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Therapeutic dosage of ozone inhibits autophagy and apoptosis of nerve roots in a chemically induced radiculoneuritis rat model

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Abstract. – OBJECTIVE: Radiculoneuritis characterizes by the neurogenic pain along the back of patients. This study aims to investigate the therapeutic effects of ozone on radiculoneuritis and the associated mechanisms in rat models.

MATERIALS AND METHODS: A chemical radiculoneuritis rat model was successfully established. The rats were divided into 3 groups, including radiculoneuritis Model rats group (Model group, n=18), Ozone therapy group (n=18), and Normal control group (n=18). Ozone was administered at a dosage of 1 mg/kg/day. The electron microscope was used to observe autophagosomes in the cytoplasm. Immunohistochemistry assay was performed to examine cleaved caspase 3 and double-labeled immunofluorescence assay was used to detect light chain 3B (LC3B) and neuronal nuclear antigen (NeuN) expression. Quantitative Real-time PCR (RT-PCR) and Western blot were employed to evaluate the expression of LC3B, Beclin 1, phosphodiesterase 2A (PDE2A), and nuclear factor-kB p65 (NF-kBp65).

RESULTS: Ozone significantly decreased autophagosomes formation and inhibited autophagy of nerve root cells in radiculoneuritis rat model. Ozone significantly decreased levels of autophagosomes initiator, LC3B, compared to Model group ($p<0.05$). Ozone significantly decreased cleaved caspase 3 expressions and alleviated apoptosis of nerve root cells compared to that of Model group ($p<0.05$). According to RT-PCR and Western blot assay, ozone significantly suppressed LC3B and Beclin 1 expression compared to that of Model group ($p<0.05$). Ozone significantly decreased PDE2A and NF-kB p65 expression compared to that of the Model group ($p<0.05$).

CONCLUSIONS: Therapeutic dosage of ozone inhibits autophagy by suppressing LC3B and Beclin 1 expression and reduces apoptosis by blocking NF-kB signaling pathway.

Key Words:
Radiculoneuritis, Autophagy, Apoptosis, Ozone, Nerve root.

Introduction

Radiculoneuritis characterizes by the neurogenic pain along the back of patients, radiating into the foot and legs, with tingling and numbness in the legs[1,2]. The radiculoneuritis is the most common symptom of patients with peripheral Lyme disease[3]. Radiculoneuritis in clinical mainly was defined as the inflammation of the dorsal root ganglia and the nerve roots following with the inflammatory perineuritis or infiltrate[4]. The radiculoneuritis patients always illustrate the electrophysiological abnormalities, slowed nerve conduction, widespread axonal damage[5]. In clinical, the radiculoneuritis is difficult to be treated and has been become a medical problem, which is needed to be resolved urgently. Although many rehabilitation and the oral pharmacological treatments have been used to the radiculoneuritis therapy, the long-time effects are relatively lower in clinical. Ozone therapy has been considered as the effective or alternative treatment option for the patient with back pain[6]. To date, a few mechanisms for the roles of ozone have been discovered, such as anti-inflammatory, analgesic, injury-inductive, and oxidant effects[7]. Meanwhile, the ozone also plays important roles in treating Buruli ulcer and exhibits a clinical excellent outcome[8]. Dall'Olio et al[9] also reported that ozone could

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treat the herniated lumbar disc in patients with nerve root compression. Therefore, we speculated that the ozone might be applied to treat the radiculoneuritis. In this study, we attempted to investigate the effects of ozone on the radiculoneuritis and the associated mechanisms. Therefore, according to the previously published reports[10], we established a chemically induced radiculoneuritis rat model. The expressions of autophagy mediators or apoptosis biomarkers surrounding the nerve roots were evaluated in the radiculoneuritis model and compared to the associated biomarkers in the other rat groups.

Materials and Methods

Animals

A total of 54 specific pathogen free (SPF) Wistar rats weighting from 250 to 300 g (irrespective of sex) were purchased from Experimental Animal Center of Shandong Medical University, Jinan, China. The rats were maintained in a condition of 12 h light/12 h dark cycle at a temperature of 25 ± 2°C. The Wistar rats freely accessed to standard commercial diet (CLEA Japan Inc., Shizuoka, Japan) and water. This study was performed following the guidance of Care and Use of Laboratory Animals of National Institute of Health (NIH). The animal experiments were approved by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China.

Trial Grouping

All of the rats were randomly divided into 3 groups, including Normal control group (n=18), radiculoneuritis rat models without any treatment (Model group, n=18) and Model group treated with ozone (Ozone therapy group, n=18). Meanwhile, each group was divided into 3 subgroups, including 8 h group (n=6), 1 day group (n=6) and 3 days group (n=6).

Establishment of Radiculoneuritis Rat Model

The rats were dorsally depilated 2-day before the surgery and operation[11]. On the day of operation, the rats were anesthetized by intraperitoneal injection with the 10% chloral hydrate and final dose of 0.35 ml/100 g body weight. A size of 2 cm midline incision was prepared on the dorsal skin following the strict aseptic conditions utilizing the fourth lumbar spinous process as the center. The muscles of the rats were dissected to the paraspinal muscles bluntly, ant right articular. The lamina process of the fifth and fourth lumbar spinous processes was isolated and removed. Then, the L5 nerve root was precisely isolated and exposed under a microscope. The hemostasis and oxidation cellulose paper (0.5 diameter) was treated with 50 µl complete Freund's adjuvant (CFA, Sigma-Aldrich, St. Louis, MO, USA) for 24 h, and rinsed by using the sterile saline. Then, the paper was placed on the L4/L5 nerve root and dorsal root ganglia. For the control group, the CFA was substituted with 50 µl of physiological saline. Finally, the incision was closed by using the sutures layer by layer. After the operation, in order to prevent the infection of rats, the 40000 units of gentamicin was injected intramuscularly daily for 4 days. Briefly, the detailed process was conducted due to the previous study[10]. The detailed processes for establishing radiculoneuritis rat model were listed as Figure 1.

