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Effects of 2.5 s mouse nerve growth factor on regeneration of injured sciatic nerves in mice and rats

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KEY WORDS nerve growth factors; nerve regeneration; sciatic nerve; nerve crush; skeletal muscle; neural conduction

AIM: To study the promoting activity of 2.5 s murine nerve growth factor (NGF) for the regeneration of injuried sciatic nerve. **METHODS**: The sciatic nerves of mice and rats were crushed. The axon counts and differential axon counts were made. Evoked potential latency (NMEPL) was recorded at sites of proximal (Pro) and distal (Dis) segment of soleus muscles. **RESULTS**: In mice, NGF im 0.5 – 1 kBU · kg^{-1} increased the axon counts of regenerated nerves 20 d after crush; 2 – 4 kBU · kg^{-1} simultaneously alleviated the denervated atrophy of soleus muscles. In rats, NGF im increased the axon counts at dose of 1 kBU · kg^{-1} for 40 d, 2 kBU · kg^{-1} for 30 d and 40 d, and 4 kBU · kg^{-1} for 20 d, 30 d and 40 d after crush; the number of large axons of regenerated nerves were also increased by NGF im 2 kBU · kg^{-1} for 40 d and 4 kBU · kg^{-1} for 30 d and 40 d; Pro NMEPL was shortened by NGF 1, 2, and 4 kBU · kg^{-1}, Dis-NMEPL was shortened by NGF 2 and 4 kBU · kg^{-1} for 40 d. **CONCLUSION**: NGF im evidently promotes the regeneration of injuried sciatic nerve, and attenuates the skeletal muscular atrophy caused by nerve injury.

Nerve growth factor (NGF) was an essential factor for survival of sensory and sympathetic nerves[1]. It promoted the regeneration of injured motor nerves[2-8]. Nevertheless, most of the experiments were done by direct application of NGF to location of injury on the nerves. The present experiment was designed to observe the promoting activity for regeneration of injured sciatic nerves after systematic administration of NGF.

MATERIALS AND METHODS

The 2.5 s mouse NGF with a purity of 99.9 % and 1 biological unit (1 BU) = 2 ng was provided by Lanzhou Institute of Biological Products, Ministry of Public Health, China. It was dissolved in normal saline before use. Kunming strain mice, 19.0 ± 1.2 g (♂) were purchased from Animal Breeding Center of Lanzhou Institute of Biological Products. Wistar rats, 220 ± 18 g (♂ ♀) were supplied by Experimental Animal Center of Xi-an Medical University.

Sciatic nerves injury in mice Mice (n = 70) were randomly divided into 7 groups; sham surgery group, model control group, NGF 0.5, 1, 2, 4, and 8 kBU · kg^{-1} · d^{-1} groups. Under ether anesthesia, the right sciatic nerve midway between sciatic notch and popliteal fossa was crushed[3] with forceps for 1 min, which caused an injury with entirely broken axon but intact epineurium. The sciatic nerves in sham operating mice were not crushed. After operation, NGF was injected in left hind leg (normal side) for 20 d. The sham mice and model control mice were injected with normal saline 5 mL · kg^{-1} in the same sites. The mice were decapitated on d 21 after operation. The samples of right sciatic nerves were taken at sites of 5 mm proximal and distal to the injured center, fixed in 100 mL · L^{-1} formalin normal saline, stained with osmic acid, embedded in paraffin, and cut into 5 μm sections. Photomicrograph was taken at magnification × 400, and axons were counted. The regeneration ratio of axon was calculated from a ratio of distal axon counts to proximal axon counts. Soleus muscles were weighed. The extent of muscle atrophy was expressed by the ratio of weight of soleus of injured side (right) to normal side (left).

