling and immunohistochemistry

Three months after the surgery the animals were deeply anaesthetized with chloralhydrate. On the operated side the ventral ramus of the left L4 spinal nerve was sectioned and the proximal stump of the nerve covered with few crystals of Fast Blue (FB, Illing Plastics GmbH, Breuberg, Germany).

Three days after application of fluorescent dyes the animals were anaesthetized and perfused transcardially with 4% paraformaldehyde in a 0.1 mol/l phosphate buffer (pH 7.4).

The lumbar portion of the spinal cords, with the reimplanted ventral root were removed and kept in fixative for 4 h. The tissues were then immersed in 30% sucrose. Serial 25 μm thick cryostat sections were cut, mounted on gelatinized slides and examined in an Olympus BX50 fluorescence microscope (Olympus, Tokyo, Japan).

The retrogradely labelled cells were counted. To avoid double counting the same neuron present in two consecutive sections the retrogradely labelled neurons were mapped with the

aid of an Olympus (Olympus, Tokyo, Japan) drawing tube, and their locations were compared to that of labelled motoneurons in the previous section.

Three spinal cords from each group were processed further for choline acetyltransferase (ChAT) immunohistochemistry. Sections processed for ChAT immunohistochemistry were preincubated in 3% normal goat serum for 1 h, then incubated with a polyclonal goat anti-ChAT antibody (Chemicon, Hofheim, Germany, 1:200) overnight at 4 °C. The immune reaction was completed using the avidin– biotin technique (reagents were purchased from Vector Laboratories, Burlingame, CA, USA) and finally tyramide-amplified with the Cyanine3 TSA kit (Tyramide Signal Amplification, PerkinElmer, Zaventem, Belgium). The number of ChAT-stained motoneurons was also determined in the pools where retrogradely-labelled cells were found both on the operated and control sides. Some sections were stained with cresyl violet to assess the morphology of the spinal cord. Sections were photographed using an Olympus DP70 digital camera mounted on the microscope.

The retrograde labelling and the immunohistochemical process was carried out in the C6 avulsion/reimplantation model on the same way. The only difference was in Group IV, where the distal portion of the implanted peripheral nerve was sectioned and labelled.

Muscle regeneration model

Male Wistar rats (250 gr) were used in the muscle regeneration model. The left sciatic nerve was exposed at the proximal third of the thigh by splitting the gluteal muscles. In the first group of animals, approx. a 12 mm long nerve segment was resected and used as an autologous nerve graft. The coaptation sites were sutured by 10/0 nylon epineural sutures. In the second group of rats reinnervation was achieved by making simple sutures at the proximal cutting level. In the third group of animals approx. 12 mm long nerve segment was removed without nerve reconstruction. Based on preliminary experiments, 3 month after microsurgery the soleus muscles of denervated, sutured and grafted animals were removed and compared to the contralateral normal muscles. In the fourth group of (grafted) rats, muscle necrosis was induced by the injection of notexin (20 μg im.). 1, 3, 5, 7, 10, 21 28 and

35 days after the injection, soleus muscles of both the injected and the contralateral hindlimbs were removed and weighed (Dux *et al.*, 1993).

Morphology: Cryostat sections of 15 µm thickness were stained with haematoxylin-eosin (HE) for light microscopy. Some samples of regenerated, grafted soleus muscles were also processed for standard electronmicroscopy (EM). Ultrathin sections cut in an ultramicrotome were investigated with a JEOL JEM-1010 (Jeol,Tokyo,Japan) electron microscope.

Dynamics of motor endplate formation: Control and regenerated muscles were stained by the method of Tago based on the Acethylcholinesterase-activity (AChE) of the endplate (Tago *et al.*, 1986)

Statistical analysis

The non-parametric Mann-Whitney U test, the one-way ANOVA test and paired *t*-test were used to compare the data.

4. Results:

4a: Clinical results:

Clinical results of the typical treatment of C5-C6-C7 avulsion injuries:
In this section typical avulsion injury cases are presented with varying symptoms and clinical approach.

Treatment of left C5-C6 obstetric brachial plexus root avulsion injury

Status:
Erb Duchenne paresis on the left side at delivery, no spontaneous recovery of shoulder abduction and elbow flexion after an observation period of three months.

Surgery:
1. Supraclavicular exposure, neurotization of the accessory spinal nerve to suprascapular nerve in order to restore shoulder abduction at the age of 3 months.
2. At the age of 10 months Th3-Th4 neurotization of the intercostal nerve to the musculocutaneous nerve in order to restore biceps function. In both cases the use of sural nerve graft was necessary.

Result:
Active elbow flexion range of motion 0–160 degree, motor activity M5, shoulder abduction 110 degree motor activity M5 on the left side 5 years after surgery (Fig.4).

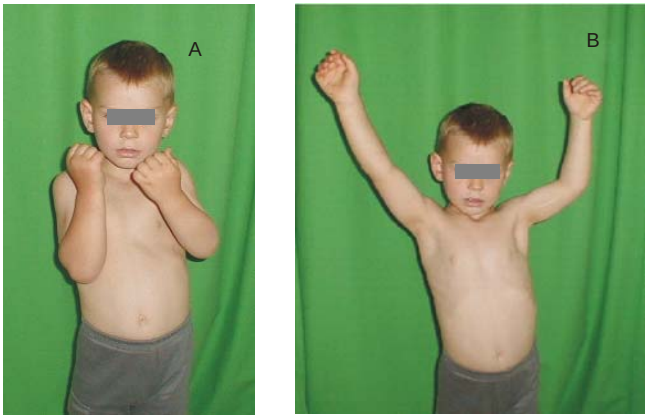

Figure 4. Elbow flexion (A) and shoulder abduction (B) on the left side 5 years after surgery

Conclusion:

In the diagnosis of obstetric brachial plexus injury evaluation of the clinical features and the associated injuries, in addition to the examination of motion according to Katona`s method (Katona, 1997) and assessment of video records are sufficient. Indications for surgical intervention can be set up according to the Tassin criteria (Gilbert *et al.*, 1987). To alleviate the severe symptoms caused by the neonatal injury it is firmly suggested to perform an early surgical correction by the age of three months (Kirjavainen *et al.*, 2007). The intercostal neurotization at the age of 6-12 months, as a second-stage procedure can be performed. In the course of intercostal neurotization motor nerves exposed in the midaxillary line are used, with a graft in all cases (Hentz & Meyer, 1991).

Treatment of a C5-C6-C7 right side brachial plexus avulsion injury, sustained at 1.5 years of age from a traffic accident

Status:

Brachial plexus root avulsion injury from road traffic accident is extreme rare at this age of life. The right handed 1.5 years old girl had no voluntary activity in proximal arm muscles, the humerus in internal rotation, the forearm in prone position was seen. No active wrist,

thumb and finger extension was found. Sensation was normal over the palmar surface of the hand. MRI finding was: C5-C6-C7 root avulsion.

Surgery:

1. Supraclavicular exposure, accessory spinal nerve to supraclavicular nerve neurotization to restore shoulder abduction.
2. Th3-Th4 intercostal nerve to musculocutaneus nerve neurotization to restore biceps function.
3. Distal humeral osteotomy for positioning of the forearm.
4. Boyes surgery to restore selective hand movements because of high radial nerve injury.

Result:

Voluntary elbow flexion, thumb extension and palm pinch after the series of surgeries (Fig.5).

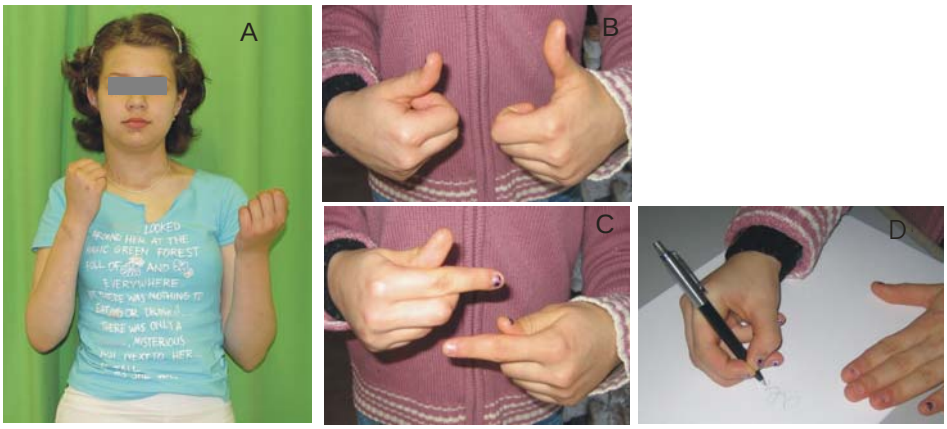

Figure 5. Elbow flexion (A), thumb extension (B), index extension (C), palm pinch,writing (D). Functional results 13 years after surgery

Conclusion:

In superior, middle trunk avulsion injuries, the accessory spinal to suprascapular nerve, or intercostal to musculocutaneus nerve neurotization is recommended. If intercostal neurotization is carried out an osteotomy of the humerus is necessary later to establish the optimal plane of elbow flexion.This is performed on the distal metaphysis. Boyes' surgery should be chosen in childhood for the treatment of proximal radial nerve palsy. It is true, that there are more efficient surgical processes for pro-supination and wrist extension than this,

but with Boyes' procedure the creation of isolated thumb and forefinger motion, key, and palm grasp can be achieved.

C5-C6-C7 Left sided avulsion injury in an adult patient, sustained in a traffic accident

Clinical feature:

A 27 year old left-handed man was involved in a road traffic accident with C5-C6-C7 root avulsed injury. Shoulder girdle and biceps muscles were paralyzed. Neurosurgical therapy was not a standard procedure for brachial plexus lesions at the time of the injury. After an observation period of six months no recovery of nerve function was found.

Surgery:

1. Spinal accessory to supraclavicular nerve neurotization
2. Th3-Th4 intercostal to musculocutaneous nerve neurotization to restore biceps function.

Result:

Shoulder stability and voluntary elbow flexion, range of motion 0–90 degree, muscle activity M3, 2 years after surgery (Fig.6).

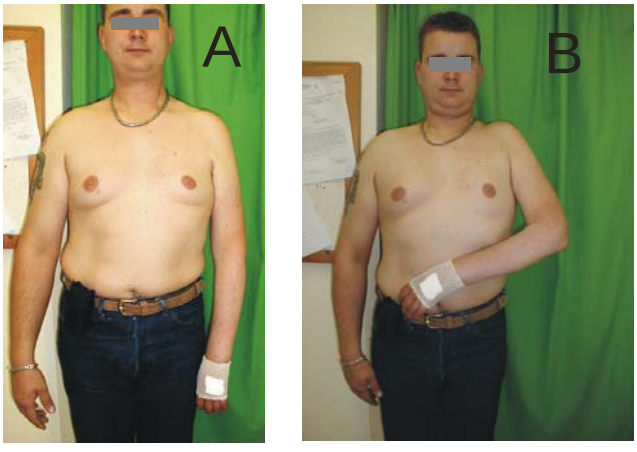

Figure 6. Shoulder stability (A) and elbow flexion (B) 2 years after surgery

Conclusion:

In adulthood, the biceps muscle denervation period is much longer after neurotization, because the large distance between the proximal functioning nerve stump and the muscle. Therefore the results are modest, even if we perform so-called distal intercostal neurotization following the motor nerves to the parasternal region. It is better to apply the phrenic or the lower part of the accessory spinal nerve.

This can be performed by partial transfer of the motor branch of the ulnar nerve. If adequate conditions are present, functional free muscle flap transfer gives a better outcome (Shin *et al.*, 2004).

Series of surgeries to restore valuable hand function 9 years after having suffered a C5-C6-(C7) right sided avulsion injury in an adult patient

Status:

19 years old right handed man was involved in a motorbike accident with C5-C6-(C7) root avulsion injury combination with brain contusion, maxillar fracture and shock. Flail of shoulder, disability of elbow flexion with valuable hand function was found 9 years after the injury.

Surgery:

1. Glenohumeral arthrodesis to restore shoulder stability.
2. Greater pectoral to biceps brachii muscle motor transfer, applying fascia lata strip
3. Steindler flexor plasty for augmentation of elbow flection

Result:

The abduction is 60 degree motor power M5, elbow flexion 10–100 degree motor power M5 2 years after surgeries (Fig.7).

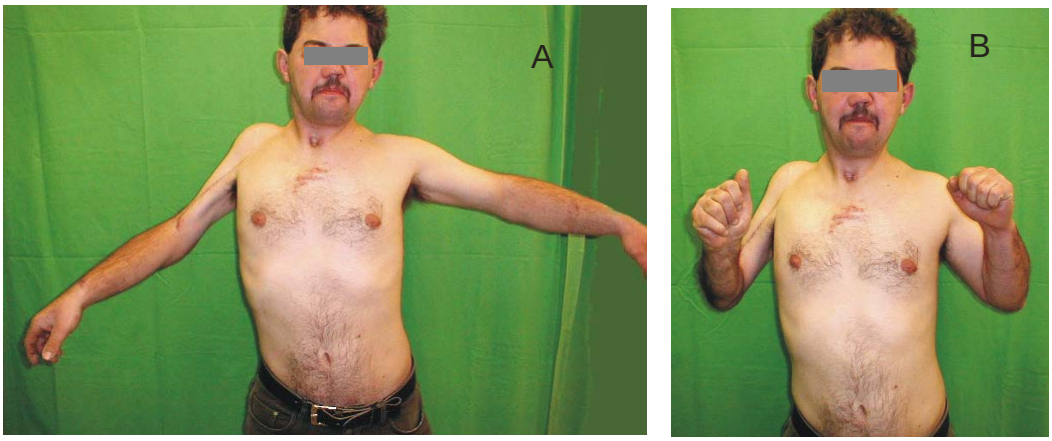

Figure 7. Upper extremity abduction after glenohumeral arthrodesis (A), elbow flexion after greater pectoral muscle and Steidler flexorplasty (B).

Conclusion:

After C5-C6-C7 avulsion injury, valuable hand function is wasted because of instability of the glenohumeral joint, and the loss of elbow flexion. Arthrodesis of the glenohumeral joint restores the balance of the trunk, and furthermore assures approximately 60 degrees of abduction. The previously applied uni- or rather bipolar latissimus dorsi flaps to restore biceps function gave poor results. Transfer of greater pectoral muscle assured higher amplitude and muscle strength. If a Steindler procedure is performed after GH arthrodesis, the positioning of the forearm flexors is remarkably important because the humerus is not able to accommodate losses in pro-supination with an axial rotation (Brunelli *et al.*, 1995; Beaton *et al.*, 1995; Saul *et al.*, 2003).

Surgery to enhance shoulder stability, and to restore elbow flexion 16 years after a C5-C6-(C7) obstetric brachial plexus avulsion injury

Status:

Erb Duchenne paresis developed at delivery because of shoulder dystocia on the left side. Shoulder girdle muscles atrophy, flail glenohumerale joint and disability of biceps muscle function were found at the age of 16. In contrast valueable hand function was seen: good wrist balance, total finger motion and skin sensibility were present.

Surgery:

1. Functioning gracilis free muscle flap transfer to biceps

Result:

Shoulder stability and voluntary elbow flexion was present 6 month after the surgery. Range of motion: 0–150 degree, motor activity M4 (Fig.8).

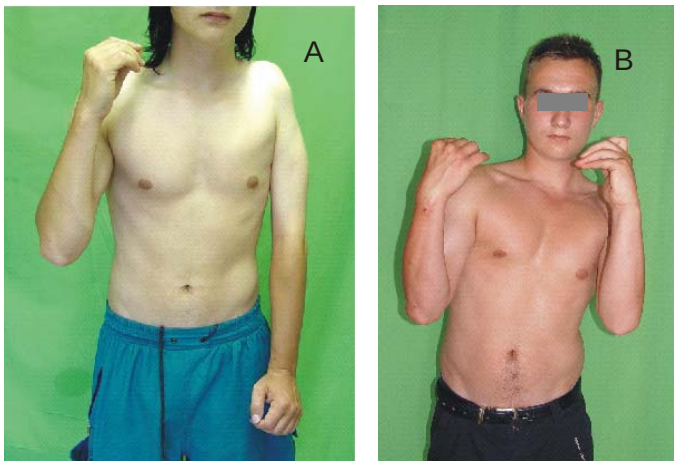

Figure 8. Elbow flexion before (A) and after (B) surgery

Conclusion:

In the presence of conditions requiring microvascular transplantation, the most rapid and efficient intervention is a functional gracilis free muscle flap transfer. Motion amplitude of the gracilis muscle assures the best function. In cases of high energy injury, angiographic evaluation is important before the planning of the surgery. The muscle artery is sutured onto the thoraco-acromial artery, and the vein onto the cephalic vein. The nerve can be sutured onto the lower part of the accessory spinal or phrenic nerve (Baliarsing *et al.*, 2002; Hattori *et al.*, 2002; Pintér *et al.*, 2007).

4b Experimental results

Avulsion model

Determination of the average number of retrogradely labelled motoneurons at the L4 and C6 spinal levels

First, the number of resident motoneurons in the L4 and C6 motoneuron pools was assessed applying by retrograde tracers to the ventral ramus of the L4 and C6 spinal nerves. The average number of retrogradely labelled motoneurons was 1164±29 (S.E.M.), in the L4 and 875,5±20,7 (S.E.M.) in the C6 spinal segment. The labelled motoneurons were localized mainly in the lateral motoneuron column of the spinal segment

Riluzole treatment increases the number of surviving motoneurons in rats after reimplantation of the ventral root in avulsed L4 and C6 spinal root

The effect of riluzole treatment following L4 avulsion and reimplantation was studied in the next series of experiments. In animals where riluzole was applied immediately after L4 avulsion and reimplantation, 763±36 (S.E.M.) 65% retrogradely labelled motoneurons were found, indicating that more than half of the total population of L4 motoneurones survived and these cells were able to grow axons into the L4 ventral root. (Table 2)

Intact L4 motoneurons	1164±29 (S.E.M.)
L4 avulsion and reimplantation	20.4±1.6 (S.E.M.)
L4 avulsion and reimplantation + Riluzole treatment started immediately after surgery	763±36 (S.E.M.)

Table 2. Number of retrogradely labelled motoneurons at the L4 spinal segment in various experimental groups

In animals treated with riluzole immediately after C6 avulsion and reimplantation 573,5 ±8,63 (S.E.M.) retrogradely labelled motoneurons were found indicating that 65.5 % of the total population of C6 motoneurons survived (Fig.9).

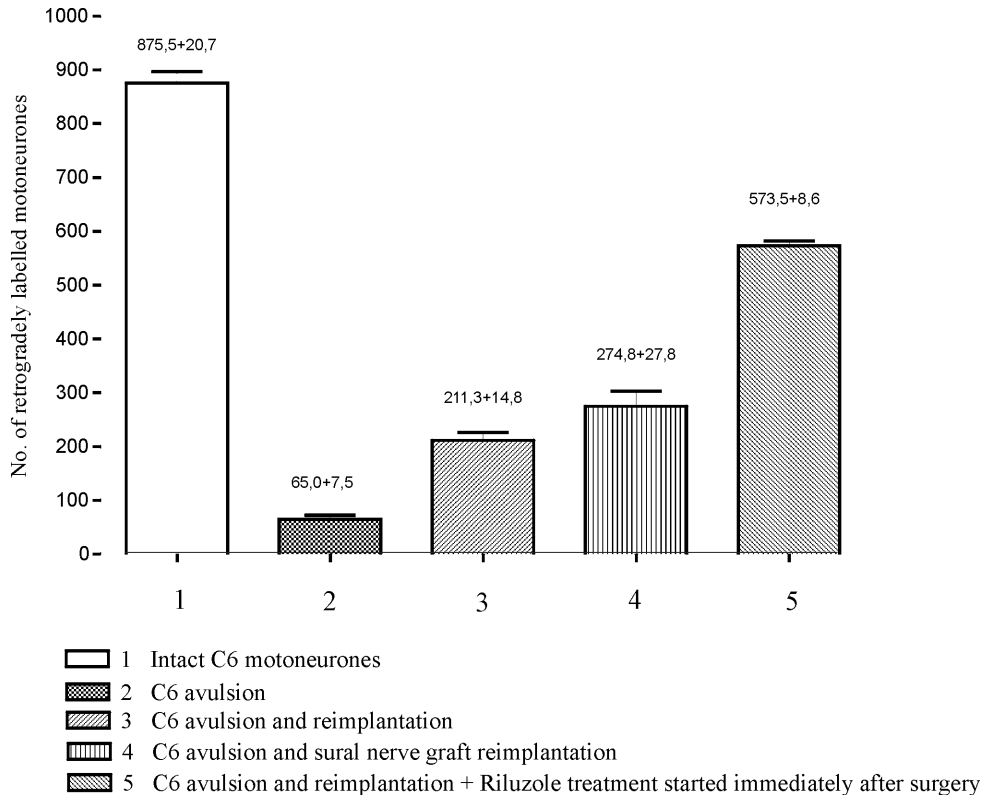

Figure 9. Motoneuron numbers after surgical procedure at the C6 level Bar chart shows the number of retrogradely labelled neurons in various procedures. Note that 65,5% of the motoneurons found in the intact C6 motoneuron pool (1) are rescued by riluzole treatment following C6 ventral root avulsion and reimplantation (5) The results of the avulsed root reimlantation (3) and sural nerve graft implantation (4) did not differ significantly from each other (P>0.05, ANOVA).