Ozone Administration

We used the ozone, a three-oxygen molecule (O_3)[12]. For the Ozone therapy group, 50 µl of 30 µg/ml (which is a therapeutic concentration) ozone (Medozon compact, Herrmann Apparatebau GmbH, Germany) was epidurally administrated via the indwelling P10 catheter for 3 days till the rats were sacrificed. For the Model group, the rats were only treated with the normal air. The rats were sacrificed at 8 h, 1 day, and 3 days post the operation, respectively.

Electron Microscope Analysis

The nerve tissues were fixed by using the 5% glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered solution (PBS, ZSGB Bio. Tech., Beijing, China) at pH of 7.4 for 5 h. Then, the nerve tissues were also fixed in the 1% osmium tetraoxide (OsO4) in phosphate-buffered saline (PBS) at pH 7.4 and 4°C for 2 h. The tissues were then dehydrated in the graded ethanol (Sigma-Aldrich, St. Louis, MO, USA) and embedded in the Araldite (Sigma-Aldrich, St. Louis, MO, USA). After that, the nerve tissues were sliced into sections by using the ultra microtome (Mode: OMU3, Leica Reichert, Germany) and stained by using the lead citrated and the uranyl acetate. Finally, the sections were examined by using the electron microscope (Mode: JEM1200EX, JEOL, Tokyo, Japan) according to the previously reported study[13].

Immunohistochemistry Assay

The nerve roots were isolated and fixed with the 4% paraformaldehyde (Sangon Biotech, Shan-

ghai, China) for 20 min. After washing with PBS for 3 times (3 min per time), the nerve root tissues were sliced into sections and the endogenous peroxidase in the sections was inactivated by using 3% hydrogen peroxide at room temperature for 10 min. Then, the section was blocked with 5% bovine serum albumin (BSA) for 20 min and washed with PBS for 3 times. The nerve root sections were incubated with rabbit anti-rat monoclonal antibody (1:2000, Catalogue No. MAB835; RD Systems, Minneapolis, MN, USA) at 4°C overnight. Then, the nerve root sections were incubated with the Biotin-conjugated goat anti-rabbit IgG (1:2000, Catalogue No. BAF008, RD Systems, Minneapolis, MN, USA) for 1 h at room temperature. Finally, the nerve root sections were washed with PBS for three times, and immersed in the alkaline phosphatase labeled streptavidin (ZSGB Bio. Inc. Co., Beijing, China), and rinsed in the distilled water. The images of stained nerve root sections were observed by using an inverted fluorescence microscope (Model: IX51; Olympus, Tokyo, Japan) and captured with the image-scanning system (Model: BH-2, Olympus, Japan).

Double Labeled Immunofluorescence Assay

The microtubule-related protein light chain 3B (LC3B) and neuronal nuclear antigen (NeuN) were stained by using the double immunofluorescence assay. The never root tissues were treated, inactivated and sliced into the sections as above described. The sections were incubated with 200 μl 10% goat serum (Hyclone, South Logan, UT, USA) in a humidified chamber at room temperature. Then, the sections were incubated by using rabbit anti-rat LC3B polyclonal antibody (1:2000; Catalogue No. ab48394, Abcam Biotech., Cambridge, MA, USA) and mouse anti-rat NeuN monoclonal antibody (1:3000; Catalogue No. ab104224, Abcam Biotech. Cambridge, MA, USA) at 4°C overnight. The LC3B and NeuN were double-stained and observed by using the Alex Fluor 647 red conjugated goat anti-rabbit IgG (Catalogue No. ab150079; 1:1000; Abcam Biotech., Cambridge, MA, USA) and fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG (Catalogue No. ab6785; 1:1000; Abcam Biotech. Cambridge, MA, USA). The nucleus was stained by using the 4'6-diami-