Sciatic nerve injury in rats Rats (n = 130) were divided into 3 groups (decapitated on d 20, d 30, and d 40 after injury). In each group, 40 rats were randomly divided into 4 subgroups: NGF 0, 1, 2, and 4 kBU · kg^{-1} · d^{-1} subgroups. Under anesthesia with ip urethane 1.0 g · kg^{-1}, the right sciatic nerve at site of 6 mm distal to sciatic notch was crushed[3]. The other 10 rats were used as reference group and their right sciatic nerves were exposed but uncrushed. After operation, NGF was injected in left hind leg for 20, 30, or 40 d. The reference rats and model control rats were injected im with saline 5 mL · kg^{-1} in

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the same sites. The evoked nerve muscle potential (NMEP) latency, reflecting the conduction velocity of sciatic nerve, was determined on d 20, d 30, and d 40 after surgery. The both sides of sciatic nerves were exposed under anesthesia with ip urethane 1.2 g · kg^{-1}. The small branches of sciatic nerve above injured sites were trimmed to prevent shortcircuit and the part of gastrocnemius muscle was cut off to explore the soleus muscle. The platinum-tipped bipolar stimulating electrode was suspended on the trunk of sciatic nerve at sciatic notch. Two needle-shaped bipolar recording electrodes were inserted into the proximal and distal segments of soleus muscle 3 and 4 cm, respectively, from stimulating electrode. The ground electrode was inserted into the place between stimulating and recording sites. The potential was evoked at site proximal to injury by computer-controlled stimulator using two-fold threshold voltage (0.4 V in 30 d or 40 d group and 0.8 V in 20 d group) with 0.1 ms wave width and 210 ms intervals. The evoked potential distal to injury was preamplified, A/D-transformed and recorded by MS-302 three-channel physiologic signal recording system and treated by 486 IBM computer. The scan velocity was 2000 mm · s^{-1}. Stimulating signal was produced and output from a stimulator controlled by MS-302 three-channel physiologic signal recording system. After determination of NMEP latency, the samples were taken separately at 5 mm proximal and 10 mm distal to the injured center. Axon count was made under a magnification of × 400 and further calculated according to the total cross area of sciatic nerve from a photo at magnification of × 100. The directly counted axons were more than 1/2 of total axons. The reference rats were decapitated 20 d after operation and the morphological examination was made as above.

The axons distal to the injury were further divided into small (≤6 μm) and large (>6 μm).

RESULTS

Regeneration of injured sciatic nerve in mice The ratio of axon count at the proximal to that at the distal segment of sciatic nerves was 95 ± 5 % in the reference group, and was 45 ± 10 % in the control group. The ratio of weight of soleus muscles of right (injured) to that of left (normal) leg was 99 ± 2 % in operating sham group, and was 59 ± 8 % in control group. NGF 0.5 and 1 kBU · kg^{-1} · d^{-1} increased the ratio of axon counts (distal/proximal), but had no effect on the ratio of weight of soleus muscles (right/left). NGF 2 – 4 kBU · kg^{-1} · d^{-1} increased the ratio of axon counts and the ratio of weight of soleus muscles. But NGF 8 kBU · kg^{-1} · d^{-1} showed less potent (Tab 1).

Ratio of axon counts of injured sciatic nerve in rats The ratio of axon counts of distal to proximal segments was 91 ± 8 % in the reference group. The ratio of axon counts in injured nerves was much lower than that of the reference group on d 20, d 30, or d 40 after injury, and gradually increased as time went on (Tab 2).

Tab 1. Effect of NGF on the regeneration of injured sciatic nerves in mice. $n = 10$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Groups	NGF/ kBU · kg^{-1} · d^{-1}	Ratio of axon counts/ %	Weight ratio of soleus muscles/ %
Sham	–	95 ± 5^c	99 ± 2^c
Control	–	45 ± 10	59 ± 8
NGF	0.5	63 ± 8^c	60 ± 11^a
NGF	1	63 ± 16^b	66 ± 6^a
NGF	2	84 ± 13^c	73 ± 11^c
NGF	4	84 ± 9^c	73 ± 15^b
NGF	8	78 ± 12^c	61 ± 7^a

On 20 d after injury, the ratio of axon counts in the subgroups with NGF 1 and 2 kBU · kg^{-1} · d^{-1} seemed to be higher than that of the control subgroup ($P > 0.05$) in the subgroup with NGF 4 kBU · kg^{-1} · d^{-1}, the ratio of axon counts was increased ($P < 0.05$).

On 30 and 40 d after injury, the ratios of axon counts were increased by 38.7 % and 19.4 %, respectively in the subgroups with NGF 2 kBU · kg^{-1} ($P < 0.01$ and 0.05 *vs* control); and much higher in the subgroups with NGF 4 kBU · kg^{-1} (Tab 2), but slightly increased ($P > 0.05$) in the subgroups NGF 1 kBU · kg^{-1}. On d 40 after injury, the total axon counts distal to injury in the subgroups with NGF 1 kBU · kg^{-1} were increased ($P < 0.05$ *vs* control).