Reimplantation of avulsed roots or peripheral nerve graft rescues injured motoneurons

In those animals where the avulsed C6 root was reimplanted into the dorsolateral part of the spinal cord, the number of surviving motoneurons was 211.3±14.8 (S.E.M.; 24,1 %),(Fig.9).

In the other group where the peripheral nerve autograft was reimplanted after C6 avulsion and the other end was implanted into the neighbouring muscle, the number of retrogradely labelled motoneurons was 274,6 ±27.8, (S.E.M.; 31,4 %) No significant difference was found between the results of the two surgical procedures. This can be of high clinical importance in human cases. From a surgical-technical point of view it is more simple and safer to implant a peripheral nerve than to reimplant an avulsed root (Fig.9).

Delayed riluzole administration is able to rescue injured motoneurons at the L4 level

The effect of riluzole treatment starting at various time points following L4 avulsion and reimplantation was studied in the next series of experiments. Riluzole treatment started either immediately or 5, 10, 14 and 16 days after the operation and lasted for 3 weeks. In animals where riluzole was applied immediately after L4 avulsion and reimplantation, 763±36 (S.E.M.) retrogradely labelled motoneurons were found indicating that more than half of the total population of L4 motoneurons survived and was able to grow axons into the L4 ventral root. Riluzole treatment commencing 5 or 10 days after L4 avulsion and reimplantation resulted in similar numbers of retrogradely labelled motoneurons [815±50.6 (S.E.M.) and 772±39.1 (S.E.M.), respectively]. Although the number of surviving motoneurons appeared somewhat higher in these two groups, there was no significant difference in surviving motoneuron numbers compared to the group where riluzole treatment started immediately after operation. In contrast, significant decrease in the number of retrogradely labelled motoneurons was noticed when riluzole treatment started 14 or 16 days after L4 avulsion and reimplantation (Fig.10). In these cases fewer retrogradely labelled motoneurons were found [67±3.9 (S.E.M.) and 52±3 (S.E.M.), respectively]. The numbers of retrogradely labelled motoneurons in these latter groups were not significantly different from each other, but they were different from the ones obseved in the avulsion + reimplantation only group.

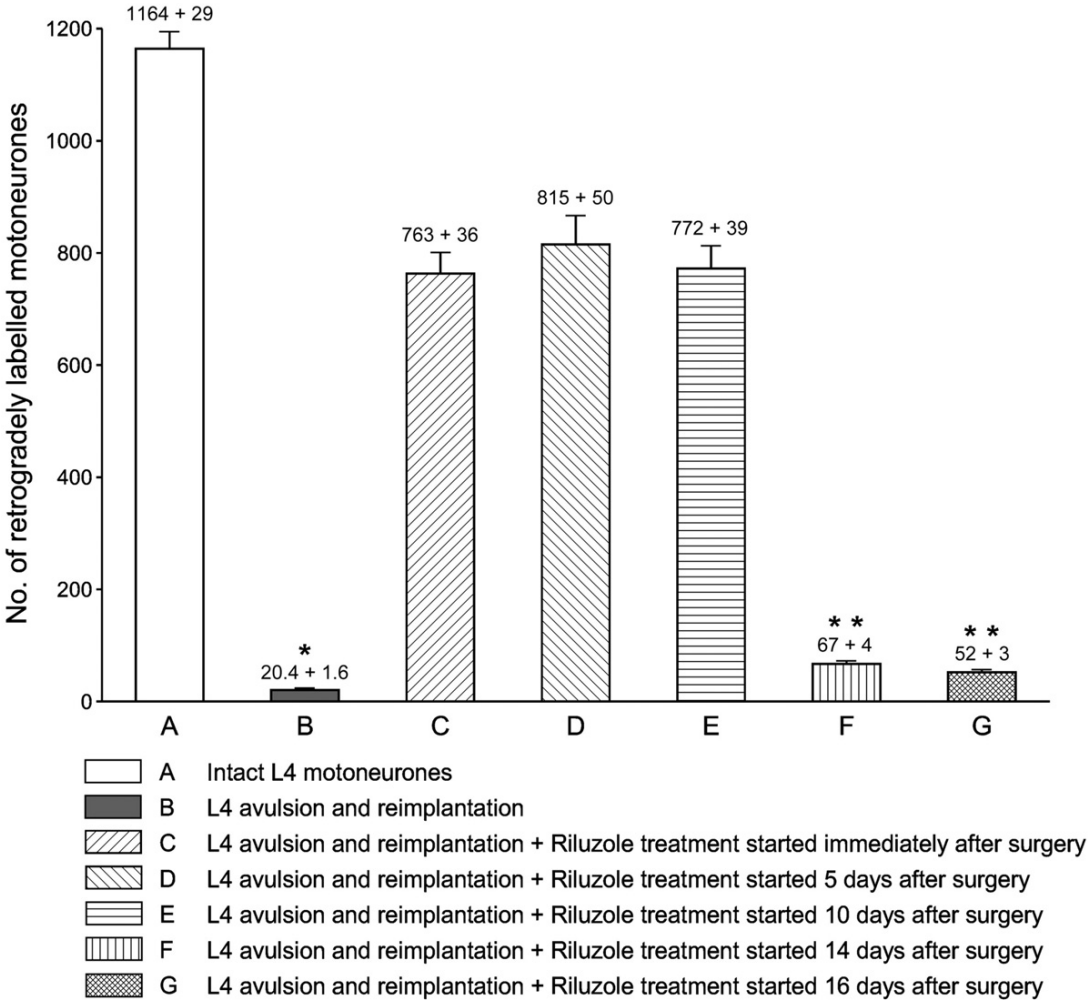

Figure 10. Bar chart shows the number of retrogradely labelled neurons in various experiments. Note that 65% of the motoneurons found in the intact L4 motoneuron pool (A) are rescued by riluzole treatment following L4 ventral root avulsion and reimplantation (C) when compared with survival of injured motoneurons without treatment (B). Similar survival was found when riluzole treatment started 5 or 10 days after avulsion injury (D and E), but the survival dramatically decreased when riluzole treatment started 14 or 16 days after L4 ventral root avulsion (F and G). * Significant difference between B and A, C, D, E (Mann-Whitney *U* test, *P*=0.029). ** Significant difference between B and F, G and between F, G and C, D, E (Mann-Whitney *U* test, *P*=0.016).

Expression of ChAT in injured and regenerating motoneurons

We compared the expression of ChAT a motoneuron marker with that of FB-labelled reinnervating cells. In control rats all the retrogradely labelled motoneurons in the dorsolateral motoneuron pool were ChAT immunoreactive. There were occasionally ChAT immunoreactive motoneurons in the ventromedial pool only which remained unlabelled with FB (Fig. 11A–B). Similar colocalization was found in groups, which received the first riluzole treatment immediately, 5 or 10 days following avulsion and reimplantation, however, in these animals there were some ChAT immunoreactive cells which were not retrogradely labelled. Accordingly, in these animals the proportion of ChAT positive motoneurons on the operated side (% of operated/control side) was somewhat higher than that of FB-labelled motoneurons (% of operated side/intact pool) [5 days delay: FB^+ vs. $ChAT^+$ = 70±4.3 vs. 90.7±2.1 (%±S.E.M.); 10 days delay: FB^+ vs. $ChAT^+$ = 66.3±3.3 vs. 85.4±2.4 (%±S.E.M.)]. In animals with more delayed start of riluzole treatment (14 or 16 days after operation) considerable numbers of ChAT positive motoneurons were located in the motoneuron pools but only few of these were retrogradely labelled, i.e. the proportion of ChAT positive neurons on the operated side was significantly higher than that of FB-labelled motoneurons [14 days delay: FB^+ vs. $ChAT^+$ =5.76±0.34 vs. 41.4±2.1 (%±S.E.M.); 16 days' delay: FB^+ vs. $ChAT^+$ =4.5±0.26 vs. 38.3±2.5 (S.E.M.); Fig.11 I–J,]. However, the $ChAT^+$ motoneurons on the operated side appeared degenerated with less-developed dendritic tree and displayed weaker ChAT imunoreactivity than $ChAT^+$ motoneurons on the intact side (Fig.11 L–M).

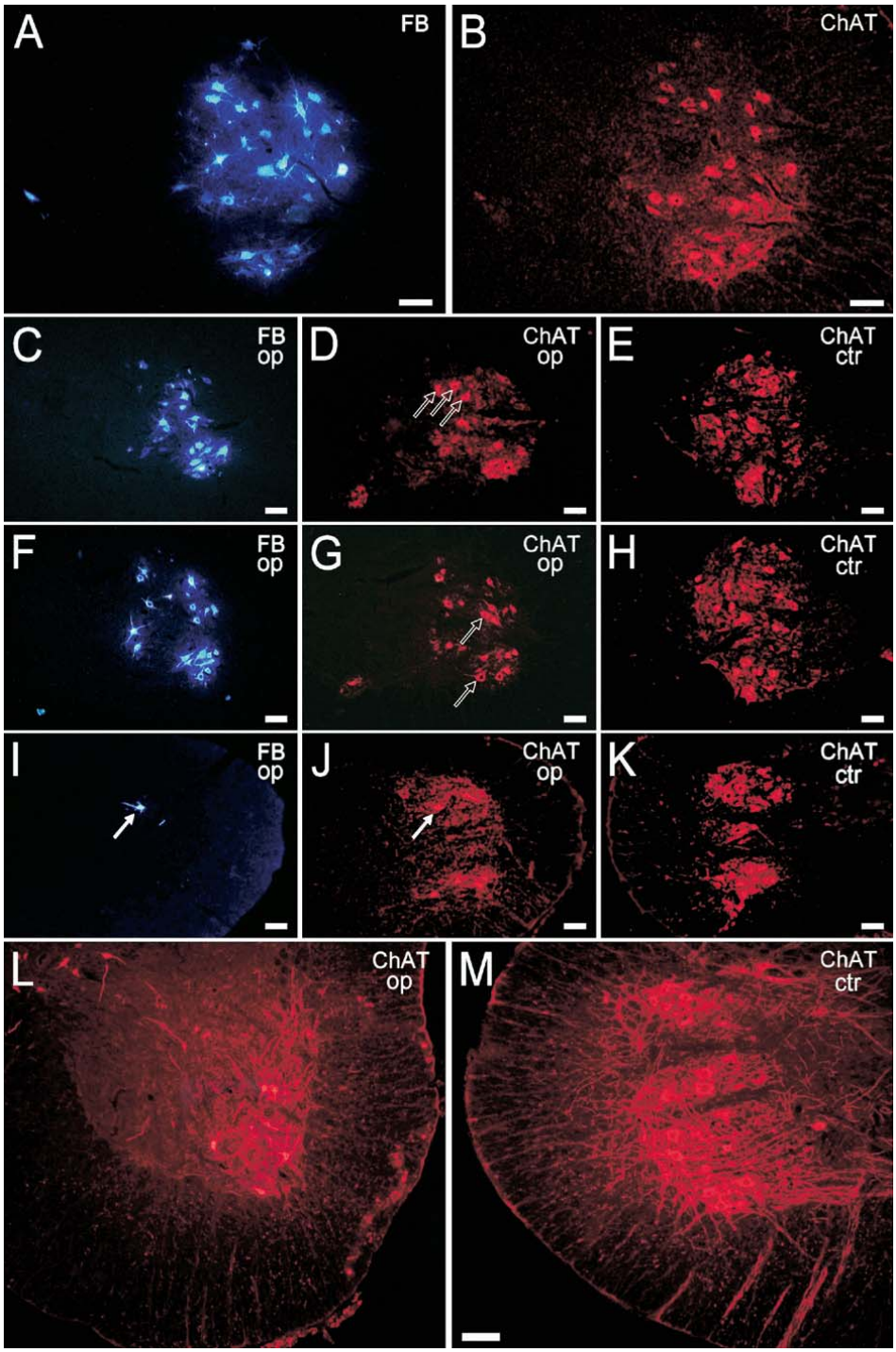

Fig. 11. Transverse sections of spinal cord taken from (A and B): intact L4 spinal cord segment with retrogradely labeled motoneurons and ChAT immunoreactive neurons, respectively. (C–E) Spinal cord with ventral root avulsion and reimplantation followed by riluzole treatment 5 days after surgery (empty arrows in D point to $ChAT^{+}/FB^{-}$ cells). (F–H) Spinal cord with ventral root avulsion and reimplantation followed by riluzole treatment 10 days after surgery (empty arrows in G point to $ChAT^{+}/FB^{-}$ cells). (I–K) Spinal cord with ventral root avulsion and reimplantation followed by riluzole treatment 16 days after surgery. Surviving and

reinnervating motoneurons were retrogradely labeled with FB and the same sections processed for ChAT immunohistochemistry. Note the low number of retrogradely labeled cells in I and the somewhat higher number of surviving cells of the same field in J (arrow points to the same cell). (L, M) These figures show that the surviving ChAT positive cells (riluzole treatment was delayed for 16 days after reimplantation) appear degenerated with shrunken dendritic trees on the operated side (L) as compared with the motoneurons on the intact side of the same section (M). In E, H and K ChAT immunoreactive neurons on the control side are shown to demonstrate the difference between operated and intact sides of the same section. Scale bar=100 μm.

Muscle regeneration model

Muscle recovery not complete at 3 months after reinnervation

Based on muscle weight and morphology (HE, AChE-staining), muscle recovery was not complete after 3 months (Fig.12, Fig.13). These results according to Ijkema-Paassen *et al.*, (2001b) who described that even after 21 weeks, both muscle- and endplate-morphology were still abnormal.

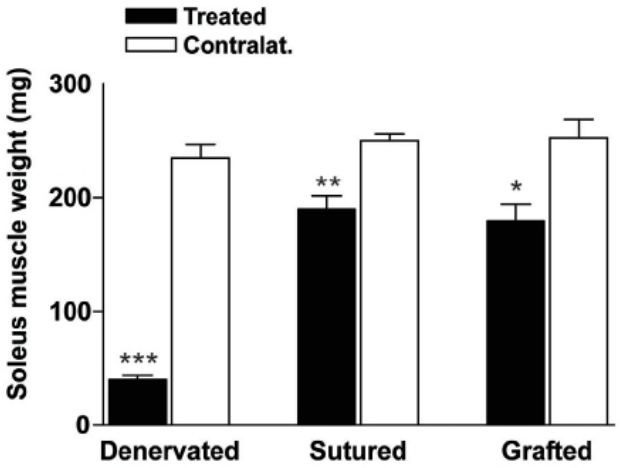

Figure12. Muscle weights of denervated and reinnervated (sutured, grafted) soleus muscles at 3 months after operation

Colums represent mean values ±S.E.M. of data obtained from 3–4 animals in each group, asterisks show significant differences compared to untreated contralateral muscles (Paired *t*-test, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). Reinnervated muscles differed significantly from their contralateral ones, and also from the denervated muscles ($P < 0.001$, ANOVA). Muscle weights of the sutured and grafted muscles did not differ significantly from each other ($P > 0.05$, ANOVA).

The morphology of sutured and grafted soleus muscles is similar at the level of light microscopy

After 3 months of reinnervation, sutured and grafted soleus muscles did not show remarkable differencies at the level of light microscopy (Fig 13 BFCG). Therefore, we used grafted muscles for inducing regeneration since this latter technique proved to be clinically more relevant.

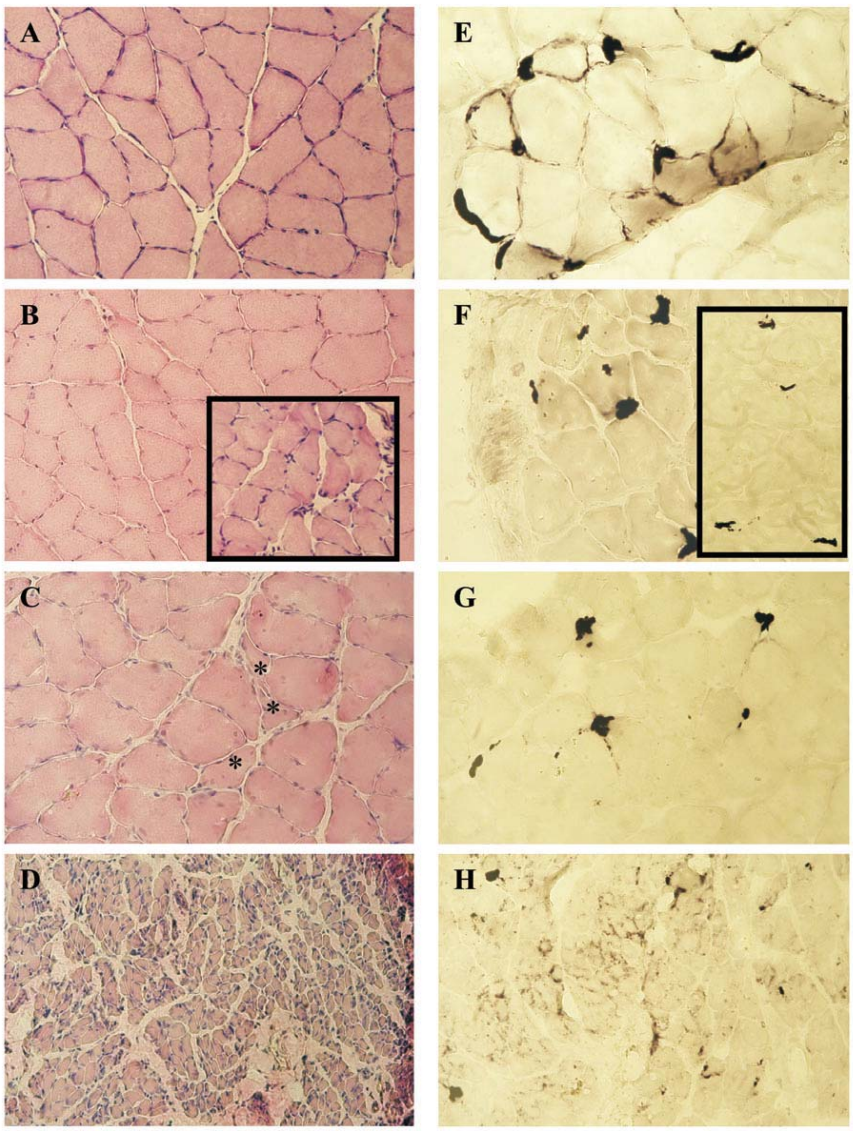

Figure 13. Fiber and motor endplate morphology of normal, reinnervated and denervated soleus muscles of rat 3 months after microsurgery.

After removal of the muscles, cryostat sections of 15µm thickness were either stained with haematoxylin-eosin (HE) or the motor endplate formation was checked by staining for acetylcholinesterase (AChE) activity of the endplates. HE staining of normal A, grafted B, sutured C and denervated D muscles, respectively. AChE staining of normal E, grafted F, sutured G and denervated H muscles, respectively. In grafted muscles we detected atrophied fibers and more connective tissue (B insert) besides fibers of close to normal morphology (B). This difference was also evident in the variability of the size of motor endplates (F and F insert).The morphology of sutured muscles was similar to that of grafted muscles, here we show regions with atrophied fibers interspersed among normal ones (C asterisks) with variable endplate morphology (G). Denervated muscles showed general atrophy (D) and only diffuse, if any, AChE-activity (H). Magnification 200x.

Notexin-induced regeneration of reinnervated (grafted) muscles show differencies compared to that of normal muscles.

After 3-5 days of regeneration, connective tissue seemed to be more abundant troughtout the regeneration process accompanied by pronounced variability of fibre size. Although motor endplates reappeared at similar time (on day 5 after necrosis) than those of normal regenerated muscles, their morphology seemed to be more variable even at late stages of regeneration (Fig.14, Fig.15).

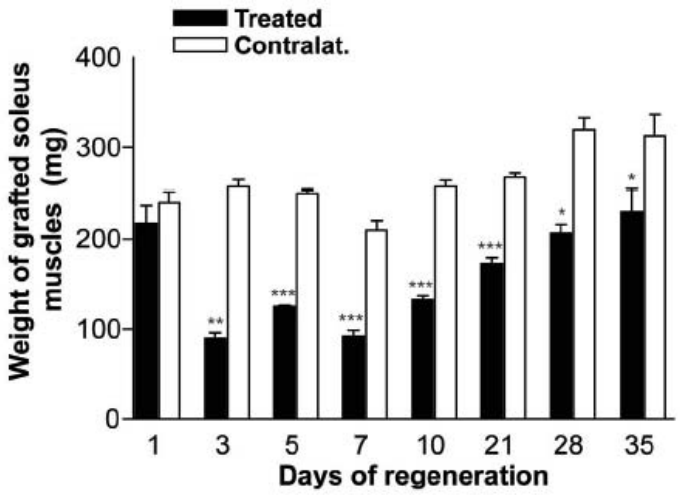

Figure 14 Muscle weights of reinnervated (grafted) soleus muscles regenerating from notexin-induced necrosis

Columns represent mean values ±S.E.M. of data obtained from 3–4 animals at each stage of regeneration (1–28: days after notexin administration), asterisks show significant differences compared to untreated contralateral muscles (Paired *t*-test, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). By day 3 after notexin treatment, the muscles became significantly smaller than their untreated counterparts. Thereafter, muscle weights increased until the end of the examined period of regeneration, but even at that time they were smaller than the contralateral ones.

