

Research on recovery function of two drugs combination on rat sciatic nerve injury regeneration model

Liang Ju¹, Xia Zhang¹, Tong Zhang² and Jianping Zheng^{3*}

¹Department of Orthopaedics, Yishui Central Hospital, Linyi, Shandong, China

²Department Radiology Jinan Central Hospital Shandong Province, China

³Department of Orthopaedics, Xiangyang Central Hospital Hubei Province, China

Abstract: This paper aims to study the recovery function of two drugs combination on rat sciatic nerve injury regeneration model. Sixty rats were divided into groups randomly and averagely. All animals after dividing left sciatic nerve were given epineurium-interrupted suture for constructing peripheral nerve injury model. Muscle on operation side in medication administration team was injected 0.5 ml drug while the contrast group was given equal amount of normal saline. Sciatic nerve function evaluation and nerve electrophysiology index detection were conducted after operation at fixed period. We drew materials for morphological observation 12 weeks after operation. The results showed that group with independent administration of nerve growth factor (NGF) and nimodipine (ND) in large dose was superior than group in small dose in nerve electro physiology index ($P < 0.05$) and group with combination administration of NGF and ND in large dose was also superior than group in small dose ($P < 0.05$). In addition, regeneration effect of combination administration group was better than that of independent administration group when using same dose. The larger the dose was, the better the effect was. We can conclude that two-drug combination can promote recovery function on rat sciatic nerve injury regeneration model.

Keywords: Combination, nerve regeneration, NGF.

INTRODUCTOIN

Although development of medical technology and improvement of microsurgical technique perform certain promotion function on treatment of nerve injury disease in recent years, there is no satisfactory result of clinical function evaluation. Moreover, nerve injury caused by various factors present a rise trend. Perpheral nerve injury will lead to dysfunction of sensory nerve, such as sciatica induced by protrusion of lumbar intervertebral disc (Yuyang *et al.*, 2011; Xueyong *et al.*, 2012; Jiajia *et al.*, 2013), lower limbs dysfunction of apoplexy hemiparalysis patient (Zhongshu *et al.*, 2013; Jiajia *et al.*, 2013), root pain of nerve root type cervical spondylopathy (Xiaoliang, 2013) etc. That severely affects normal life activity. Therefore, medical field spread research on effect of drug combination on nerve injury recovery. Nimodipine injection has recovery function on perpheral nerve injury regeneration model. Its function mechanism may be realized by inhibition of nerve scar and improvement of blood supply by expansion of blood vessel (Yuyang *et al.*, 2011) and thus we carried out the following research.

MATERIALS AND METHODS

Materials

The experiment needed sixty adult male rats weighing of

200g \pm 10g. The following materials were also needed: microinstrument such as operating microscope, 16 channels physiological recorder, inverted phase contrast microscope, freezing microtome, microscope, suture line, scalpel, normal saline, rubber band, rat board, pentobarbital sodium solution, paraffin embedding machine, paraffin slicing machine, ND injection, photochemical reaction bonding technique (as a innovated research achievement, photochemical reaction bonding technique can realize suture-free bonding between tissues and it was developed by Harvard University in 2005 and obtained patent (Henry *et al.*, 2009; O'Neill *et al.*, 2009; O'Neill *et al.*, 2009).), rat NGF injection, etc.

Methods

Preparation of sciatic nerve injury model

Pentobarbital sodium was added into 0.9% normal saline in concentration of 10mg/ml. Rats were injected pentobarbital sodium in enterocoelia for anesthesia (40mg/kg weight). Rats were fixed on rat board by rubber band. We adopted regular disinfection and draping and cut a longitudinal incision on backside of right thigh. Scalpel was used to cut skin to bluntly separate muscle. Right side sciatic nerve was showed in spatium intermusculare of femoral posterolateral. Horizontal cut was conducted 5 mm away from sciatic nerve. Then we used microscopic no damage suture line to suture epineurium along with blood vessel trending on the surface of epineurium and then close incision by photochemical reaction suture. Rats after operation feed

*Corresponding author: e-mail: zipzxyy@163.com

in standard animal room under same condition. The temperature in quiet and sterile feeding room was maintained in 25°C. Rats in feeding period were given fodder and water regularly. The growth condition of animals was observed everyday.

Grouping

Sixty rats were divided into 4 groups randomly and each group had 15 rats: two groups of combination administration in small and large dose (NGFS+NDS, NGFL+NDL), one group of large dose of NGF combined with small dose of ND (NGFL+NDS), three groups of large dose of ND combined with small dose of NGF (NDL+ NGFS); group of instant medication administration after operation, group of medication administration 24h after operation and group of medication administration 48 h after operation. Operation side muscle of medication administration group was injected 0.5ml drug the next day for one week. The contrast group was given equal amount of normal saline.

