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The value of an operating microscope in peripheral nerve repair

An experimental study using a rat model of tibial nerve grafting

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Summary. *The aim of this study was to test the hypothesis that the use of an operating microscope improves the results of peripheral nerve repair. Tibial nerve grafting was carried out on 48 Fischer rats divided into 2 groups: in one, a loupe was used, and in the other a surgical microscope. At 5 months after grafting, recovery was evaluated by functional, electromyographic, and morphometric tests. The mean motor nerve conduction velocity was 26.77 ± 9.37 m/sec in the group where the loupe was used compared with 44.19 ± 11.36 m/s when the microscope group was used. The soleus muscle weight and the diameter of myelinated fibres also confirmed better regeneration in the microscope group. These results clearly indicate that it is essential to use the microscope for peripheral nerve repair.*

Résumé. *Le but de l'étude est de faire tester l'hypothèse que l'aide du microscope améliore les résultats de la chirurgie du nerf périphérique. La greffe du nerf tibial a été pratiquée sur 48 rats Fischer, repartis en deux groupes, l'un avec l'aide de la loupe et l'autre avec l'aide du microscope. Cinq mois après la greffe les résultats ont été soumis à une évaluation fonctionnelle, électromyographique et morphométrique. Les moyennes de la vitesse de la transmission motrice du nerf dans le groupe assisté par la loupe et celui assisté par le microscope étaient 26.77 ± 9.37 m/s et 44.19 ± 11.36 m/s respectivement. L'équivalent du test clinique, le poids du muscle soleus ainsi que les mésures du diamètre des fibres myelinisées ont aussi démontré une meilleure régénération, dans le groupe assisté par microscope. Ces resultats montrent clairement que l'aide du microscope est essentielle pour la réfection du nerf périphérique.*

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Introduction

There is controversy about the value of using an operating microscope for peripheral nerve repair. Some authors have not reported any significant improvement in their results when using a microscope compared with the use of loupes, or even without magnification [3, 5]. However, others have no doubt about the value of the microscope, but have not produced evidence to support their opinion [4, 8].

The recovery of peripheral nerve injuries sustained in war and treated with microneurosurgical techniques have shown significant improvement compared to the results reported in World War two and the Vietnam war [7]. Although we have presumed that this improvement was due to the use of the microscope this could not be confirmed by comparing other series with our own.

We have tested the hypothesis that the use of a microscope improves the results of peripheral nerve repair by using a model of tibial nerve grafting in rats.

Materials and methods

Animal model and the surgical procedure

Forty-eight male Fischer rats weighing 320 g to 370 g provided by the Medical School Breeding Laboratory, Rijeka, Croatia were divided into 2 groups (n = 24): in one the loupe and, in the other, the microscope was used. Subsequently each group was divided into two subgroups (n = 12) according to the magnification used (2.5×, 5× with loupe, and 6× and 40× with the microscope). The animals were housed in cages with flat floors and allowed as much rat chow and water as they needed.

All procedures were performed under anaesthesia with Ketamine 50 mg/kg IP (Kela, Hoogstraten, Belgium) and Valium 5 mg/kg IP, (T. Hoffmann-La Roche Ltd, Basel, Switzerland). The right tibial nerve was exposed; a segment 1 cm long was removed and then used again to bridge the gap. In the first group, indirect suture was performed with the use of the loupe magnification, in subgroup 1A with Keeler Galilean loupes 2.5×/420 mm, and in subgroup IB with a Keeler panoramic telescope 5×/500 mm. In the second group, a Leica M 651 microscope was used with magnification of 16× in subgroup 2A, and 40× in subgroup 2B. Coaptation was maintained with Nylon 10-0 epineural sutures (Deknatel, Queens Village, New York, USA).

Functional and electromyographic assessment of nerve regeneration

Recovery was assessed at 5 months after grafting by two researchers working independently. Scoring from 0 to 3 was used, according to Millesi [8]; a test was carried out with the animals climbing on the Rivlin and Tator plate, inclined at the angle of 62° [6], to correspond to a clinical test.