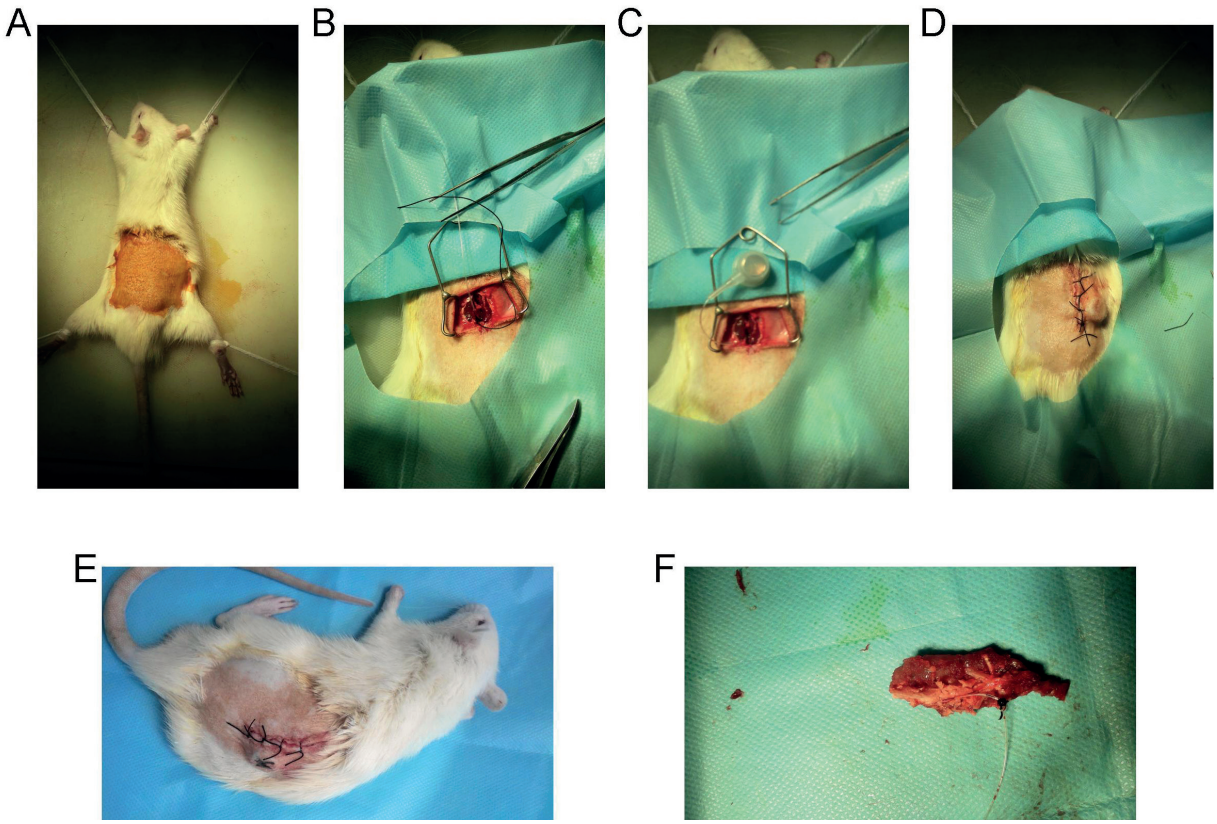

Figure 1. Detailed processes for establishing radiculoneuritis rat model. ***A,*** Fixation and shaving. ***B,*** Paving towel and enlarging the opening. ***C,*** Drug implantation. ***D,*** Wound suture. ***E,*** Wound healing. ***F,*** Nerve root tissues isolation.

dino-2-phenylindole (DAPI) staining solution (Beyotime Biotech., Shanghai, China). The double immunofluorescence images were captured and observed by using the laser confocal scanning microscopy (Mode: SP5, Leica Inc. Ltd., Germany).

Quantitative Real-time PCR (RT-PCR)

The nerve root tissues were lysed by using the tissue homogenate machine (Scientz Bio. Tech. Inc., Ningbo, China) and tissue total protein lysis buffer (ShineGene, Molecular Biotech. Inc., Shanghai, China) according to the instructions of manufacturer. The total RNAs were extracted by using the cell lysis buffer (ShineGene, Molecular Biotech. Inc., Shanghai, China). Then, the extracted RNAs were reversely transcribed by using reverse transcription kit (Catalogue No: 18080-051, Western Biotech., Chongqing, China) to synthesize complementary DNAs (cDNAs), which was used for the quantitative Real-time PCR (RT-PCR) assay. The PCR primers for Beclin 1, LC3B, phosphodiesterase 2A (PDE2A), nuclear factor-kB p65 (NF-kBp65) and β-actin were synthesized by Western Biotech. Inc. Co. (Chongqing, China) (Table I). SYBR Green I Real-time PCR kit (Catalogue No. 218073, Western Biotech., Chongqing, China) and Real-time PCR instrument (Mode: FTC2000, Funglyn Biotech., Toronto, Ontario, Canada) were used to amplify target genes due to the instructions of manufacturer. Amplification conditions for quantitative RT-PCR were listed as the followings: 94°C for 4 min, followed by 35 cycles of 94°C for 20 s, 60°C for 30 s and 72°C for 30 s, and terminated at 72°C for 10 min. All of the reactions were performed for at least six repeats with the final volume of 50 µl. The $2^{-\Delta\Delta Ct}$ method was used to analyze for RT-PCR findings.

Western Blot Assay

The nerve root tissues were lysed by using the tissue homogenate machine (Mode: DY89-I, Scientz Bio. Tech. Inc., Ningbo, China) and tissue total protein lysis buffer (Catalogue No. ZB1082, ShineGene, Molecular Biotech. Inc., Shanghai, China) according to the instructions of manufacturer. Protein lysates were separating by using the 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, ShineGene, Molecular Biotech. Inc., Shanghai, China), and electrotransferred onto polyvinylidene difluoride (PVDF) (Millipore, Boston, MA, USA). PVDF membranes were blocked by using the 5% defatted milk in phosphate-buffered saline (PBS, containing 0.05% Tween-20 solution and pH 7.5, ShineGene, Molecular Biotech. Inc., Shanghai, China), and incubated with the rabbit anti-rat LC3B polyclonal antibody (1: 2000; Catalogue No. ab48394, Abcam Biotech., Cambridge, MA, USA), rabbit anti-rat Beclin 1 polyclonal antibody (1:2000; Catalogue No. ab62557, Abcam Biotech.), rabbit anti-rat PDE2A polyclonal antibody (1:2000, Catalogue No. ab14604, Abcam Biotech.), rabbit anti-rat NF-kB p65 polyclonal antibody (1:2000, Catalogue No. ab16502) and rabbit anti-rat GAPDH monoclonal antibody (1:3000, Catalogue No. ab181602) at 4°C overnight. Then, PVDF membranes were incubated with 1:3000 horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Catalogue No. ab205718, Abcam Biotech., Cambridge, MA, USA). The signals of proteins were visualized with enhanced chemiluminescence kit (ECL) (ShineGene, Molecular Biotech. Inc., Shanghai, China). Finally, the Western blot bands were captured and analyzed by utilizing UVP gel image scanning system (Labwor-

Table I. Primers for the quantitative RT-PCR.

Genes		Sequences	Gene ID	Length (bp)
Beclin 1	Forwards	TGGAGTCCCTGACAGACAAATC	114558	137
	Reverse	TTCCACCTCTTCTTTGAACTGC		
LC3B	Forwards	AGAGCGATACAAGGGTGAGAAG	64862	141
	Reverse	AGGAGGAAGAAGGCTTGGTTAG		
PDE2A	Forwards	CCTATCAAGAACGAGAACCAAGAG	81743	137
	Reverse	GAGTGAGCGATGCTAATGCC		
NF-kBp65	Forwards	TCTGTTTCCCCTCATCTTTCC	309165	168
	Reverse	GGGTGCGTCTTAGTGGTATCTG		
β-actin	Forwards	TCTGTTTCCCCTCATCTTTCC	55576	150
	Reverse	GGGTGCGTCTTAGTGGTATCTG		

LC3B: microtubule-related protein light chain 3B, NeuN: neuronal nuclear antigen, RT-PCR: real-time PCR, PDE2A: phosphodiesterase 2A, NF-kB p65: nuclear factor kB p65.

