Molecular mechanism of electroacupuncture treatment on oxaliplatin-induced peripheral neurotoxicity in rats

Du Shanshan,1,* Tingting Yang,1,* Sun Qiang,2 and Zhang Lin1

Abstract
Oxaliplatin (L-OHP) has been widely used in the treatment of various tumors, especially in colorectal cancer. The mechanism of peripheral neurotoxicity induced by L-OHP (OIPN) is unclear and current therapeutic options only serve to alleviate the symptoms rather than prevent OIPN. To observe the effects of electroacupuncture (EA) stimulation on OIPN in rats, rats were randomly divided into the control group, OIPN model group, and OIPN + EA group. To establish OIPN rat models, a single intraperitoneal injection with 10 mg/kg L-OHP in the OIPN model rats. Rats in the control group received a single intraperitoneal injection with 0.9% lactose. From the third day after establishing OIPN models, the rats were treated with EA. Cold allodynia and heat sensitivity were assessed using the acetone drop and the Hargreaves method, respectively. Pathological changes in nerves were detected using hematoxylin and eosin (H&E) staining and transmission electron microscopy. Related mRNA and protein expression levels were measured by real-time polymerase chain reaction (PCR) and western blotting from 14 days after establishing OIPN models. Our results showed that L-OHP significantly increased the sensitivity to cold allodynia and nervous injury, which were ameliorated after EA treatment. The expression of glucocorticoid receptor alpha (GR-α) and B-cell lymphoma 2 (Bcl-2) was significantly decreased and that of Bax was significantly increased in sciatic nerve of OIPN model rats compared with control rats. However, EA treatment significantly inhibited L-OHP-induced protein expressions in rats. Moreover, compared with the control rats, the nucleus NF-κBp65 levels were significantly increased, while the cytoplasm NF-κBp65 levels were significantly decreased, which were reversed by EA treatment. In conclusion, EA treatment may reduce peripheral neurotoxicity induced by L-OHP through regulating related protein expression.

Keywords
electroacupuncture, molecular mechanism, oxaliplatin, peripheral neurotoxicity

Introduction
As the incidence of tumor increases, oxaliplatin (L-OHP) has been widely used in the treatment of various tumors, especially in colorectal cancer. It can effectively inhibit DNA synthesis through the formation of DNA intrastrand cross-linking.1 Currently, L-OHP combined with leucovorin and 5-fluorouracil has become first-line chemotherapy regimens for advanced colorectal cancer.2 L-OHP...
has the characteristics of good efficacy and low toxicity, but the side effect of peripheral neurotoxicity, which limits its application in clinics.\textsuperscript{3,4} There are two types of neurotoxicity induced by L-OHP, one is acute neurotoxicity occurring within 24 h after infusion, and the other usually occurs after 24 h of infusion, which is cumulative chronic neurotoxicity.

The mechanism of peripheral neurotoxicity induced by L-OHP is unclear and current therapeutic options only serve to alleviate the symptoms rather than prevent peripheral neurotoxicity induced by L-OHP (OIPN). L-OHP can delay the inactivation of voltage-gated Na\(^{+}\) channel, resulting in the increase of Na\(^{+}\) influx, and reduce the gap between the resting membrane potential and the threshold potential, which is related to the alteration of Na\(^{+}\) channel through oxalate-dependent regulation of Ca\(^{2+}\) signaling.\textsuperscript{5,6} The mechanism of L-OHP-induced chronic neurotoxicity may be involved in the inhibition of the nucleolar rRNA synthesis in sensory neurons of dorsal root ganglia, causing dysfunction of protein synthesis and abnormal morphological changes and functional impairment of sensory neuron organelles.\textsuperscript{7,8} L-OHP also causes axonal transport and dysfunction of peripheral nerve growth factor (NGF) secretion, which is important in the neuronal protein synthesis, resulting in the damage to the structure and function of the soma and the peripheral nerve.\textsuperscript{9} Moreover, oxidative stress is another mechanism contributed to OIPN by formation of DNA adducts and destruction of mitochondrial respiratory chain leading to mitochondrial damage.\textsuperscript{10}

