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Research paper

Effect of mutated defensin NP-1 on sciatic nerve regeneration after transection—A pivot study

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HIGHLIGHTS

- This is the second paper discussing the role of defensins in nerve regeneration.
- Functional and morphological observation was firstly made for this drug's effect.
- The mechanism of it's role was discussed after reviewing lots of papers.

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ABSTRACT

Defensins are small cationic peptides that constitute the first line of defense against pathogens and are involved in immune regulation. In this study, their role in peripheral nerve regeneration was investigated. Rat sciatic nerves were transected and the two nerve stumps were bridged by a chitin conduit with a gap of 5 mm between the stumps. The animals were injected intramuscularly with mutated rabbit neutrophil peptide 1 (defensin mNP-1), the positive control nerve growth factor (NGF) or the negative control saline, for 7 consecutive days after repair. After 6 weeks, the sciatic functional index (SFI), MNCV (motor nerve conductive velocity) and morphological parameters including myelinated fiber amounts, fiber diameter, axon diameter, myelin thickness and *G*-ratio were measured. Compared to the SFI of saline group, the NGF and mNP-1 groups had an increase of 18.3% and 18.8%, respectively. The numbers of myelinated fibers in the distal nerve of NGF and mNP-1 groups were 1.45- and 1.32-fold higher than in the saline group. The MNCVs of NGF and mNP-1 groups were 7.3 and 4.4 times of that of saline group. Fiber diameter, axon diameter, myelin thickness and *G*-ratio in the NGF and mNP-1 groups were also significantly higher than those of saline group. Our results demonstrate that, like NGF, the defensin mNP-1 can promote regeneration after a peripheral nerve cut.

1. Main manuscript

Defensins are a family of antimicrobial peptides that are considered to be important antibiotic-like effectors of innate immunity [1–3]. These peptides display a broad spectrum of anti-microbial activity against bacteria, yeasts, fungi and viruses, but also participate in the modulation of immune responses in mammals, for example enhancing phagocytosis [4]. Their antimicrobial effect was believed to be mainly related with the disruption of the microbial membrane. The polar topology of defensins with spatially separated charged and hydrophobic regions allows them to insert themselves into the phospholipid membranes [1]. The consequent disruption of membrane integrity and function ultimately leads to the lysis of microorganisms. Apart from this, their role in innate and adaptive antimicrobial immunity also contributes to antimicrobial effect. For example human neutrophil-derived α-defensins (HNPs) are capable of enhancing phagocytosis by mouse macrophages and chemotactiv for human monocytes and Tcells. Previous studies were mostly concerned with their antimicrobial effect, because this would be a potential anti-inflammatory reagent [5]. But their role in restoring the functions of injured nerve was scarcely investigated. Nozdrachev et al. [6] assessed the effects of defensin on the growth rate and functional characteristics of regenerating nerve fibers 3 weeks after nerve repair. Their results showed