Detection of sciatic nerve index

We designed a walkway of 50cm length, 10 cm width and 10cm height. Before function detection, a large white paper in length of 60cm was paved on walkway. We adopted dyeing method to record the effect of two drugs on rat's nerve in order to make the experiment more straight and obvious. Rats walked on the walkway freely. A period of time later, the fin of rats was stained by black dye. We asked the rats to walk on the white paper, observed the footprints on the paper and recorded footprints on two sides. Three parameters were recorded: footprint length (PL), that is, the longest distance of footprint; footprint width (TS), that is, the distance between the first toe to the fifth toe; inter-toes distance (IT), that is, the distance between second toe to the forth toe. The recorded three parameters were substituted into Bain formula (E-test side, N-normal side), then $SFI = 109.5(ETS-NTS) / NTS - 38.3(EPL-NP L) / NPL + 13.3(EIT-NIT) / NIT - 8.8$. The calculation result of 0 meant normal and -100 meant complete loss of nerve function.

Electrophysiological index record

Sixteen channels physiological recorder was used. Two stimulating electrodes were placed on near-end of dividing nerve and the position that nervus peroneus communis crossing peroneal muscle with a distance of 2 cm. And amplitude and incubation period of muscle action potential (MAP) of two stimulating points was also recorded. Then the nerve conduction velocity (NCV) was calculated out according to the difference and distance of incubation period.

STATISTICAL ANALYSIS

We can known by analysis on the data by SPSS13.0

statistical software that, in four combinations of the medication combination administration groups of NGF and nimodipine, the groups of large and small dose were all superior than the contrast groups on sciatic nerve function index ($P < 0.05$). However, they had no obvious significance compared to the groups of applying NGF or ND independently. But they were superior to the contrast group on nerve electrophysiology index ($P < 0.05$). And the combination medication administration groups of nerve growth and ND in large dose was superior to small dose of administration ($P < 0.05$). And groups of combination administration in large and small dose were all superior to independent administration groups of NGF or ND in same dose ($P < 0.05$). The difference between groups has statistical meaning ($P < 0.05$).

RESULTS

Sciatic eunctional index (SFI) detection

After modeling, sciatic functional index (SFI) of all groups were all close to complete block value 100. SFI value of the medication administration groups had no obvious difference with the contrast group ($P > 0.05$) two weeks after operation. SFI of animals in the contrast groups gradually decreased 4 or 12 weeks after operation, which showed that SFI was recovered. SFI level of medication administration groups of NGF in large and small dose and group of ND in large dose at all time points were all obviously lower than that of the contrast group (as shown in table 1). Compared to the medication administration group of NGF in small dose, SFI level of the medication administration group of NGF in large dose decreased obviously 8 and 12 weeks after operation ($P < 0.05$).

Sciatic nerve electrophysiological index detection

Electrophysiological index detection was conducted 4, 8 and 12 weeks after operation. As time went on, NCV and MAP of animals in the contrast group gradually increased, which showed that sciatic nerve electrophysiological index was gradually improved. The medication administration group increased obviously compared to the contrast group (as shown in Table 2). NCV recovery situation of medication administration group of NGF in large and small dose and group of ND in large dose was obviously better than the contrast group. NCV recovery situation of group of ND in small dose was obviously better than the contrast group after 12 weeks. In addition, NCV level at all time points was also obviously higher than the contrast group. NCV recovery situation of group of two drugs in large dose was better than small dose group 8 and 12 weeks after operation. Compared to the medication administration group of NGF in small dose, NCV level of the medication administration group of NGF in large dose obviously rose 8 and 12 weeks after operation ($P < 0.05$). MAP recovery situation of group of

NGF in large dose only showed obvious difference with the contrast group 8 and 12 weeks after operation.

rapid recovery and growth of injured nerve and ensure its regeneration fiber function is always the hotspot of peripheral nerve repair basis and clinical research. So far,

Table 1: SFI detection (n=13)

| Groups | 2 weeks after operation | 4 weeks after operation | 8 weeks after operation | 12 weeks after operation |
|-------------------|-------------------------|-------------------------|-------------------------|--------------------------|
| NGF in small dose | 94.83±2.49 | 73.57±6.64 | 49.13±3.64 | 32.82±3.82 |
| NGF in large dose | 95.62±3.45 | 69.61±3.26 | 42.33±2.54 | 28.75±2.82 |
| ND in small dose | 96.73±2.46 | 75.55±4.63 | 50.25±4.74 | 29.95±4.62 |
| ND in large dose | 95.61±3.75 | 70.71±4.45 | 47.13±4.58 | 26.95±4.62 |
| Contrast group | 95.51±1.76 | 81.02±3.62 | 55.91±3.53 | 35.08±3.13 |