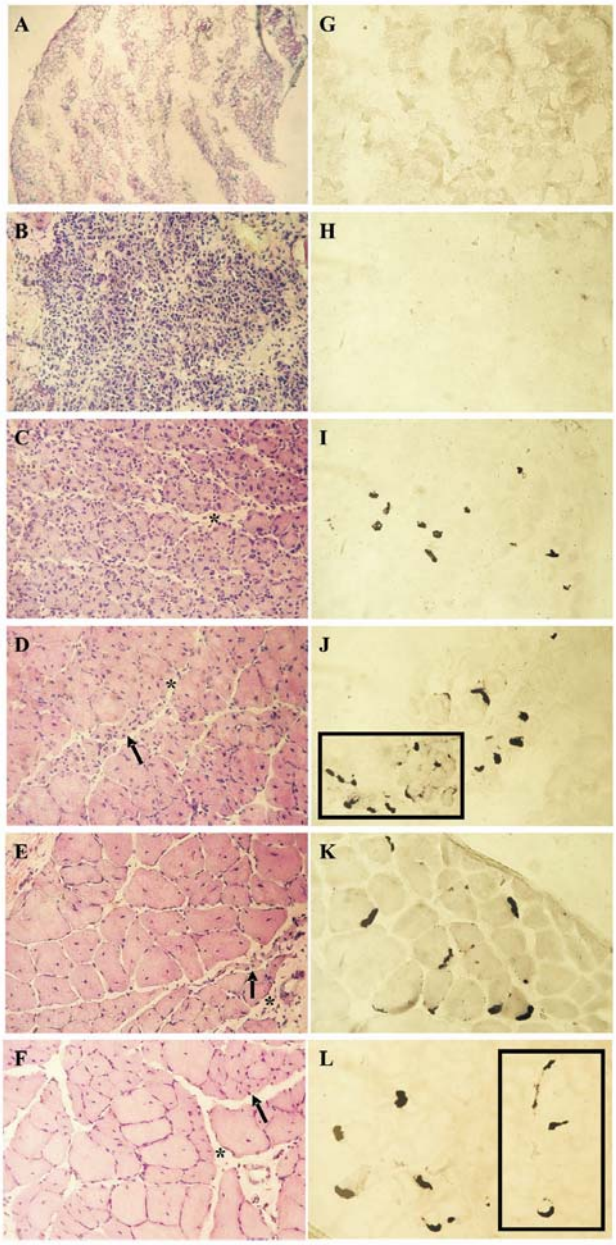

Figure 15. Fiber and motor endplate morphology of reinnervated (grafted) rat soleus muscles regenerating from notexin-induced necrosis.

After removal of the muscles, cryostat sections of 15 µm thickness were either stained with haematoxylin-eosin (HE) or the motor endplate formation was checked by staining for acetylcholinesterase (AChE) activity of the endplates. A–F: HE staining of muscles 1, 3, 5, 10, 28, and 35 days after notexin injection, respectively. G–L: AChE staining of muscles 1, 3, 5, 10, 28, and 35 days after notexin injection, respectively. Notexin induced complete necrosis by day 1 (A) destroying virtually all fibers. Three days after notexin injection mononucleated cells filled up the injured muscle (B). Most of the mononucleated cells had already fused to form new myotubes by day 5 (C). From this stage on, however, connective tissue seemed to be more abundant throughout the

regeneration process (C–F, asteriks). Abnormal and pronounced variability of the fibre size is the characteristic feature of the 7- and 10 day-regenerated muscles (D, arrow shows smaller fibers). Even after 28 and 35 days of regeneration, the fibre size variability was still present (E, F arrows show smaller fibers) and more than 80% of the fibers still contained centrally located nuclei. Notexin treatment destroyed all the motor endplates by day 1 (G) and any signal of motor enplate formation was not seen until day 3 (H). The first new motor endplates reappeared by day 5 after necrosis (I), at a similar time to those of normally innervated regenerated muscles. However, their morphology seemed to be more variable even at late stages of regeneration (J, K, L) showing smaller and in some cases fragmented motor endplates (J, L inserts). Magnification 200x, except Fig. 15A (magnification 40x).

Regenerated grafted muscle shows abnormalities at the ultrastructural level

At the ultrastructural level, regenerated grafted muscle showed serious abnormalities of mitochondria by 35 days after notexin treatment (Fig 16). Since the ultrastructural morphology of the regeneration has not been characterized completely yet, we do not know whether this finding is the consequence of the impaired reinnervation or it is also a characteristic feature of the normal regeneration.

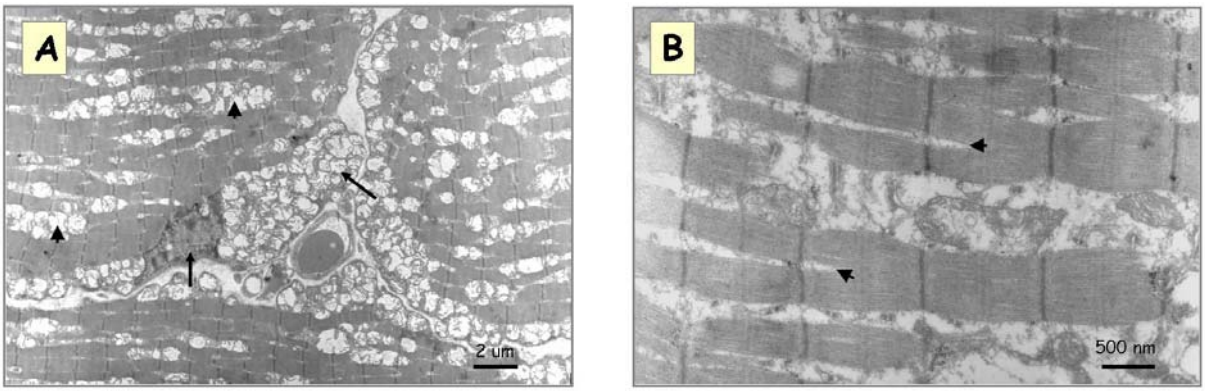

Figure 16 Ultrastructural morphology of a 35 day-regenerated, reinnervated (grafted) soleus muscle.

A Note the abnormal morphology of the mitochondria (arrowheads), many of them are phagocytosed by macrophages (arrow). **B** Besides normal sarcomeric arrangement, note the ”splitting” of some myofibrils (arrowheads).

5. Discussion

Avulsion of the nerve roots is usually caused by road traffic accident in adults, and obstetric complications in children. They represent usually a major component of severe brachial plexus injuries (Bahm *et al.*, 2007)

These conditions are widely regarded as untreatable. Until recently the current microsurgical treatment was based on nerve transfer and neurotization with limited functional recovery (Merrell *et al.*, 2001). As a palliative procedure, tendon transfer is used to restore at least some of the lost functions.

Numerous experimental models and clinical surgery results have shown that axons of motoneurons can regrow into the reimplanted spinal root and induce functional recovery.

After avulsion of the anterior root, the axons of the motoneurons are able to grow from the CNS (Cullheim *et al.*, 1999) towards the periphery. Scar tissue evolving after trauma is made up of CNS cell elements (oligodendrocytes and astrocytes), but few axons are able to grow and penetrate it. By microscopic examination of the scar, a trabecular system made of astrocyte spurs, leptomeningeal cells invading the white matter were found, whereas in the expanded extracellular space, large amounts of collagen is deposited. Axons leading to ventral roots as well as (during the first three weeks) inflammatory cells can also be found. The Schwann cells do not enter the spinal cord, in the CNS area the axons are surrounded by a myelin-pod of oligodendrocyte origin, i.e. more numerous glial spurs expand into the proximal part of the ventral root (Hallin *et al.*, 1999).

The reimplantation represents an interesting surgical strategy for the treatment of brachial plexus injuries but it is not always possible because of the physical distance between the spinal cord and the avulsed rootlets. Some experiments have shown that this gap can be bridged by the use of a synthetic tube (Liu *et al.*, 1997; Liu *et al.*, 1998; Kassar-Duchossoy *et al.*, 2001), or by peripheral nerve grafts and this procedure reportedly enhances motoneuron survival (Bertelli & Mira, 1994; Gu *et al.*, 2004).

After peripheral nerve injury, most of the motoneurons die in newborn mammals, whereas they survive in the adult spinal cord (Koliatsos *et al.*, 1994; Greensmith & Vrbová, 1996)
However, after avulsion of an anterior root, motoneuron death occurs in adult animals, too.
The hypothesis is that axotomy performed close to the cell body severely affects the motoneuron's integrity and increases the vulnerability of the cell to excitotoxic effects. Possible reasons for cell death include the traumatic insult to the nervous tissue, hypoxia-ischemia, toxins and hypoglycaemia. The usual process, how cell-destructive mechanisms lead to the death of the cell can be conduted in the followings: Changes in cell-membrane permeability induces cytoskeleton desintegration, activation of catabolic enzymes, free radicals are released and levels of intracellular calcium increases. During the excitotoxic reaction, similar changes can be observed. Excitotoxicity is a term used to express the neurotoxic ability of glutamate and similar excitatory aminoacids (e.g. aspartate, NMDA, cysteine, homocysteine). Regardless of the cause of neuronal cell death, this process seems to be common (Choi, 1992), and the excitotoxicity can possibly be held responsible for the injury of the motoneurons after avulsion as well (Mentis *et al.*, 1993).

Riluzole the most potent antiexcitotoxic drug is applied in the treatment of ALS. Riluzole blocks the volgate activated Na^+-, K^+- and Ca^{2+} -channels and inhibits the presynaptic glutamate release (Doble, 1996).
In our earlier study, it has been proven that riluzole is able to rescue injured motoneurons destined to die following an avulsion injury.
The present results confirm and expand our earlier experimental findings (Nógrádi and Vrbová, 2001) that injured adult motoneurons destined to die due to avulsion of their axons in the ventral root can be rescued by treatment with riluzole. The rescued motoneurons not only survive but a considerable number of these cells extend their axons into the reimplanted ventral root. These axons regenerate, reach their target muscles and are able to improve the recovery of the denervated muscles and the locomotor performance of the denervated hind limb (Nógrádi and Vrbová, 2001). In addition to confirming these earlier findings the present study shows that treatment with riluzole can be delayed for up to 10 days while remains still effective in rescuing almost the same number of reinnervating motoneurons as when the treatment started immediately after the operation. Only when riluzole treatment started 14–16

days after the operation did no longer induce the repopulation of endoneuronal sheaths with axons of regenerating motoneurons.

Although in these delayed treatment groups the reinnervating motoneuron numbers were very low, they were still 2.5- and 3-times higher than in the untreated group. Functional data indicated that this relatively low motoneuron number was able to prevent the complete atrophy of the affected muscles. The present results showed that after avulsion and reimplantation of the ventral root some cholinergic neurons, that have features of motoneurons were present in the spinal cord even 3 months after the operation, but after treatment with riluzole delayed for more than 10 days they were no longer able to extend axons into the L4 ventral root. This is in agreement with the results of Gu et al. (2004) where many surviving motoneurons were located in the cervical spinal cord after avulsion and reimplantation of the ventral root, but only some of these were able to extend their axons into the reimplanted ventral root. Recently Hoang et al. (2006) have clearly shown that only 53% of surviving preganglionic parasympathetic neurons and 64% of surviving motoneurons in the L6 spinal segment reinnervate the avulsed and reimplanted L6 ventral root.

The present finding shows a very abrupt decline of the ability of the motoneurons to extend their axons into the ventral root after treatment with riluzole delayed for more than 10 days. It is possible that treatment with riluzole not only rescues the motoneurons from cell death but maintains these cells in a condition that enables them to regenerate their axons given the right conditions. Apparently, after a delay of 14–16 days the number of reinnervating motoneurons dramatically drops while still numerous ChAT immunoreactive motoneurons are present in the ventral horn. Accordingly, there is a clear division between neural survival and reinnervation, i.e. not all surviving ($ChAT^{+}$) neurons are able to send their axons into the vacated endoneural sheaths. The present finding that surviving cholinergic cells are found in the ventral horn even 3 months after injury suggests that given the appropriate stimulus such as having access to a fresh, recently axotomized nerve conduit may induce these dormant motoneurons to regenerate. It is possible that riluzole treatment combined with a fresh conduit may then be effective for even longer periods of time. Future experiments should be able to elucidate the optimal conditions for the survival and function of damaged neurons. The present results show that treatment with riluzole is able to induce damaged motoneurons to survive and regenerate their axons up to 10 days after injury, are promising and with

refinements of the technique they inspire hope that it may be possible to restore function of various peripheral nerve injuries close to the cell body even after long delays of surgical intervention.

In the reinnervation study our aim was to characterize the muscle regeneration capacity of reinnervated muscles. Up to now, no data were available in the literature in this regard. It is well known that denervated muscles have impaired regenerative capacity, which is most pronounced during the second phase of regeneration, i.e. the newly formed primitive fibers are not able to differentiate to reach their normal size (Sesodia & Cullen, 1991). Still, it is debated whether satellite cells, the main sources of muscle regeneration (Mauro, 1961; Bischoff, 1993; Asakura *et al.*, 2002) are inactivated by denervation (Maier *et al.*, 2002), or on the contrary, they become more active upon denervation (Nnodim, 2001; Wang *et al.*, 2002).

Our morphological results are in line with the original hypothesis that the regenerative capacities of reinnervated muscles might be impaired. One reason could be that since reinnervation is not complete, satellite cells of more atrophied regions might be of lower activity than those of the normal ones. However, the question seems to be even more complicated. Ijkema-Paassen described that reinnervated muscles show high proportion of endplates of abnormal morphology (Ijkema-Paassen *et al.*, 2001a; Ijkema-Paassen *et al.*, 2001b), which we could also confirm in our experiments. Yet we do not know whether this pattern of "abnormality" will be recapitulated in the course of regeneration when the newly formed endplates are established after complete necrosis. In theory, it cannot be ruled out that the necrotized muscle sends cues for the reinnervating axons which might modify the reinnervation pattern of the regenerating reinnervated muscle. If so, it could well be that the fiber-type composition also shows changes after regeneration of reinnervated muscles. Investigation of the myosin and SERCA (Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase) isoforms at both mRNA and protein levels and their expression pattern in tissue sections, compared to normally innervated regenerated muscles (Zádor *et al.*, 1998; Mendler *et al.*, 1998a; Zádor *et al.*, 2001), might at least partly answer this question. Indeed, we found very recently a dramatic slow to fast fiber-type transition in reinnervated soleus muscles and an even more fast transformation of the fibers after regeneration with the presence of the fastest

IIB fibers as well. These findings- together with morphological data- strengthen our hypothesis that regeneration is impaired in reinnervated muscles. The change of complex cues coming either from axons and/or from regenerating muscles might be responsible for this phenomenon. Possible misregulation might involve myogenic regulatory factors (MRFs) and myostatin (Mendler *et al.*, 1998b; Mendler *et al.*, 2000), or any element of the calcium-calcineurin signaling cascade of which investigations are among our further plans.

On the other hand, investigation of high sciatic nerve transection gives the model of mixed nerve reinnervation, together with the further impairment of ambient muscles. To exclude the possible role of mechanical inactivity of the whole hindlimb as well as the mixed reinnervation of soleus muscle, we have just developed a selective reinnervation model which might ensure more standard condition for analyzing regeneration process after reinnervation of the specific soleus nerve.

Clinical consequences:

It is clinically very relevant that rescued motoneurons of the affected spinal segment(s) following brachial plexus injury can be efficiently guided to the denervated muscles. For future therapeutic considerations it seems feasible, that patients with severed brachial plexus injury should first undergo riluzole treatment and then the connection between the injured and rescued motoneurons and the peripheral nerves is established. Our experimental studies suggest that it is not the method of connecting the spinal cord to the peripheral targets but the number of regenerating motoneurons what determines the functional outcome of such a reconstructive surgery.

6. The following new findings are presented in this thesis:

1. We have worked out a therapeutic strategy to induce as much functional recovery in the various forms of brachial plexus injury as possible. Further functional improvement is possible only if the results of our experimental studies are introduced in clinical use.
2. It has been shown that avulsed cervical and lumbar motoneurons destined to die can be rescued to the same extent when applying riluzole immediately following the injury.
3. Injured motoneurons survive for approx. 12-14 days following avulsion and they are able to reinnervate peripheral targets even if riluzole traetment is delayed up to 10 days.
4. There is no difference in motoneuron reinnervation capacity in cases when the avulsed ventral root was reimplanted or a peripheral nerve bridge was established.
5. We have presented evidence that there is no difference in muscle regeneration when motor axons were guided to the muscle via a direct peripheral nerve suture or nerve graft.
6. The regeneration following notexin induced muscle necrosis after reinnervation, is morphologically different from normal muscle regeneration.

7. Acknowledgments

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8. References

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9. Appendix

I, II, III, IV, V.

A felső végtag fedett érsérüléseinek diagnosztikája és kezelése

Érkezett: 1994. november 8.

SIMONKA JÁNOS AURÉL DR.[1], NAGY ENDRE DR.[2], VÖRÖS ERIKA DR.[2], CSÁSZÁR JÓZSEF DR.[1], PINTÉR SÁNDOR DR.[1]

Összefoglalás

A szerzők a fedett érsérülések diagnosztikai problémáit, az ellátás és az utánvizsgálatok lehetőségeit i: mertetik négy eset bemutatásával. A felső végtag fedett érsérülései ritkán fordulnak elő. Törések, ficamok é egyéb sérülések szövődményeként találkozhatunk a kórképpel. A klinikai tünetek az esetek egy részében korá ráirányítják a figyelmet a sérülésre, míg gyakran az érsérülés rejtett maradhat. A korai diagnózis felállításában különböző típusú (Doppler, duplex Doppler, color Doppler, B-módú) ultrahangvizsgálat és az angiográfia segí het. A sürgős és szakszerű ellátás biztosítja a további károsodások megelőzését és a jó funkció helyreállítását.

J. A. SIMONKA, E. NAGY, ERIKA VÖRÖS, J. CSÁSZÁR, S. PINTÉR: *Diagnosis and treatment of close vascular injury on the upper extremity.*

According to four cases autors introduce the diagnostic problems, the possible treatment and the way c follow-up in the cases of closed vascular injuries. The closed vascular injury on the upper extremities are quit rare, occurs following fractures and dislocations as a complication.

Clinical symptoms could lead to the diagnosis in a few cases, but in most of the cases difficult to discov the problem.

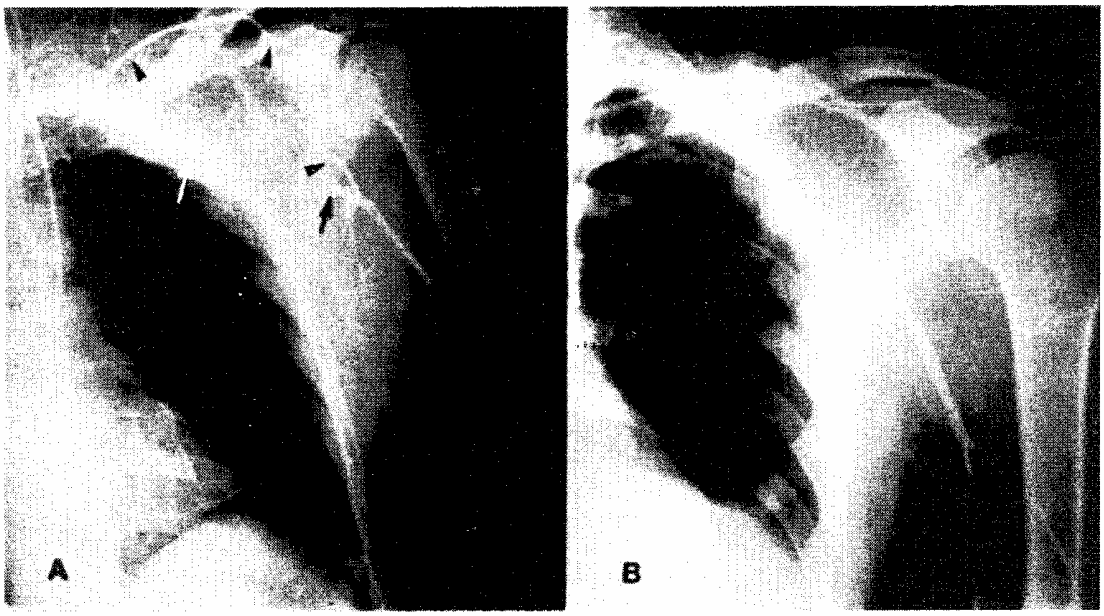
The ultrasound (Doppler, duplex Doppler, color Doppler, B-mode) and the angiography could help in th early diagnosis. The urgent and skilled management of this problem is key to the success.

Bevezetés

A perifériás érsérülések ellátása a traumatológus, az érsebész, vagy az általános sebés feladata. Intézményünkben az érsérüléseket a traumatológusok látják el. Mint általában a ha tárterületeken, a diagnosztikai problémákon kívül, az ellátás szakmai irányelvei sem mindi és mindenütt tisztázottak. Különösen nagy nehézséget okozhat a fedett érsérülések kórismé zése és megfelelő kezelése. A diagnosztikában a fizikális vizsgálat eredményei következté ben felmerülő gyanújelek alapján végzett hagyományos radiológiai vizsgálatok mellett a kor szerű új módszerek pontosabban mutatják ki az érsérülést és kisebb megterhelést jelentene a sérültek számára. A felső végtag fedett érsérüléseinek problémakörét néhány eset bemuta tásával kívánjuk szemléltetni.

