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Effect of epothilone B on the expression of neuroproteins after anastomosis of the sciatic nerve transection in the rat

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Abstract

Peripheral nerve injury (PNI) is a common condition that leads to the partial loss of function in the sensory, motor, and autonomic nervous systems. The peripheral nervous system has an inherent capacity to regenerate after injury and re-innervate its target organs, but full functional recovery is rare. In recent years, there has been growing interest in identifying drugs that can promote axonal regeneration and outgrowth following PNI. Epothilone B (EpoB) is an FDA-approved antineoplastic agent that promotes tubulin polymerization and enhances the stability of microtubules. Recently, the regenerative effects of EpoB in the central nervous system have garnered attention, but its potential therapeutic effects on peripheral nerve regeneration remain underexplored. This study utilized a sciatic nerve transection and anastomosis model in rats to evaluate the effects of EpoB on neuroprotein expression following nerve injury. Behavioral analysis, Masson's trichrome staining, and immunofluorescence staining were conducted to assess the impact of EpoB on sciatic nerve regeneration. Over time, motor recovery and muscle reinnervation were observed, with Sciatic Functional Index (SFI) scores higher in the EpoB-treated group compared to the vehicle group. The expression of fibronectin (FN) was significantly lower in the EpoB group, while the expression of Tau, neurofilament-M (NF-M), and growth-associated protein-43 (GAP-43) was significantly higher. In conclusion, EpoB treatment significantly increases the expression of Tau, NF-M, and GAP-43, suggesting a positive effect on axonal regeneration and repair.

Keywords Sciatic nerve transection anastomosis, Peripheral nerve injury, Epothilone B, Fibronectin, Tau, neurofilament-M, Growth associated protein-43, Sciatic functional index, Rats, Axonal regeneration

Introduction

PNIs are common clinical conditions associated with high rates of disability and often poor functional outcomes [1]. Injured peripheral nerves have the ability to regenerate and reinnervate their target organs [2–6]. Following a PNI, a series of key events are triggered to facilitate nerve regeneration. These include Wallerian degeneration, where axonal segments distal to the lesion site degenerate [7]. Schwann cells, which play a role in peripheral nerve regeneration, undergo significant reprogramming in response to the injury. Myelin and Remak Schwann cells transform into repair Schwann cells that

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promote axonal regeneration, target reinnervation, and myelin clearance [8–9]. This transformation is regulated by the transcription factor c-Jun, which is essential for the dedifferentiation of Schwann cells, their proliferation, and the formation of regeneration tracks known as bands of Bungner [9–10]. Additionally, Schwann cells are involved in the clearance of myelin debris, a process that is critical for maintaining the regenerative environment and enabling axon growth [11]. However, the regenerative potential of the peripheral nervous system diminishes with aging and chronic denervation, as c-Jun expression decreases, leading to a reduced regenerative capacity [10, 12]. Microtubule-stabilizing agents have shown promise in enhancing regeneration in both traumatic and neurodegenerative injuries [13–14]. Microtubules are important in neurons, including transport, cellular polarity, cell motility, mitosis, cytokinesis and neuron growth [13]. The polymerization of microtubules, along with actin filaments in growth cones, promotes neurite outgrowth and axon extension [15–17].

Epothilone B (EpoB), a microtubule-stabilizing agent, induces neurite outgrowth and axon extension in rodent models by increasing microtubule polymerization and stabilization at the growth cone [18–19]. The US Food and Drug Administration (FDA) has approved EpoB for the treatment of cancer. In addition, EpoB has been shown to improve axonal regeneration in rodent models of brain and spinal cord injury. It reduces fibrotic scarring [20], which exacerbates axonal outgrowth after stroke or traumatic CNS injury [21]. It can cross the blood-brain barrier and has differential effects on the microtubule (MT) cytoskeleton in neurons and fibroblasts. In neurons, EpoB induces rapid MT polymerization at the tips of neurites, promotes axon extension in the central nervous system, and shows promise for clinical applications [19]. It has gained prominence for its regenerative effects on the central nervous system. However, research has only just begun to investigate the potential therapeutic effects of EpoB on peripheral nerve regeneration. A recent study found that acellular nerve allografts (ANAs) loaded with 0.1 nM EpoB can effectively reconstruct the transected sciatic nerve in rats, probably by enhancing axonal sprouting and extension [22]. EpoB can promote axonal regeneration after peripheral nerve injury by enhancing the migration of SCs, with this activity being controlled by PI3K/Akt signaling-mediated autophagy in SCs [23]. EpoB treatment may be therapeutically useful for improving corneal reinnervation and restoring sensitivity after corneal injury. In the present study, it was suggested that after injury, lesion scars and poor axon growth prevent axon regeneration, and EpoB promotes axon growth throughout the scar area by inducing concerted MT polymerization in the axon tip [24]. The antibodies used in this study were selected based on their specificity for proteins involved in neuronal function and plasticity. FN is a key extracellular matrix protein known to influence cell adhesion, migration, and survival, particularly in the context of neuronal regeneration. Tau is a microtubule-associated protein critical for maintaining neuronal stability and implicated in neurodegenerative diseases. NF-M is a major component of neurofilament networks, important for axonal transport and neuronal integrity. GAP-43 is a marker of axonal growth and plasticity, widely used to assess changes in neuronal growth and regeneration. In our study, we investigated the effects of EpoB on the expression of fibronectin (FN), Tau, neurofilament-M (NF-M), growth-associated protein-43 (GAP-43) in rat sciatic nerve after sciatic nerve transection anastomosis. To explore the mechanism of EpoB promoting peripheral nerve regeneration.