Table 2: Sciatic nerve electro physiology index detection (n=13)

| Group | NCV (m/s) | | | MAP (m/s) | | |
|-------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
| | 4 weeks after operation | 8 weeks after operation | 12 weeks after operation | 4 weeks after operation | 8 weeks after operation | 12 weeks after operation |
| NGF in small dose | 10.86±0.87 | 22.35±2.53 | 38.96±2.14 | 5.86±0.23 | 13.65±0.92 | 23.89±2.37 |
| NGF in large dose | 12.83±0.77 | 26.75±2.53 | 44.96±1.74 | 6.05±0.26 | 15.76±1.03 | 25.92±2.47 |
| ND in small dose | 9.88±0.83 | 20.15±1.53 | 39.66±2.14 | 5.86±0.23 | 13.11±0.92 | 20.89±2.42 |
| ND in large dose | 11.82±0.77 | 24.62±2.57 | 45.95±2.24 | 6.77±0.23 | 14.76±1.03 | 24.72±3.47 |
| Contrast group | 8.45±0.36 | 18.36±1.25 | 29.48±1.22 | 5.67±0.21 | 11±0.75 | 20.64±0.98 |

Morphological observation

Regenerated axon and intensive distribution of myelinated fiber could be observed in all groups from rat sciatic nerve light microscope observation. Myelinated fiber in the contrast group was relatively less. In addition, myelin sheath was thin and the form presentation was inconsistent. Myelinated fiber in small dose group was in large amount. And myelin sheath was thin and the form was regular. Myeline fiber in large dose group had the maximum amount and distributed intensively. They were much close to normal nerve tissue. table 3 showed the result after image analysis. The result showed that regeneration amount of myeline fiber in-group of NGF in large dose increased obviously compared to the contrast group ($P<0.01$).

DISCUSSION

In recent years, it is a problem to be solved to find a more effective, safe and simple means to promote injury recovery with the increase of morbidity of peripheral nerve injury. Nerve has self-repair function after injury. However, the effect of self-repair function is often unsatisfactory. Nerve injury can induce serious result such as sciatica induced by protrusion of lumbar intervertebral disc (Xueyong *et al.*, 2012; Jiajia *et al.*, 2013), lower limbs dysfunction of apoplexy hemi paralysis patient (Zhongshu *et al.*, 2013; Jiajia *et al.*, 2013), root pain of nerve root type cervical spondylopathy (Xiaoliang, 2013), etc. Therefore, a kind of effective drug that can promote

drug therapy is still one of main means for peripheral nerve injury. The used drugs can be divided into three kinds: one is drugs that can promote growth speed of axon and maturity of regenerated axon such as neurotrophic factors and hormone matter; second is drugs that can inhibit cicatrix formation such as prednisolone, protease inhibitor, hyaluronic acid, calcium channel blockers, etc; third is drugs that can improve local blood supply such as some Chinese herba preparation for promoting blood circulation to remove blood stasis, interleukin, etc.

Table 3: Counting of myelinated fiber

| Group | Counting of myelinated fiber |
|-------------------|------------------------------|
| NGF in small dose | 2482±227 (n=13) |
| NGF in large dose | 2815±257 (n=10) |
| ND in small dose | 2487±427 (n=12) |
| ND in large dose | 2576±269 (n=14) |
| Contrast group | 2387±127 (n=12) |

Experiments at home and abroad and clinical research proves that: NGF and ND can respectively promote regeneration recovery of peripheral nerve effectively (Linkun and Yincheng, 2010). However, previous experiments mostly emphasized research on single element. So far, research on drug combination on treatment of spinal cord injury have made great progress. Methylprednisolone, representative drug of corticosteroids, is the only one drug that approved by U.S. Food and Drug Administration to cure spinal cord injury

and applied broadly. Research found that combination of methylprednisolone and NEP1 for curing spinal cord injury can reduce extent of disease, relieve white matter fiber demyelinating lesion and remit spinal cord electrophysiological disorders caused by latter half transection of spinal cord. That has stronger effect on promoting repair reaction of nerve cell after spinal cord injury and is more beneficial for axon regeneration compared to independent medication administration. Combination of vitamin C in large dose and MP in small dose have obvious effect on spinal cord injury treatment and is superior than single application of MP and vitamin C. However, research on combination medication administration on regeneration of peripheral nerve injury is rare.

CONCLUSION

The recovery effect of two drugs combination was better than independent use of NGF or ND in equal amount. In addition, the recovery effect was related to the concentration of medication. The promotion function of medication combination on rat sciatic nerve injury regeneration was superior than the independent use of drugs in equal amount. And medication combination of NGF in small dose and ND in large dose produced obvious synergistic effect.

We can speculate that the appearance of this synergistic effect was because of the generation of three effects such as promoting growth speed of axon, reducing formation of scar tissue and increasing blood supply (Yuyang *et al.*, 2011).

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