Curcumin promotes nerve regeneration and functional recovery in rat model of nerve crush injury

Junxiong Ma¹, Jun Liu¹, Hailong Yu, Qi Wang, Yu Chen, Liangbi Xiang*

Department of Orthopedics, General Hospital of Shenyang Military Area Command of Chinese PLA, Shenyang, Liaoning, China

HIGHLIGHTS

- Curcumin promotes nerve regeneration after crush nerve injuries.
- Curcumin accelerates motor functional recovery after crush nerve injuries.
- High dose of curcumin shows better performance than low doses.

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ABSTRACT

Background and aim: Curcumin at 100 mg/kg has been shown to have a protective effect on crush nerve injury. However, it is unclear whether the protective effect of curcumin on nerve injury is dose dependent. The present study was designed to investigate such a possibility. Methods: The rats subjected to crush nerve injury were intraperitoneally administrated daily for 4 weeks with curcumin (50 mg/kg, 100 mg/kg and 300 mg/kg), or 100 μg/kg mecobalam or normal saline, respectively. The axonal regeneration was investigated by retrograde labeling and morphometric analysis. The motor functional recovery was evaluated by electrophysiological studies, behavioral tests and histological appearance of the target muscles. Results: Our data showed that curcumin and mecobalam achieved better nerve regeneration and functional recovery than vehicle group. In addition, high doses of curcumin (100 mg/kg and 300 mg/kg) showed better performance in promoting nerve regeneration and functional recovery than low dose of curcumin (50 mg/kg). Conclusions: Curcumin is capable of promoting nerve regeneration after nerve injuries, highlighting the therapeutic values of curcumin as a neuroprotective drug for peripheral nerve repair applications.

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1. Introduction

Peripheral nerve injury is a serious health concern for society, which always results in restricted activity or life-long disability [4]. Although microsurgical treatments for nerve injuries have been improved over the past decades, the outcome of peripheral nerve injury repair remains unsatisfactory. Commonly, microsurgical repair is required for the architectural reconstruction of the injured nerve, while neuroprotective drugs are used to promote nerve regeneration in the treatment of peripheral nerve injuries. Therefore, searching for effective drugs for promoting nerve regeneration, especially the naturally occurring ones, has gained extensive attention.

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a naturally occurring compound which is extracted from the roots of Curcuma longa (Zingiberaceae). Curcumin exhibits a variety of pharmacologic properties, including anti-inflammatory, anti-carcinogenic, and anti-oxidant [17]. In recent years, the potential therapeutic values of curcumin in the treatment of neurodegenerative disease have been increasingly recognized [8,10,16,3]. In vitro studies show that curcumin protected PC12 cells against amyloid-induced toxicity [8], and protected against glutamate excitotoxicity in rat cerebral cortical neurons [10]. In vivo studies have shown that curcumin can protect rat brain against ischemia injury by reducing the expression of apoptotic genes [16]. In addition, curcumin is capable of inhibiting apoptosis and neuronal loss, and improving motor function after spinal cord injury in rats [3]. All those studies indicate the neuroprotective capacities of curcumin, and raise the possibility that curcumin may promote nerve regeneration after peripheral nerve injuries. In most recent studies, curcumin at 100 mg/kg has been shown a protective effect on crush nerve injury, indicating the possibility of using curcumin as a neuroprotective agent in the

* Corresponding author. Tel.: +86 024 28856247; fax: +86 024 28856247.
E-mail addresses: xiangbiyi963@163.com, xiangbiyi963@sina.com (L. Xiang).
1 These authors contribute equally to this work.

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treatment of nerve injuries [5,6]. However, it is unclear whether the protective effect of curcumin on nerve injury is dose dependent. In addition, the optimal dose of curcumin for promoting nerve regeneration and functional recovery has not been identified for peripheral nerve injury. Therefore, the present study was designed to investigate the effect of curcumin at different doses on nerve regeneration and functional recovery in a rat model of crush sciatic nerve injury.