Materials and methods

Animals

All animal experiments adhered to the guidelines of the Chinese Association for Laboratory Animal Sciences. Fifty-four female Sprague-Dawley rats, aged 6–8 weeks, were purchased from Cloud-Clone Corp. (SYXK(E)2023 – 0135, Wuhan, China). The vendor confirmed that the animals were specific pathogen-free (SPF) through screening for pathogens, ensuring they were free from infectious agents that could impact the results. Every effort was made to minimize the number and suffering of the animals used in the following experiments in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No.85 – 23, revised 1986).

PNI model establishment and drug administration

Surgical procedures were performed according to the guiding principles for experimental animal Wellness issued by the Ministry of Science and Technology of the People's Republic of China and the ARRIVE guideline [25]. Fifty-four SPF SD rats were randomly divided into three groups: Control group ($n = 6$), Vehicle group ($n = 24$) and EpoB group ($n = 24$). 40 mg/kg of 1% pentobarbital sodium was i.p. injected into animals to achieve anesthesia. The rats were disinfected bilaterally with iodophor and fixed in the prone position on the operating table. The rat skin was incised along the surface of the sciatic nerve, then the skin was separated with surgical forceps and the muscle was gently separated with a tiny glass needle to expose the sciatic nerve. This tool was chosen for its thin tip, which enables precise manipulation and minimizes mechanical damage to both the surrounding tissue and the nerve during the surgical process. And the sciatic nerve was cleanly transected at the mid-thigh level, with no portion excised. The epineurium of these rats was then precisely sutured with a 9 – 0 microsuture

by end-to-end suture without torsion of the nerve, with a total of six sutures. To avoid excessive tension that could compromise nerve integrity, the suturing was performed under low tension, ensuring that the nerve ends were aligned without causing any mechanical stress. Finally, the skin incision was closed. The whole procedure was performed under aseptic conditions. They then received an intraperitoneal injection of EpoB at 0.75 mg/kg (Dalian Meilun Biology Technology Co., Ltd., China) or vehicle (1:1 mixture of dimethyl sulfoxide and saline) at 1, 15 days post-injury [19]. The drug was administered via intraperitoneal injection on days 1 and 15 post-repair to target the inflammatory and axonal regeneration phases, respectively. The rats in the control group were only deeply anesthetized and injected intraperitoneally with 1:1 mixture of dimethyl sulfoxide and saline; no surgical procedures were performed. Post-operative analgesia was provided to all animals to minimize pain and distress. The analgesic agent (buprenorphine) was administered subcutaneously at a dose of 0.05 mg/kg every 12 h for 3 days following surgery.

Behavioral analysis

SFI

We made a cuboid test channel of 50 cm in length, 8.5 cm in width, and 10 cm in height. The number of animals per group ($n = 6$) has been specified and the number of prints analyzed (minimum of 6 footprints per animal). The behavior of the rats was assessed at 1,2, 3and 4 weeks post-injury using an alley test. For a short period of time, animals could walk down a wooden aisle with a dark target on its other side. We covered their feet with acrylic paint and white paper and observed their walking behavior. This made it possible to visualize anatomical landmarks in the resulting footprints. The Sciatic Functional Index (SFI) was calculated by analyzing the prints [26]. This was repeated three times to make sure that the footprints could be clearly seen. SFI = 109.5 (ETS-NTS)/NTS-38.3 (EPL-NPL)/NPL + 13.3 (EIT-NIT)/NIT-8.8 where EPL is the experimental paw length, NPL is the normal paw length, ETS is the experimental toe spread, NTS is the normal toe spread, EIT is the experimental intermediary toe spread, and NIT is the normal intermediary toe spread. A score of SFI = 0 is normal and a score of SFI = -100 is completely damaged.

Euthanasia protocol

Euthanasia of rats was performed using pentobarbital sodium (300 mg/kg, intraperitoneal injection). Following the injection, animals were monitored for the absence of vital signs, including heartbeat, respiration, and reflexes. Death was confirmed by the lack of response to a toe pinch and cessation of all vital functions. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and adhered to ethical guidelines for animal welfare.