Diameter of axons of regenerating sciatic nerves In injured groups (including control subgroup and NGF subgroups), the axons were small in diameter, and myelin sheath was thin, which were consistent with the characteristic of regenerating nerves (Fig 1, Plate 1).

The large axons of sciatic nerve counted 10.4 % (on d 20), 18.9 % (on d 30), and 24.2 % (on d 40) in control subgroups. On d 20 after operation, the large axon counts were not much influenced by the 3 doses of NGF. On d 30, the large axon counts were increased in the subgroups with NGF 4 kBU · kg^{-1}; and on d 40 the large axon counts were increased in the subgroups with NGF 2 and 4 kBU · kg^{-1} *vs* control (Tab 2).

Tab 2. Effect of NGF on regeneration of injured sciatic nerves in rats. $n = 10$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Groups	NGF kBU·kg^{-1}	Axon counts		Ratio/% (distal/ proximal)	Differential count of regeneration axons	
		Proximal	Distal		Large	Small
Reference		10 222 ± 500	9 333 ± 724	91 ± 8	4 304 ± 757	5 029 ± 1 017
			On d 20 after injury			
Control	–	10 652 ± 932	4 015 ± 1 436	38 ± 13	419 ± 216	3 626 ± 1 346
NGF	1	10 579 ± 796	4 746 ± 1 569^a	45 ± 14^a	484 ± 291^a	4 262 ± 1 386^a
NGF	2	10 695 ± 780	4 723 ± 635^a	44 ± 7^a	452 ± 150^a	4 271 ± 664^a
NGF	4	10 973 ± 1 143	5 842 ± 1 391^b	53 ± 12^b	498 ± 160^a	5 344 ± 1 295^b
			On d 30 after injury			
Control	–	10 539 ± 1 131	4 887 ± 1 494	46 ± 12	923 ± 318	3 972 ± 1 308
NGF	1	11 575 ± 1 140	6 174 ± 1 484^a	54 ± 12^a	1 044 ± 669^a	5 130 ± 1 107^a
NGF	2	10 143 ± 1 490	6 697 ± 1 616^b	63 ± 12^c	1 354 ± 594^a	5 344 ± 1 092^b
NGF	4	10 685 ± 1 193	7 294 ± 1 218^c	68 ± 10^c	1 636 ± 780^b	5 658 ± 1 111^c
			On d 40 after injury			
Control	–	9 981 ± 803	6 209 ± 838	62 ± 11	1 504 ± 433	4 716 ± 733
NGF	1	10 786 ± 1 216	7 334 ± 1 123^b	69 ± 12^a	1 670 ± 531^a	5 364 ± 1 178^a
NGF	2	10 002 ± 634	7 404 ± 877^c	75 ± 9^b	2 263 ± 597^c	5 173 ± 789^a
NGF	4	10 487 ± 948	7 724 ± 1 009^c	74 ± 7^b	2 327 ± 677^c	5 395 ± 718^a

Conduction velocity of regenerating sciatic nerves On d 20 after injury, the conduction velocity of sciatic nerves on injured side in control subgroup was evidently slower than that of normal side as shown in the prolonged NMEP latency. It did not recover to normal level even up to 40 d after injury (Tab 3).

On d 20 and d 30 after injury, the NMEP latency recorded in the distal segment of soleus muscles was shortened by the 3 doses of NGF used but in the proximal segment of soleus muscles, only NGF 4

Tab 3. Effect of NGF on conduction velocity of regeneration sciatic nerves in rats. $n = 10$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. SM: soleus muscles.