2. Materials and methods

2.1. Surgical procedures and drug treatments

All procedures were reviewed and approved by the Institutional Ethical Committee of the Fourth Military Medical University (FMMU) prior to the experiment. Forty eight young adult male Sprague-Dawley rats (provided by the Laboratory Animal Center of the FMMU, body weight 200–220 g) were used. The animals were anesthetized by an intraperitoneal injection of sodium pentobarbital solution (40 mg/kg body weight). The left sciatic nerve was exposed. Forty rats were subjected to nerve crush injury and the remaining 8 rats were subjected to wound closure and served as sham surgery group. For the crush nerve injury, the exposed sciatic nerves (at the site 5 mm to the bifurcation) were crushed using a pair of forceps for 10 s × 3 times with an interval of 10 s, and then the skin was sutured with 4-0 stitches. Following surgery, the rats with crush injury were randomly divided into 5 groups (n = 8 in each group), and were intraperitoneally administrated daily for 4 weeks with 50 mg/kg (Cur50 group), 100 mg/kg (Cur100 group) or 300 mg/kg curcumin (Cur300 group), or 100 µg/kg mecobalamin (Eisai, Tokyo, Japan; positive group) or saline (vehicle group), respectively. The administration of curcumin (Sigma, St. Louis, MO, USA), mecobalamin and saline was started immediately after nerve injury. In addition, curcumin and mecobalamin were dissolved in saline, and equal volume of saline with or without curcumin (or mecobalamin) was injected in each group.

2.2. Behavioral analysis

Walking track analysis was performed on all rats every week after surgery. Prior to surgery, the rats’ hind paws were painted with non-toxic finger paint and the rats were trained to walk down a wooden track (50 cm × 7 cm) into a darkened goal box. Their paw prints were recorded until five measurable footprints were collected. The sciatic functional index (SFI) was calculated as follows:

\[
SFI = \left( -38.3 \times \frac{EPL - NPL}{NPL} \right) + \left( 109.5 \times \frac{ETS - NTS}{NTS} \right) + \left( 13.3 \times \frac{EIT - NIT}{NIT} \right) - 8.8
\]

Print length (PL) represents the distance from the heel to the top of the third toe, intermediary toe spread (IT) is the distance between the second to the fourth toe, and toe spread (TS) is the distance from the first and the fifth toe. NPL, NIT and NTS represent the PL, IT and TS recorded from the non-operated foot. EPL, EIT, ETS represent the PL, IT and TS recorded from the experimental, operated foot [1].

2.3. Hot plate test

Hot plate test was used to investigate sensory functional recovery following a previous report with minor modification [14]. In brief, animals in each group were housed in individual plastic boxes and were allowed to accommodate the environment for 24 h before testing. The rats were then positioned to stand with the operated hind paw on a hot plate at 56 °C. The time elapsed from the onset of hotplate contact to hind paw withdraw was measured with a stop-watch and was recorded as withdrawal reflex (WRL). The affected limbs were tested 4 times, with an interval of 5 min between consecutive tests. The hot plate was removed to prevent tissue damage if no paw withdraw was observed after 12 s, and the WRL was recorded as 12 s.

2.4. Mechanical withdrawal thresholds

The paw withdrawal threshold in response to a mechanical stimulus was measured at four weeks after crush injury and treatment in rats using a series of von Frey filaments. Forces applied ranged from 47 mN to 156 mN. The rats were placed in a plastic cage on a metal mesh floor and were allowed to get used to this set up prior to testing. von Frey filaments were applied to the mid-plantar surface of the left hindpaw for 6–8 s. Filaments were applied in ascending order and the smallest filament eliciting a foot withdrawal response was considered the threshold stimulus [9].

2.5. Electrophysiological analysis

Electrophysiological studies were performed every week after surgery. The sciatic nerve was exposed after anesthesia was induced. A bipolar stimulating electrode was placed under the sciatic nerve at the site 10 mm proximal to the crushed site. A recording electrode was placed in the gastrocnemius muscle to record the compound muscle action potential (CMAPs). The CMAPs in the contralateral un-operated side were measured and recorded as control value. The CMAP peak amplitude, CMAP latency of onset, and nerve conduction velocity (NCV) values were calculated [1].

2.6. Retrograde labeling

Retrograde labeling was performed at 4 weeks after surgery. The operated sciatic nerve trunk at the site of nerve bifurcation was exposed and received an injection of 2 µL of 4% FG (Biotium Inc., CA, USA) solution, and the incision was then sutured. Three days later, the rats were fixed by transcardiac perfusion with 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer. The L4, L5 and L6 of the spinal cords were then harvested, post-fixed in buffered 4% paraformaldehyde for an additional 12 h, immersed in an anti-freezing agent (in 30% sucrose) overnight at 4 °C, and processed for freezing-sectioning on a cryostat. Transverse sections (thickness: 25 µm) were prepared from the spinal cords [1].