Tissue collection and processing

40 mg/kg of 1% pentobarbital sodium was i.p. injected into animals to achieve anesthesia and perfused through the heart with 0.9% saline (37 °C; 300 ml) followed by 4% paraformaldehyde in 0.01 M phosphate buffer (pH 7.4) at 4 °C (300 ml). The sciatic nerves were dissected at the mid-thigh region, approximately 1 cm on either side of the nerve anastomosis site, and postfixed in 4% paraformaldehyde at 4 °C for 6 h. For paraffin sectioning, the tissues were dehydrated through a graded series of alcohols. They were permeabilized with xylene, embedded in paraffin, and sectioned. The nerve sections were prepared in the longitudinal direction to assess the protein expression along the nerve fibers.

Masson's trichrome staining

The sciatic nerve side was fixed in 4% formaldehyde solution for 48 h. The tissue samples were dehydrated in an ethanol gradient (70%, 80%, 90%, and 100% ethanol, 30 min each), embedded in paraffin at 60 °C for 3 h, and sectioned into 5–10 μm slices. The sections were dewaxed in xylene for 10 min, then rehydrated in a descending ethanol gradient (100%, 90%, 80%, 70%) and rinsed in distilled water. The sections were then stained with Masson's stain, dehydrated in anhydrous ethanol and sealed in neutral resin. The sections were then examined under the microscope.

Immunofluorescence staining

The following primary antibodies were used: rabbit anti-FN polyclonal antibody (1:100,15613-1-AP; PTG), rabbit anti-NF-M polyclonal antibody (1:100, 25805-1-AP; PTG), rabbit anti-GAP43 recombinant monoclonal antibody (1:200, ET1610-94; HUABIO), rabbit anti-Tau recombinant monoclonal antibody (1:200, ET1612-44; HUABIO). The secondary antibodies were Cyanine 3-conjugated goat anti-mouse IgG (CY3,1:200; Servicebio), Cyanine 3-conjugated goat anti-rabbit IgG (CY3,1:200; Servicebio). Immunofluorescence staining was performed as described previously. Briefly, paraffin-embedded sections were deparaffinized, rehydrated and washed three times with 0.01 M phosphate-buffered saline (PBS). The tissue sections were treated with citric acid buffer (0.01 M, pH 6.0), antigen retrieval was performed by microwave irradiation at full power for 10 min and allowed to cool naturally to room temperature. After three washes with 0.01 M PBS, the slides were incubated with 0.01 M PBS containing 0.05% Triton X-100 for 15 min at room temperature, washed three times with 0.01 M PBS and incubated with 5% normal goat serum for 30 min at 37 °C to block non-specific staining.

Incubation with the appropriate primary antibodies was performed approximately 12–16 h at 4 °C. After repeated washing with 0.01 M PBS, the sections were incubated with the appropriate secondary antibodies for 30 min at 37 °C in the dark. The nuclei were counterstained with DAPI (1:100) for 5 min at room temperature, then washed 3 times with 0.01 M PBS (pH 7.4) for 5 min each to remove excess dye. After washing, the slides were mounted with a coverslip and examined under a upright microscope (OLYMPUS CK31, Olympus, Japan). DAPI dye was used for nuclear staining with ultraviolet excitation. The excitation wavelength was 330–380 nm, and the emission wavelength was 420 nm, emitting blue fluorescence. CY3 dye was used to label specific molecules, with an excitation wavelength of 510–560 nm and an emission wavelength of 590 nm, emitting red fluorescence.

Image J analysis of immunofluorescence images

Protein expression was analyzed using Image J software. In the semi-quantitative analysis of immunofluorescence images, Image J software was used to measure the average fluorescence intensity. The formula for calculating the average fluorescence intensity is Mean fluorescence intensity (Mean) = Integrated density (IntDen) / Area of the region of interest (ROI). First open your image by selecting File > Open. Using Image > Color > RGB Merge to isolate the red fluorescence channel by unchecking the green and blue channels. Next, go to Analyze > Measure to calculate the average fluorescence intensity, shown under the Mean column in the results window. Finally, save the results by selecting File > Save as in the results window.

Statistical analysis

Data are expressed as mean ± SD. GraphPad Prism software was used for analysis. The Kolmogorov-Smirnov test was applied to determine the data's normality. One-way analysis of variance was used to compare differences in quantitative measurements between multiple groups. For each sample, all assays were repeated at least three times. Statistical significance was defined as $P < 0.05$.

Results

EpoB promotes functional recovery following PNI

SFI scores ranging from 0 (normal function) to 100 (complete dysfunction) are used to assess motor recovery after sciatic nerve injury. Motor recovery over the 4 weeks was assessed using a gait trajectory analysis, which showed that there was a progressive improvement in the SFI over time. (Fig. 1). SFI did not differ significantly between the vehicle and EpoB groups with in the first 2 weeks. At 3 and 4 weeks after injury and treatment, the SFI in the EpoB group was elevated relative to the vehicle group ($P < 0.05$). The relationship with the observation time is short, with the passage of time, the functional recovery may be more obvious. But it suggesting functional recovery was achieved by the EpoB treatment.