Esetismertetések

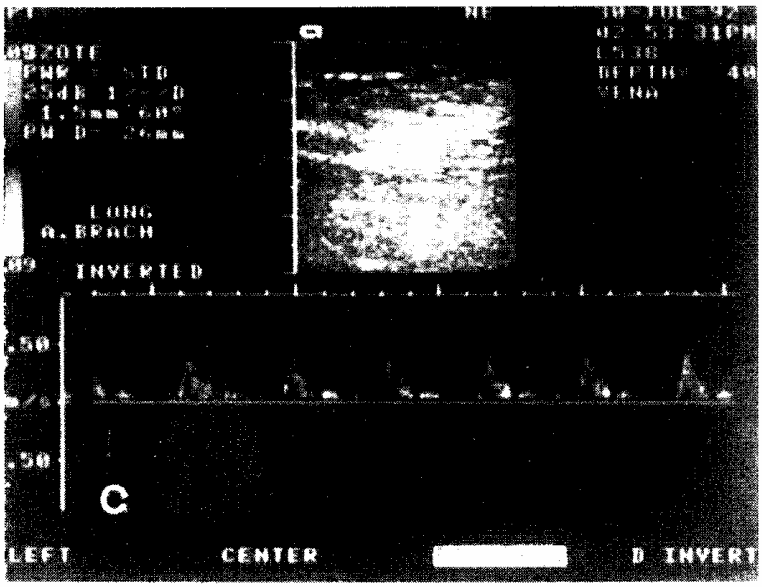
(D. J.) 33 éves férfit személygépkocsi ütötte el. Nyílt jobb lábszár törését külső rögzítő vel (fixateur externe), a bal oldali nyílt mediális és laterális tibia condylus törését lemezze stabilizáltuk, az artéria poplitea sérülését megvarrtuk. A bal felső végtagon a plexus brachiali és az artéria subclavia sérülés tüneteit észleltük. Disztális pulzus nem volt tapintható, a vég tagon sem mozgás, sem érzés nem volt kimutatható. Az angiogramon az a. axillaris szakadá sa és az azt áthidaló gyér kollateralis érhálózat látszott (1.a. ábra).



1. a) ábra. A bal a. axillaris szakadását (nyilak) gyér kollaterális hidalja át (nyílhegyek).

1. b) ábra. Az artéria végek egyesítése után az a. axillaris végig normális lumentágasságú.

A primer műtét során az artéria axillaris szakadását direkt varrattal egyesítettük. Ez jól látható a kontroll angiogramon (1.b. ábra). A plexus brachialison a C7-8 és Th1 gyök a ge-



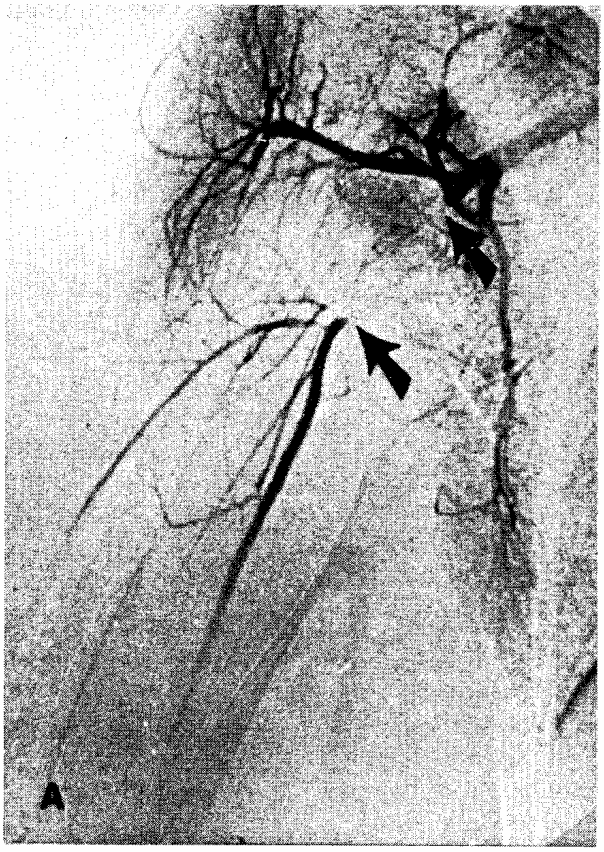
1. c) ábra.
Öt és fél év múlva a color Doppler vizsgálat poststenoticus jellegű alacsony áramlási sebességgörbét mutat az a. brachialisban. (A szabályos végtagi artériás áramlási görbe a relative magas perifériás ellenállás miatt trifázisos, systolés sebesség maximuma 1 m/sec körüli. A szűkület utáni érszakaszban mérve a spektrumgörbe kiszélesedik, a systolés csúcssebességet lassabban éri el, a sebességmaximum kisebb, a trifázisos jelleg általában megszűnik.)

rincvelőből történt kiszakadás miatt nem volt rekonstruálható, csupán a felső truncus (C5-6) szakadását varrtuk meg mikroszkóp alatt 10-0-ás fonallal. A keringés visszatért, a radialis pulzus jól tapinthatóvá vált. A plexus brachialis regenerációja után a váll abdukciója 0-20 fok, a könyök flexiója 0-80 fok, az izomerő gyenge. A kézen az izomműködés nem tért vissza,

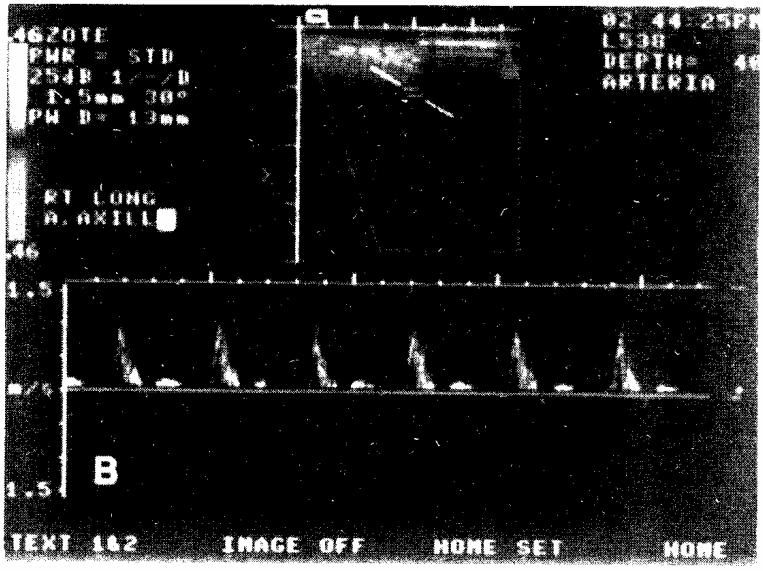
csupán csekély védő szenzibilitás mutatható ki. Az öt és fél évvel később elvégzett színkódolt Doppler ultrahangvizsgálat során alacsony áramlási sebességet találtunk a bal a. brachialison, a görbe jellege poststenoticus áramlásra utalt (1.c. ábra).

(H.I.) A 16 éves fiatalember jobb karját kutya harapta meg, és a karját rángató kutyával dulakodott. Vizsgálatakor a felső végtagon számos kisebb-nagyobb harapásos nyom és seb volt látható. Az axilláris régió feszesen duzzadt volt. Perifériás pulzus nem volt észlelhető, a Doppler-vizsgálat a végtag disztális artériáiban észlelhető, de az ellenoldalinál gyengébb keringést mutatott. A végtag keringése megtartott volt, érzészavar nem volt észlelhető. A jobb felső végtag DSA vizsgálata során (2.a. ábra) az artéria axillarison 4 cm hosszú kontraszttelődési hiány látszott. A sürgősséggel végzett műtét során a sérült intimájú szakaszt rezekáltuk, a hiányt véna saphena magna grafttal pótoltuk. Az érvarratot mikroszkóp alatt 6-0-ás csomós öltésekkel végeztük. A végtag keringése helyreállt. A kontroll ultrahangvizsgálat során az a. axillarisban szabályos áramlást detektáltunk (2.b. ábra). A rekonstrukció után a végtag funkciója mind a mozgás, mind az érzés tekintetében teljes értékű.

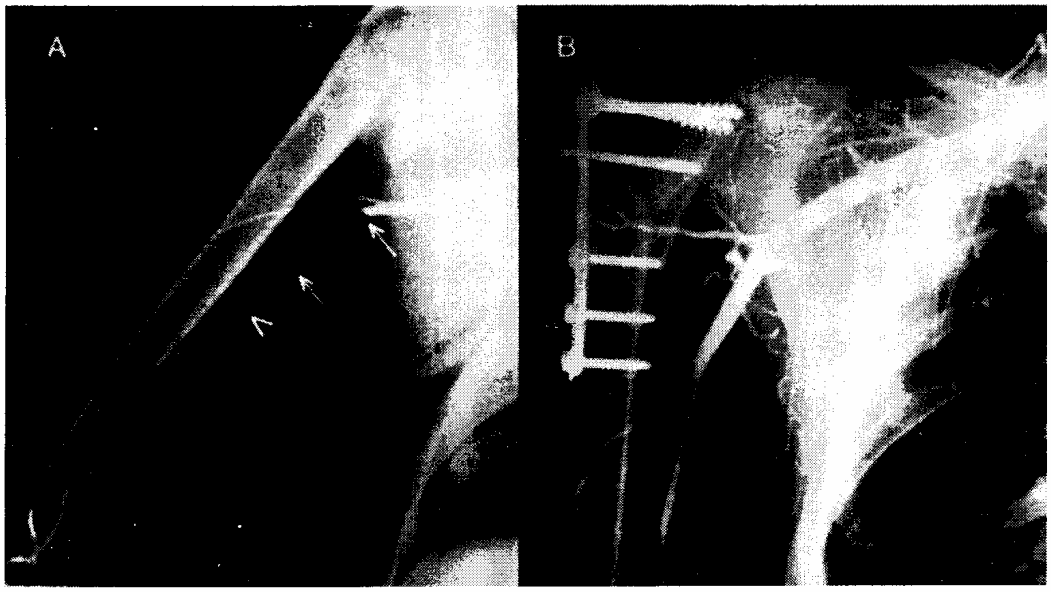
(N. Z.) 25 éves férfi személygépkocsi utasaként sérült. Beszállításakor a duzzadt jobb felkaron krepitációt észleltünk a pulzus nem volt tapintható, a végtag hűvös volt.



2. a) ábra. Jól látható a jobb a. axillaris folytonossági hiánya (nyilak) és a gyér telődésű disztális artériák.



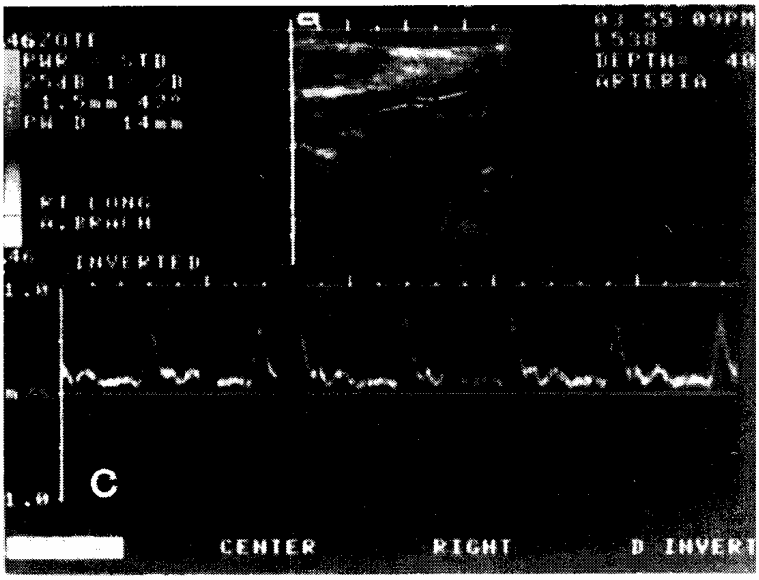
2. b) ábra. A jobb a. axillarisban szabályos háromfázisos sebességgörbe detektálható.



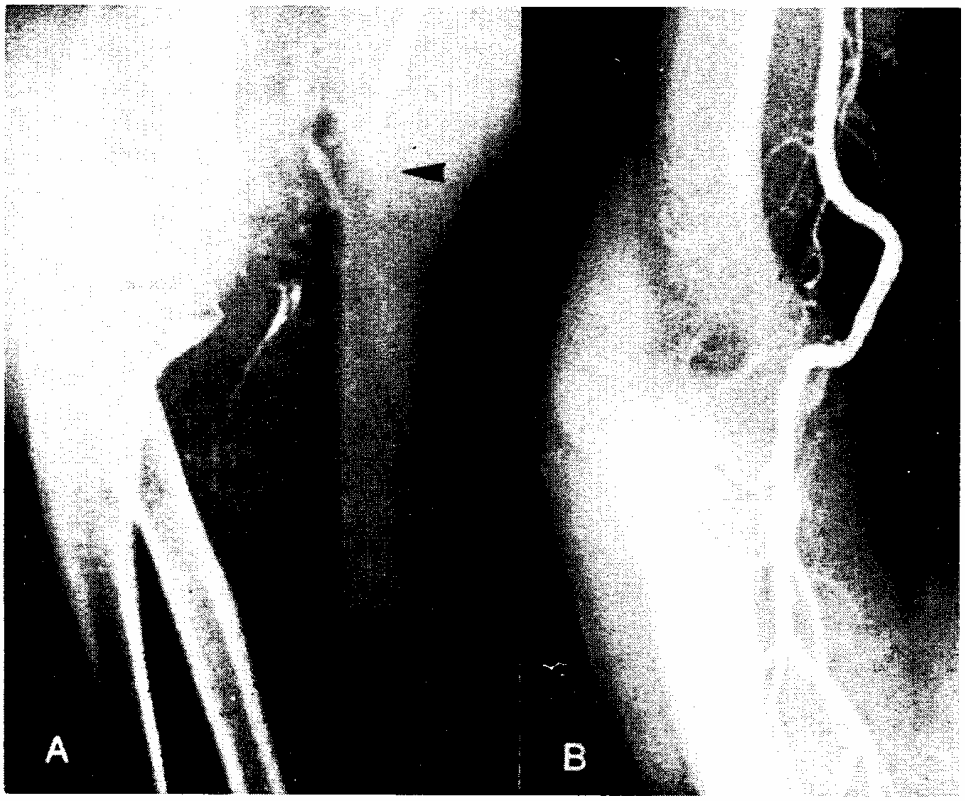
3. a) ábra. A jobb a. brachialis telődési hiánya (nyilak) után csak igen gyér telődés látható (nyílhegy).

3. b) ábra. Az artéria brachialis keringése a rekonstrukció után helyreállt.

A kétirányú jobb váll felvételen a humerus collum chirurgicumán ferde törést láttunk a tuberculum maius és egy ék kitörésével. A végtag keringési elégtelensége miatt Seldinger-módszerrel angiográfiás vizsgálatot végeztünk, amely az arteria brachialis folytonosságának megszakadását mutatta a törés magasságában (3. a. ábra). Az azonnali műtét során előbb T-lemezzel rögzítettük a törést, majd feltártuk az arteria brachialist. Az ér sérült trombotizált szakaszát, amelyet csak az adven-titia tartott össze, re-zekáltuk, majd az eret feszülés nélkül mikroszkóp alatt 6-0-ás Prolene csomós öltésekkel egyesítettük. A végtag keringése helyreállt. A posztoperatív 3. napon kialakult haemopneumothorax becsövezése és tartós szívása után a tüdő expandált, állapota rendeződött. A kontroll angiográfiás felvételen az arteria brachialis jól feltelődött (3.b. ábra). A váll mozgásai teljes mértékben visszatértek. Az 5 évvel később készült ultrahangvizsgálat során az érben normális áramlási viszonyokat találtunk (3.c. ábra).

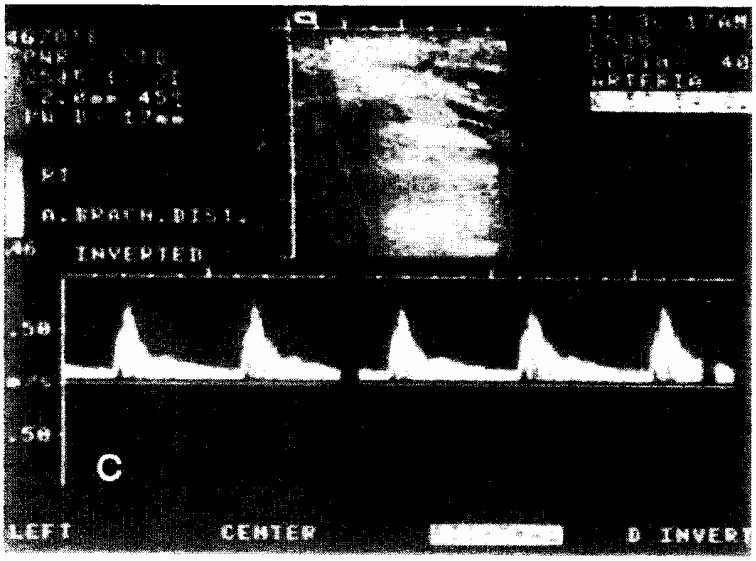


3. c) ábra. Öt évvel később az a. brachialis áramlási görbéje teljesen szabályos volt.



4. a) ábra. A jobb a. brachialis folytonossága a könyökhajlat fölött megszakadt, disztális telődés nem látható.

4. b) ábra. A kontroll angiográfián jól látszik a lágyrészduzzanatot megkerülő érszakasz (beültetett véna graft).



4. c) ábra. Az ellenőrző ultrahangvizsgálat az érben szabályos áramlást mutatott.

(T. A.) 43 éves férfi lovas kocsiról esett le, jobb könyöke kificamodott. Beszállításakor a jobb könyök deformált volt, a végtagon pulzust nem észleltünk. A repozíció után DSA-vizsgálatot végeztünk, amely az arteria brachialis szakadását mutatta (4.a. ábra). Az azonnal elvégzett műtét során a sérült részt rezekáltuk, majd az ér folytonosságát saphena graft beültetésével állítottuk helyre. Az alkaron kialakult compartment szindróma miatt fasciotomiát végeztünk, a bőrt csak lazán közelítettük. Az így visszamaradt alkari bőrhiányt részvastag rácsplasztikával fedtük. A végtag funkciója teljes egészében visszatért, keringése kifogástalan. A kontroll angiográfián jól ábrázolódott a lágyrészduzzanatot megkerülő érszakasz (4.b. ábra). Az ellenőrző ultrahangvizsgálattal az érben szabályos áramlást észleltünk (4.c. ábra).

Megbeszélés

A felső végtag érsérülései a végtagsérülések csaknem felét teszik ki [4]. A sérültek közel 80%-a férfi és döntően a munkaképes korosztályból kerül ki. A sérülések nagyobb része nyílt sérülés, amely nyilvánvaló tünetekkel jár, a diagnózis felállítása nem nehéz [5]. A fedett érsérüléseket tompa trauma, vongálódás, valamint törések okozta elmozdulás miatt szakadás vagy az éles csontvég direkt vágása okozhatja. A fedett érsérülések ritkán fordulnak elő [6], Bunt és mtsai 9%-osnak találták előfordulási arányát [3]. A felső végtagon válltáji törések, ficamok, supracondylaris humerus törések és könyökficamok kapcsán jönnek létre.

A válltáji sérülések között a legsúlyosabb a felső végtag kiszakadásával járó tünetegyüttes, amely az artéria subclavia szakadása mellett leggyakrabban clavicula-törésel, a plexus brachialis (C5-8, T1) sérülésével társul. A klinikai tünetek súlyosak, gyakran polytraumatizáció részét képezik és felvetődik a végtag megmenthetőségének kérdése is. Az érsérülést viszonylag egyszerűen direkt varrattal vagy autológ graft beültetésével el lehet látni, azonban a plexus brachialis sérülés, különösen gyökkiszakadás esetén megkérdőjelezi a végtag funkcionális értékét [6]. A rekonstrukciós műtét során az érvarrat mellett a plexus brachialis mikrosebészeti ellátása is primeren javasolt.

A vállízület ficama és súlyos vongálódása az arteria axillaris fedett sérülését okozhatja, gyakran csak az intima megszakadása és felpödrődése, valamint másodlagos trombózis révén következik be az ér elzáródása [4]. A kollaterális keringés miatt a klinikai tünetek rejtettek, sokszor a radialis pulzáció gyengén tapintható. Ilyenkor a diagnózis felállítása és az akut ellátás késhet, a teljes funkció elérése érdekében azonban, az érsérülés korai rekonstrukciója szükséges. Az érszakasz feltárása technikailag nehéz lehet, ekkor a m. pectoralis maior inának átvágására is sor kerülhet.

A humerus törések kapcsán az arteria brachialis sérülését, valamelyik törtdarab szilánkja okozza, de ritkábban a nagyfokú elmozdulás miatt is sérülhet. A klinikai tünetek, valamint a sérülés helyén keletkező hematoma utalnak az ér sérülésére, melynek ellátására a csonttörés stabil rögzítése után kerülhet sor [7]. Éles szélű csontszilánk okozta sérülés esetén az érintett érszakasz mobilizációja után direkt varrat is végezhető, míg roncsolt ér esetén graft alkalmazása szükséges.

A könyöktájon az érsérülések fedetten könyökficamhoz, vagy supracondylaris töréshez társulhatnak [3, 4]. A klinikai tünetek szembetűnőek: a kézen és alkaron kifejezett ischaemiás tünetek észlelhetők, a radialis és ulnaris pulzus nem tapintható, gyakran compartment szindróma tünetei is igen korán kifejlődnek [2]. A diagnosztikus nehézséget az arteria brachialis kompressziója jelentheti, a törés vagy ficam helyretétele után is meglevő klinikai tünetek azonban a pontos kórisme felállítását és a sebészi beavatkozás mielőbbi elvégzését teszik szükségessé.