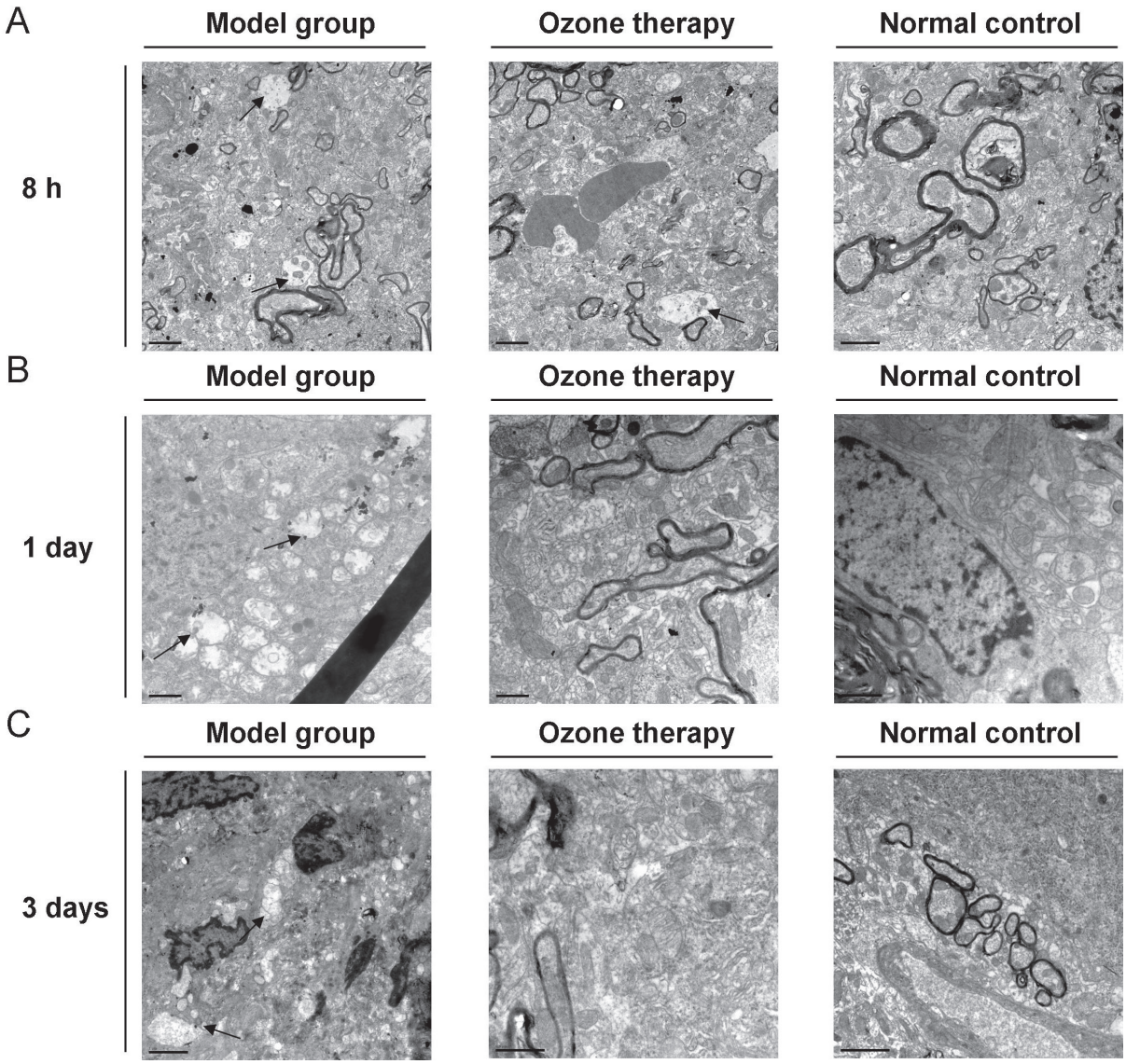

Figure 2. Electron microscope analysis for the autophagosomes in the cytoplasm of Model group, Ozone therapy group and Normal control group. ***A,*** Autophagosomes evaluation at 8 h post the operation. ***B,*** Autophagosomes evaluation at 1 day after the operation. ***C,*** Autophagosomes evaluation at 3 days after the operation. The *black arrows* represent the autophagosomes in the cytoplasm. Magnification, 10000×.

ks 4.6, Bio-Rad Laboratories, Hercules, CA, USA).

Statistical Analysis

Data were recorded as mean ± standard deviation (SD) and were analyzed by using SPSS software 20.0 (IBM, Armonk, NY, USA). All the data were obtained from at least six independent tests or experiments. Student's *t*-test was utilized for the statistical analysis between two groups. Tukey's post hoc test was used to validate ANOVA for comparing measurement data between groups. A statistical significance was defined when $p<0.05$.

Results

Ozone Inhibited the Autophagy of Nerve Root Cells in Model Rats

The autophagy in the nerve root cells of radiculoneuritis rat model was examined by using the electron microscopy to observe the autophagosomes in the cytoplasm. The results indicated that a few autophagosomes containing the abnormal structures (such as double membrane) and many cytoplasmic organelles were discovered in the nerve root cells of radiculoneuritis rat model at 8 h (Figure 2A), 1 day (Figure