2.7. Morphometric analysis

The regenerated nerves were harvested and fixed in 3% glutaraldehyde. The samples were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.3), dehydrated in ethanol, and embedded in resin. The distal portion of the samples were prepared into semi-thin (thickness: 1.0 µm) and ultra-thin (thickness: 50.0 nm) sections. The semi-thin sections were stained with a 1% toluidine blue/1% borax solution prepared in distilled water and examined under a light microscope (AH3, Olympus, Tokyo, Japan). Ultra-thin sections were stained with uranyl acetate and lead citrate, and were examined under a transmission electron microscope (H-600, HITACHI, Tokyo, Japan). Morphometric evaluations were blind conducted by examiners. In each group, axonal regeneration was estimated by the total number of myelinated axons per nerve transverse section (\(M_{ax} \)) and the mean diameter of nerve fibers [1]. The degree of myelination was estimated by the axon to fiber diameter ratio (G-ratio).
2.8. Histological analysis of target muscles

Four weeks after surgery, the gastrocnemius muscles in the limbs from the operated rats were harvested from the belly of muscle. The muscle samples were post-fixed with formalin, embedded in paraffin, and underwent Masson trichrome staining. For each sample, photographs were taken from three randomly-chosen fields and a Leica software package was used to measure the transverse section area of the muscle fibers. Both the total area and the muscle fiber area were measured and the percentage of the latter to the former was calculated [1].

2.9. Statistical analysis

All data are expressed as the mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to compare mean values with the SPSS13.0 software package (SPSS Inc., Chicago, IL, USA). If there was a significant overall difference among groups, Tukey post hoc test was used to make pair-wise comparisons. Values of p < 0.05 were considered statistically significant.

3. Results

3.1. Curcumin promotes nerve regeneration after crush nerve injuries

At the end time-point (4 week after surgery), the number of FG-positive motoneurons in three curcumin groups and mecobalamin group were significantly higher than that in vehicle group (p < 0.05 for Cur50, p < 0.01 for Cur100, Cur300 and mecobalamin; Figs. 1A–E and 2A). In addition, the number of FG-positive neurons

![Fig. 1. Representative micrographs following fluoro-gold (FG) retrograde tracing (A–E), transmission electron microscopy (F–J), and target muscles (K–O) in the vehicle group (A, F, K), mecobalamin group (B, G, L), curcumin at 50 mg/kg (C, H, M), 100 mg/kg (D, I, N), and 300 mg/kg (E, J, O) at 4 weeks after surgery.]

![Fig. 2. Statistical analysis of FG retrograde labeling (A), morphometric analysis (B), mechanical withdraw threshold (C), and thermal withdraw threshold (D). *p < 0.05 and ^p < 0.01 for comparison with vehicle group. ¥p < 0.05 for comparison with curcumin group at 50 mg/kg. Æp < 0.05 for comparison with sham-surgery group.]
in Cur100, Cur300 and mecobalamin groups were in the similar range (p > 0.05, Figs. 1A–E and 2A), which were significantly higher than that in the Cur50 group (p < 0.05, Figs. 1A–E and 2A). The number of FG-positive neurons in sham-surgery group was significantly higher than that in the remaining groups (p < 0.05, Fig. 2A).

Four week after surgery, the number of myelinated axons per nerve transverse section and the mean diameter of nerve fibers in Cur50, Cur100, Cur300 and mecobalamin groups were in the similar range, which were significantly higher than that in vehicle group (p < 0.05 for Cur50, p < 0.01 for Cur100, Cur300 and mecobalamin; Table 1 and Fig. 1F–J). In addition, the values of G-ratio (an indicator of myelination) were in the similar range in all groups (p > 0.05; Table 1 and Fig. 1F–J). Furthermore, the morphometric indices in sham-surgery group were significantly better than those in the remaining groups (p < 0.05, Table 1).

3.2. Curcumin promoted motor functional recovery after crush injuries

At 1 week and 2 week post-operatively, the SFI values in the saline group, three curcumin groups, and mecobalamin group were in the similar range (p > 0.05, Table 2). At 3 and 4 weeks after treatment, the SFI values in curcumin groups (Cur50, Cur100 and Cur300) and mecobalamin group were significantly higher than that in saline group (p < 0.05 for Cur50, p < 0.01 for Cur100, Cur300 and mecobalamin; Table 2), indicating that curcumin and mecobalamin achieved better motor functional recovery than vehicle after crush injury in rats. In addition, the SFI values in the Cur100, Cur300 and mecobalamin groups were in the similar range, which were significantly higher than those in the Cur50 group at 3 and 4 weeks after treatment (p < 0.05, Table 2). Additionally, the SFI values in sham-surgery group were significantly better than those in the remaining groups (p < 0.05, Table 2).