EpoB reduced scarring

In the control group, the nerve fibers were neatly and densely arranged with a clear morphological structure. In the vehicle group, the nerve fibers are disorganized and sparse, the morphology is disrupted, the nuclei are hyperchromatic, the nerve fiber count is markedly decreased, and the scar is markedly hypertrophic. After treatment with EpoB, the structure of the nerve fibers

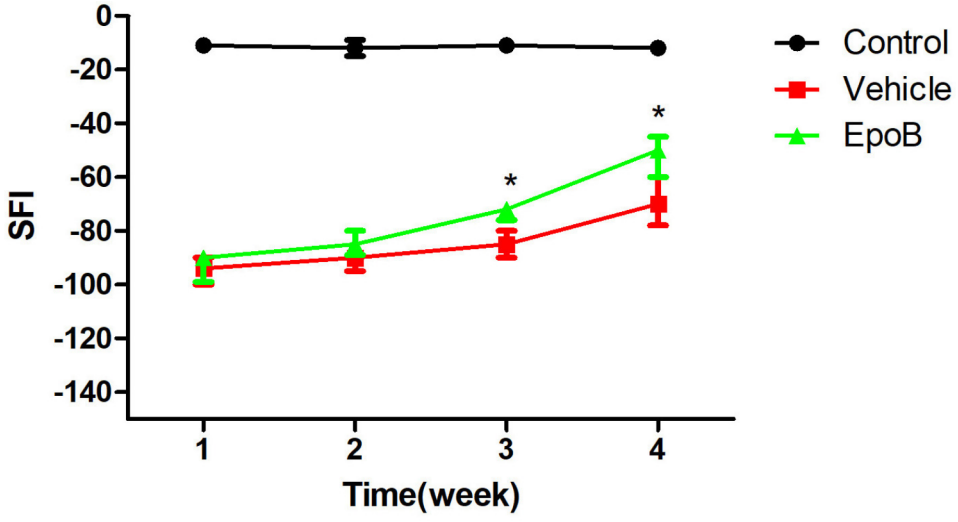

Fig. 1 Assessment of motor function recovery using sciatic functional index (SFI) calculation. The SFI values of each group at 1, 2, 3, and 4 weeks after surgery. *$p < 0.05$ for comparison with vehicle group. ($n = 6$ per group)

was clearer and more complete, and scarring was significantly reduced. (Fig. 2).

Effect of EpoB on protein expression of and FN, Tau, NF-M and GAP-43

Immunofluorescence labeling was then performed, and the Cy3 fluorescence signals were detected to identify immunoreactive FN, Tau, NF-M and GAP-43 protein. The levels of fibronectin were lower in the control group and significantly increased in the vehicle and EpoB group. The levels of FN were lower in the control group and significantly increased at 3 days ($P<0.01$), 7 days ($P<0.05$), 14 days ($P<0.05$) and 28 days ($P<0.001$) after sciatic nerve transection in the vehicle group. But there is no statistically significant between the vehicle group and EpoB group at 3,7,14 days. Compared with the vehicle group, the FN levels were lower in the EpoB group at 28 days ($P<0.05$) (Fig. 3, 4 A). The levels of Tau were lower in the control group and significantly increased at 7 days ($P<0.05$), 14 days ($P<0.05$) and 28 days ($P<0.01$), no statistically significant at 3 days after sciatic nerve transection. There is no statistically significant between the vehicle group and EpoB group at 7,14 days. Compared with the vehicle group, the Tau levels were stronger in the EpoB group at 28 days ($P<0.05$) (Figs. 3 and 4B). The levels of NF-M were lower in the control group and significantly increased at 7 days ($P<0.01$), 14 days ($P<0.001$) and 28 days ($P<0.001$), no statistically significant at 3 days after sciatic nerve transection. There is no statistically significant between the vehicle group and EpoB group at 7 days. Compared with the vehicle group, the NF-M levels were stronger in the EpoB group at 14 days ($P<0.05$) and 28 days ($P<0.05$) (Fig. 3, 4 C). The levels of GAP-43 were lower in the control group and significantly increased at 7 days ($P<0.05$), 14 days ($P<0.01$) and 28 days ($P<0.001$), no statistically significant at 3 days after