Groups	NGF kBU·kg^{-1}	NMEP latency recorded in Proximal segment of SM Times/ms		Prolonged/ %	NMEP latency recorded in Distal segment of SM Times/ms		Prolonged/ %
		Injured	Normal		Injured	Normal	
			On d 20 after injury				
Control		6.88 ± 1.43	1.20 ± 0.05	473	12.80 ± 4.00	1.54 ± 0.10	730
NGF	1	6.34 ± 1.25^a	1.25 ± 0.11^a	407	8.53 ± 1.95^c	1.56 ± 0.09^a	447
NGF	2	6.31 ± 1.14^a	1.24 ± 0.08^a	409	8.18 ± 2.34^c	1.58 ± 0.18^a	418
NGF	4	5.77 ± 0.56^b	1.21 ± 0.12^a	377	8.00 ± 1.86^c	1.54 ± 0.11^a	420
			On d 30 after injury				
Control		3.34 ± 0.67	1.21 ± 0.09	176	4.52 ± 0.92	1.62 ± 0.17	179
NGF	1	2.85 ± 0.51^a	1.26 ± 0.07^a	126	3.86 ± 0.38^b	1.59 ± 0.10^a	143
NGF	2	2.81 ± 0.39^a	1.23 ± 0.11^a	128	3.85 ± 0.14^b	1.60 ± 0.18^a	141
NGF	4	2.79 ± 0.33^b	1.26 ± 0.10^a	121	3.66 ± 0.41^b	1.56 ± 0.17^a	135
			On d 40 after injury				
Control		2.59 ± 0.31	1.38 ± 0.22	88	3.45 ± 0.39	1.78 ± 0.21	94
NGF	1	2.28 ± 0.31^b	1.42 ± 0.15^a	61	3.09 ± 0.36^a	1.77 ± 0.14^a	75
NGF	2	2.26 ± 0.25^b	1.45 ± 0.17^a	56	3.04 ± 0.36^b	1.78 ± 0.23^a	71
NGF	4	2.31 ± 0.21^b	1.43 ± 0.17^a	62	2.88 ± 0.34^c	1.88 ± 0.13^a	53

kBU · kg^{-1} accelerated the conduction velocity *vs* control.

On d 40 after injury, the NMEP latency recorded in the proximal segment of soleus muscles was shortened by the 3 doses of NGF. In the distal segment of soleus muscles, NMEP latency was shortened by NGF 2 and 4 kBU · kg^{-1} *vs* control (Tab 3).

DISCUSSION

NGF was shown having the activity to promote the regeneration of injured peripheral nerves including sensory, sympathetic, and motor nerves when locally administrated[1-8]. In order to demonstrate whether NGF has the therapeutic action on injury of peripheral nerves, sciatic nerve was crushed and NGF was injected im on uninjured side to exclude the local activity in present experiment. The results showed that NGF 1, 2, and 4 kBU·kg^{-1}·d^{-1} evidently increased the axon counts, elevated the ratio of large axons, accelerated the conduction of regenerating sciatic nerves in rats. It was demonstrated that NGF possessed the promoting activity for regeneration of peripheral nerves.

Differential count of axons showed that NGF increased the ratio of large axons, and electrophysiologic evaluation demonstrated that NGF accelerated the conduction of the regenerated sciatic nerves in rats. These findings suggest that NGF has an accelerating effect on the maturation of regenerating nerves.

Nutritive activity is also one of the functions of peripheral nerves. The atrophy of soleus muscles on injured side was attenuated by NGF in mice. This result could be attributed to the acceleration of regeneration of injured nerves.

Over high dose of NGF (8 kBU·kg^{-1}·d^{-1}, in mice) showed less potent activity, suggesting that NGF possess the most suitable dose in treating injury of peripheral nerves, and that attention should be paid to this feature in clinical use of NGF.

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小鼠 2.5 s 神经生长因子对大鼠和小鼠受损坐骨神经再生的影响

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关键词 神经生长因子; 神经再生; 坐骨神经; 神经损伤; 骨骼肌; 神经传导

目的: 证实 NGF 促进坐骨神经再生的作用. **方法**: 夹断小鼠和大鼠坐骨神经轴索, 测再生轴索计数及分类, 在比目鱼肌远(Dis)、近(Pro)端测神经-肌肉电潜伏期(NMEPL). **结果**: 小鼠 NGF im 0.5 – 1 kBU·kg^{-1} 20 d 增加轴索再生率, 2 – 4 kBU·kg^{-1} 减轻比目鱼肌萎缩. 大鼠 NGF im 1 (40 d), 2 (30 和 40 d), 4 (20, 30, 40 d) kBU·kg^{-1} 均显著增加损伤神经的轴索再生率; 高、中剂量增加粗轴索计数; 各剂量均缩短 Dis-NMEPL (20 d, 30 d) 和 Pro-NMEPL (40 d); 高剂量在各时点使两者均缩短. **结论**: NGF im 明显促进大鼠和小鼠坐骨神经损伤后再生并减轻骨骼肌萎缩.