Electroacupuncture (EA) is a modified acupuncture technique that induced analgesic effects on neuropathic pain have been reported in several clinical studies.\textsuperscript{11–13} Previously studies found that EA at specific acupoints can improve the clinical symptoms of peripheral neuropathy,\textsuperscript{14,15} which may be related to the improvement of the peripheral nerve conduction velocity, and some patients can even achieve healing. Although it has not been studied whether or how EA treatment of OIPN, the possible mechanisms including (1) the electric field of acupuncture is beneficial to the regeneration of injured nerve and the improvement of ion channel function\textsuperscript{16}; (2) the electric field of acupuncture can promote the growth of nerve growth–associated proteins\textsuperscript{17}; and (3) the electric field of acupuncture can promote the expression of S-100 protein in Schwann cells.\textsuperscript{18} Moreover, EA can also inhibit the abnormal pain induced by cold stimulation by regulating the gamma-aminobutyric acid (GABA) receptor in spinal cord of rats.\textsuperscript{19} The transcription factor NF-\(\kappa\)B is a critical regulator of immune and inflammatory responses. In mammals, the NF-\(\kappa\)B/Rel family comprises five members: p50, p52, p65 (Rel-A), c-Rel, and Rel-B proteins, which form homo- or heterodimers and remain as an inactive complex with the inhibitory molecules called IkB proteins in resting cells. The most abundant form of NF-\(\kappa\)B activated by pathologic stimuli via the canonical pathway is the p65:p50 heterodimer. Disproportionate increase in activated p65 and subsequent transactivation of effector molecules are integral to the pathogenesis of many chronic diseases such as the rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and even neurodegenerative pathologies. Hence, the NF-\(\kappa\)Bp65 signaling pathway has been a pivotal point for intense drug discovery and development.

Although clinical predictors for both acute and chronic OIPN have been suggested, to the best of our knowledge, no reliable genetic or molecular biomarkers have been identified to date. The glucocorticoid receptor alpha (GR-\(\alpha\)) is an active regulator in inflammatory responses that plays an important role in neuropathic pain induced by nerve injury\textsuperscript{20} and binds to and inhibits expression of NF-\(\kappa\)Bp65.\textsuperscript{21} In this study, we investigate whether EA can improve peripheral neurotoxicity induced by a single injection of L-OHP in rats and the underlying molecular mechanism involved in GR-\(\alpha\) NF-\(\kappa\)B signaling.

**Materials and methods**

**Sample size**

Sample size was determined by the power analysis and the calculation was made by the software G Power (Faul, Erdfelder, Lang, and Buchner, 2007) for sample size calculation.

**Experimental OIPN model.** Male Sprague Dawley rats (age, 4 weeks; weight, 250–300 g) were obtained from Shanghai Laboratory Animal Company (Shanghai, China) had a dark/light cycle of 12/12 h in the animal facility at 25°C (humidity, 60%–70%) with available water and food freely, and were randomly divided into three groups: control groups
(n=5), OIPN groups (n=5), and OIPN + EA treatment groups (n=5). In control groups, rats were single injected in left lower abdominal cavity with 0.9% lactose, once a day for 14 days. In OIPN groups, rats were single injected in left lower abdominal cavity with 10 mg/kg L-OHP diluted with 0.9% lactose, once a day for 14 days. From the third day after establishing OIPN models, the rats were treated by EA, 20 min each time, once a day for 12 days as previously described. Briefly, a pair of stainless steel acupuncture needles was inserted into the bilateral ST36, LI10, GB30, and LI12 acupoints with constant rectangular current pulses (2 Hz, 0.3 ms pulse duration). The stimulating current ranged from 1 to 3 mA: 1 mA, nuclear and cytoplasmic protein was extracted with NE-PER™ Nuclear and Cytoplasmic besides RNA was extracted using TRIzol reagent during the first 5 min, 2 mA during the second 5 min, and 3 mA for the remaining 10 min. All experimental protocols were approved by the Animal Care Committee of the Putuo Hospital, Shanghai University of Traditional Chinese Medicine. From the 14th day after establishing OIPN models, rats were anesthetized with chloral hydrate, and the rat nerves, which size was 0.5 cm³, were collected and stored at −80°C. Sciatic nerve was used for the anesthesia purpose. The dose of chloral hydrate was 400 mg/kg with concentration of 5% i.p. The choice of anesthetic agent depends on the procedure to be performed, research aims, and other factors such as animal age. Consultation with area veterinarian with questions about drug selection was also done. This model is of great clinical interest as it ranks among the most common dose limiting toxicities of oxaliplatin (OXA) administration with an obvious impact on the outcome of cancer patients. In addition, it has a unique spectrum of clinical presentation, being manifested with two distinct syndromes: the acute neurotoxicity that appears soon after OXA administration and is usually transient, and the chronic cumulative syndrome that resembles the characteristics of all platinum compounds.