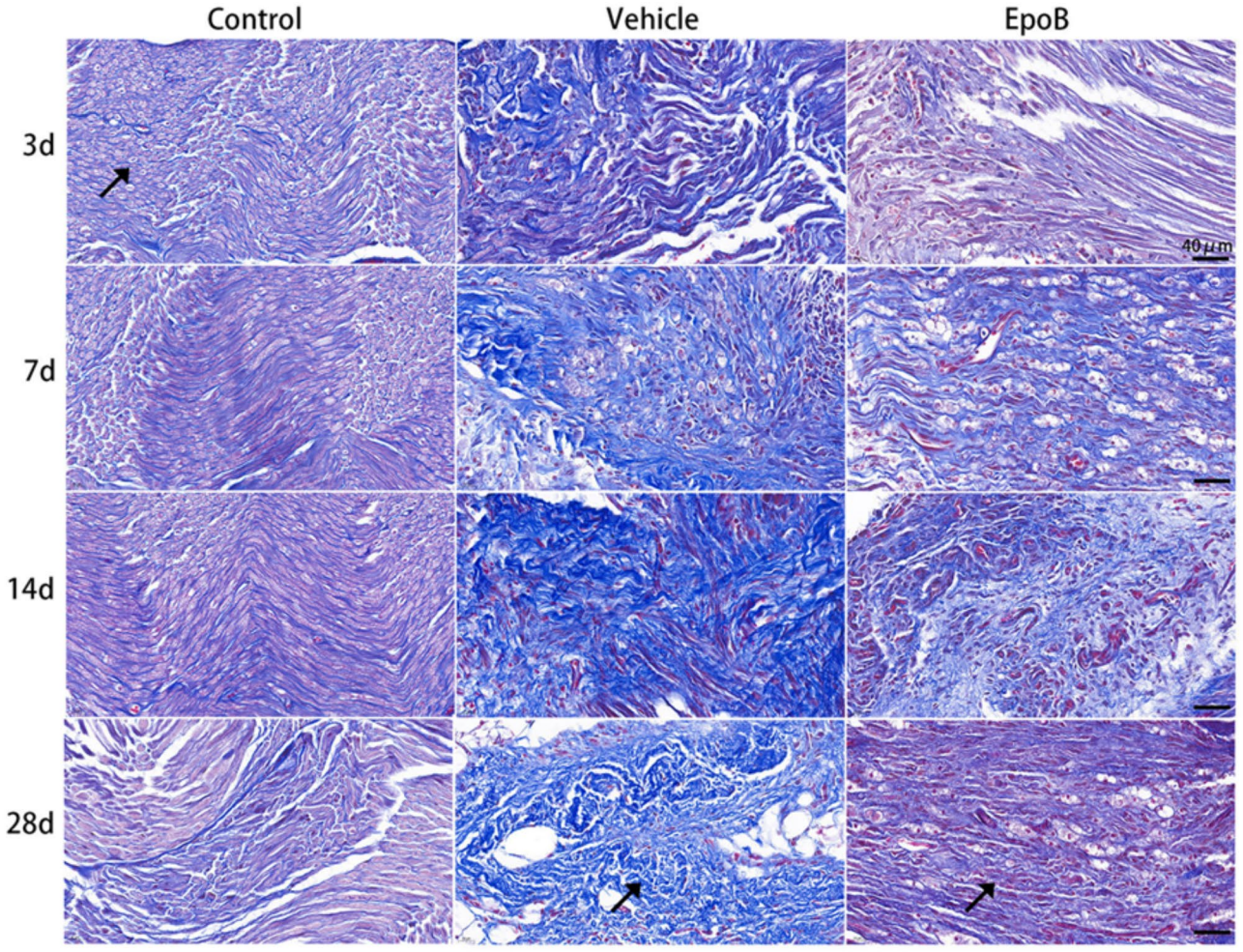

Fig. 2 Masson's trichrome staining of the sciatic nerve site at different time points. Blue staining indicates collagenous material, and red or dark red staining indicates nervous tissue. In the control group, the arrows point to normal nerve tissue with organized nerve fibers. In the vehicle group, the arrows indicate areas with significant scarring and disorganized nerve fibers after injury. In the EpoB group, the arrows highlight areas with clear nerve fibers and reduced scarring

