JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2013. Vol. 7(Spl. Edn.), p. 375-380

Histological Study of Peripheral Nerve Regeneration Repaired in different Size by Artery Sleeve Briding

Yan Liu [1], Haoyu Liu [1],Xueman Luy [2], Zhan Zhang [1] and Biao Liu[1]*

[1]Department of Hand Surgery, [2]Department of Ophthalmology,
China-Japan United Hospital of Jilin University, Jilin Province, China.

(Received: 03 March 2013; accepted: 14 April 2013)

The mechanism of peripheral nerve regeneration is complex, its mechanism of regeneration after repaired in different size is indefinite. The experiment was done to explore the mechanism and reserve of nerve function of the peripheral nerve regeneration when the distal nerve stump is repaired by a smaller proximal end with artery sleeve briding, and to investigate the best situation which benefits for the reserve of nerve function. 10 Wistar rats served as the donors of artery for briding. 80 Wistar rats were randomized into the 4 groups (n=20 per group). The group A was the control group. In group B, the right sciatic nerves were cut off at the level of 1.5cm distal to infrapiriform foramen, and received epineurium suture. In group C and D, the right sciatic nerves were cut off at the same level and 1cm segment of the common fibular nerves were removed from the proximal ends, the remaining were repaired with epineurium suture in group C, and with 3mm gap artery sleeve bridging in group D. Observation under operation microscope of the nerve repaired site, different types of stainings, including HE staining, toluidine blue staining,Marsland-Luxolfastblue staining, and TEM examinations were used at the time of 16w after operation to observe the morphological features of regenerated nerve. The results shows that there do have amplification effect in the group repaired in different size with artery sleeve briding with obviously larger number of the regenerated axons in the distal segment than the proximal, And the regenerated axons are mature more regular, although they are smaller in diameter compared with other groups. The functional reserve of the nerve regeneration exists after repaired when the proximal end is smaller than the distal in size. And the method of small gap bridging provides a better situation for the nerve amplification.

Key words: Sciatic nerve, artery sleeve briding, functional reserve, histology.

Backgroud

Although peripheral nerve repair skills improve, repair method innovation, but clinically, recovery effect after peripheral nerve repair is still not satisfactory(Höke *et al*.,2006).Especially for brachial plexus root avulsion, nerve transposition is difficult because of the limited number of[1] donor nerves, and the dynamic volume of donor nerve fibers are also less. The proximal donor nerves are often smaller and less than the distal receptors after nerve anastomosis. In this situation, what happens during nerve regeneration and whether the less proximal nerve fibers can restore the distal nerve function are indefinite yet.

Based on the above issues, this experimental study was carried out to explore the mechanism and reserve of nerve function of the peripheral nerve regeneration when the distal nerve stump is repaired by a smaller proximal end with artery sleeve briding,, and to investigate the best situation which benefits for the reserve of nerve function.

* To whom all correspondence should be addressed.
E-mail: liubiao0998@sina.com. Tel: 13578869800

MATERALS AND METHODS

Grouping for experimental animals

We used a total of 90 adult, maleWistar rats (body weight 200-250g, aging 2 months).10 Wistar rats served as the donors of arteris for briding. 80 Wistar rats were randomized into the 4 groups (n=20 per group).

For all surgical procedures, general anesthesia was achieved by1% 40 mg/kg intraperitoneal injection of ketamine. Surgeries were carried out under aseptic conditions. Aorta were cut served as sheathed artery from the 10 donor rats and put in physiological saline. For the other 80 rats, the right sciatic nerve was exposed through a longitudinal incision made in the lateral aspect of the thigh.