**Blood biochemical indicators and routine blood indexes**

Rats were anesthetized with 1% pentobarbital sodium (0.4 mg/kg) and were sacrificed 6 h after the last EA treatment. A blood sample (2 mL) was collected from the aorta ventralis and homogenized with heparin. The blood samples were analyzed with the MEK-6018 automated hematology analyzer for the following indicators: white blood cell (WBC) count, hemoglobin (Hb) level, and blood platelet (PLT) count. The Reitman–Frankel method was used to detect the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and the sarcosine oxidase method was used to detect the serum creatinine (Cr) concentration.

**Hematoxylin and eosin staining and histological observation of the sciatic nerve and coccygeal nerve.** For light microscopic observation, the samples of rat nerves were fixed with 4% formaldehyde and dehydrated in ascending concentrations of alcohol ranging from 75% to 100%. The sections were stained with hematoxylin and eosin (H&E) for histological observation. For transmission electron microscopic observation, nerve tissues were fixed with pre-cooled (4°C) 3% glutaraldehyde, and this was followed by fixation with 1% osmic acid and staining with uranyl acetate and lead citrate.

**Cold allodynia test.** The cold allodynia test was performed on post-modeling day 14, according to the protocol of Flatters and Bennett. Briefly, rats were placed on a metal net with a transparent glass cover (50 cm × 50 cm × 50 cm). After acclimatization in the test environment for 10 min, a drop of acetone (50 μL) was placed at the center of the plantar hind paw. The responses of the rats were evaluated and scored as follows: 0 = no response, 1 = quick withdrawal, flick or stamp of the paw, 2 = prolonged withdrawal or repeated flicking, and 3 = repeated flicking of the paw with licking directed at the ventral side of the paw. Acetone was applied three times to each paw with an interval of 5 min between each application (six applications in total). The cumulative scores were calculated by summing the six scores for each rat, with the scores ranging from 0 to 18, and 18 representing the highest sensitivity to cold stimulation, the lowest pain threshold, and the most intensive pain.

**Heat sensitivity test.** The heat sensitivity test was performed on post-modeling day 14. The luminous power used was 36 W. A canister was placed on top of the light pain threshold detector, which was tightly attached to the base. A rat was placed in the canister with the tail in the center of the photoelectric-controlled probe, and the recording...
was discontinued when the rat’s tail moved. Each rat was tested three times, and the average value of the recordings was calculated.

**Real-time polymerase chain reaction.** RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) to obtain cell lysate. Afterward, PrimeScript reagent kit of reverse reaction (DRR037A; TaKaRa) was used to carry out reverse transcription (RT) reaction on RNA in accordance with protocols of manufacturer. General and real-time RT-polymerase chain reaction (PCR) amplification was performed using HotStartTaq polymerase (TaKaRa) and SYBR Premix Ex Taq TM II kit (TaKaRa, Mountain View, CA, US), respectively, according to manufacturer’s instruction. Expression levels are given as ratios to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers used in this study were subsequently shown: GR-α-F, 5'-AATGGGCAAGGCAGTAC-3’ and GR-α-R, 5’-CAGGAGCAAGCAGAGCAG-3’; GAPDH-F, 5’-GGAGTCTACTGGCGTCTTCAC-3’ and GAPDH-R, 5’-ATGAGCCCTTCCACGATGC-3’. The assays were repeated in triplicate.