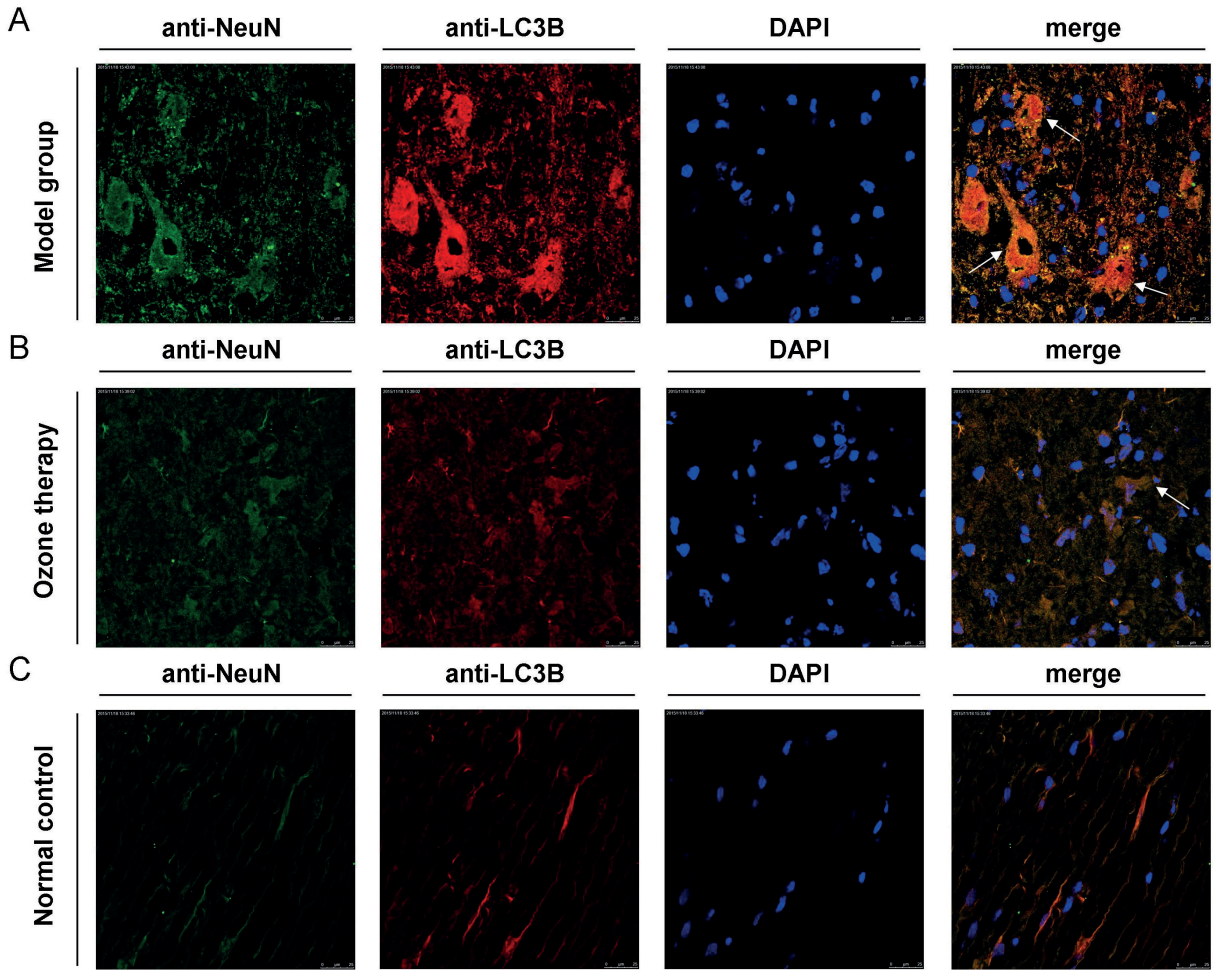

Figure 3. Double labeled immunofluorescence analysis for the expression and distribution of autophagosomes initiator, LC3B. ***A,*** LC3B expression and distribution of Model group. ***B,*** LC3B expression and distribution of Ozone therapy group ***C,*** LC3B expression and distribution of Normal control group. The green staining cells represent NeuN positive cells, red staining cells represent LC3B positive cells, blue staining represents the nucleus, the orange staining cells represent the merge cells. The *white arrows* represent co-localization of NeuN and LC3B. LC3B: microtubule-related protein light chain 3B, NeuN: neuronal nuclear antigen.

2B), and 3 days (Figure 2C) after the operation. Meanwhile, when the radiculoneuritis rat model was treated with ozone, the amounts of autophagosomes were significantly decreased compared to the Model group (Figure 2). Moreover, the autophagosomes amounts in Ozone therapy group even decreased to the amounts of Normal control group (Figure 2).

Ozone Decreased Levels of Autophagosomes Initiators

LC3B mainly distributes in autophagosomes and is essential for maturation of autophagosomes[14]. Therefore, in order to verify the electron microscopy results, the expression of LC3B was evaluated by using double-labeled immunofluorescence assay. The results showed that many LC3B staining regions (orange staining) appeared in the cytoplasm of nerve root cells of Model rats (Figure 3A). However, the ozone therapy significantly declined the size of LC3B staining regions compared to that of the Model rats (Figure 3B). Meanwhile, there were even no LC3B staining regions in nerve root cells of Normal control rats (Figure 3C).

Ozone Alleviated Apoptosis of Nerve Root Cells of Radiculoneuritis Rat Model

The apoptosis key marker[15], cleaved caspase 3 (activated form of caspase 3) was examined in