The group A was the control groupÿwith only surgicalexposure of the right sciatic nerve. In group B, the right sciatic nerves were cut at the level of 1.5cm distal to infrapiriform foramen using sharp microsurgery scissors, and then repaired using the epineural suturing technique with a single 10-0 nylon suture. In group C and D, the right sciatic nerves were cut at the same level and 1cm segment of the common fibular nerves was removed from each proximal end. The proximal ends remaining of group C were repaired with the distal stumps using a single 9-0 nylon suture by epineural suturing technique. Each remaining proximal end and distal stump of group D was then drawn into either end of an 8 mm long sheathed artery and left a 3mm gap betweenthe two ends. Two 10-0 nylon suture were applied at each side of the anastomosis

In all groups at 16 weeks after the operation, animals were sacrificed and examined for further testing.

Muscle mass

Gastrocnemius muscle harvests were performed through a longitudinal incision on the posterior aspect of the leg. The overlying skin was dissected free of the gastrocnemius muscle. Using 6–10×magnification, the gastrocnemius was isolated and freed along the muscular tissue planes from insertion to origin. It was removed en block and immediately weighed. The contralateral, uninjured muscle was also harvested to control for variation in size between individual rats. And calculation of the triceps Cuadros index (TSCI) was taken (TSCI= triceps surae muscle weight / body weight of an animal x 100). Then the muscles were fixed in 10% neutral buffered formalin, embedded in paraffin, sections, carried with HE staining. Observation was taken under light microscope.

Walking tracks

Walking tracks were conducted as previously described (Bain *et al*., 1989; Brown *et al*., 1989). Briefly, the walking track consists of an 8.2×42cm track darkened at one end with a length of exposed X-ray film placed on the bottom of the track. The hind feet of the rat were coated with X-ray film developer and then the rat was allowed to walk down the track. The hind footprints appeared as the developer reacted with the film. Measurements of footprints from walking tracks were used to calculate a tibial function index as described previously (Bain *et al*., 1989; deMedinaceli *et al*., 1982).

Histological evaluation

The regenerated nerve specimens were sampled proximal and distal 5mm to the anastomosis. Nerve cross sections proximal and distal 5mm to the anastomosis were stained with hematoxylin and eosin (HE), toluidine blue, and observed by a light microscopy. The average diameter of axon,myelinated nerve fiber number and thickness of myelin sheath in the proximal and distal 5mm of regenerated nerves was estimated using an image analysis program (i-solution, IMT, Korea) from the captured light microscopy pictures respectively.

Nerve Longitudinal sections of the same segment of regenerated nerve were stained with MarslandNLuxolfastblue(LFB), observation was taken about the density and arrangement of nerve fiber and axons in the distal compared with the proximal of the regenerated nerve under light microscopy.

To observe more detailed axon and myelin sheath regeneration inside the tubes, the specimens were also cut into ultrathin sections with 50-60 nm thickness. The sections were stained with lead citrate and uranyl acetate, and then examined by a transmission electron microscope (TEM; Model H-600, Hitachi, Japan).

We used SPSS13.0 statistical analysis software for statistical analysis, using multiple experimental groups mean Q ‘ inspection, set the level of 95%, $P<0.05$ had statistical significance.

RESULTS

Muscle mass

All triceps surae muscles of the experimental rats have atrophied of varying degrees. Muscles in group B and D were full, resilient, and in pink colors, while those in group C were atrophy obviously, thin, and pale. The TSCI and triceps surae muscle cross-sectional area was lower in group C than in group B, and D ($P < 0.05$); Those in group B and D showed no significant difference ($P > 0.1$), but were less than group A ($P < 0.05$).(Table 1.Figure 1)

Walking tracks

Not all the experimental rats were involved in the measurements of footprints because the toe body autophagy phenomenon happened in some experimental rats. The results were showed in the Table 2. The SFI was lower in group C than in group B, and D ($P < 0.05$); The SFI in group B and D showed no significant difference ($P > 0.1$), but were less than group A ($P < 0.05$). (Table 2)