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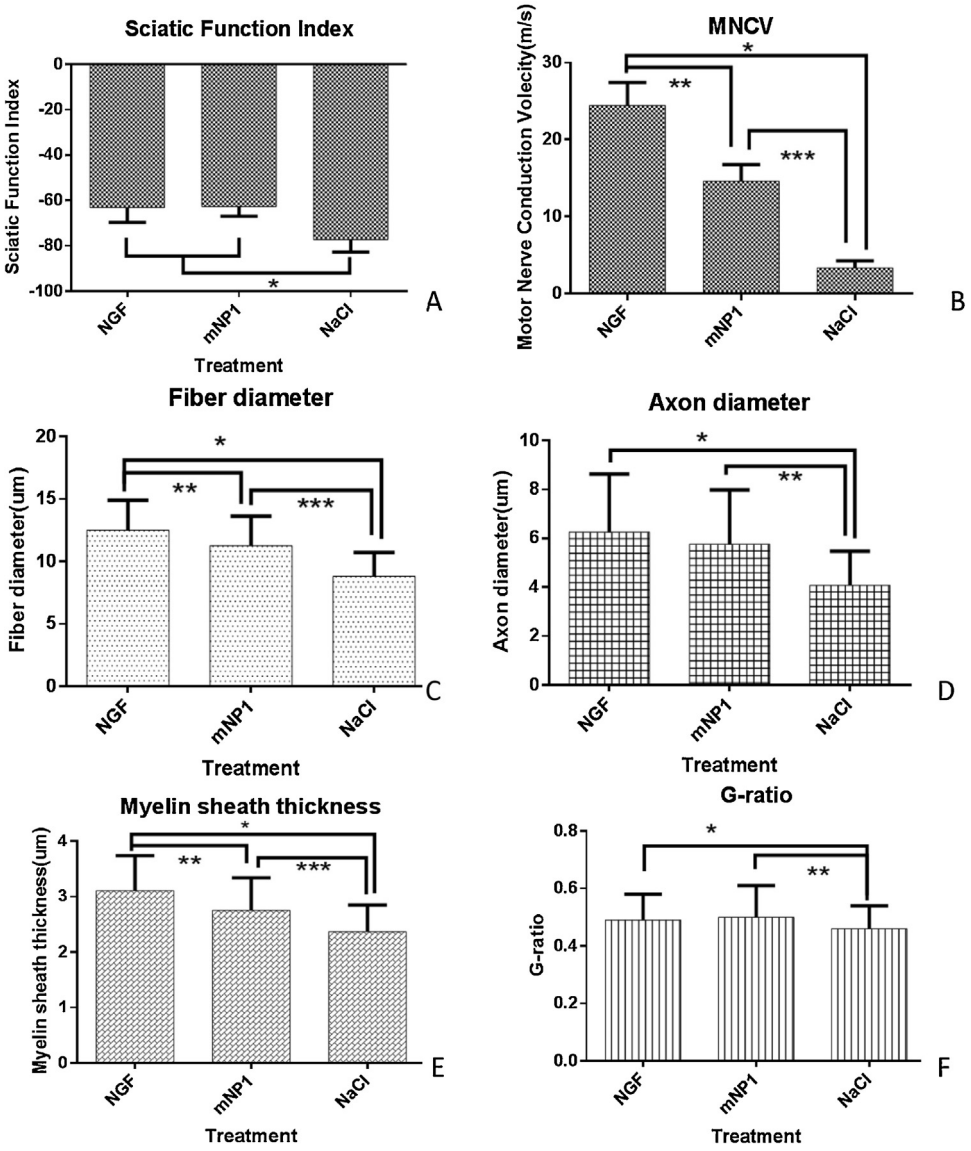

Fig. 1. The sciatic function index (A), motor nerve conduction velocity (B), fiber diameter (C), axon diameter (D), myelin sheath thickness (E) and *G*-ratio (F) of NGF, mNP-1 and saline groups of rats after 6 weeks' recovery. (*, ** and *** indicates that difference exists between the two groups).

that defensins had positive effects on nerve regeneration. However, in their experiment, only total action potentials of injured nerve and the distance of nerve spike conductivity were measured. In this study, we performed functional test of the affected limb and observed the morphological changes of regenerated nerves. Our aim was to identify whether defensins could promote nerve regeneration after neurorhaphy.

Eighteen female SD rats, body weight around 220 g, were anesthetized after approval of Animal Ethical Committee at Peking University People's Hospital. The sciatic nerve in the hind limb was exposed and transected at 1 cm proximal to its bifurcation into the common peroneal nerve and the tibial nerve. Then the biodegradable chitin conduits were used to bridge the ends of proximal nerve stump and distal nerve stump. The gap between the two nerve ends was kept at 5 mm. After that, the wound were closed by layers. The eighteen rats were divided equally into three groups and treated with respective solution daily for seven days starting on the day of surgery. For the NGF group, 1 μg recombinant murine β-NGF was injected intramuscularly for each rat daily. For the mNP-1 treatment group, 25 μg mNP-1 was injected intramuscularly for each rat daily. The 6 controls were given placebo (sodium chloride) simultaneously in an identical manner. All the rats were allowed for recovery for 6 weeks after the surgery. The mutated rabbit neutrophils peptide-1 (mNP-1) was produced by Institute of Genetics and Developmental Biology, Chinese Academy of Science, and detected by anti-microbial activity [7]. This mNP-1 gene was a mature α-defensin NP-1 gene from rabbit with an additional initiator codon in the 5′-terminus. It was produced in nitrate reductase (NR)-deficient *C. ellipsoidea* (*nrm-4*).

Walking track analysis was performed for all the animals at 6 weeks post-surgery. Animals were allowed conditioning trials in a confined walking track (10 × 60 cm) darkened at one end. White paper that had the appropriate dimensions was placed on the bottom of the track. The rat's hind limbs were dipped into black ink before the animal was placed at the entrance of the walking track. Foot prints appeared immediately on the paper when the rat walked down the track. Prints for measurement were chosen at the time of walking, based on clarity and completeness at a point when the rat was walking briskly. Paired footprint parameters for print length (distance from heel to toe, PL), toe spread (distance from first to fifth toe, TS) and intermediary toe spread (distance from second to fourth toe, IT) were recorded for the left normal