Electromyographic recordings across the nerve graft were made using a computer-assisted machine (TD-50, Medelec, Surrey, UK). During anaesthesia, the sciatic and tibial nerves and the gastrocnemius muscle were exposed in both legs. The earth was placed at the animal's tail and two sets of recordings taken. In the first set at the grafted side, a right angle bipolar stimulating electrode (Nicolet Biomedical, Madison, Wisconsin, USA) was placed on the sciatic nerve above the operated segment (point A) and a concentric needle recording electrode (Medelec, Surrey, UK) in the proximal part of the medial head of the gastrocnemius muscle. In the second set of recordings the stimulating electrode was moved to the tibial nerve below the graft (point B). The distance between point A and point B was measured, and ranged between 2 cm and 2.5 cm. Motor nerve conduction velocity was calculated as a quotient of the AB distance, and the difference in distal latencies of the compound muscle action potential were evoked at points A and B. The unoperated left leg served as control.

Muscle weight and morphometric nerve studies

Following electromyographic recordings, both tibial nerves and the soleus muscles were removed. The weights of the soleus muscle on the grafted side were expressed as a percentage of the normal side.

The tibial nerves were fixed in 3% glutaraldehyde solution and post-fixed in osmium tetroxide. The segment 5 mm distal to the second suture line was stained with toluidine blue, and cut in 1 μm thick sections for light microscopy. At 1000× magnification, 6 representative fields per nerve were chosen by an observer who was not aware of the situation and evaluated with a digital image-analysis system linked to morphometric software (VAMS, Zagreb, Croatia). The following indices were calculated: myelinated fibre density (total number of myelinated fibres per square mm), myelinated fibre diameter, myelinated fibre percentage area (percentage of neural tissue).

Statistical analysis

The distribution frequency data of motor nerve conduction velocity, motor nerve conduction velocity percentage, the clinical test equivalent, soleus muscle weight, myelinated fibre density, myelinated fibre diameter, and myelinated fibre percentage area, were first determined. Subsequently, statistical analyses of the differences in group means was calculated by the one-way analysis of variance test (1W-ANOVA) and the independent sample t-test. The significance level was set at $P < 0.01$.

Results

A well synchronised compound muscle action potential wave of biphasic or triphasic shape was obtained in each rat, with a mean distal motor latency of 1.78 ± 0.70 ms and a mean duration of 2.42 ± 1.08 ms. Motor nerve conduction velocity in the group operated on with loupe magnification compared to the group with the microscope were means of 26.77 ± 9.37 m/s and 44.19 ± 11.36 m/s, respectively (Table 1). The corresponding motor nerve conduction velocity percentage of the normal side was 39.37 ± 16.20 and 63.98 ± 24.18, respectively. The mean equivalent clinical test and soleus muscle weight percentages were significantly higher in the microscope group. The mean myelinated fibre diameter and myelinated fibre percentage area were also significantly higher in the microscope group, as opposed to the mean myelinated fibre density.

The results of physiological, electromyographic and morphometric assessment have not shown significant differences in the subgroups in both the loupe and microscope groups.

Discussion

Nerve grafting is the most commonly used way of repairing nerve injuries in both civil and wartime practice [7]. The criteria for a succesful primary neurorrhaphy are a clean transection, minimal crushing and stretching of the nerve, a satisfactory general condition of the patient and an expert microsurgeon with a fully equipped operating room. These conditions are, however, seldom met.