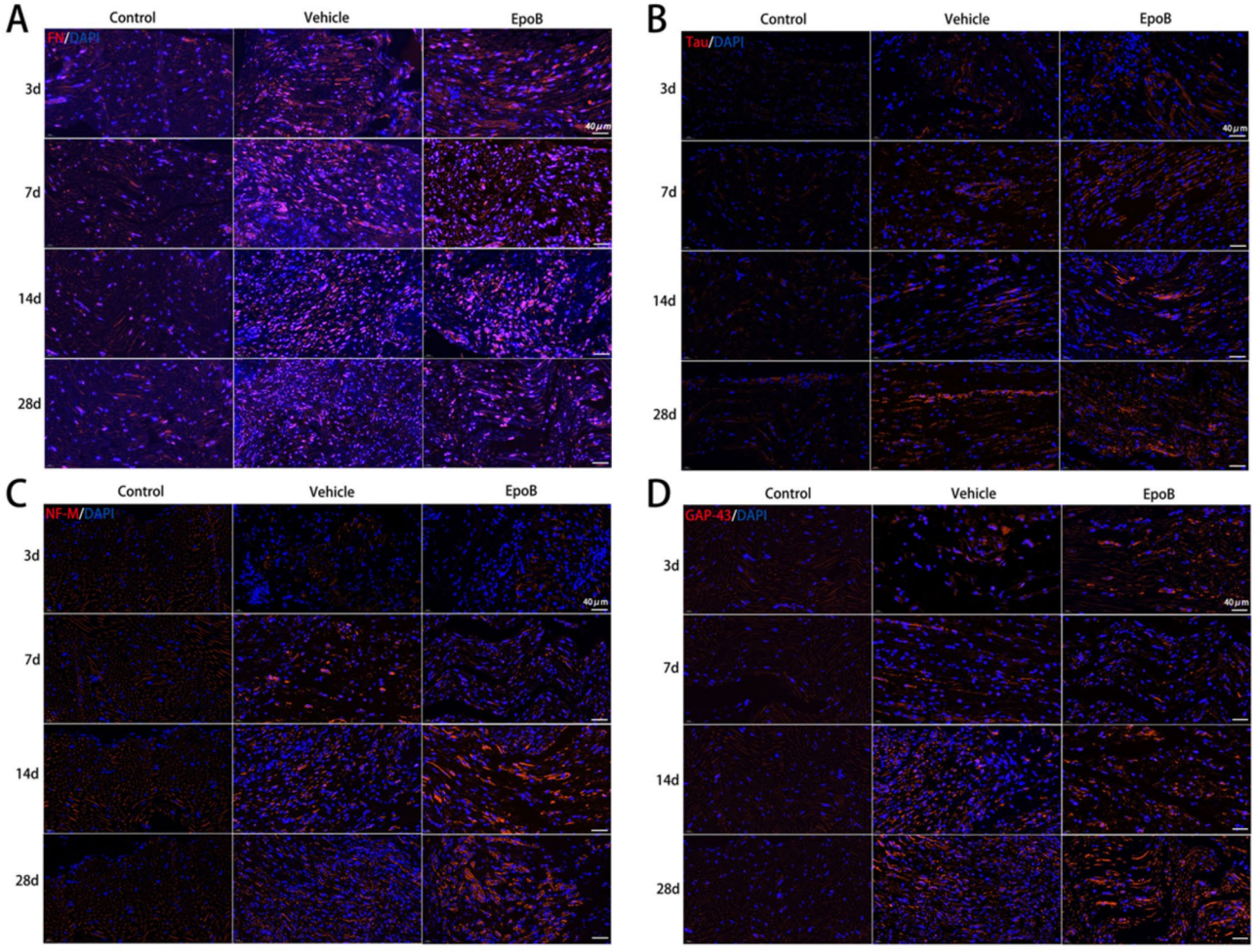

Fig. 3 FN, Tau, NF-M, GAP-43 protein expression after sciatic nerve transection in control, vehicle, EpoB group at 3, 7, 14 and 28 days. (**A**) The FN levels were lower in the EpoB group at 28 days, FN (red). (**B**) The Tau levels were stronger in the EpoB group at 28 days, Tau (red). (**C**) The NF-M levels were stronger in the EpoB group at 14, 28 days, NF-M (red). (**D**) The GAP-43 levels were stronger in the EpoB group at 28 days, GAP-43 (red). DAPI (blue); EpoB: Epothilone B; FN: Fibronectin; NF-M: Neurofilament-M; GAP-43: Growth associated protein-43; DAPI:4,6-diamidino-2-phenylindole; Scale bars: 40 μ m. rabbit anti-FN polyclonal antibody (1:100), rabbit anti-NF-M polyclonal antibody (1:100), rabbit anti-GAP43 recombinant monoclonal antibody (1:200), rabbit anti-Tau recombinant monoclonal antibody (1:200), cyanine 3-conjugated goat anti-mouse IgG (CY3,1:200), cyanine 3-conjugated goat anti-rabbit IgG (CY3,1:200)

sciatic nerve transection. There is no statistically significant between the vehicle group and EpoB group at 7, 14 days. Compared with the vehicle group, the GAP-43 levels were stronger in the EpoB group at 28 days ($P < 0.05$) (Figs. 3 and 4D). EpoB treatment leads to a significant increase in Tau, NF-M, and GAP-43 protein expression, suggesting positive effect on axonal regeneration.

Discussion

Peripheral nerve injury (PNI) and its clinical significance

PNI is highly prevalent in clinical settings, leading to irreversible tissue atrophy and dysfunction. It has been recognized as a significant burden on affected individuals, their families, and society [27]. Despite recent advances in peripheral nerve repair, the prognosis for PNI patients remains poor. Surgical procedures aimed at restoring the anatomical continuity of the injured peripheral nerve have been developed, but they show limited effectiveness in improving regeneration. Following injury, axonal outgrowth is regulated by various factors, including immune cell infiltration, phenotypic transformation of Schwann cells (SCs), and neurovascular regeneration [28]. Macrophages, neutrophils, endothelial cells, and SCs play crucial roles in different stages of the peripheral nerve repair process. SCs proliferate, form the myelin sheath along peripheral nerves, and are responsible for the formation of the bands of Bungner. Bungner's bands are cellular columns formed by Schwann cells in the injured nerve, which are essential for guiding regenerating axons. These bands are created by the reorganization of myelin and Remak Schwann cells distal to the injury site. Repair Schwann cells, derived from myelin and Remak cells,