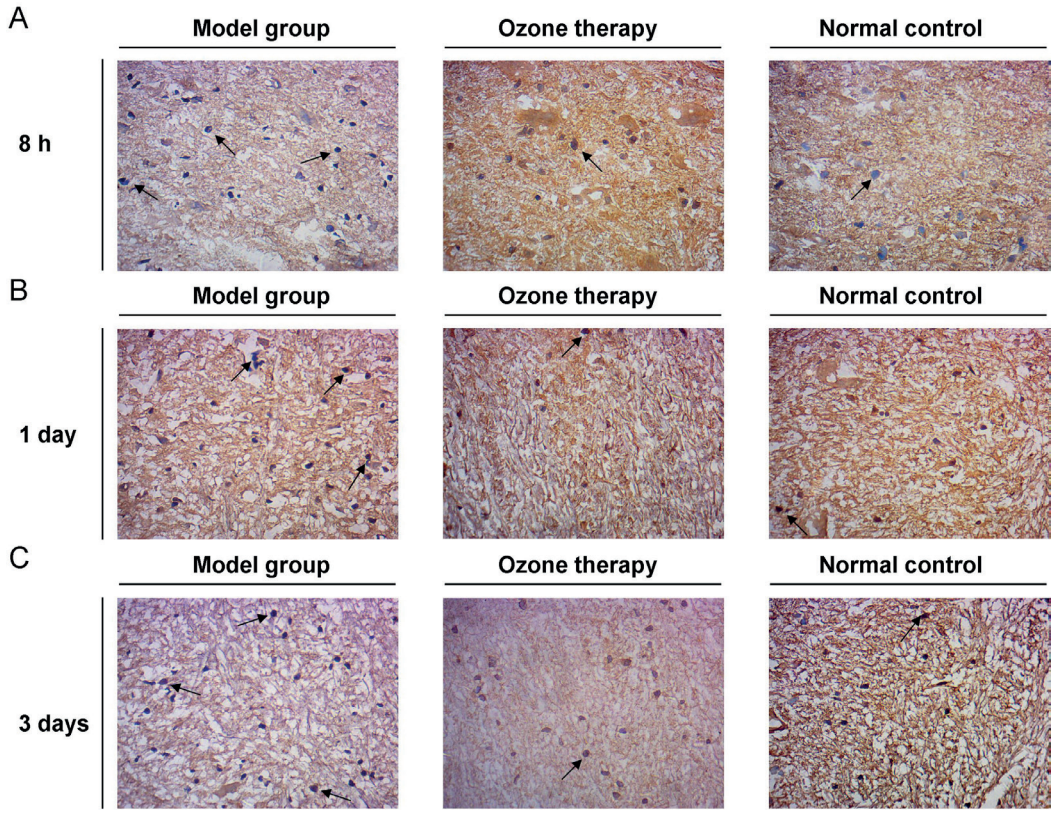

Figure 4. Immunohistochemistry assay for the cleaved caspase 3 expression in Model group, Ozone therapy group and Normal control group. ***A,*** Cleaved caspase 3 expression at 8 h post the operation. ***B,*** Cleaved caspase 3 expression at 1 day after the operation. ***C,*** Cleaved caspase 3 expression at 3 days after the operation. The *black arrows* represent the cleaved caspase 3 positively stained cells. Magnification, 200×

nerve root cells by using immunohistochemistry assay. The results indicated that there were many cleaved caspase 3 positive stained cells in nerve root cells of Model rats at 8 h (Figure 4A), 1 day (Figure 4B), and 3 days (Figure 4C), respectively. However, the ozone therapy markedly decreased the amounts of cleaved caspase 3 positive stained cells of radiculoneuritis rats compared to that of Model rats (Figure 4), which even achieves to the levels of Normal control rats.

Ozone Suppressed Autophagy by Activating LC3B/Beclin 1 Pathway

Both LC3B and Beclin 1 are important initiators for autophagosomes formation[16]. Therefore, the LC3B and Beclin 1 expressions were determined by using the Western blot assay and quantitative RT-PCR, respectively. The RT-PCR results indicated that both Beclin 1 (Figure 5A) and LC3B (Figure 5B) mRNA levels in Ozone therapy group were significantly decreased compared to that of Model group, at 8 h, 1 day, and 3 days after the operation, respectively ($p<0.05$). Also, the Western blot assay also indicated that LC3B (Figure 6B) and Beclin 1 (Figure 6C) expressions in Ozone therapy group were also significantly decreased compared to Model group ($p<0.05$).

Ozone Inhibited Apoptosis by Triggering NF-kB p65 Signaling Pathway

The apoptosis associated biomarkers, PED2A NF-kB p65, were also detected in radiculoneuritis rat models by using the Western blot assay and quantitative RT-PCR, respectively. The results indicated that PDE2A mRNA levels (Figure 5C) and NF-kB p65 mRNA levels (Figure 5D) were significantly decreased in Ozone therapy group compared to the Model group ($p<0.05$). The Western blot assay (Figure 7A) results also showed that the PDE2A protein expression in Ozone therapy group was significantly decreased compared to the Model group (Figure 7B, $p<0.05$). Meanwhile, the NF-kB p65 protein expression was also significantly decreased in Ozone therapy