3.3. Curcumin promoted sensation recovery after crush injuries

The withdrawal threshold of the operated hindpaw significantly decreased after nerve crush, indicating the presence of mechanical allodynia after crush injury. At 4 weeks after treatment, the withdrawal threshold in curcumin groups (Cur50, Cur100 and Cur300) and mecobalamin group were significantly higher than that in vehicle group (p < 0.05, Fig. 2C), indicating that curcumin and mecobalamin achieved better sensory functional recovery than vehicle group after crush injury in rats. In addition, the withdrawal threshold in the Cur100, Cur300 and mecobalamin groups were in the similar range, which were significantly higher than those in the Cur50 group at 4 weeks after treatment (p < 0.05, Fig. 2C). Furthermore, the withdrawal threshold in sham-surgery group were significantly shorter than those in the remaining groups (p < 0.05, Fig. 2C).

Table 1

<table>
<thead>
<tr>
<th>Morphometric values</th>
<th>Sham-surgery group</th>
<th>Vehicle group</th>
<th>Mecobalamin group</th>
<th>Curcumin groups</th>
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<td>300 mg/kg</td>
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<tr>
<td>Total number of myelinated axons per cross section (×10⁴)</td>
<td>0.526 ± 0.014</td>
<td>0.258 ± 0.063***</td>
<td>0.317 ± 0.042***</td>
<td>0.294 ± 0.031***</td>
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<tr>
<td>Mean diameter of myelinated axons (µm)</td>
<td>4.865 ± 0.651</td>
<td>3.462 ± 0.251***</td>
<td>3.965 ± 0.246***</td>
<td>3.726 ± 0.361***</td>
</tr>
<tr>
<td>G-ratio (axon to fiber diameter ratio)</td>
<td>0.562 ± 0.114</td>
<td>0.722 ± 0.134***</td>
<td>0.696 ± 0.152***</td>
<td>0.712 ± 0.081***</td>
</tr>
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</table>

*** p < 0.05 for comparison with vehicle group.
**** p < 0.01 for comparison with vehicle group.
**** p < 0.05 for comparison with curcumin group at 50 mg/kg.
**** p < 0.05 for comparison with sham-surgery group.

The CMAP latency of onset, the peak amplitude of CMAP, and NCV in three curcumin groups and mecobalamin group were significantly better than that in vehicle group at the 4 week end point (p < 0.05, Table 3). In addition, the Cur100 and Cur300 groups showed a shorter CMAP latency of onset, higher amplitude of CMAP, and faster NCV than those in the Cur50 group (p < 0.05, Table 3). The electrophysiological values in sham-surgery group were significantly better than those in the remaining groups (p < 0.05, Table 3).

The atrophy of gastrocnemius muscle after nerve injury was significantly reversed by application of curcumin or mecobalamin. The average percentage of muscle fiber area in the Cur100, Cur300 and mecobalamin groups were significantly higher than that in the Cur50 and vehicle groups 4 weeks after ES (p < 0.05, Figs. 1K–O and 2B). In addition, the average percentage of muscle fiber area in sham-surgery group was significantly better than those in the remaining groups (p < 0.05, Fig. 2B).

Table 2

<table>
<thead>
<tr>
<th>Weeks after surgery</th>
<th>Sham-surgery group</th>
<th>Vehicle group</th>
<th>Mecobalamin group</th>
<th>Curcumin groups</th>
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<tr>
<td>1</td>
<td>– 1.78 ± 0.25</td>
<td>– 76.41 ± 5.38***</td>
<td>– 74.63 ± 7.82***</td>
<td>– 75.11 ± 7.86***</td>
</tr>
<tr>
<td>2</td>
<td>– 0.84 ± 0.12</td>
<td>– 69.54 ± 6.72***</td>
<td>– 66.46 ± 6.28***</td>
<td>– 67.49 ± 7.25***</td>
</tr>
<tr>
<td>3</td>
<td>– 2.23 ± 0.36</td>
<td>– 55.36 ± 5.72***</td>
<td>– 49.84 ± 4.91***</td>
<td>– 52.81 ± 5.37***</td>
</tr>
<tr>
<td>4</td>
<td>– 1.29 ± 0.22</td>
<td>– 50.92 ± 4.76***</td>
<td>– 41.62 ± 3.55***</td>
<td>– 46.73 ± 4.45***</td>
</tr>
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*** p < 0.05 for comparison with vehicle group.
**** p < 0.01 for comparison with vehicle group.
**** p < 0.05 for comparison with curcumin group at 50 mg/kg.
**** p < 0.05 for comparison with sham-surgery group.
The hot plate test was used to assess the recovery of thermal nociceptive sensation. The WRL in the sham-surgery group was 1.5 ± 0.2 s, which were significantly lower than that in the remaining groups (p < 0.05, Fig. 2D). The WRL in three curcumin groups and mecobalamin group were significantly lower than that in vehicle group at the 4 week end point (p < 0.05, Fig. 2D). In addition, the WRL values in the Cur100, Cur300 and mecobalamin groups were in the similar range, which were significantly lower than those in the Cur50 group at 4 weeks after treatment (p < 0.05, Fig. 2D). Furthermore, the withdrawal threshold in sham-surgery group were significantly shorter than those in the remaining groups (p < 0.05, Fig. 2D).