A fedett érsérülések kórisméjének felállításában a klinikai tünetek gondos elemzésén túl a Doppler ultrahangnak, az angiográfiának vagy a DSA vizsgálatnak van szerepe [8, 9].

Az ultrahang noninvazív módszer, így alkalmazása különösen ideális, azonban ép bőrfelület szükséges a megfelelő kontaktus biztosítására. Az angiográfia, méginkább a DSA lehetővé teszi az érsérülés egyértelmű igazolását, helyének, jellegének pontos meghatározását és a kollaterális rendszer kimutatását [1, 9]. Mindezek hasznos segítséget nyújtanak a műtét megtervezéséhez.

Érvarrat vagy érgraft sebészi kivitelezése szabad szemmel, lupe vagy mikroszkóp használatával csomós vagy tovafutó öltésekkel végezhető, az operációs mikroszkóp használata megkönnyíti és pontosabbá teszi az ér egyesítését. Az érvarrat után klinikai gyakorlatunkban makromolekuláris folyadék infúzió (2x250 ml Fluidex), valamint thrombocita aggregációt gátló gyógyszerek (2x1/2 amp Aspisol és 1x1/2 tabl. Colfarit) alkalmazása elegendőnek bizonyult. Heparinizálást nem végeztünk.

Az érvarratok posztoperatív funkcióját korábban angiográfiával ellenőriztük, a műtét utáni kontrollvizsgálatra ma már mindenképpen a noninvazív Doppler vagy a color Doppler ultrahang a választandó módszer. Alkalmazásával meghatározható az áramlás iránya és sebessége [10, 11]. Ezekből a paraméterekből és az áramlási görbe alakjából az esetleges szűkületekre, elzáródásokra, keringési rendellenességekre egyértelmű következtetéseket vonhatunk le. Az ultrahangvizsgálat ezenkívül szövődmények (álaneurysma vagy perivascularis haematoma, stb.) kimutatására is alkalmas.

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A plexus brachialis MR-vizsgálata

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ÖSSZEFOGLALÁS: A plexus brachialis képi megjelenítésére, különösen a traumatológiában, egyre nagyobb az igény. A szerzők rövid anatómiai áttekintés után 6 egészséges egyénben és 10 sérültben végzett MR-vizsgálataik tapasztalatairól számolnak be. A praeganglionalis szakasz ábrázolására az axialis, a postganglionalis szakasz megjelenítésére a coronalis, esetenként a sagittalis síkú scaneket tartják a legalkalmasabbnak.

KULCSSZAVAK: Plexus brachialis, praeganglionalis laesio, postganglionalis laesio, MR-vizsgálat

MR imaging of brachial plexus

SUMMARY: The demand for imaging of the brachial plexus, particularly in traumatology, is becoming ever stronger. After a short anatomical review, the authors report on their experience during examinations of 6 healthy persons and 10 injured patients. Axial scans were found to be most appropriate for imaging of the preganglional part, and coronal or sagittal scans for imaging of the postganglionalpart of the brachial plexus.

KEY WORDS: brachial plexus, preganglional lesion, postganglional lesion, MR imaging

BEVEZETÉS

A perifériás idegek képi megjelenítése a szakemberek régi vágya. Bármennyire jogos volt is az igény, a hagyományos röntgentechnika nem tudta ezt kielégíteni, hiszen a konvencionális röntgenfelvételen az elenyésző sugárelnyelődési különbségek miatt a perifériás idegek nem különíthetők el a környező lágyrészektől. Az ultrahang- és a CT-vizsgálatok elterjedésével egyes idegképletek megjelenítése már lehetővé vált (3, 5), de az igazi áttörést az MR orvosi alkalmazása jelentette (6). E módszerrel eltérő protonsűrűségük és relaxációs idejük alapján jól elkülöníthetők egymástól az egyes lágyrészstruktúrák, így a perifériás idegek is.

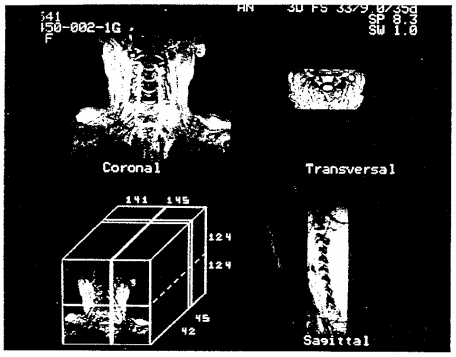
Napjainkban a súlyos közlekedési balesetek számának rohamos növekedésével egyre gyakrabban találkozhatunk a plexus brachialis valamilyen szintű sérülésével. Ezek egyrésze, különösen a postganglionalis szakaszt érintők, eredményesen kezelhetők primaer vagy halasztott idegvarrat, decompressio vagy egyéb más, az ingerületvezetést helyreállító beavatkozással (8). Reménytkeltők az eredmények a szülési trauma kapcsán létrejött újszülöttkori plexus brachialis sérülések ellátásában is (9). A megfelelő terápia megtervezéséhez jelentős segítséget nyújt, ha pontosan ismerjük az idegsérülés helyét (praeganglionalis, postganglionalis) és természetét (részleges vagy komplett szakadás, kompresszió) (2, 4, 10). Az irodalmi adatok alapján úgy tűnik, hogy ezekre a kérdésekre részben vagy egészben választ tud adni a brachialis MR-vizsgálata.

Munkánk célja az volt, hogy feltérképezzük a rendelkezésünkre álló 0,5 Teslás MR-készülék lehetőségeit a plexus brachialis prae- és postganglionalis szakaszának ábrázolásában. Meghatároztuk azokat a síkokat és szekvenciákat, amelyekkel a legtöbb képi információt kaphatjuk a plexus brachialis állapotáról. Megkíséreltük megállapítani a sérülés helyét és jellegét.

ANYAG ÉS MÓDSZER

A vizsgálatokat 1 Teslás GIREX V Dlx (Elscint) MR-készülékkel végeztük. Hat önként vállalkozó egészséges és 10 klinikailag plexus sérültnek tartott személyt vizsgáltunk. A háton fekvő beteg nyakát úgy helyeztük el a nyaktekercsben, hogy a lordosis és a kényszertartásból eredő esetleges scoliosis minél inkább kiegyenlítődjön. A lehetőleg minél kényelmesebb helyzet biztosítása után T2 jellegű gyors szekvenciás axialis scaneket készítettünk a plexus brachialis eredésének megfelelő discus rések magasságában, majd coronalis síkú, T1

jellegű gyors szekvenciás 3D adatgyújtést végeztünk 1 mm-es rétegvastagsággal. Az átlagosan 60 szelet elkészítése az általunk használt készülék esetén mintegy 20–25 percet vett igénybe. A T1 jellegű axialis, coronalis és szükség esetén sagittalis képeket a 3D aquisitio nyers adataiból rekonstruáltuk (1. ábra). A distalisabb szakasz megjelenítésére olykor újabb, ferde coronalis beállítást kellett alkalmaznunk.



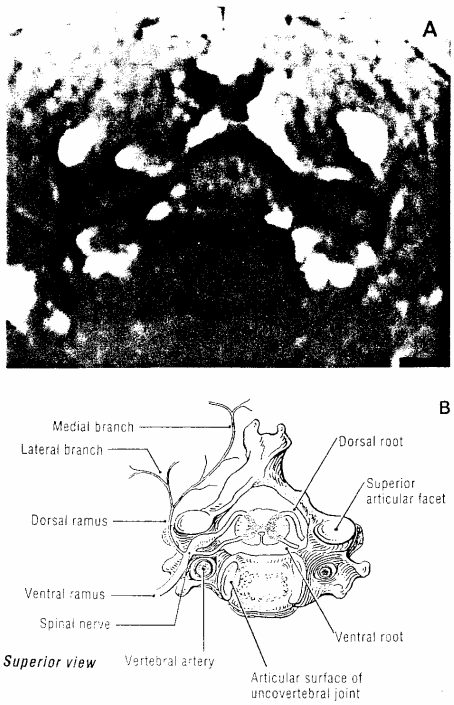
1. ábra: *3D gyorsszekvenciás adatgyűjtés coronalis, transversalis és sagittalis rekonstrukciós képei*

EREDMÉNYEK

A 6 egészséges és 10 klinikailag plexus brachialis sérültnek tartott személy vizsgálata során minden esetben értékelhető minőségű képeket kaptunk. A képminőség elsősorban a beteg mozdulatlanságától függött, illetve attól, hogy mennyire tudtuk a nyaki lordosist és az esetleges scoliosist kiegyenlíteni. A 6 egészséges személyben minden esetben azonosítani tudtuk a prea- (2. ábra) és postganglionalis szakasz (3. ábra) részeit a clavicula szintjéig. A distalisabb szakasz a kiegészítő ferde coronalis síkú metszeteken jól, többnyire megítélhető volt (4. ábra). A 10 sérült személynél háromban találtunk praeganglionalis laesiót (5. ábra), kettőben pedig annak gyanújeleit. Egy betegben láttuk a postganglionalis sérülés biztos jelét (6. ábra). A maradék 4 betegben nem találtunk plexus brachialis sérülésre utaló morfológiai eltéréseket. A képek minőségét nem rontotta, illetve a betegnek nem okozott kellemetlen érzést a társuló humerus törések esetében előzőleg behelyezett rögzítő fém még akkor sem, ha az mágnesezhető anyagból készült.

MEGBESZÉLÉS

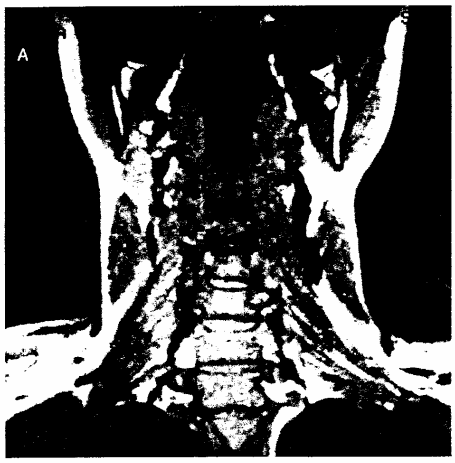
Az egyes lágyrészstruktúrák elkülönítésére, beleértve a perifériás idegeket is, jól alkalmazható

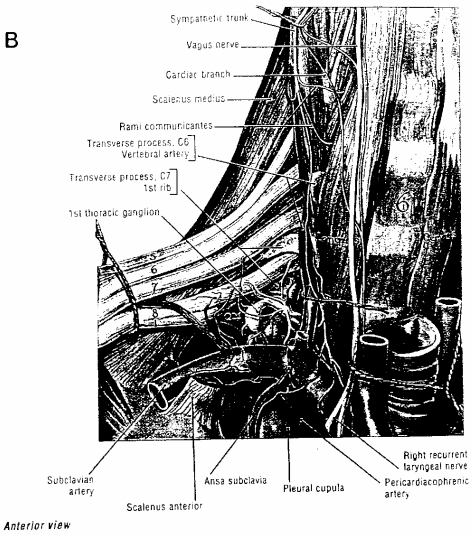


2. a), b) ábra: *A T1 súlyozott axialis metszeteken (A) pontosan tanulmányozhatók az anatómiai ábrán (B) megjelölt struktúrák. (Az anatómiai ábrák Anne M. R. Agur „Grant's Atlas of Anatomy" című könyvének IX. kiadásából származnak – Williams & Wilkins, ISBN 0-683-09429-7)*

képalkotó eljárás az MR-vizsgálat. Annak megértéséhez, hogy a plexus brachialis egyes szakaszainak ábrázolása mégsem egyszerű feladat, rövid anatómiai áttekintésre van szükség (1, 11).

A plexus brachialis mindkét oldalon a négy alsó cervicalis és az első thoracalis ideg ventralis ágának összefonódásából jön létre. A fonat a hiatus scalenin előlépve a kulcscsont feletti szakaszon truncusukat, a kulcscsont alatt pedig átrendeződve fasciculusokat alkot. A hiatus scaleniben haladva az a. subclavia felett és részben mögött helyezkedik el, majd a clavicula alatt a fossa axillarisban a fasciculusok az árok lateralis falán húzódó a. axillarist medialisan, lateralisan és hátulról ölelik körül. A truncusok és fasciculusok részt vesznek a mell-, a felületes hát- és a vállövi izmok beidegzésében. A fasciculusok folytatásaként kialakuló idegek pedig a szabad felső végtagot látják el. E bonyolult átrendeződés közben a plexus fentről lefelé ventrolateralis irányban halad, miközben enyhe S alakot ír le.





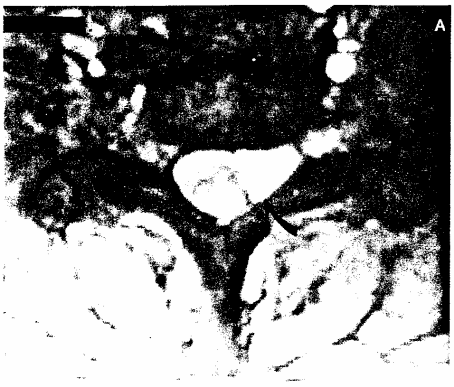
3. a), b) ábra: *A T1 súlyozott coronalis síkú metszet (A) részletdús és jól azonosítható képet ad az anatómiai ábrán (B) látható képletekről*

Pusztán lefutása miatt sem könnyű dolog az idegfonat MR-megjelenítése. Mivel csupán néhány mm átmérőjú idegképletekről van szó, amelyeknek a környező izmoktól való elkülönítését csak a perineuralis zsír teszi lehetővé (12), az adatgyűjtésnek kellően vékony szeletekben kell történnie. Ez azonban csökkenti a hasznos jel erősségét és rontja a jel-zaj viszonyt. Ha növeljük az aquisitios időt, részben a zaj is nő, részben a mozgási artefactumok (a. subclavia, a. axillaris pulsatioja, légzési mozgások) rontják a kép minőségét. További

4. ábra: *A plexus brachialis clavicula alatti szakasza a ferde coronalis scaneken ábrázolható legjobban*

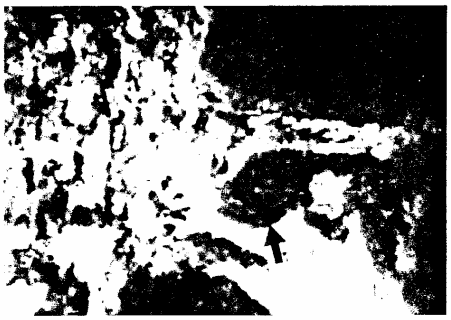
problémát jelent a gyakran meglévő scoliosisos kényszertartás és az esetleg fokozott lordosis. Így, különösen ha tekintetbe vesszük a 0,5 T-ás készülék adta szerényebb lehetőségeket, világos, hogy csak bizonyos kompromisszumos megoldásokat alkalmazhatunk. A fellelhető irodalmi ajánlások a többnyire 1,5 T-ás készülékek birtokában 3–5 mm szeletvastagságú, különböző síkú T1, illetve T2 súlyozott képeket részesítik előnyben (12, 13), amelyek kétségkívül jó képminőséget biztosítanak. A vizsgálati idő lerövidítése érdekében saját gyakorlatunkban a vékony rétegű T1 jellegű 3D adatgyűjtést alkalmaztunk a multiplanaris ábrázolásra. T2 jellegű képeket csak axialis síkban készítettünk a plexus brachialis eredésének megfelelő discus rések síkjában. Így sikerült elérnünk azt, hogy a mai viszonyok között már lassúnak számító készülékünkkel, mindössze 30 perc körüli vizsgálati idővel, két különböző szekvenciában több képsíkban tudtuk ábrázolni a plexus brachialist. Ez az aránylag rövid vizsgálati idő tette lehetővé, hogy az elég nehezen kooperáló plexus sérültek esetében is viszonylag jó minőségű képeket nyerhettünk. A képeken nagy biztonsággal megítélhető a praeganglionalis szakasz állapota, bár sajnos éppen ez a sérüléstípus a legkevésbé gyógyítható forma. A helyreállító sebészet szempontjából nagyobb jelentőségű postganglionalis sérülés kimutatása nehezebb és időigényesebb (7), hiszen a neurológiai eltérések sokszor igen összetettek, nem jelzik pontosan a laesio várható szintjét. A postganglionalis nyaki szakasz ábrázolására a coronalis, míg a clavicula mögött és alatt futó szakasz megítélésére a sagittalis, esetenként a ferde coronalis síkú metszeteket találtuk a legalkalmasabbaknak.

Vizsgálataink eredményei és az irodalmi ada-



5. a), b) ábra: *T2 jellegű gyorsszekvenciás axialis (A) és ferde coronalis (B) metszetek egyértelműen demonstrálják (nyilak) a praeganglionalis sérülés következtében létrejött két szegmentumra kiterjedő pseudomeningokelét*

tok alapján megállapítottuk, hogy a plexus brachialis képi megjelenítésére alkalmas az MR-vizsgálat. Megfelelő technikával már 0,5 T-ás készülékkel is diagnosztikus értékű képeket nyerhetünk. A sérülések jellegének (szakadás, részleges



6. ábra: *A T1 jelű másodlagosan rekonstruált coronalis metszeteken egyértelmű a clavicula mögötti postganglionalis szakasz sérülését követő perineuralis vérömleny (nyíl)*

szakadás, kompresszió) egyértelmű meghatározása vizsgálataink során nem volt sikeres. A sebészi megoldás szempontjából nagyobb jelentőségű postganglionalis sérülések kimutatása általában nehezebb, időigényesebb feladat. Ennek ellenére kedvező tapasztalataink alapján javasoljuk plexus brachialis sérülés gyanújakor az MR-vizsgálat elvégzését.

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Az MR cholangiographia értéke a ductus choledochus kövek igazolásában

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AJR 167; 1441–1445, 1996.

A szerzők egy új T2 súlyozású turbo spin echo szekvencia (HASTE) értékét vizsgálják a ductus choledochusban levő kövek diagnosztikájában. 23 choledochus kövességre gyanús betegnél MR, UH és endoszkópos cholangiographia történt. Az MR-vizsgálatokat 1,5T berendezéssel végezték sagittális, koronális és axiális síkokban. A vizsgálatot követően 3D képeken ábrázolták a teljes epeúti rendszert. Az MR-vizsgálatok értékének elemzésekor a choledochusban látható kövek alakját, számát, elhelyezkedését, valamint a choledochus tágasságát elemezték, és az eredményeket összehasonlították a másik két vizsgáló módszerrel. A 23 beteg közül az endoszkópos cholangiographia – mint gold standard – 15 betegnél igazolt kövességet. Az MR 14, míg az UH 9 esetben volt eredményes a kövek kimutatásában. 12 betegnél a cholangiographiával megegyezően az MR- és UH-vizsgálatok igazolták a közös epevezeték tágulatát. A szerzők szerint a HASTE szekvenciával végzett MR cholangiographia eredményes, noninvazív módszer, az endoszkópos cholangiographiát szükség esetén jól helyettesíti.

Puskás Tamás dr.

Az izolált pancreas sérülés CT-diagnosztikája

FISHER, J. H. és mtsai (Dept. of Radiology University of Washington School of Medicine Seattle WA 98104)
AJR 167; 1152, 1996.

A fedett hasi traumák 3–12%-ban a pancreas is megsérül és ezek 10–25%-a halálos kimenetelű. A korai halál oka leggyakrabban a parenhymás szervek vérzése. A 48 órán túli halál okai között szerepel a pancreas vezeték sérülése, és ennek egyáltalán nem, vagy késői felismerése. A szerzők egy eset ismertetése kapcsán összefoglalják a pancreas izolált sérüléseinek jellegzetes CT megjelenési formáit. A se. amylase érték sokszor félrevezető. Hasi traumákat követően magasabb se. amylase érték mellett csak az esetek 10%-ban találtak pancreas érintettséget. Ugyanakkor a pancreast is érintő sérüléseknél a se. amylase 40%-ban normális értékű volt. A gyakorlatban a pancreas sérülései sokszor az egyéb szervek traumás eltérései miatt végzett sürgősségi műtétek során kerülnek felfedezésre. A korai felismerés részben a túlélés, részben a késői szövődmények megelőzése szempontjából fontos. A natív és iv. kontrasztanyag adása után végzett CT-vizsgálatok szenzitivitása és specifitása a pancreas sérülések esetében magasabb mint 80%. A traumát követő 12 órán belül a CT-vizsgálatok sokszor negatív eredményűek. Amennyiben a beteg állapota a továbbiakban is gyanús pancreas érintettségre úgy a CT-vizsgálatot meg kell ismételni. A jellemző CT-eltérések a következők: a mirigyállomány folyamatosságának megszakadása, annak megnagyobbodása, inhomogén szerkezete. A bursa omentalisban, illetve a pancreas és a v. lienalis közti folyadékgyülem, az elülső perirenális fascia megvastagodása. Ezek az elváltozások sok esetben a traumás pancreatitis képére is jellemzőek. Szükségesnek tartják megemlíteni, hogy a pontos diagnózist jelentősen befolyásolja a vizsgálati technika, annak helyesen megválasztott időpontja, valamint a vizsgálat értékelését végző orvos jártassága.

Puskás Tamás dr.