**Western blotting.** Total protein extraction was performed with radioimmunoprecipitation assay (RIPA) lysis buffer and then centrifuged at 12,000g for 20 min at 4°C. Nuclear and cytoplasmic protein was extracted with NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Waltham, MA, USA) and then centrifuged at 16,000g for 20 min at 4°C. Proteins in cell lysates were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to the polyvinylidene fluoride membrane (PVDF) (Bio-Rad, Hercules, CA, USA). Blots were blocked using skim milk for 1h, and then incubated with GR-α (1:1000, Abcam, Eugene, OR, USA), Bcl-2 (1:300; Santa Cruz Biotechnology, Dallas, TX, USA), Bax (1:300; Santa Cruz Biotechnology), NF-κB (1:1000, Cell Signaling Technology, Danvers, MA, USA), H3 (1:1000, Cell Signaling Technology), or GAPDH (1:2000, Cell Signaling Technology) primary antibodies at 4°C overnight. Afterward, the peroxidase-conjugated secondary antibodies (1:1000, Beyotime Biotechnology, China) were further used for 2h at room temperature. Blots were analyzed using an enhanced chemiluminescence detection kit (Pierce, Rockford, IL, USA). The measurements were repeated for three times.

**Statistical analysis.** Results were shown as mean values ± standard deviation (SD). Statistical analysis was performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Comparison was analyzed by one-way analysis of variance followed by Tukey’s post hoc test. A P value of less than 0.05 was considered statistically significant.

**Results**

**Changes in blood biochemical indicators and routine blood indexes after EA treatment**

After the EA treatment, the WBC count was 7.19 ± 2.64 (10^9/L), 5.79 ± 1.88 (10^9/L), and 7.63 ± 2.43 (10^9/L); the Hb level was 128.6 ± 38.88, 113.0 ± 35.79, and 126.1 ± 37.41 g/L; the PLT value was 1461.2 ± 214.64 (10^9/L), 1552.8 ± 264.35 (10^9/L), and 1491.4 ± 169.56 (10^9/L); the ALT value was 23.4 ± 6.87, 28.2 ± 7.02, and 24.7 ± 5.95 IU/L; the AST value was 82.5 ± 26.22, 91.1 ± 23.73, and 76.4 ± 26.26 IU/L; and the CR value was 14.1 ± 5.78, 17.4 ± 4.97, and 16.3 ± 5.91 mmol/L in the control group, model group, and EA group, respectively. There was no significant difference in these parameters between the three groups (P > 0.05, Table 1).

**Pathological changes in the nervous system after EA treatment**

In the sciatic nerve control group, well-arranged nerve fibers and normal myelin sheath could be observed. After administration of L-OHP, we observed some nerve fiber swelling and transection injuries in the sciatic nerves, as well as demyelinating changes in vacuoles in the model group. EA treatment ameliorated demyelination, as more normal myelin was found in the EA treatment group. In the coccygeal nerve control group, nerve fibers were tightly arranged and equally distributed with normal myelin sheath. In the model group, a considerable number of demyelinated sites of coccygeal nerves with dissolved myelin sheath were observed, and nerve fiber swellings and transection injuries were also observed. These pathological changes were ameliorated after EA treatment, which resulted in only a few demyelinated and dissolved myelin sheaths, as well as a mild decrease in the number of sheath cells (Figure 1). These results suggest that L-OHP-induced nerve injury can be inhibited by EA treatment. Pathological changes in the coccygeal nerves under transmission electron
microscopic observation showed that the nerves were well myelinated with a relatively consolidated, dense myelin structure of certain thickness; mitochondria are abundant without obvious swelling and with only few fractures of mitochondrial cristae; and in the cytoplasm, certain organelles with only few vacuoles are observed in the control group. We observed only a sparse amount of myelin in the coccygeal nerves after L-OHP administration, with variations in the morphology and thickness of myelin. Besides, the number of mitochondria had significantly decreased, and some of