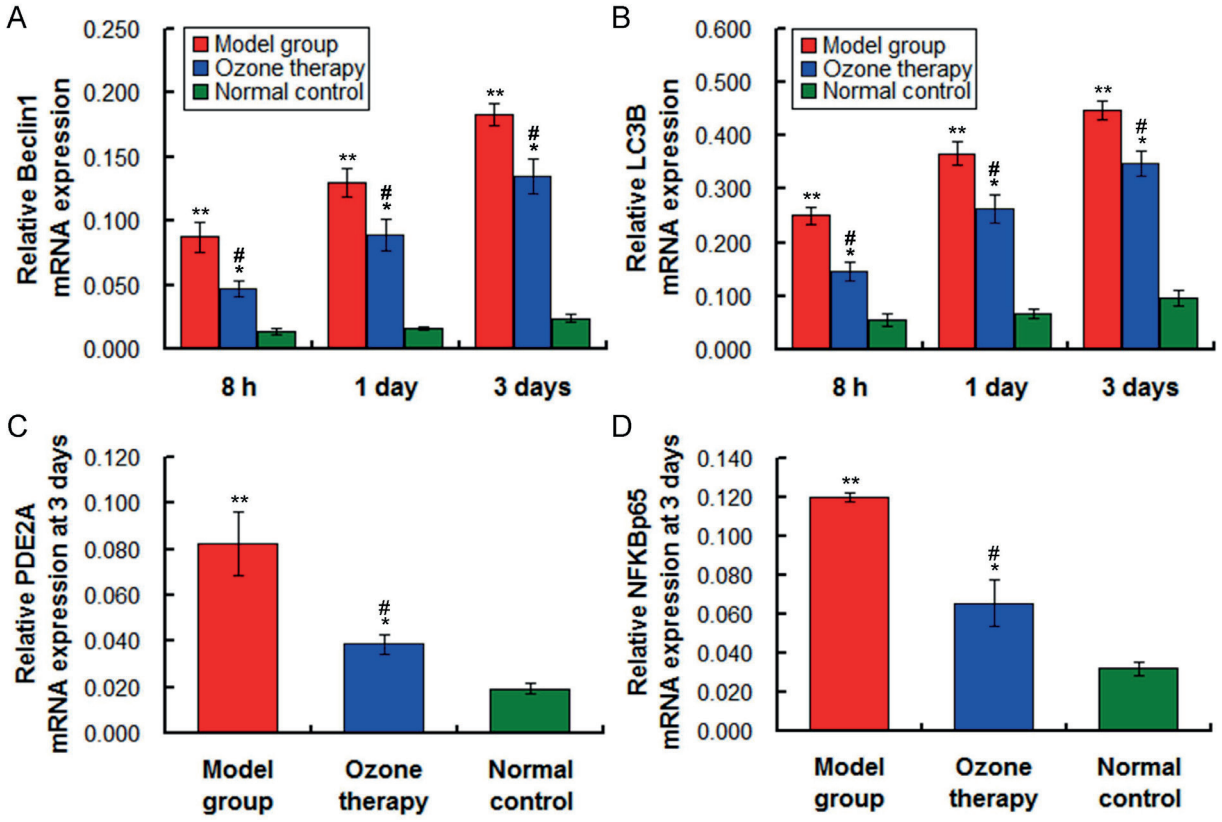

Figure 5. Autophagy and apoptosis biomarkers examination by using quantitative RT-PCR. ***A,*** RT-PCR assay for the Beclin 1 mRNA expression. ***B,*** RT-PCR assay for the LC3B mRNA expression. ***C,*** RT-PCR assay for the PDE2A mRNA expression. ***D,*** RT-PCR assay for the NF-kB p65 mRNA expression. $^{*}p<0.05$, $^{**}p<0.01$ *vs.* Normal control group. $^{\#}p<0.05$ *vs.* Model group. LC3B: microtubule-related protein light chain 3B, RT-PCR: Real-time PCR, PDE2A: phosphodiesterase 2A, NF-kB p65: nuclear factor kB p65.

group compared to the Model group (Figure 7C, $p<0.05$).

Discussion

The radiculoneuritis is the critical factor for the pathological processes for inducing the radicular pain in the lumbar disc herniation patients[17]. The radiculoneuritis is caused by the chemical radiculitis or pain, which is induced by the exposed nucleus pulpous and rupture of annulus fibrosus of nerve roots[18]. Actually, the microcirculation and venous occlusion associated diseases block the nutrients and the oxygen supply to the tissues, which result in the metabolites accumulation. Therefore, the key points to treat the radiculoneuritis are to supply the nutrients, improve the microcirculation, suppress the inflammatory response of the nerve roots, and decrease the inflammatory chemicals accumulation in nerve roots[10,19].

Ozone mainly illustrates by the O_3 form is an oxidant gas playing the roles of immunomodulating, anti-inflammation, anti-septic characteristics, and analgesic properties[20]. The ozone always conducts the functions of anti-nociceptive or anti-neuropathic pain in disc herniation and degenerative disc[21]. The previous researches reported that the ozone therapy could improve the microcirculation[22] and also supply the oxygen and nutrients[23]. Therefore, the ozone therapy could remove the accumulated inflammatory factor, improve blood circulation, and so alleviate the pain. With the above functions, it is speculated that the ozone may be an optimal treatment for the radiculoneuritis.

We successfully established a chemical radiculoneuritis rat model according to the previous study[10]. The autophagy associated ultrastructures and biomarkers and the apoptotic indicators in the nerve roots tissues were examined at 8 h, 1 day, and 3 days post the models were prepared. The autophagosomes formation

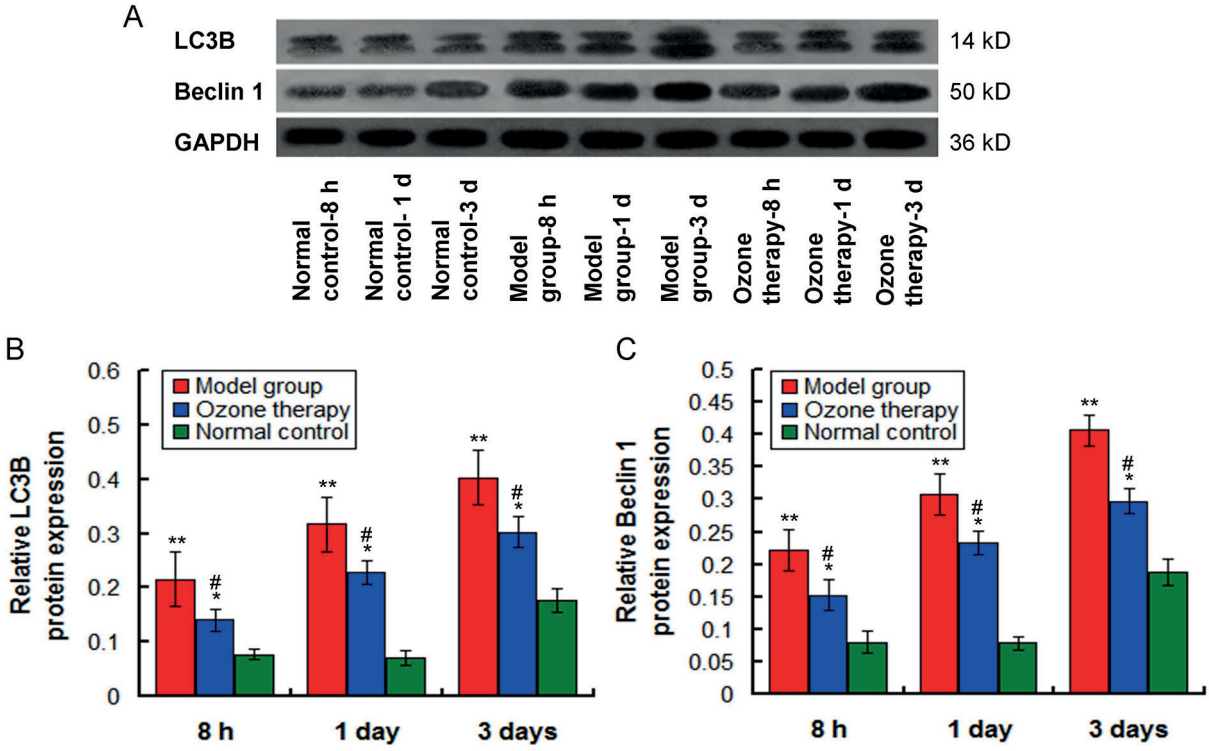

Figure 6. Autophagy biomarkers examination by using Western blot assay. ***A,*** Western blot images for the LC3B and Beclin 1 protein expression. ***B,*** Statistical analysis for the LC3B expression. ***C,*** Statistical analysis for the Beclin 1 expression. $^{*}p<0.05$, $^{**}p<0.01$ *vs.* Normal control group. $^{\#}p<0.05$ *vs.* Model group. LC3B: microtubule-related protein light chain 3B.