Histological evaluation

In each experimental group, the proximal nerves had different extent of retrograde reactions, as follows: a small amount of the proximal axons, myelin disintegration, and absorption. In group B, the regenerating nerve arrangement was not regular, especially at the anastomotic suture site. The regenerated nerve fibers were more mature, most of which were larger diameter nerve fibers. The diameter of the nerve trunk distal and proximal to the anastomosis was similar. In group C, most regenerated nerve fibers were small. Some were unmyelinated, and arranged in disorder obviously. The diameter of the nerve trunk distal to the anastomosis was smaller than that of the distal nerve trunk of group B because the less amount of regenerated nerve fibers. In group D, the distal regenerating nerve fibers arranged relatively regular, uniform, and with matured axonal myelination, but the diameter of the regenerating nerve fibers was smaller compared with that in group B. The nerve trunk distal to the anastomosis increased in quantity and diameter significantly compared with the proximal trunk, but similar to the distal trunk of group B. (Table 3.Figure 2, 3, 4)

In the sections of nerves after LFB staining, nerve fibers were black, and axons were

Table1. Triceps surae test parameter values ($\overline{X}$ ±s)

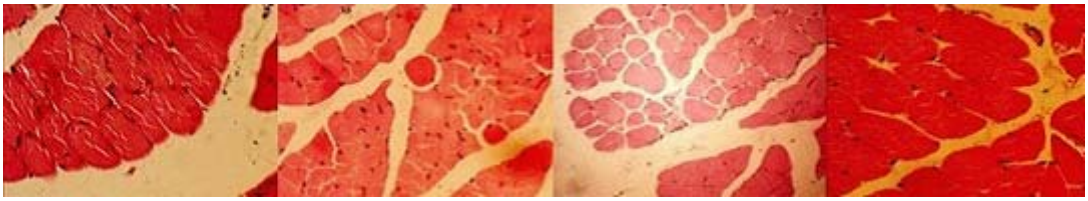
Group	TSCIMuscle fiber cross-sectional area (μm²)	
A	0.6353±0.061559	5.36±26.51
B	0.5123±0.0495(1)	517.92±23.17(1)
C	0.3181±0.0264(1)(2)	412.25±20.86(1)(2)
D	0.5094±0.0359(1)(2)(4)	511.33±25.75(1)(3)(4)

1) Group B,C,D compared with A, P<0.05
2) Group C compared with B, P<0.05
3) Group D compared with B, P<0.1
4) Group D compared with C, P<0.05

Table 2. Footprint analysis of sciatic nerve function index

Group	nSFIPLTSIT	
A	10	-12.2±7.529.0±8.38.4±2.17.2±1.5
B	13	-41.3±9.2(1)32.9±7.4(1)7.5±1.9(1)5.3±1.2(1)
C	10	-64.7±5.8(1)(2)37.2±2.0(1)(2)6.3±2.8(1)(2)3.7±0.8(1)(2)
D	14	-53.1±6.4(1)(3)(4)36.1±9.7(1)(3)(4)6.9±3.2(1)(2)(4)4.6±2.9(1)(3)(4)

1) Group B,C,D compared with A, P<0.05
2) Group C compared with B, P<0.05
3) Group D compared with B, P<0.1
4) Group D compared with C, P<0.05



Group A Group B Group C Group D

Fig. 1. Cross sections of gastrocnemius after HE staining (×40)