4. Discussion

The present study investigated the possibility of developing curcumin as a potential neuroprotective agent for the treatment of peripheral nerve injuries. We found that curcumin was capable of promoting nerve regeneration and motor functional recovery after crush nerve injury in rats. The nerve regeneration achieved by curcumin at high doses (100 mg/kg and 300 mg/kg) was equivalent to that by mecobalamin, which is a commonly used neuroprotective agent for neuronal degenerative diseases. Further studies showed that high dose of curcumin (100 mg/kg and 300 mg/kg) achieved better nerve regeneration than low dose of curcumin (50 mg/kg). All the findings indicate the therapeutic potential of curcumin as a neuroprotective agent in the treatment of peripheral nerve injuries. However, curcumin was administrated immediately after nerve injury in the present study. It is desirable to know whether delayed administration of curcumin could promote nerve regeneration and functional recovery after injury, which needs further investigations in future studies.

Curcumin is capable of enhancing nerve regeneration after peripheral nerve regeneration in the present study. In many previous studies, the neuroprotective effect of curcumin has been increasingly recognized in the central nervous system [13,11,15]. In mouse models of Parkinson’s disease, curcumin can protect dopaminergic neurons against neuronal death through inhibiting the c-Jun-N-terminal kinase (JNK) signaling pathway, which suppresses the increase in caspase-3 in dopaminergic neurons [15]. In transgenic Alzheimer mouse, curcumin has been found to inhibit the formation of amyloid-oligomers and fibrils, reduce oxidative damage and amyloid burden in the brain region [11,2]. All those findings, together with the findings in the present study, highlight the therapeutic values of curcumin as a neuroprotective agent for neuronal diseases in both central and peripheral nervous systems.

Electrophysiology study was performed to further investigate the motor functional recovery after curcumin treatment. The CAMP amplitude is directly proportional to the number of nerve fibers innervating the target muscle, which allows a further evaluation of motor functions [1]. In the present study, a significant shorter CAMP latency, faster NCV and higher CAMP peak amplitude were recorded in curcumin and mecobalamin groups than that in vehicle group, indicating a better motor functional recovery in curcumin and mecobalamin groups. In addition, the atrophy of the denervated muscles after nerve injuries was partially reversed by curcumin, and the behavioral appearances were significantly improved by curcumin, indicating that curcumin is able to promote motor functional recovery after crush nerve injuries. Further investigations showed that curcumin also improved both mechanical and thermal sensation recovery, highlights the therapeutic value in the treatment of crush nerve injury.

Thus far, many previous studies have been performed to investigate the pharmacokinetic of curcumin in vivo. In rats, oral administration of curcumin (500 mg/kg) yielded a peak plasma level of 0.06 ± 0.01 μg/ml within 42 min and an intravenous injection of curcumin (10 mg/kg) resulted in a peak plasma level of 0.36 ± 0.05 μg/ml [12]. However, it is pity that no information was available for an intraperitoneal injection of curcumin at 100 mg/kg in rats. A study in mice showed that an intraperitoneal injection of curcumin (100 mg/kg) yielded a peak plasma level of 2.25 μg/ml within 15 min, which declined rapidly within 1 h [7]. Although the pharmacokinetics of curcumin in rats might be similar to that in mice when it was given in the same way by an intraperitoneal injection, the pharmacokinetics of curcumin in rats needs to be identified when it was intraperitoneally administrated at 100 mg/kg in future studies.

In conclusion, curcumin was capable of promoting nerve regeneration and accelerating motor functional recovery. In addition, curcumin at high doses shows a better performance than that at low dose in promoting nerve regeneration. All those evidences suggest that curcumin has the potential to be a neuroprotective agent for nerve injury repair applications.

Conflict of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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