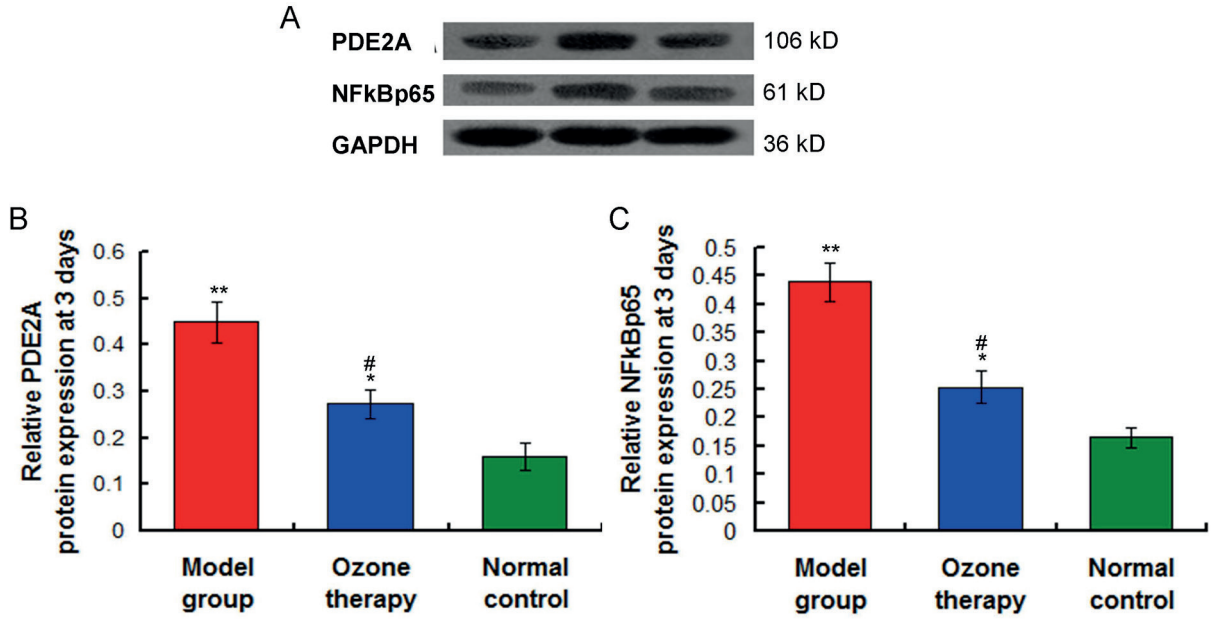

Figure 7. Apoptosis biomarkers evaluation by using Western blot assay. ***A,*** Western blot images for the PDE2A and NF-kB p65 protein expression. ***B,*** Statistical analysis for the PDE2A expression. ***C,*** Statistical analysis for the NF-kB p65 expression. $^{*}p<0.05$, $^{**}p<0.01$ *vs.* Normal control group. $^{\#}p<0.05$ *vs.* Model group. PDE2A: phosphodiesterase 2A, NF-kB p65: nuclear factor kB p65.

was obvious, suggesting that the autophagy involves in the radiculoneuritis. Meanwhile, the ozone significantly decreased autophagosomes levels in local nerve root tissues of model rats according to the electron microscopy results. Moreover, the autophagosomes initiators, such as LC3B and Beclin 1[16], were also examined in the nerve tissues of rats. The results indicated that the ozone therapy significantly suppressed the enhanced levels of LC3B and Beclin 1 in nerve root tissues of Model rats, which suggests that ozone therapy could inhibit the autophagy of nerve root tissues. The apoptosis signaling pathway biomarker, cleaved caspase 3[24], was also examined. The immunohistochemistry assay results showed that there was plenty of cleaved caspase 3 positively stained nerve root cells in the Model group at 8 h, 1 day, and 3 days post-operation, respectively. However, the ozone therapy significantly declined the amounts of cleaved caspase 3 positively stained cells compared to the Model group. This result suggests that ozone inhibits the nerve root cells apoptosis by activating and cleaving the caspase 3, which is consistent with the previous study[25] reporting that ozone was inhibiting apoptosis. Furthermore, to clarify the mechanism for the apoptosis, the apoptosis-associated biomarkers, PDE2A and NF-kB p65, were also examined by using both RT-PCR and Western blot assay, respectively. The results showed that both PDE2A and NF-kB 65 expressions in Ozone therapy group were significantly decreased compared to Model group. Therefore, we believed that the ozone inhibits the apoptosis by activating the NF-kB signaling pathway.

Conclusions

We successfully established a chemical radiculoneuritis rat model. We discovered that the ozone therapy significantly inhibits the expression of autophagy biomarkers (LC3B and Beclin 1) and apoptosis key indicator, cleaved caspase 3, in the nerve root cells of radiculoneuritis rat model. Meanwhile, ozone suppresses the apoptosis of nerve root cells by inactivating the NF-kB p65 expression in radiculoneuritis rat model. Finally, the therapeutic concentration of ozone could inhibit autophagy by suppressing LC3B and Beclin 1 expression and could reduce apoptosis by blocking the NF-kB signaling pathway.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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