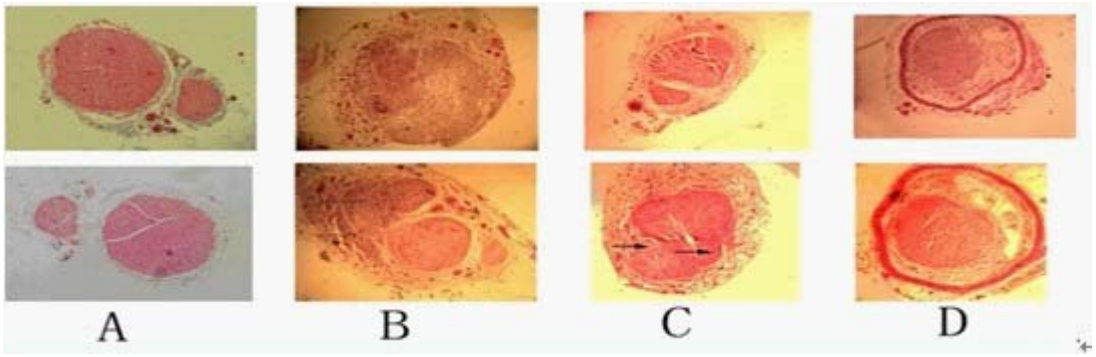


Fig. 2. HE staining of the nerve cross sections distal (figure below) and proximal (figure beyond) to the anastomotic stoma 16 weeks after operation. Outleakage of regenerated nerve fibers were marked with arrows (x40)

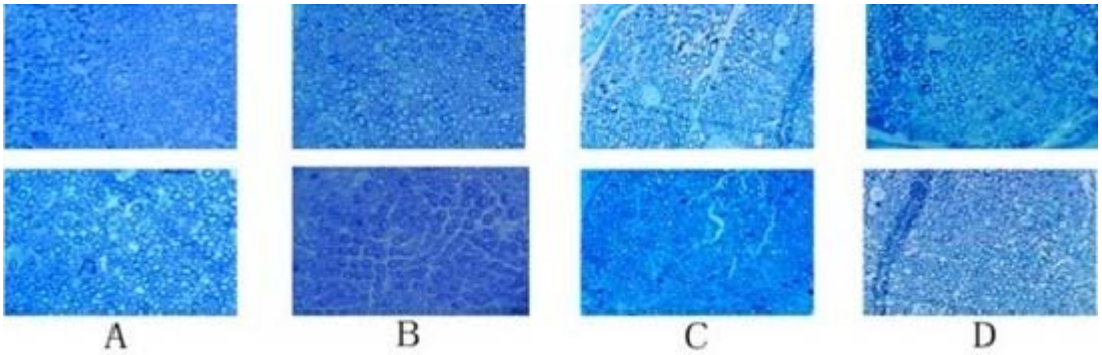
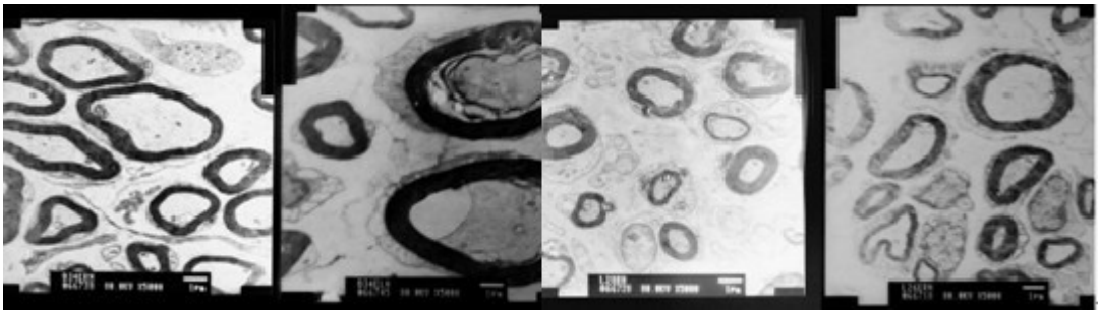


Fig. 3. Toluidine blue staining of the nerve cross sections distal (figure below) and proximal (figure beyond) to the anastomotic stoma 16 weeks after operation (×400)



Group A Group B Group C Group D

Fig. 4. Ultrastructure of the regenerated nerves showed by TEM examinations 16 weeks after operation

Group A Group B Group C Group D

Fig. 5. LFB staining of the nerve longitudinal sections of regenerated nerves 16 weeks after operation (×100)

Table 3. The morphological features of regenerated nerve fibers (±s, n=20)