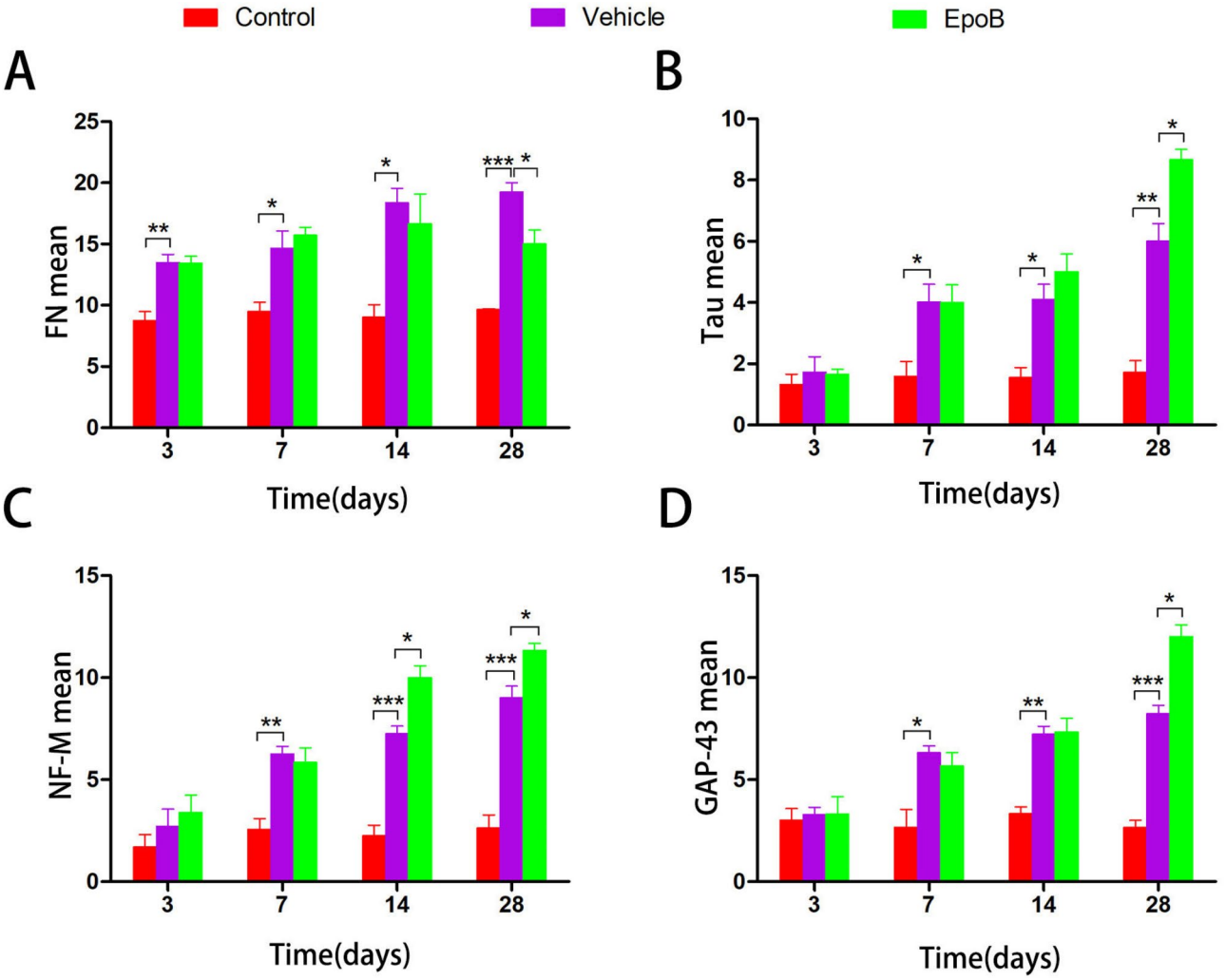

Fig. 4 Image J analysis of the mean immunofluorescence intensity of protein FN, Tau, NF-M and GAP-43 expression. Data are summarized from three independent experiments, and values are shown as the mean ± SD. Differences between groups were tested using one-way analysis of variance. *$P < 0.05$, **$P < 0.01$, ***$p < 0.001$

undergo morphological changes, including elongation and branching, to support axonal regeneration and remyelination. They play a crucial role in nerve repair [29].

Microtubule stabilization in axonal regeneration

Microtubule stabilization plays a crucial role in axon regeneration by maintaining structural integrity, facilitating efficient intracellular transport, and enhancing growth cone dynamics. Stabilized microtubules provide a solid scaffold for axonal elongation, ensuring that the cytoskeletal framework remains intact and resistant to fragmentation following injury. Furthermore, they actively support the growth cone's ability to navigate its environment, which is essential for proper axonal guidance and target reinnervation [30–32]. Our findings align with previous studies that indicate microtubule-targeting agents promote axonal outgrowth by reinforcing these mechanisms. Specifically, by stabilizing the microtubule network, the treatment likely improved axonal transport efficiency and enhanced the growth cone's structural and functional capabilities. This strengthening of the cytoskeletal framework may have contributed to both the observed axonal regeneration and the subsequent functional recovery in our model.