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QUARTERLY

Neural impacts on the regeneration of skeletal muscles★✪

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Key words: skeletal muscle, denervation, reinnervation, notexin-induced regeneration

The regeneration of skeletal muscles is a suitable model to study the development and differentiation of contractile tissues. Neural effects are one of the key factors in the regulation of this process. In the present work, effects of different reinnervation protocols (suture or grafting) were studied upon the regenerative capacity of rat soleus muscles treated with the venom of the Australian tiger snake, notexin, which is known to induce complete necrosis and subsequent regeneration of muscles. Morphological and motor endplate analysis indicated that the regenerative capacity of denervated, and thereafter surgically reinnervated muscles remains impaired compared to that of normally innervated muscles, showing differences in the muscle size, fiber type pattern and motor endplate structure, even 35 days after the notexin injection. A lack or deficiency of secreted neural factors, deterioration of satellite cells and/or incomplete recovery of the sutured or grafted nerves may be the cause of these discrepancies in the regeneration process.

The functional diversity of skeletal muscle fibers is deeply rooted in their innervation pattern (Pette & Staron, 1990; Pette & Vrbová, 1992). Loss of innervation not only by experimental denervation, but also by accidents leads to general morphological and physiological deterioration of the affected fibers (Sunderland & Ray, 1950; Gutmann & Zelena, 1962; Borisov *et al.,* 2001; Germinario *et al.,* 2002). The superficial localization, mass and mechanical activities expose skeletal muscles and their motoneurons to

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Abbreviations: TNF-α, tumor necrosis factor-α; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase; HE, haematoxylin-eosin; AChE, acetylcholinesterase.

different types of injuries. Injury of the brachial plexus may occur in newborns (during delivery) as well as in young adults in (motorcycle) accidents (Mackinnon, 1993; Alnot, 1995). Microsurgical protocols allow early nerve reconstruction aiming at regaining active muscle function soon after the injury. The injured part of the nerve is either replaced by a peripheral nerve (grafting), or less frequently by simple suture, or in some cases the so-called free muscle transplantation is recommended as an alternative (Berger & Brenner, 1995; Doi *et al.,* 1998). Even after a good nerve reconstruction, the muscle function remains impaired in high-level mixed-type nerve injuries. The reason for this impairment can be in part the relatively late neurotisation (3–6 months after the injury), by which time the denervated muscle fibers are severely atrophied. Moreover, the regeneration of the nerve and the reinnervation of the muscles normally need weeks or months, and afterwards adaptic axon losses may disable the perfect reinnervation (Alnot, 1995).

Injured skeletal muscle fibers undergo almost complete regeneration provided their satellite cell content was unharmed. The experimentally induced muscle regeneration processes are suitable for characterization of the events and regulation during muscle development and differentiation (Lefaucheur & Sebille, 1995; Saito & Nonaka, 1994). The Australian tiger snake venom notexin is one of the most frequently used inducers of muscle necrosis for subsequent regeneration studies (Harris *et al.,* 1975; Harris & Johnson, 1978; Preston *et al.,* 1990; Grubb *et al.,* 1991; Davis *et al.,* 1991; Vater *et al.,* 1992).

The regeneration process following notexin administration has been thoroughly characterized in the last decade, in normal and dystrophic skeletal muscles (Sewry *et al.,* 1992; Dux *et al.,* 1993; Wilson *et al.,* 1994a; 1994b; Zádor *et al.,* 1996; 1998; 1999; 2001; Mendler *et al.,* 1998a; 1998b; 2000). Molecular events of the regeneration process, such as expression of myogenic regulatory factors and some growth factors (myostatin, TNF-α), formation of new motor endplates with reinnervation, expression and re-establishment of myosin and SERCA isoform distribution were analysed. Introduction of antisense RNAs or expression vectors into the regenerating muscles allowed the modulation of the regeneration model (Zádor *et al.,* 2002; Zádor & Wuytack, 2003).

The regenerated muscle achieved its normal morphology by the 28th day post notexin injection, although some nuclei were still in a central position in the fibers (Harris *et al.,* 1975; Harris & Johnson, 1978; Whalen *et al.,* 1990; Sesodia & Cullen, 1991). In regenerated soleus muscles the typical slow myosin isoform and sarcoplasmic reticulum structure were recovered (Whalen *et al.,* 1990; Sesodia & Cullen, 1991), although myosin and SERCA isoform composition become more uniform (Davis *et al.,* 1991; Mendler *et al.,* 1998b; Zádor *et al.,* 1998). The complete recovery of the metabolic capacity in regenerated muscles ensured the background for functional activity (Sesodia *et al.,* 1994). Although electrophysiological studies indicated that regenerated muscles were able to produce normal action potentials and contractions as soon as the newly formed motor endplates obtained their mature form (Grubb *et al.,* 1991; Whalen *et al.,* 1990), in the functional sense the recovery did not seem to be complete. Louboutin *et al.* (1995) reported that the amplitudes of contractions in the regenerated muscle remained strongly dependent on the external Ca^{2+} concentration, a feature typical of neonatal muscles, instead of normal adult muscle fibers.

In the present work we explored the notexin-induced muscle regeneration of denervated/reinnervated rat soleus. In this first phase of experiments morphological changes were characterized at the light and electron microscopic levels. Moreover, the dynamics of motor endplate formation was followed during the regeneration of reinner-

vated muscles, compared to those regenerating with normal innervation. We also compared the effects of different nerve reconstruction techniques (suture or grafting) at the morphological level.

MATERIALS AND METHODS

Treatment of animals. Adult male Wistar rats (250–280 g) were anesthetized with intraperitoneal injection of Nembutal. The left sciatic nerve was exposed at the proximal part of the thigh by splitting the gluteal muscle.

Reinnervation protocols. In the first group of animals, an approx. 12 mm nerve segment was resected and used as an autologous nerve graft. The coaptation sites were sutured by 10/0 nylon epineural sutures. The second group of rats was reinnervated by making simple suture at the proximal cutting level.

Control animals. In the third group of animals, a nerve segment of more than 30 mm was removed without doing nerve reconstruction.

Based on preliminary experiments, at 3 months after microsurgery the soleus muscles of the denervated, sutured or grafted animals from both the uni- and contralateral sides were removed and further processed for morphological analysis. Each group of animals contained at least 3–4 animals.

Induction of regeneration. In the fourth group of rats, that had been reinnervated by grafting, muscle necrosis was induced to the soleus muscle by intramuscular injection of 20 μg of notexin in physiological NaCl solution. This amount of notexin was chosen since it is known to induce complete necrosis to the muscle (Mendler *et al.*, 1998a) (see Fig. 3A). At 1, 3, 5, 7, 10, 21, 28 and 35 days after injection, animals were sacrified by an overdose of Nembutal injection, and soleus muscles of both the injected and the contralateral hindlimbs were removed, weighed and further processed for morphological analysis. At each stage of regeneration 3–4 animals were examined.

Preparation and staining of tissue sections. Soleus muscles of all groups of animals (denervated, reinnervated by graft or by suture, reinnervated and regenerated muscles) were processed for light microscopical analysis. Cryostat sections of 15 μm thickness were either stained with haematoxylin-eosin (HE) or the motor endplate formation was checked by using the method of Tago (Tago *et al.*, 1986) staining the acetylcholinesterase (AChE) activity of the endplates. Some samples of grafted-regenerated soleus muscles were also processed for standard electron-microscopy (EM).

RESULTS AND DISCUSSION

In denervated rat soleus muscles we detected pronounced and uniform atrophy (Figs. 1 and 2D) and only diffuse, if any,

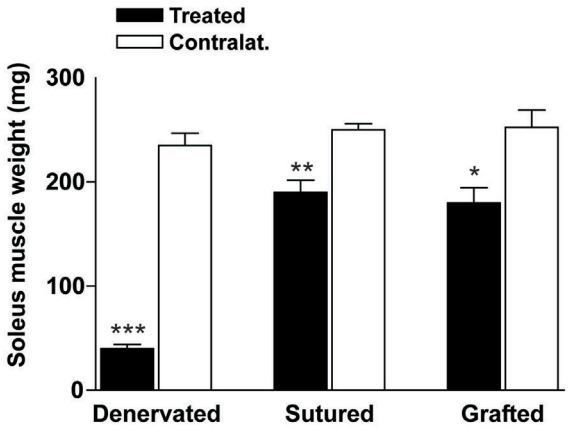

Figure 1. Muscle weights of denervated and reinnervated (sutured or grafted) rat soleus muscles 3 months after microsurgery.

Colums represent mean values ±S.E.M. of data obtained from 3–4 animals in each group, asterisks show significant differences compared to untreated contralateral muscles (Paired *t*-test, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$**).** Reinnervated muscles differed significantly from their contralateral ones, and also from the denervated muscles ($P < 0.001$, ANOVA). Muscle weights of the sutured and grafted muscles did not differ significantly from each other ($P > 0.05$, ANOVA).

AChE activity showing no motor endplate formation (Fig. 2H). The weights of reinnervated soleus muscles were significantly higher than of the denervated ones ($P < 0.001$, ANOVA), but they did not reach the values of the contralateral untreated muscles even after 3 months of reinnervation (Fig. 1). In all of the investigated reinnervated muscles we found atrophied fibers either in groups, characterized by more pronounced connective tissue as well (Fig. 2B insert), or interspersed (Fig. 2C) among fibers which had close to normal diameter and morphology (Fig. 2A, B, C). In line with these findings, the morphology of motor endplates was variable, they were smaller and of unmatured character in the regions of the atrophied fibers (Fig. 2F insert, G). Others also described (Ijkema-Paassen *et al.*, 2001b, Wang *et al.,* 2002) that rat soleus muscles did not regain their normal size and endplate morphology even after 21 weeks of reinnervation. Moreover, the normally slow type muscle was transformed into a predominantly fast phenotype (Ijkema-Paassen *et al.*, 2001a; 2001b; Wang *et al.,* 2002). The fast type tibialis anterior muscles showed better recovery in all the aspects investigated, suggesting that the fiber type composition, and consequently, the initial innervation pattern of a given muscle can ultimately influence the efficiency of the reinnervation later on. However, there were no data in the literature whether different microsurgical techniques, i.e. suture *versus* graft have different effects on muscle recovery. We found that the weights of sutured and grafted muscles did not differ significantly from each other ($P > 0.05$, ANOVA; Fig. 1) and the morphology was similar in both cases, at least at the light microscopical level (Fig. 2B, C, F, G). Therefore, we used grafted muscles for the regeneration studies since this technique proved to be clinically more relevant.

The regeneration process of grafted muscles showed differences compared to that of normal muscles, although – similar to the normally innervated ones – notexin induced

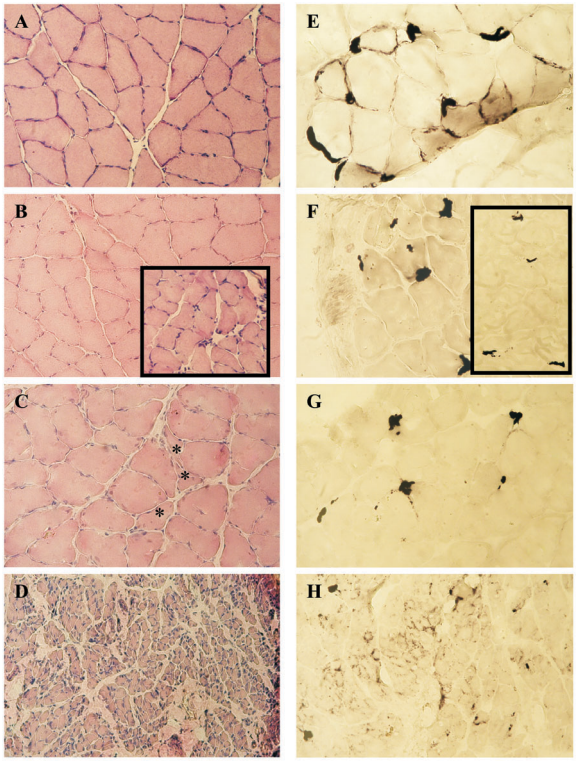

Figure 2. Fiber and motor endplate morphology of normal, reinnervated and denervated rat soleus muscles 3 months after microsurgery.

After removal of the muscles, cryostat sections of 15 μm thickness were either stained with haematoxylin-eosin (HE) or the motor endplate formation was checked by staining for acetylcholinesterase (AChE) activity of the endplates. **A–D**: HE staining of normal, grafted, sutured and denervated muscles, respectively. **E–H**: AChE staining of normal, grafted, sutured and denervated muscles, respectively. In grafted muscles we detected atrophied fibers and more connective tissue (B insert) besides fibers of close to normal morphology (B). This difference was also evident in the variability of the size of motor endplates (F and F insert). The morphology of sutured muscles was similar to that of grafted muscles, here we show regions with atrophied fibers interspersed among normal ones (C asterisks) with variable endplate morphology (G). Denervated muscles showed general atrophy (D) and only diffuse, if any, AChE-activity (H). Magnification 200×.

complete necrosis by day 1 (Fig. 3A). By this time muscle weights did not decrease (Fig. 4) probably because of the pronounced oedema. Three days after notexin injection the muscles were significantly smaller than the un-

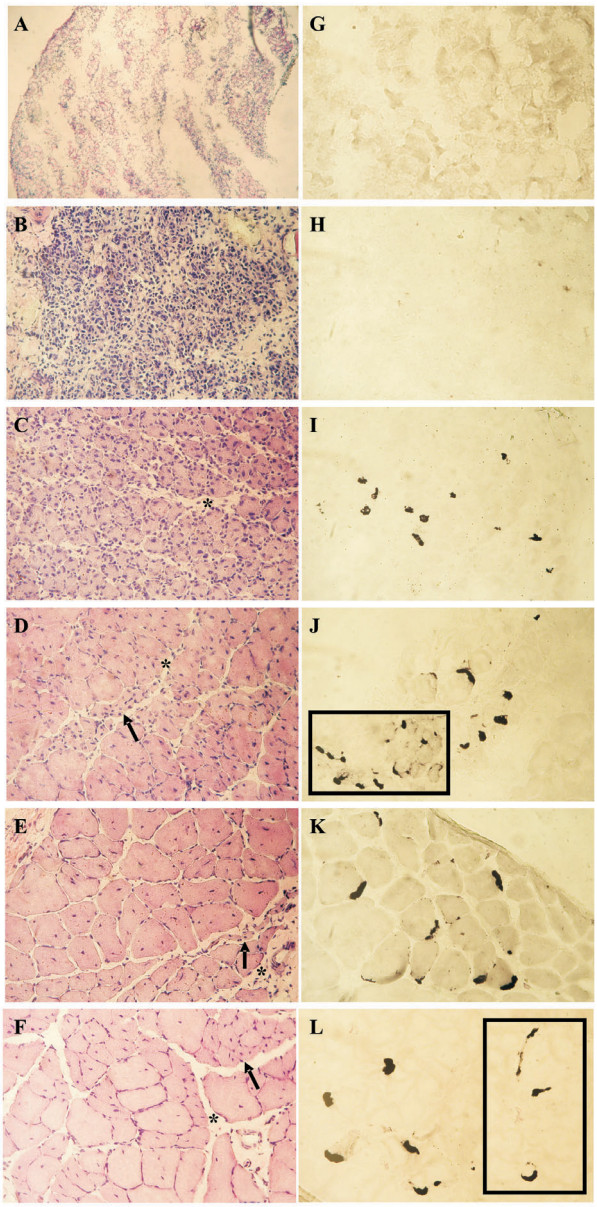

Figure 3. Fiber and motor endplate morphology of reinnervated (grafted) rat soleus muscles regenerating from notexin-induced necrosis.

After removal of the muscles, cryostat sections of 15 μm thickness were either stained with haematoxylin-eosin (HE) or the motor endplate formation was checked by staining for acetylcholinesterase (AChE) activity of the endplates. **A–F**: HE staining of muscles 1, 3, 5, 10, 28, and 35 days after notexin injection, respectively. **G–L**: AChE staining of muscles 1, 3, 5, 10, 28, and 35 days after notexin injection, respectively. Notexin induced complete necrosis by day 1 (A) destroying virtually all fibers. Three days after notexin injection mononucleated cells filled up the injured muscle (B). Most of the mononucleated cells had already fused to form new myotubes by day 5 (C). From this stage on, however, connective tissue seemed to be more abundant throughout the regeneration process (C–F, asteriks). Abnormal and pronounced variability of the fibre size is the characteristic feature of the 7- and 10 day-regenerated muscles (D, arrow shows smaller fibers). Even after 28 and 35 days of regeneration, the fibre size variability was still present (E, F arrows show smaller fibers) and more than 80% of the fibers still contained centrally located nuclei. Notexin treatment destroyed all the motor endplates by day 1 (G) and we did not see any signal of motor enplate formation until day 3 (H). The first new motor endplates reappeared by day 5 after necrosis (I), at a similar time to those of normally innervated regenerated muscles. However, their morphology seemed to be more variable even at late stages of regeneration (J, K, L) showing smaller and in some cases fragmented motor endplates (J, L inserts). Magnification 200×, except Fig. 3A (magnification 40×).

treated ones (Fig. 4), and mononucleated cells filled up the whole cross-section area of the injured muscle (Fig. 3B). Like in the normally innervated muscles, most of the mononucleated cells had already fused to form new myotubes by day 5 (Fig. 3C). From this stage on, however, connective tissue seemed to be more abundant in the reinnervated muscles throughout the regeneration process (Fig. 3C–F). Abnormal and pronounced variability of the fibre size was the characteristic feature of the 7 and 10 day-regenerated/reinnervated muscles (Fig. 3D). Even after 28 and 35 days of regeneration (Fig. 3E, F), the fibre size variability was still present and more than 80% of the fibers still contained centrally located nuclei, much more than found in normally innervated regenerated muscles (Mendler *et al.*, 1998a). These findings, together with the significantly lower muscle weights at this late stage of regeneration (Fig. 4), suggest that a significant number of fibers were not able to perfectly regenerate/differentiate.

Although notexin treatment destroyed all the motor endplates by day 1 (Fig. 3G, H), the first new motor endplates reappeared at a similar time (by day 5 after necrosis, Fig. 3I) as those of normally innervated regenerated muscles. However, their morphology seemed

to be more variable even at late stages of regeneration showing smaller and in some cases fragmented motor endplates coupled to smaller/less regenerated fibers (Fig. 3J, K, L with inserts).

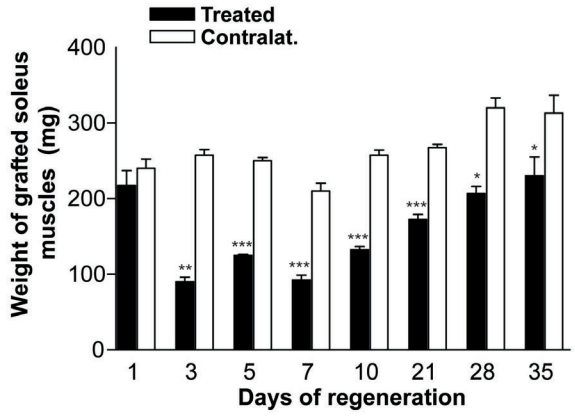

Figure 4. Muscle weights of reinnervated (grafted) rat soleus muscles regenerating from notexin-induced necrosis.

Columns represent mean values ±S.E.M. of data obtained from 3–4 animals at each stage of regeneration (1–28: days after notexin administration), asterisks show significant differences compared to untreated contralateral muscles (Paired *t*-test, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$). By day 3 after notexin treatment, the muscles became significantly smaller than their untreated counterparts. Thereafter, muscle weights increased until the end of the examined period of regeneration, but even at that time they were smaller than the contralateral ones.

At the ultrastructural level, grafted regenerated muscles showed serious abnormalities of mitochondria by day 35 after notexin treatment (not shown).

In this work, our aim was to characterize the muscle regeneration capacity of reinnervated muscles. Up to now, no data were available in the literature in this regard. It is well known that denervated muscles have impaired regenerative capacity, which is most pronounced during the second phase of regeneration, i.e. the newly formed primitive fibers are not able to differentiate to reach their normal size (Sesodia & Cullen, 1991). Still, it is debated whether satellite cells, the main sources of muscle regeneration (Mauro, 1961; Bishoff, 1993; Asakura *et al.*, 2002), are inactivated by denervation (Maier *et al.*, 2002), or on the contrary, they become more active upon denervation (Nnodim, 2001). This author found in the specific androgen dependent levator ani muscle of male rats that the activation of satellite cells caused by denervation could be prevented by castration, indicating that the effects of denervation can be modulated by other (humoral) factors as well.

Our morphological results are in line with the original hypothesis that the regenerative capacities of reinnervated muscles might be impaired. One reason could be that since reinnervation is not complete, satellite cells of more atrophied regions might be of lower activity (number?) than those of the normal ones. However, the question seems to be even more complicated. As cited earlier, other groups (Ijkema-Paassen *et al.*, 2001b) described that reinnervated muscles show high proportion of endplates of abnormal morphology, which we could also confirm in our experiments. Yet we do not know whether this pattern of "abnormality" will be recapitulated in the course of regeneration when the newly formed endplates are established after complete necrosis. In theory, it cannot be ruled out that the necrotized muscle sends cues for the reinnervating axons which might modify the reinnervation pattern of the regenerating reinnervated muscle. If so, it could well be that the fiber-type composition also shows changes after regeneration of reinnervated muscles. Investigation of the myosin and SERCA isoforms at both mRNA and protein levels and their expression pattern in tissue sections, compared to normally innervated regenerated muscles (Zádor *et al.*, 1998; 1999; Mendler *et al.*, 1998b), would at least partly answer this question. Moreover, the regulatory molecules like myogenic regulatory factors or myostatin (Mendler *et al.*, 1998a; 2000; Zádor *et al.*, 1999) involved in the differ-

entiation of the fibers, or any element of the calcium-calcineurin signaling cascade may also show changes, which we would also like to explore in further experiments.