Table 1. WBC, Hb, PLT, ALT, AST and Cr values after EA treatment.

|                  | Control          | L-OHP            | L-OHP + EA        |
|------------------|------------------|------------------|-------------------|
| **WBC (10^9/L)** | 7.19 ± 2.64      | 5.79 ± 1.88      | 7.63 ± 2.43       |
| **Hb (g/L)**     | 128.6 ± 38.88    | 113.0 ± 35.79    | 126.1 ± 37.41     |
| **PLT (10^9/L)** | 1461.2 ± 214.64  | 1552.8 ± 264.35  | 1491.4 ± 169.56   |
| **ALT (IU/L)**   | 23.4 ± 6.87      | 28.2 ± 7.02      | 24.7 ± 5.95       |
| **AST (IU/L)**   | 82.5 ± 26.22     | 91.1 ± 23.73     | 76.4 ± 26.26      |
| **Cr (mmol/L)**  | 14.1 ± 5.78      | 17.4 ± 4.97      | 16.3 ± 5.91       |

EA: electroacupuncture; WBC: white blood cell; Hb: hemoglobin; PLT: blood platelet; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: serum creatinine.

Figure 1. (a) Effects of EA on nerve injury in L-OHP-induced rats. From the 14th day after establishing OIPN models, sciatic and coccygeal nerve tissues were stained with hematoxylin and eosin (H&E) for histological observation. Scale bar: 100 μm. (b) The morphological observations of dorsal root ganglia. (c) Effects of EA on the pathological changes of coccygeal nerves in L-OHP-induced rats. (d) Neurophysiology data: panel A represents the average waveform in the time domain; panel B represents the mean power of the data; and panel C represents phase locking factor of the data.
the mitochondrial cristae were fractured. Nuclear chromatin was aggregated, the number of cytoplasmic organelles had decreased, and the number of vacuoles had increased. In contrast, EA treatment induced an increase in the amount of myelin sheath, and the morphology of the myelin sheath was more consistent. In addition, EA treatment increased the number of mitochondria, decreased the amount of mitochondrial crista fractures and vacuoles, and increased the number of cytoplasmic organelles (Figure 1 (a)).

Effect of EA on cold allodynia and heat sensitivity

The sensitivity to cold allodynia varied between the groups after EA treatment. It was 2.2 ± 1.48 in the control group and 4.5 ± 1.78 in the EA group, while it increased to 8.5 ± 4.79 in the model group. Sensitivity to cold allodynia in the model group and EA group was significantly higher than that in the control group, whereas that in the EA group was significantly lower than that in the model group (Table 2). After EA treatment, the heat sensitivity was 5.45 ± 1.33, 4.63 ± 1.23, and 4.66 ± 1.46 s in the normal, model, and EA group, respectively (Table 2). We did not detect any difference in heat sensitivity after EA treatment, which means that EA treatment did not influence heat sensitivity in the rats.

Effects of EA on the expressions of GR and NF-B p65 in OIPN model rats

From the 14th day after establishing OIPN models, the mRNA and protein expressions of GR-α were measured by real-time PCR and western blotting, respectively. As shown in Figure 2(a) and (b), L-OHP treatment significantly decreased the mRNA and protein expressions of GR-α by 73.2% and 64.9% in sciatic nerve of rats. However, EA treatment significantly increased the mRNA expression of GR-α by 114.9% and 85.7% compared with L-OHP-induced rats (Figure 2(a) and (b)). From the 14th day after establishing OIPN models, the protein expression of cytoplasmic NF-κBp65 was significantly decreased by L-OHP treatment, while that of nuclear NF-κBp65 was significantly increased by L-OHP treatment compared with control rats (Figure 2(c) and (d)). However, EA treatment significantly increased the protein expression of cytoplasmic NF-κBp65, and decreased that of nuclear NF-κBp65 in sciatic nerve of L-OHP-induced rats (Figure 2(c) and (d)).