EpoB in peripheral nerve regeneration

EpoB is an antineoplastic and microtubule stabilizing macrolide that crosses the blood–brain barrier (BBB), has been approved for cancer therapy. In present many studies have identified the regenerative effect of EpoB in the central nervous system [19–20]. EpoB improves axonal regeneration and attenuate fibrotic scarring after spinal cord injury [19]. However, the potential therapeutic effects of EpoB on peripheral nerve regeneration has only just begun to be investigated and the effect of EpoB on peripheral nerves has received very little attention to

date. Zhou et al. reported that EpoB promotes sensorimotor recovery and axon regeneration following sciatic nerve crush injury in rats [23]. Omar et al. reported that EpoB is a microtubule-stabilizing agent with neuroprotective properties [22]. But in our study, SD rats received an intraperitoneal injection of EpoB at 0.75 mg/kg at 1, 15 days post-injury and employed the sciatic nerve transection anastomosis model. The expression changes of nerve growth related proteins after sciatic nerve transection were detected; it is further confirmed that EpoB can promote nerve regeneration and functional recovery.

Fibronectin (FN), Tau, NF-M, and GAP-43 in nerve regeneration

Fibronectin (FN) is one of the extracellular matrix (ECM) of peripheral nerves, which is non-collagen glycoproteins [33]. The ECM and SCs are associated with the nerve regeneration environment under both physiological and pathological conditions. Fibronectin can promote SC growth and motility [34]. However, with the increase of ECM, scar formation becomes a barrier and hinders nerve regeneration. FN is involved in cell adhesion and is essential for synaptic formation and repair in the nervous system. Tau protein is a very soluble microtubule-associated protein encoded by the gene tau. Tau is most abundant in neurons of the central nervous system. Tau is also found in the peripheral nervous system [35–36]. Zha et al. suggest that the expression and phosphorylation of tau may be involved in the repair of peripheral nerves, and that the modulation of the phosphorylated form of the tau protein may contribute to the regeneration of peripheral nerves [35]. Yi et al. suggest that tau modulates Schwann cell proliferation, migration and differentiation [36]. Growth-associated protein 43 (GAP-43), a neuronal protein known for its important role in axon guidance [37]. GAP-43 is normally involved in neurite outgrowth and axonal growth. It has been termed a growth-associated protein because its synthesis is upregulated during axonal regeneration [38]. Neurofilaments (NFs) are the most abundant component of mature neurons, forming the cytoskeleton with actin and microtubules [39]. Specifically expressed in the nervous system, neurofilament M (NF-M) proteins are critical in the regulation of axonal transport.

Functional recovery and limitations

The SFI scores in our study were higher in the EpoB-treated group, indicating partial locomotor recovery and muscle reinnervation. Given that changes in protein expression were observed at 28 days post-treatment, further studies with longer observation periods may provide a clearer understanding of the long-term functional benefits of EpoB treatment.

In our study after EpoB treatment, SFI scores were higher, indicating partial locomotor recovery and muscle reinnervation; scarring was significantly reduced; FN protein expression were dramatically lower; in addition tau, NF-M and GAP-43 protein expression was greater. But this study had several limitations: the difference between EpoB group and vehicle group is not particularly obvious; these changes were only evident at 28 days after two injections related to the time and measurement of administration drug; no quantification was performed in this study. In addition, the control group only received anesthesia and an intraperitoneal injection, without undergoing surgery. The control group should have undergone the same procedure as the experimental group, with nerve exposure but no transection. This would account for potential confounding effects from the surgical manipulation itself, such as tissue disruption and inflammation. The lack of a sham surgery makes it difficult to differentiate between the effects of nerve injury and those caused by the surgery. Future studies should include a sham surgery group for more accurate results. Although this study provides promising insights into the therapeutic potential of EpoB for peripheral nerve injury, further investigations into the optimal treatment regimen and long-term outcomes are warranted. Additionally, future studies should explore the mechanistic pathways underlying EpoB's effects, including potential interactions with Schwann cells, immune cells, and other signaling pathways involved in nerve regeneration.

Conclusion

Collectively, our results suggest that EpoB treatment leads to a significant increase in Tau, NF-M, and GAP-43 protein expression, suggesting a delayed but positive effect on axonal regeneration and repair. Our study helps to elucidate the importance of EpoB in enhancing regeneration and functional recovery in cases of PNI.

Abbreviations

EpoB	Epothilone B
PNI	Peripheral nerve injury
FN	Fibronectin
NF-M	Neurofilament-M
GAP-43	Growth-associated protein-43
SFI	Sciatic Functional Index

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Author contributions

Wei Zhao. Kun-xiu Song and Bing-dong Ma. Yong-tao Liu wrote the main manuscript text and Guang-chao Sun Yong Chai.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All experiments involving rats were approved by the Ethics Committee for Animal Care and Use of Binzhou Medical University in China.

Competing interests
The authors declare no competing interests.

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