We would like to thank Dr. Ernő Zádor for his helpful advices in planning the experiments.

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DELAYED RILUZOLE TREATMENT IS ABLE TO RESCUE INJURED RAT SPINAL MOTONEURONS

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Abstract—The effect of delayed 2-amino-6-trifluoromethoxy-benzothiazole (riluzole) treatment on injured motoneurons was studied. The L4 ventral root of adult rats was avulsed and reimplanted into the spinal cord. Immediately after the operation or with a delay of 5, 10, 14 or 16 days animals were treated with riluzole (n=5 in each group) while another four animals remained untreated. Three months after the operation the fluorescent dye Fast Blue was applied to the proximal end of the cut ventral ramus of the L4 spinal nerve to retrogradely label reinnervating neurons. Three days later the spinal cords were processed for counting the retrogradely labeled cells and choline acetyltransferase immunohistochemistry was performed to reveal the cholinergic cells in the spinal cords. In untreated animals there were 20.4±1.6 (±S.E.M.) retrogradely labeled neurons while in animals treated with riluzole immediately or 5 and 10 days after ventral root avulsion the number of labeled motoneurons ranged between 763±36 and 815±50 (S.E.M.). Riluzole treatment starting at 14 and 16 days after injury resulted in significantly lower number of reinnervating motoneurons (67±4 and 52±3 S.E.M., respectively). Thus, riluzole dramatically enhanced the survival and reinnervating capacity of injured motoneurons not only when treatment started immediately after injury but also in cases when riluzole treatment was delayed for up to 10 days.

These results suggest that motoneurons destined to die after ventral root avulsion are programmed to survive for some time after injury and riluzole is able to rescue them during this period of time.

Key words: reinnervation, spinal motoneuron, survival, ventral root avulsion.

There is much evidence that reducing the excitatory input to injured neurons enhances their chances of survival. In the case of motoneurons NMDA receptor antagonists are known to rescue cells destined to die after neonatal nerve injury (Mentis and Vrbová, 1993). Moreover, adult injured motoneurons can also be rescued by reducing excitation by 2-amino-6-trifluoromethoxy-benzothiazole (riluzole) (Nógrádi and Vrbová, 2001).

Riluzole is a compound that acts to block voltage-activated Na^+, K^+ and Ca^{2+} channels and to inhibit presynaptic glutamate release (Doble, 1996). Riluzole reportedly reduces the damage to neurons caused by ischemia in the spinal cord (Lang-Lazdunski et al., 1999) and protects motoneurons destined to die *in vitro* after exposure to glutamate agonists (Estevez et al., 1995). Moreover, riluzole apparently increased survival of a subset of ALS patients with bulbar onset (Bensimon et al., 1994; Meininger et al., 1997) and it is still the most promising drug for the treatment of ALS (Gordon, 2005; McGeer and McGeer, 2005).

Although adult motoneurons do not die if their axons are injured at some distance from the cell body, they are unable to survive injury caused close to the cell body by avulsion of their axons shortly after they leave the spinal cord via the ventral roots (Greensmith and Vrbová, 1996). Ventral root avulsion causes death of almost all neonatal and adult motoneurons damaged in this way (Chai et al., 2000; Chan et al., 2001, 2002; Gu et al., 2004; Nógrádi and Vrbová, 1996, 2001). However, these cells do not die instantaneously, and some of them can be rescued if the ventral root is re-inserted into the spinal cord (Chai et al., 2000; Chan et al., 2002; Nógrádi and Vrbová, 1996, 2001). Few axons of the damaged motoneurons then enter this conduit and are able to regenerate all the way to the muscle (Cullheim et al., 2002; Nógrádi and Vrbová, 1996; Bergerot et al., 2004). It could be that the extent of the rescue of the damaged motoneurons depends on the speed at which they die, or on the delay that prevents their axons from reaching the reimplanted ventral root. Several attempts have been made to rescue adult motoneurons with avulsed axons, including therapy with neurotrophic factors (Blits et al., 2004; Novikov et al., 1995; Haninec et al., 2003; Wu et al., 2003). Since the damaged motoneurons are likely to die as a result of their increased sensitivity to excitatory influences they are thought to be rescued by neutralizing excitatory effects. We have in a previous study treated animals immediately after ventral root avulsion and its reimplantation with systemic administration of riluzole (Nógrádi and Vrbová, 2001). This treatment rescued the vast majority of the injured motoneurons, and allowed their axons to regenerate into the implant. In these studies the treatment of riluzole was started immediately after the injury was inflicted, probably before the program for cell death was set in motion. Therefore most of the cells

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Abbreviations: ChAT, choline acetyltransferase; EDL, extensor digitorum longus; FB, Fast Blue; riluzole, 2-amino-6-trifluoromethoxy-benzothiazole; TA, tibialis anterior.

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were able to regenerate their axons into the implanted ventral root, and avoid cell death.

Both from a clinical and theoretical point of view it is important to establish whether it is possible to rescue the injured cells at a later time after injury. From previous results it appears that in the rat, damaged neurons may survive in the CNS for up to 15–16 days (Koliatsos et al., 1994). The aim of our study was to see whether these 'surviving' cells can be rescued and are able to perform their normal function, i.e. to reinnervate the reimplanted ventral root.

Our results are encouraging, for they show that for quite some time the injured cells are capable of recovery if protected by riluzole.

EXPERIMENTAL PROCEDURES

Altogether 32 Sprague–Dawley rats (weight at time of surgery: 180–200 g) were used in this study. In 29 animals the left L4 ventral root was avulsed from the cord and then the L4 ventral root was reimplanted dorsolaterally into the spinal cord. Four of these animals remained untreated while the other five groups of animals were treated with riluzole for 3 weeks. Three intact animals were used for counting the L4 motoneuron pool. The operated animals survived for 3 months and then the ventral ramus of the left L4 spinal nerve was labeled with the fluorescent dye Fast Blue (FB, Illing Plastics GmbH, Breuberg, Germany).

The experiments were carried out with the approval of the Committee for Animal Experiments, University of Szeged regarding the care and use of animals for experimental procedures. All the procedures were carried out according to the Helsinki Declaration on Animal Rights. Adequate care was taken to minimize pain and discomfort. Efforts were made to minimize the number of animals used.

Surgery

All the operations were carried out under deep chloral hydrate anesthesia (4%, 1 ml/100 g body weight) and sterile precautions. Laminectomy was performed at the level of T13–L1, the dura was opened and the left L4 ventral root was pulled out leaving the dorsal roots intact. Then the cut end of the ventral root was gently inserted into the dorsolateral part of the spinal cord (Nógrádi and Vrbová, 1996, 2001). The spinal cord was covered with the remaining dura, the wound was closed and the animals were allowed to recover.

Riluzole treatment

Animals were treated with riluzole (kind gift of Tocris Cookson Ltd., Langford, UK; 4 mg/kg) for 3 weeks. Riluzole treatment started either immediately on the day of surgery or 5, 10, 14 and 16 days following surgery ($n=5$ in each group). The drug was injected intraperitoneally daily for 1 week and every second day for the next 2 weeks. Four animals remained untreated. This treatment protocol was based on the successful riluzole treatment described in our earlier paper (Nógrádi and Vrbová, 2001). The dose of riluzole was established from data obtained from our earlier and other laboratories' experiments (Lang-Lazdunski et al., 1999; Nógrádi and Vrbová, 2001; Schwartz and Fehlings, 2001, 2002; Wahl et al., 1993). It has also been reported that 5 mg/kg riluzole administered i.p. in rats produces a significant riluzole level in the brain (Maltese et al., 2005).

Retrograde labeling and immunohistochemistry

Three months after the surgery the animals were deeply anesthetized with chloral hydrate. On the operated side the ventral ramus of the left L4 spinal nerve was sectioned and the proximal stump of the nerve covered with few crystals of FB. Three days after the application of fluorescent dye the animals were reanaesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 mol/l phosphate buffer. The lumbar part of the spinal cords, with the reimplanted ventral root was removed and kept in fixative for 4 h. The tissues were then immersed in 30% sucrose. Serial 25 μm thick cryostat sections were cut, mounted on gelatinized slides and examined in an Olympus BX50 fluorescence microscope (Olympus, Tokyo, Japan). The number of retrogradely labeled cells was counted. To avoid double counting of the same neuron present in two consecutive sections, the retrogradely labeled neurons were mapped with the aid of an Olympus drawing tube, and their location was compared with that of labeled neurons in the previous section. All sections from the L4 motoneuron pool were used.

Three spinal cords from each group were then further processed for choline acetyltransferase (ChAT) immunohistochemistry. Sections processed for ChAT immunohistochemistry were preincubated in 3% normal goat serum for 1 h, then incubated with a polyclonal goat anti-ChAT antibody (Chemicon, Hofheim, Germany, 1:200) overnight at 4 °C. The immune reaction was completed by using the avidin–biotin technique (reagents were purchased from Vector Laboratories, Burlingame, CA, USA) and finally tyramide-amplified with the Cyanine3 TSA kit (Tyramide Signal Amplification, PerkinElmer, Zaventem, Belgium). The number of ChAT-stained motoneurons in the pools where retrogradely-labeled cells were found was also determined both on the operated and control sides. Some sections were stained with Cresyl Violet to assess the morphology of the spinal cord. Sections were photographed using an Olympus DP70 digital camera mounted on the microscope. Digital images were resized and their contrast and brightness adjusted.

After completing the experiments the extensor digitorum longus (EDL) and tibialis anterior (TA) muscles were removed and weighed.

Statistical analysis

The non-parametric Mann-Whitney *U* test and the one-way ANOVA test were used to compare the groups of data.

RESULTS

Behavioral observations and muscle weights

All the animals survived the surgery and the subsequent riluzole treatment.

The behavior of the operated animals was monitored every week. Initially all animals developed a partial paralysis in the operated hindlimb. Operated animals that were not treated with riluzole dragged their hindlimb during walking and were unable to dorsiflex their ankle joint at any time. In contrast all the animals treated with riluzole before 14 days after surgery started to recover from paralysis during the 3rd and 4th week following surgery, but complete recovery took several more weeks. By the end of the survival period they were able to walk almost normally and during locomotion flexed their ankle joint. None of these treated rats dragged their operated hind leg during movement.

Table 1 summarizes the results of the muscle weight losses of EDL and TA muscles. The weights are expressed as a percentage of the muscle on the control unoperated side. It is apparent from the table that the decrease in muscle weights was much more severe in the untreated

Table 1. Muscle weight loss following riluzole treatment

Delay of riluzole treatment	Immediate	5 Days	10 Days	14 Days	16 Days	Untreated
EDL weight loss (SEM)	9±1.5	9.8±1.16	9.9±2	24.3±1.3	21.7±1.8	39.1±4.5
TA weight loss (SEM)	15.4±1.8	17±1.15	18.5±2.2	30.7±2.8	29.3±4.9	48.5±5.3

Table 1 summarizes the results of the muscle weight losses of EDL and TA muscles. The weights are expressed as a percentage of the muscle weight on the control unoperated side. It is apparent that the decrease in muscle weights was much more severe in the untreated animals then in animals that received riluzole. Note that the decrease in muscle weights was greater when treatment was delayed and started only 14–16 days after the operation.

animals than in animals that received riluzole. Note that the decrease in muscle weights was greater when treatment was delayed and started only 14–16 days after the operation.

General observations on the morphology of spinal cords

In Cresyl Violet–stained specimens the morphology and the integrity of the spinal cords could be studied. In all experimental groups fewer motoneurons appeared to be in the operated ventral horn of the L4 segment and some gliosis could be observed at the site of root avulsion.

Motoneuron loss following L4 ventral root avulsion

First the number of resident motoneurons in the L4 motoneuron pool was assessed by retrograde labeling of the ventral ramus of the L4 spinal nerve. The average number of retogradely labeled motoneurons was 1164±29 (S.E.M.; Fig. 1). The motoneurons were localized mainly in the lateral motoneuron column of the L4 spinal segment (Fig. 2A). The avulsion and the reimplantation of the L4 ventral root resulted in a dramatic decrease in surviving motoneuron numbers, only 20±1.6 survived (Fig. 1, bar B).

These motoneurons were found throughout the length of the L4 spinal segment and a marked autofluorescence indicated the presence of gliotic scar at the place of lost motoneurons.

Survival of injured motoneurons following riluzole treatment initiated at various time points after avulsion

The effect of riluzole treatment starting at various time points following L4 avulsion and reimplantation was studied in the next series of experiments. Riluzole treatment started either immediately or 5, 10, 14 and 16 days after the operation and lasted for 3 weeks. In animals where riluzole was applied immediately after L4 avulsion and reimplantation, 763±36 (S.E.M.) retrogradely labeled motoneurons were found indicating that more than half of the total population of L4 motoneurons survived and was able to grow axons into the L4 ventral root. Riluzole treatment commencing 5 or 10 days after L4 avulsion and reimplantation resulted in similar numbers of retrogradely labeled motoneurons [815±50.6 (S.E.M.) and 772±39.1 (S.E.M.), respectively]. Although the number of surviving motoneurons appeared somewhat higher in these two groups, there was no significant difference in surviving motoneuron numbers compared with the group where riluzole treatment started immediately after operation. In contrast, significant decrease in the number of retrogradely labeled motoneurons was noticed when riluzole treatment started 14 or 16 days after L4 avulsion and reimplantation (Figs. 1 and 3). In these cases fewer retrogradely labeled motoneurons were found [67±3.9 (S.E.M.) and 52±3 (S.E.M.), respectively]. The numbers of retrogradely labeled motoneurons in these latter groups were not significantly different from each other.

Fig. 3 shows the loss of motoneurons in each group treated at various time intervals after the operation. It illustrates the steep decline in motoneuron survival between groups treated 10 and 14 days after the operation. Nevertheless, a significant difference was found between the numbers of labeled motoneurons in the untreated group and in the animals which received riluzole treatment immediately or 5 and 10 days after surgery ($P=0.016$).

Whether the inability of motoneurons to extend axons to the ventral root in spite of riluzole treatment started after 14 days of injury could be explained by motoneuron death during this time interval, or by other factors was tested by investigating whether any motoneurons were left in the appropriate part of the spinal cord after avulsion and reimplantation of the ventral root.

Expression of ChAT in injured and regenerating motoneurons

We compared the localization of ChAT with that of FB-labeled reinnervating cells. In control rats all the retrogradely labeled motoneurons in the lateral motoneuron pools were ChAT immunoreactive and there were occasionally ChAT immunoreactive motoneurons in the ventromedial pool only which remained unlabeled with FB (Fig. 2A–B). Similar colocalization was found in groups, which received the first riluzole treatment immediately or 5 and 10 days following avulsion and reimplantation, however, in these animals there were some ChAT immunoreactive cells which were not retrogradely labeled. Accordingly, in these animals the proportion of ChAT positive motoneurons on the operated side (% of operated/control side) was somewhat higher than that of FB-labeled motoneurons (% of operated side/intact pool) [5 days' delay: FB^+ vs. $ChAT^+$=70±4.3 vs. 90.7±2.1 (%±S.E.M.); 10 days' delay: FB^+ vs. $ChAT^+$=66.3±3.3 vs. 85.4±2.4 (%±S.E.M.), Fig. 4]. In animals with more delayed start of riluzole treatment (14 and 16 days after operation) considerable numbers of ChAT positive motoneurons were located in the

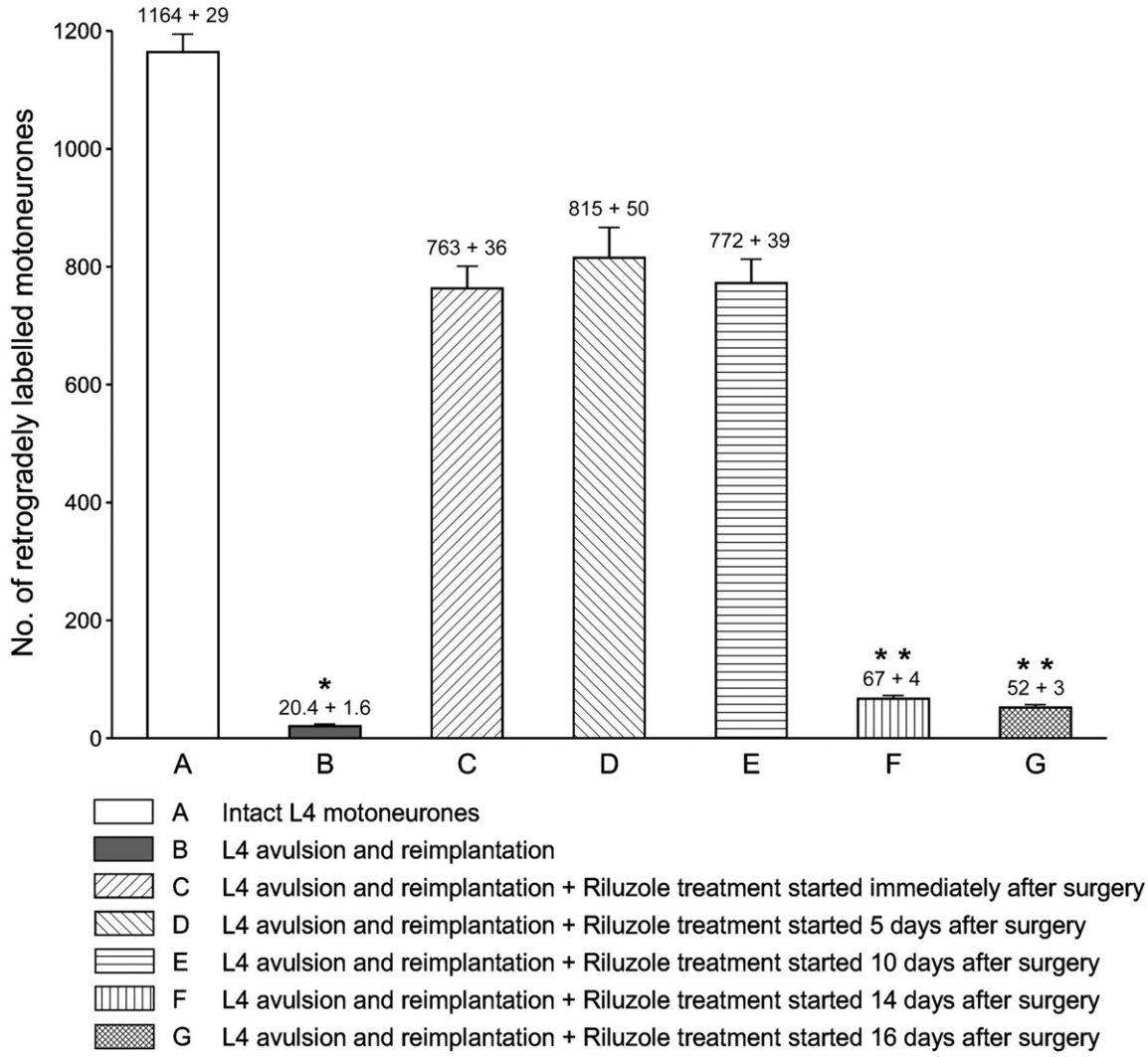

Fig. 1. Bar chart shows the number of retrogradely labeled neurons in various experiments. Note that 65% of the motoneurons found in the intact L4 motoneuron pool (A) are rescued by riluzole treatment following L4 ventral root avulsion and reimplantation (C) when compared with survival of injured motoneurons without treatment (B). Similar survival was found when riluzole treatment started 5 or 10 days after avulsion injury (D and E), but the survival dramatically decreased when riluzole treatment started 14 or 16 days after L4 ventral root avulsion (F and G). * Significant difference between B and A, C, D, E (Mann-Whitney *U* test, $P=0.029$). ** Significant difference between B and F, G and between F, G and C, D, E (Mann-Whitney *U* test, $P=0.016$).

motoneuron pools but only few of these were retrogradely labeled, i.e. the proportion of ChAT positive neurons on the operated side was significantly higher than that of FB-labeled motoneurons [14 days' delay: FB^+ vs. $ChAT^+=5.76\pm0.34$ vs. 41.4 ± 2.1 (%±S.E.M.); 16 days' delay: FB^+ vs. $ChAT^+=4.5\pm0.26$ vs. 38.3 ± 2.5 (S.E.M.); Fig. 2I–J, Fig. 4]. However, the $ChAT^+$ motoneurons on the operated side appeared degenerated with less-developed dendritic tree and displayed weaker ChAT immunoreactivity than $ChAT^+$ motoneurons on the intact side (Fig. 2L–M).