Group	quantityEquivalent diameter (every visual field)	Density (μm)	Thickness of myelin shealth (/100 μm^2)	(μm)
A	172±26	2.50±0.38	0.09±0.05	6.34±0.49
B	119±17$^{(1)}$2.24±0.27$^{(1)}$0.07±0.04$^{(1)}$	4.83±0.48$^{(1)}$		
C	97±16$^{(1)(2)}$1.83±0.38$^{(1)(2)}$0.04±0.02$^{(1)(2)}$3.37±0.28$^{(1)(2)}$			
D	136±29$^{(1)(3)(4)}$	2.09±0.31$^{(1)(3)(4)}$0.08±0.03$^{(1)(3)(4)}$4.26±0.59$^{(1)(3)(4)}$		

1) Group B,C,D compared with A, P<0.05
2) Group C compared with B, P<0.05
3) Group D compared with B, P>0.1
4) Group D compared with C, P<0.05

blue. The longitudinal sections of regenerated nerve, there was obvious amplification effect at the side of the anastomosis in group D, with greater density and quantity of myelinated nerve fibers, compared with other groups. (Figure 5)

DISCUSSION

Clinically, recovery effect after peripheralnerve repair is still not satisfactory, especially for brachial plexus root avulsion, nerve transposition is difficult because of the limited number of donor nerves, and the dynamic volume of donor nerve fibers are also less. How to use the limited donor nerves is one of the difficulties which need us to solve practically.

As preliminary experiments have shown that a small gap sleeve repair methods benefit for nervetrophic regeneration (Liu *et al*.,2011; Lu *et al*.,2008). Small gap sleeve anastomosis operation method provides for nerve regeneration a " regeneration space "(Ronald *et al*.,2010), Which can reduce the expose and leakage of nerve fibers, and the invading of fibroblast cells into nerve anastomosis and the formation of scar, avoiding the formation of stump neuroma. The certain distance between nerve stumps allowing the proximal nerve fiber play the role of regeneration in tendency and specificity, reducing the functional fascicles wrong ingrowth.

The experiment shows: There is a certain amount of functional reserve of peripheral nerves after repaired like other tissues and organs. Peripheral nerves, after rupture and repair, also exist certain functional reserve, manifested as: the proximal small nerve can significantly increase its nerve fiber numbers by regeneration, and the regenerated nerve fibers are mature and functional. The method of small gap bridging provides a better situation for the nerve amplification

The experiment explored the mechanism of peripheral nerve function reserve when the distal nerve stump is repaired by a smaller proximal end with artery sleeve briding histologically. Small gap sleeve anastomosis operation method provides for nerve regeneration a "regeneration space ", which benefits the regeneration in tendency and specificity. The distal nerve stump provides more basal membrane tubes, inducing each nerve fiber from the proximal stump to regenerate multiple axis buds extending to the distal. The regenerated nerve fibers are mature and regular arranged, although they are mainly in small diameters, and the new regenerated nerve can recover the original nerve function of target organ.

CONCLUSIONS

The functional reserve of the nerve exists after repaired when the proximal end is smaller than the distal in size. And the method of small gap bridging provides a better situation for the nerve amplification.

REFERENCES

1. Laijin Lu, Jianli Cui, XuGong., Experimental study in the function reserve of ulnar nerve in rats. *Chinese Journal of reparative and reconstructive surgery,* 2008; **9**(22):1060-1063.
2. HokeA., Mechanisms of disease: what factors limit the success of peripheral nerve regeneration in humans? *Nature CliniPractNeurol,* 2006; **2**:448-454.
3. Ronald Deumens, AhmetBozkurt, *et al*., Repairing injured peripheral nerves: Bridging the gap. *Progress in Neurobiology*, 2010; **92**(3): 245-276.
4. Yan Liu, Haoyu Liu, Zhuang Wei, Xianyu Zhang, BiaoLiu., Experimental study in the function reserve of peripheralnerve repair in different size by small gap briding. Chinese Journal of laboratory diagnosis, 15: 85-86.ed in different size by small gap briding. *Chinese Journal of laboratory diagnosis,* 2011; **15**: 85-86.