Effects of EA on Bcl-2 and Bax expression in OIPN model rats

Moreover, L-OHP treatment significantly decreased the protein expression of Bcl-2 by 87.7% and increased that of Bax by 4.57-fold compared with control in sciatic nerve of rats after 14 days (Figure 3(a) and (b)). However, EA treatment significantly increased the protein expression of Bcl-2 by 3.61-fold and decreased that of Bax by 45.4% compared with L-OHP-induced rats.

Discussion

Oxaliplatin (L-OHP) is the third generation of platinum-based chemotherapeutic drugs after cisplatin and carboplatin and is used in the first-line treatment of colorectal cancer with advantages of wide anticancer spectrum, strong curative effect, and no cross resistance with cisplatin and carboplatin.23,24 However, it has a side effect of peripheral neurotoxicity. Acupuncture has certain curative effect on the treatment of peripheral neuropathy caused by L-OHP;25 however, animal experimental studies involving the relevant effects and molecular mechanism were deficiency.

In our study, we found that administration of L-OHP for 14 days does not affect the levels of WBC, Hb, PLT, ALT, AST, or Cr. Thus, the OIPN model is safe for experimental rats. The hematological variation is the key and baseline indicator to inform about any infection or the unwanted effect;

| Table 2. Behavioral tests for detecting sensitivity to cold/heat stimulation after EA treatment. |
|---------------------------------------------|-----------------|-----------------|
| Sensitivity to cold (score)  | Control     | L-OHP          | L-OHP + EA      |
| 2.2 ± 1.48                   | 8.5 ± 4.79*   | 4.5 ± 1.78**   |
| Sensitivity to heat, latency (s) | 5.45 ± 1.33  | 4.63 ± 1.23    | 4.66 ± 1.46    |

EA: electroacupuncture.

*P < 0.01 compared with control; **P < 0.01 compared with L-OHP.
hence, the blood reports were quite all right and the model can be said successful. Sensitivity to cold stimulation in the model group was significantly higher than that in the control group, and EA treatment significantly downregulated sensitivity to cold stimulation. Thus, EA ameliorates the symptoms of crymodynia. Our study also shows that there is no difference in sensitivity to heat stimulation between the three groups, which is consistent with the results of previous studies. Indeed, a lowered threshold for cold pain is the one of the main manifestations of OIPN, and patients with OIPN experience exacerbated pain when they encounter cold stimulation. Through our observations, we found that after EA treatment, the injury of nerves and coccygeal nerves was observed while that was ameliorated after EA treatment. Neuropathic pain induced by nerve injury causes prolonged suffering and significantly decreases the quality of life in affected patients. Glucocorticoid receptor (GR) is a member of the nuclear receptor superfamily and a type of hormone-dependent transcriptional regulation factor has been verified to involve in neuropathic pain. In this study, we demonstrated that L-OHP stimulation significantly decreased the GR-α expression and cytoplasmic NF-κBp65 level, while increase of the nuclear NF-κBp65 level in sciatic nerve of rats 14 days after establishing OIPN models, which was reversed by EA treatment. Recently, NF-κB has been found to be important for maintaining redox homeostasis in healthy cells, and constant activation of NF-κB results in the development of peripheral neuropathy through increasing oxidative stress and neuroinflammation. GR-α mediated transcriptional inhibition of downstream gene NF-κB inhibits the development of neuropathic pain in spinal cord in the spared nerve injury rats. These results suggest that EA may protect against L-OHP-induced peripheral neurotoxicity through the GR-α NF-κB signaling pathway. L-OHP-induced cytotoxicity in SH-SY5Y cells through increasing reactive oxygen species (ROS) production, cytochrome C release, caspase-3 activation and DNA damage, and decreasing mitochondrial membrane potential and Bcl-2/Bax ratio. Previous study reported that EA treatment reduced neuronal apoptosis through decreasing Bax and increasing Bcl-2 expression in the spinal cords of cats following partial resection of dorsal root ganglion. In line with previous studies, our study showed that L-OHP significantly increased Bax/Bcl-2 ratio in sciatic nerve of rats 14 days after