Table 1. Electromyographic, functional, and morphometric assessment of the loupe magnification vs microscope-assisted tibial nerve graft

Group	1			2		
Subgroup	1A		1B	2A		2B
Magnification	2.5×	Loupe	5×	16×	Microscope	40×
MNCV (m/s)	26.94 ± 9.75 (NS)	26.77 ± 9.37	26.61 ± 9.40	43.12 ± 13.30	44.19 ± 11.36*	45.26 ± 9.51 (NS)
MNCV %	32.53 ± 11.18	39.37 ± 16.20	46.21 ± 17.94 (NS)	62.55 ± 24.09	63.98 ± 24.18*	65.42 ± 25.26 (NS)
CTE (score 0–3)	1.92 ± 0.67	2.00 ± 0.66	2.08 ± 0.67 (NS)	2.75 ± 0.62	2.83 ± 0.48*	2.91 ± 0.29 (NS)
SMW %	84.65 ± 0.02 (NS)	84.05 ± 0.03	83.46 ± 0.03	98.61 ± 0.02 (NS)	97.98 ± 0.02*	97.35 ± 0.02
MF-Density (No. MF/mm²)	13 178 ± 1443	13 644 ± 1446*	14 108 ± 1348 (NS)	9813 ± 998	10 195 ± 1184	10 577 ± 1009 (NS)
MF-Diameter (μm)	3.42 ± 0.41	3.68 ± 0.61	3.93 ± 0.66 (NS)	5.88 ± 0.95 (NS)	5.84 ± 0.92*	5.79 ± 0.93
MF%-Area	12.34 ± 3.40	15.01 ± 5.44	17.67 ± 5.91 (NS)	26.78 ± 7.97	27.65 ± 8.95*	28.52 ± 10.11 (NS)

MNCV-motor nerve conduction velocity; MNCV%-expressed as a percentage of normal side; CTE-clinical test equivalent; SMW%-soleus muscle weight expressed as a percentage of normal side; MF-myelinated fiber; *$p < 0.01$; NS-non significant

Two papers denying the value of using an operating microscope dealt with primary epineural repair, and therefore relate to a minority of the patients in need of repair of peripheral nerve injuries [3, 5].

The widespread opinion that peripheral nerve repair was unsuccessful was influenced by the results of the largest published war series [4]. There has been an improvement in the results since the operating microscope was introduced for the treatment of nerve injuries in war [4, 7]. Nevertheless, the value of the microscope has not been established since it is not possible to compare past [4] with present results.

Our experiment was designed to compare the effect of different optical magnifications on peripheral nerve regeneration. The model of the rat's tibial nerve graft was used to simulate clinical interfascicular grafting. Five months after operation, the shape and length of the compound muscle action potential, and the motor distal latency confirmed that the plateau of regeneration had been reached. Thereafter, 5 of the 6 parameters which were examined showed superiority of the grafting technique when the microscope was used.

Motor nerve conduction velocity is related to myelin thickness and therefore to the diameter of the myelinated fibres, and consequently reflects maturation and the accuracy of the apposition between the proximal and distal nerve fibres [1]. The equivalent clinical test assessed complex motor-unit reinnervation co-ordinated by cortically integrated sensory feedback. Hence it is a valuable index of the quality of regeneration [1, 2], although some authors suggest that muscle weight is the most precise indicator [2]. The percentage area of myelinated fibre is significantly higher in the microscope group, in spite of the fact that the diameter of myelinated fibres and their density are inversely correlated. Myelinated fibre density is inversely correlated with 5 of the parameters and therefore with functional recovery [1]. Models with multiple parameters are recommended by some investigators [2], but the results of our study suggest that electromyography is an adequate index of recovery.

Neurotropism is important in nerve regeneration, but it is does not create order out of chaos at the suture line [9]. Greater optic magnification and the satisfactory illumination provided by the operating microscope enable more precise coaptation and better mechanical alignment of the ends of the graft.

Some of problems of peripheral nerve surgery, such as insufficient amount of graft and slow regeneration of axons, remain unsolved, but our study confirms the opinion of those who advocate the use of the microscope. Major advances in peripheral nerve surgery have occurred in the last 25 years, mainly due to the introduction of magnification which allowed aggressive early exploration, atraumatic intraneural invasion and popularisation of techniques which optimally align the corresponding fascicles of severed nerves [8].

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