DISCUSSION

The present results confirm and expand our earlier experimental findings (Nógrádi and Vrbová, 2001) that injured adult motoneurons destined to die due to avulsion of their axons in the ventral root can be rescued by treatment with riluzole. The rescued motoneurons not only survive but a considerable number of these cells extend their axons into the reimplanted ventral root. These axons regenerate, reach their target muscles and are able to improve the recovery of the denervated muscles and the locomotor performance of the denervated limb (Nógrádi and Vrbová, 2001). In addition to confirming these earlier findings the present study shows that treatment with riluzole can be delayed for up to 10 days and still be effective in rescuing almost the same number of reinnervating motoneurons as when the treatment started immediately after the operation. Only when treatment started 14–16 days after the operation did riluzole no longer induce the repopulation of endoneuronal sheaths with axons of regenerating motoneurons. It was found that considerably more muscle weight was spared in these latter two groups than in

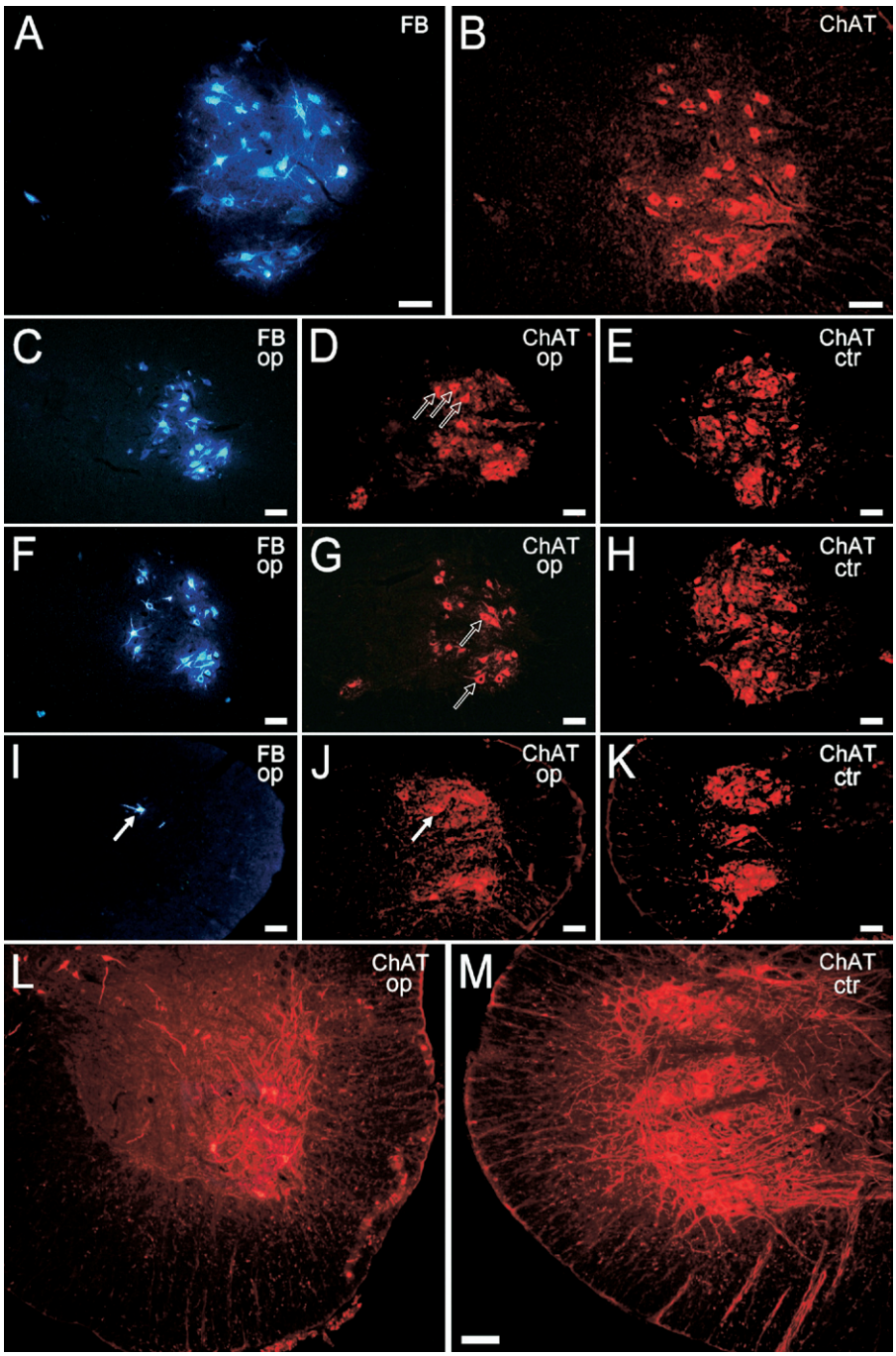

Fig. 2. Transverse sections of spinal cord taken from (A and B): intact L4 spinal cord segment with retrogradely labeled motoneurons and ChAT immunoreactive neurons, respectively. (C–E) Spinal cord with ventral root avulsion and reimplantation followed by riluzole treatment 5 days after surgery (empty arrows in D point to $ChAT^{+}/FB^{-}$ cells). (F–H) Spinal cord with ventral root avulsion and reimplantation followed by riluzole treatment 10 days after surgery (empty arrows in G point to $ChAT^{+}/FB^{-}$ cells). (I–K) Spinal cord with ventral root avulsion and reimplantation followed by riluzole treatment 16 days after surgery. Surviving and reinnervating motoneurons were retrogradely labeled with FB and the same sections processed for ChAT immunohistochemistry. Note the low number of retrogradely labeled cells in I and the somewhat higher number of surviving cells of the same field in J (arrow points to the same cell). (L, M) These figures show that the surviving ChAT positive cells (riluzole treatment was delayed for 16 days after reimplantation) appear degenerated with shrunken dendritic trees on the operated side (L) as compared with the motoneurons on the intact side of the same section (M). In E, H and K ChAT immunoreactive neurons on the control side are shown to demonstrate the difference between operated and intact sides of the same section. Scale bar=100 μm.

untreated animals. Although in these delayed treatment groups the reinnervating motoneuron numbers were very low, they were still 2.5- and 3-times higher than in the untreated group. This suggests that this relatively low motoneuron number was able to prevent the complete atrophy of the affected muscles.

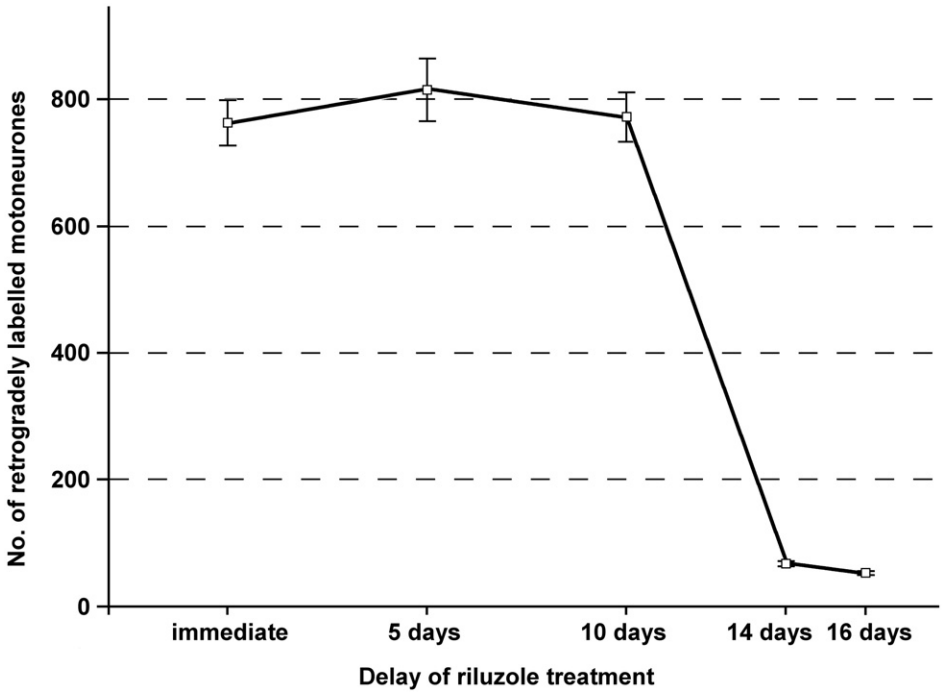

Fig. 3. The graph shows the number of retrogradely labeled motoneurons following various delay in treatment with riluzole. Note the steep decrease in the number of retrogradely labeled cells when treatment was delayed for more than 10 days.

It is possible that the failure of riluzole to induce repopulation of the reimplanted ventral root by regenerating axons after delayed treatment may be related to cell death, which has been reported to occur about 14 days after the avulsion of the ventral root (Koliatsos et al., 1994; Piehl et al., 1998; Hammarberg et al., 2000). However, in the above experiments the time course of cell death was studied after avulsion of the ventral root, but not after its avulsion and reimplantation. The present results indicate that after avulsion and reimplantation of the ventral root cholinergic neurons, that have features characteristic of motoneurons are present in the spinal cord even 3 months after the operation, but after delayed treatment with riluzole they were no longer able to extend axons into the L4 ventral root. This is in agreement with the results of Gu et al. (2004) where many surviving motoneurons were located in the cervical spinal cord after avulsion and reimplantation of the ventral root, but only some of these were able to extend their axons into the reimplanted ventral root. Recently Hoang et al. (2006) have clearly shown that only 53% of surviving preganglionic parasympathetic neurons and 64% of surviving motoneurons in the L6 spinal segment reinnervate the avulsed and reimplanted L6 ventral root.

The present finding shows a very abrupt decline of the ability of the motoneurons to extend their axons into the ventral root after treatment with riluzole. Riluzole is a potent neuroprotective drug widely used in experimental ischemic and traumatic conditions to improve functional recovery following such insults to the CNS (Lang-Lazdunski et al., 1999; Schwartz and Fehlings, 2001, 2002). Its protective action might be due to the fact that riluzole blocks Na^+ and Ca^{2+} channels and these actions reduce the excitability of the injured neurons. It is therefore possible that treatment with riluzole not only rescues the motoneurons from cell death but maintains these cells in a condition that enables them to regenerate their axons given the right condition. Apparently, after a delay of 14–16 days the number of reinnervating motoneurons dramatically drops while still numerous ChAT immunoreactive motoneurons are present in the ventral horn. Accordingly, there is a clear division between survival and reinnervation, i.e. not all surviving ($ChAT^+$) neurons are able to send their axons into the vacated endoneural sheaths.

This sudden demise of the ability of riluzole to maintain the cells in a state where they not only survive but also extend their axons into a viable conduit of the reimplanted ventral root could be explained by additional factors related to the fact, that a) a peripheral nerve conduit deteriorates with time after axotomy and is less able to support regeneration of axons after delayed reinnervation (Sulaiman and Gordon, 2000) and b) the motoneurons' ability to grow axons also declines with time. Thus the ineffectiveness of the delayed treatment is likely to be a combination of all these factors. The present finding that surviving cholinergic cells are found in the ventral horn even 3 months after injury suggests that given the appropriate stimulus such as having access to a fresh, recently axotomized nerve conduit may induce these dormant motoneurons to regenerate. It is possible that riluzole treatment combined with a fresh conduit may then be effective for even longer periods of time. Future experiments should be able to elucidate the optimal conditions for the survival and function of damaged neurons.

The present results that treatment with riluzole is able to induce damaged motoneurons to survive and regener-

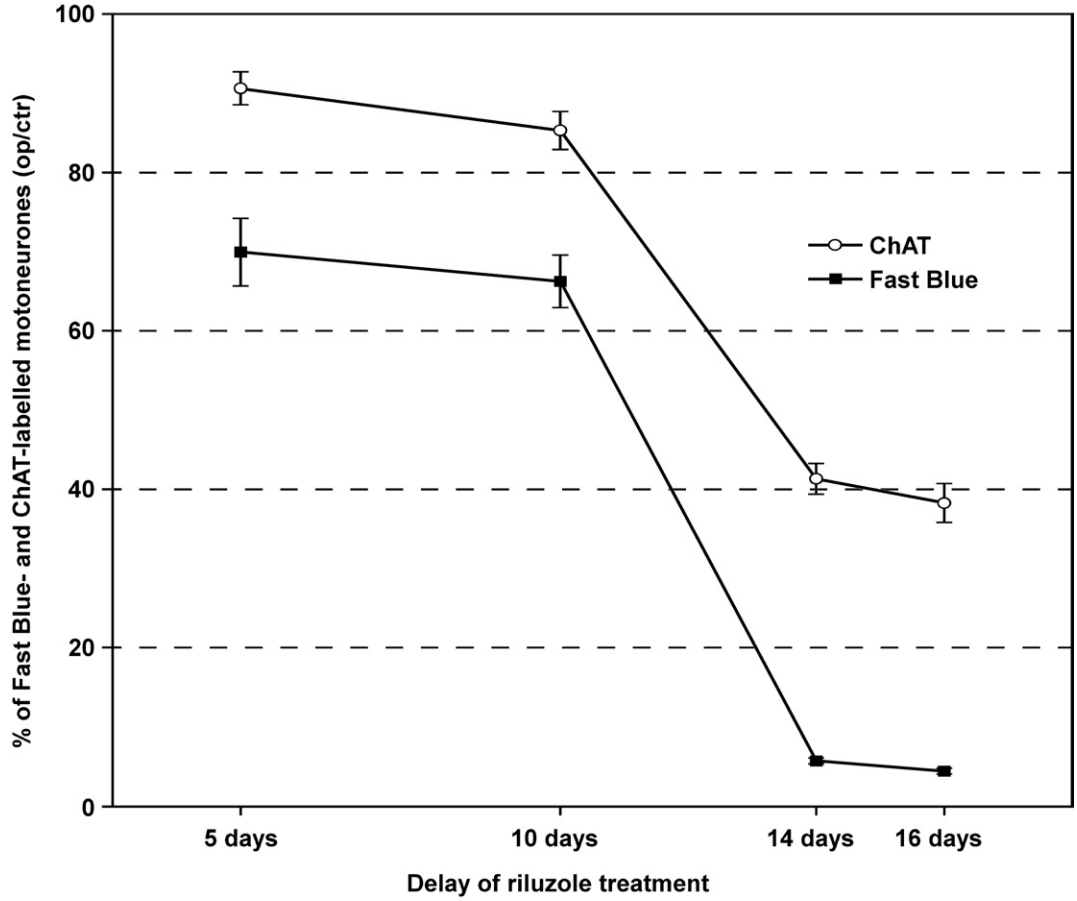

Fig. 4. The percentage of ChAT immunoreactive and retrogradely labeled motoneurons is shown (% of operated/control side for ChAT and % of operated side/intact pool data for FB-labeled cells). The higher number of ChAT positive motoneurons shows that somewhat more motoneurons survive after 5 and 10 days delay of riluzole treatment. In case of delayed treatment for 14 and 16 days many ChAT positive cells are present in the ventral horn, but they are unable to regenerate their axons into the reimplanted ventral root.

ate their axons up to 10 days after injury, are promising and with refinements of the technique they inspire hope that it may be possible to restore function of various peripheral nerve injuries close to the cell body even after long delays of surgical intervention.

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Könyök flexió helyreállítása musculus gracilis microvasculáris funkcionális izomlebeny átültetésével C5C6 (C7) szülési plexus brachialis sérülés után 16 évvel.

Dr Pintér Sándor, Dr Janka Csaba , Dr Kószó Balázs, Dr Czifra Attila , Dr Simonka János Aurél

Esetismertetés

Összefoglalás:

A C5 C6 felső plexus brachialis sérülés esetén a megmaradt jó kézfunkció csak akkor érvényesül ha a váll és a könyök stabilizáló, pozicionáló képessége helyreállítható vagy kialakítható. Felső truncus sérülés után a korai szakban a könyök flexió helyreállítására idegrekonstrukció vagy neurotizáció végezhető. Később vállízületi arthrodesis, ináthelyezés vagy funkcionális izomlebeny átültetés adhat eredményt. A szerzők egy C5 C6 (C7) szülési plexus brachialis sérülés után 16 évvel végzett műtét eredményeit mutatják be: musculus gracilis funkcionális izomlebeny transzplantációt végeztek , az izmot a nervus spinal accessorius felhasználásával neurotizálták. 10 -110 fokos könyökízületi mozgást értek el.

Kulcsszavak: plexus brachialis sérülés, funkcionális szabad izom lebeny, nervus spinal accessorius

Esetismertetés:

Anamnesis:

K.S. 16 évvel korábban bal oldali szülési plexus brachialis sérülést szenvedett. A 40. gestatios hétre 2900 gr súllyal született a köldökzsinór a nyakára volt tekerdve. Születéskor észlelték bal oldali felső tipusú (C5 C6 és részleges C7) sérülését. Korai idegreconstrukciós műtét nem történt. 16 éves korában teljes értékű kéz mellett a vállízület elülső instabilitása és a könyökflexió hiánya volt észlelhető.

Státusz:

A bal vállöv megrövidült. A musculus trapeziusban az izomerő M5. A musculus supraspinatus, a musculus infraspinatus és a musculus deltoideus atrophiás. A humerus fej subluxált. A glenohumerális ízületben és a könyökízületben is teljes passzív mozgástartomány van. Az alkar és a kéz funkciója teljes.

Terápia:

Az ellenoldali combról vett ér- és idegnyeles musculus gracilissal funkcionális szabad izom transzplantációt végzünk. Az érnyél kb. 4 cm-es volt, az ideg 5 cm-es. Az izom eltávolítása előtt az izom inas szakaszaiba öltött hosszjelző fonalat helyeztünk be. A felkaron a musculus biceps felett subcutan tunnelt készítettünk. Az áthúzott gracilis izmot a könyök felett a biceps izom distalis inába fűztük. Proximalisan a kb. 1,5 mm-es átmérőjű artériát az ennél vastagabb arteria thoracoacromiálisra kötöttük side – to – end anasztomózissal A vénát a vena cephalikával anasztomizáltuk. A proximalis izomszakaszt a kulcscsont distalis szakasza alatt áthúztuk. A hosszjelző fonal segítségével 100 fokos könyökflexiós helyzetben tensio alatt önmagához öltöttük. A jelzőfonalat eltávolítottuk. Az ideget a nervus spinal accessorius infraclavicularis szakaszára varrtuk. Az áthelyezett izom szelektív ingeráram kezelését végeztük.

Eredemény:

Műtét után 4 hónappal a könyökízületben 10 - 110 fokos aktív mozgás van. Izomerő M4. A váll stabilistása növekedett. Az önálló életvitelhez szükséges tevékenység pl. öltözködés , tisztálkodás lehetségessé vált. A felsővégtag funkciójának javulásával a fiatalember pályaválasztási lehetőségei is bővültek

1. árba 16 éves bal oldali szülési plexus brachialis sérült. Bal oldalon aktív könyökflexió nincs.

2.,3.ábra Funkcionális eredmény a musculus gracilis izomlebeny transzplantáció után 4 hónappal: A bal könyökízületben 10 – 110 fokos aktív mozgás.

Megbeszélés:

Szülési plexus brachialis sérülés létrejöhet az élveszületések 1-2 ezrelékében. Oka rendszerint téraránytalanság elhúzódó vajúdás, vállkifejtési nehézség.(5) Ritkán koraszülés és császármetszés során is létrejöhet. (1) A C5-Th1 karidegfonat sérülés általában négy csoportba sorolható: vongálódás, hegesedés, truncalis szakadás, avulsió. A klinikai képet súlyosbítja ha a köldökzsinór a nyakra vagy a vállra tekeredik. Az esetek döntő többségében elegendő a korai komplex fizioterápiás kezelés (8), kis hányadában – kb. az esetek 15 % - ban válhat szükségessé sebészi beavatkozás. Korai idegrekonstrukciós műtétet végzünk 3 – 6 hónapos korban, ami lehet neurolysis, idegvarrat, graft, vagy funkció helyreállítást ez

rendszerint neurotizáció: pl. n. spinal accessorius – n. suprascapularis, nn. intercostalis- n. musculocutaneus . (2)
Későbbi életkorban , 6 – 14 éves kor között , vállövi és felsővégtagi funkció javító beavatkozás válhat szükségessé. Ilyen műtétet végezhetünk spontán idegregeneráció után kialakult állapot , rendszerint contractura miatt és a korábban idegrekonstrukción átesett betegeknél is. A beavatkozás lehet : ínhosszabbítás, ínáthelyezés, szabad izom transzplantáció. (2) Ezekben a műtétekben közös probléma, hogy a nagyon korai időszak izomtónus zavarából eredően csont és ízületi deformitás is kialakul.
A musculus gracilis ér-idegnyeles mikrovascularis átültetésével 120 fokos flexió is elérhető. (4) Ez az izom egyebek mellett kiválóan alkalmazható kiesett biceps izom funkció pótlására (3), elegendő amplitudóval rendelkezik a könyökflexió helyreállításához. Legtöbb esetben az éranastomosist az arteria thoracoacromialisra és a vena cephalikára készítjük az ideget a nervus spinal accessorius infraclaviculais szakaszára varrjuk. Az izom az új helyén gyorsan adaptálódik. Jó funkció az izom megfelelő hosszának és feszülésének beállításával érhető el. (6)

Következtetés:

C5 C6 szülési plexus brachialis sérülés után az értékes kéz képessége korlátozódik a vállövi és könyökízületi instabilitás és aktív mozgás kiesése miatt. Ezért a korai időszakban – lehetőleg 6 hónapon belül - , idegrekonstrukciót végzünk.
Akkor is van lehetőség a funkció helyreállítására ha bármilyen okból a korai rekonstrukció nem történt meg. A vállízület dinamikus vagy statikus stabilizálása mellett pectoralis major plasztika, uni- vagy bipolaris latissimus dorsi plasztika, triceps to biceps plasztika vagy Steindler flexorplasztika révén érhető el könyökflexió.
Azonban a plexus brachialis sebészetben és a microvascularis szövetátültetésben való jártasság esetén, a musculus gracilis funkcionális izomlebeny transzplantáció gyorsabban vezethet eredményre.

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