Figure 2. Effects of EA on the expressions of GR-α and NF-κBp65 in L-OHP-induced rats. From the 14th day after establishing OIPN models, the expression of GR-α and NF-κBp65 was measured by real-time PCR (a) and western blotting (b)–(d). **P < 0.01. (e) Neurophysiological recording of sciatic nerves. From the 14th day after establishing OIPN models, the pathological changes of coccygeal nerves in L-OHP-induced rats were measured by transmission electron microscopic observation. Scale bar: 5 μm.
establishing OIPN models, which was reversed by EA treatment. Furthermore, one of the main functions of GR-α is the induction of apoptosis, either by inhibiting the expression of the Bcl-2 or by increasing the expression of Bax.30 However, the participation of GR-α in lip carcinogenesis does not involve modulation of the expression of Bax or Bcl-2.31 Thus, the direct and indirect interaction between GR-α and Bax or Bcl-2 in L-OHP-induced peripheral neuropathy needs further investigation.

Throughout the years, many different mechanisms have been proposed explaining how GR inhibits pro-inflammatory gene expression, including direct mechanisms as well as feedback loop mechanisms by GC-induced anti-inflammatory proteins. Suggestive of conserved mechanisms among nuclear receptors, the fibrate ligand-activated transcription factor peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear hormone receptor superfamily, may also exert anti-inflammatory actions by downregulating the activity of NF-κB and other pro-inflammatory transcription factors via multiple mechanisms, with some reminiscent of the ones GR is deploying. Several chronic inflammatory pathologies are associated with dysregulated phosphorylation/activation of the NF-κB:IkB complex. Hence, preventing phosphorylation using kinase inhibitors constitutes attractive therapeutic strategy. A vast number of kinase inhibitors target either the IkB kinase (IKK) complex or the adapter proteins in the NF-κB signal transduction pathway. The therapeutic potential

Figure 3. Effects of EA on Bcl-2 and Bax expression in L-OHP-induced rats. (a) and (b) From the 14th day after establishing OIPN models, the expression of Bcl-2 and Bax was measured by western blotting, ***P < 0.01. (c) Observation of sciatic nerve by TEM.
of these kinase inhibitors has been predominantly evaluated in multiple cancers. While none of these have been shown to specifically target p65, a few affect the post-translational modifications in p65 by inhibiting the associated cofactors. For example, p38 mitogen-activated protein kinase (MAPK) inhibitor has been shown to inhibit phosphorylation of the coactivator p300 and preclude acetylation on Lys310 of p65, thereby preventing DNA binding and transcriptional activity. Second-generation p38 MAPK inhibitors are being evaluated in clinical trials for chronic inflammatory diseases such as chronic obstructive pulmonary disease (COPD), Crohn’s disease, and Alzheimer’s disease. The actions of glucocorticoids are predominantly mediated through the classic GR. GRs are expressed throughout the body, but there is considerable heterogeneity in glucocorticoid sensitivity and biological responses across tissues. Ligand-activated GR induces or represses the transcription of thousands of genes through direct binding to DNA response elements, physically associating with other transcription factors, or both.

**Limitation of the study**

Animal testing is used in pharmaceutical and industrial research to predict human toxicity, and yet analysis suggests that animal models are poor predictors of drug safety in humans. Increasingly, investigators are questioning the scientific merit of animal research.

**Conclusion**

In conclusion, EA treatment may effectively inhibit nerve injury induced by L-OHP in rats through the GR-α-NF-κB signaling pathway and regulating Bax and Bcl-2. Therefore, our study may provide basic information much needed for recognizing OIPN and designing of more effective targeted therapies.

**Animal welfare**

NIH guideline for lab animals was followed while carrying this project.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical approval**

This research is approved by Gumei Community Health Service Center of Minhang District, Shanghai.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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