Melatonin Attenuates Ropivacaine-Induced Apoptosis by Inhibiting Excessive Mitophagy Through the Parkin/PINK1 Pathway in PC12 and HT22 Cells

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Abstract—Melatonin, as an endogenous circadian indoleamine secreted by the pineal gland, executes extensive biological functions, including antioxidant, anti-inflammatory, anti-tumor, and neuroprotective effects. Although melatonin has been reported to serve as a potential therapeutic against many nerve injury diseases, its effect on ropivacaine-induced neurotoxicity remains obscure. Our research aimed to explore the impact and mechanism of melatonin on ropivacaine-induced neurotoxicity. Our results showed that melatonin pretreatment protected the cell viability, morphology, and apoptosis of PC12 and HT22 cells, and it also improved ropivacaine-induced mitochondrial dysfunction and the activation of mitophagy. In addition, we found that autophagy activation with rapamycin significantly weakened the protective effect of melatonin against ropivacaine-induced apoptosis, whereas autophagy inhibition with 3-MA enhanced the effect of melatonin. We also detected the activation of Parkin and PINK1, a canonical mechanism for mitophagy regulation, and results shown that melatonin downregulated the expression of Parkin and PINK1, and upregulated Tomm20 and COXIV proteins, so that those results indicated that melatonin protected ropivacaine-induced apoptosis through suppressing excessive mitophagy by inhibiting the Parkin/PINK1 pathway. Melatonin may be a useful potential therapeutic agent against ropivacaine-induced neurotoxicity.

KEY WORDS: melatonin; autophagy; mitophagy; ropivacaine; neurotoxicity; apoptosis.
INTRODUCTION

Melatonin, as an endogenous circadian indoleamine secreted by the pineal gland, executes extensive biological functions, including antioxidant, anti-inflammatory, anti-tumor, and neuroprotective effects [1, 2]. It has been reported to exert neuroprotective effects in many studies; melatonin plays an important role in the regulation of neurogenesis and maybe a potential therapeutic for many nerve damages [3]. The present study indicated that melatonin promoted the viability and proliferation of neural stem cells, and it also enhanced the expression of neuronal markers in the PC12 cells [4, 5]. In addition, melatonin plays a protective role in many neurodegenerative diseases [6, 7]. Melatonin attenuates nerve impairment in Parkinson’s disease (PD) in vivo and in vitro experiments [8]. It also has a neuroprotective effect against Alzheimer’s disease (AD) and improved the spatial learning and memory deficits in AD by reducing the Aβ production [9]. Apart from brain neurons, melatonin protects against spinal cord injury and peripheral nerve impairment [3]. In a word, melatonin could be a possible treatment strategy for many neuronal disorders. But, the effect of melatonin on ropivacaine-induced neurotoxicity remains unclear.

Ropivacaine is widely used for peripheral nerve blocks, epidural anesthesia, spinal anesthesia, and pain management [10]. However, epidemiological studies demonstrated that ropivacaine induced neurotoxicity at the high dosage and long duration of exposure, as manifested by transient neurologic syndrome (TNS), cauda equina syndrome (CES), and delayed sacral nerve disorder [10]. Although most damages are transient, the permanent nerve injury also occurs sometimes. In recent years, the mechanism of ropivacaine-induced neurotoxicity is highly complex and there is a various pathophysiological process involved in, including intracellular calcium concentration, cell apoptosis, inflammation, and autophagy [11–14]. Wen et al. reported the inhibition of T-type calcium channel could improve ropivacaine-induced cell damage [15]. Wang and Luo et al. demonstrated apoptosis was a key point in ropivacaine-induced neurotoxicity, and the Fas/Fasl-mediated exogenous apoptosis pathway was involved in [16, 17]. In addition, Xiong et al. demonstrated ropivacaine upregulated autophagy levels in neuronal cells, and the inhibition of autophagy aggravated ropivacaine-induced neurotoxicity [10]. But, in our study, we found that the melatonin pretreatment could inhibit ropivacaine-induced apoptosis of PC12 and HT22 cells via downregulating the excessive mitophagy, which may a protective strategy against the neurotoxicity of local anesthetics.

The present study aimed to explore the impact and mechanism of melatonin on ropivacaine-induced neurotoxicity. Our study was the first to reveal that melatonin pretreatment improved cell viability, morphology, and apoptosis, reduced mitochondrial reactive oxygen species production, and increased mitochondrial membrane potential in both ropivacaine-treated PC12 and HT22 cells. Furthermore, melatonin suppressed ropivacaine-induced excessive mitophagy by reducing LC3-II/LC3-I levels, depleting p62, upregulated Tom20 and COXIV proteins, and downregulated the PINK1/Parkin pathway. And autophagy activation with rapamycin significantly weakened the protective effect of melatonin against ropivacaine-induced apoptosis, whereas autophagy inhibition with 3-MA enhanced the effect of melatonin, suggesting melatonin attenuates ropivacaine-induced apoptosis via inhibiting the excessive mitophagy.

MATERIALS AND METHODS

Reagents and Antibodies

Ropivacaine, 3-methyladenine (3-MA), and Rapamycin were from the Selleck Cham (American). Melatonin was brought from Sigma-Aldrich (St. Louis, MO, USA), the primary antibodies of LC3, p62, Beclin1, Bax, Bcl-2, PINK1, Parkin, COXIV, and Tomm20, and cleaved-caspase-3 were from the proteintech™ (Wuhan, China). The H2 DCFDA reactive oxygen species (ROS) and mitochondrial membrane potential test kits (JC-1) were from KeyGEN BioTECH (Jiangsu, China). Dylight Fluor® 488 and Dylight Fluor® 594 labeled goat anti-rabbit IgG (H + L) secondary antibodies were from Jackson ImmunoResearch (West Grove, PA, USA). The MitoTracker Red CMXRos and Hoechst 33,258 were from the Beyotime Biotechnology (Shanghai, China).

Cell Culture

The rat pheochromocytoma cell line (PC12) and hippocampal neuronal cell line (HT22) were purchased from the Cell Bank of Shanghai Institute of Chinese Academy of Sciences. These cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM, Gibco, USA) containing 10% of fetal bovine serum (FBS,
Gibco, Australia), 100 IU/ml of penicillin G sodium, and 100 mg/ml of streptomycin sulfate at 37 °C in an incubator maintained with 5% CO² at 37 °C.

**Cell Viability Assay**

The cytotoxicity of melatonin on PC12 and HT22 cells was detected by the cell counting kit-8 (CCK-8, Biosharp, Shanghai, China) assay according to the manufacturer’s instructions. PC12 cells and HT22 cells were separately seeded in the 96-well plate at a density of 5 × 10³ cells/well and then treated with PBS or various concentrations of melatonin (540 μM) in 100 μl of medium. There were six wells in each group. After incubation for 24 h, 10 μl of CCK-8 solution was added to each well, followed by incubation for 2 h at 37 °C. The absorbance was measured in a microplate reader (SpectraMax®iD3, Molecular Devices, USA) at a wavelength of 450 nm. To explore the protective effects of melatonin, cells were pretreated with different concentrations of melatonin for 2 h, then adding 1mM ropivacaine to co-culture for different times (24 h, 48 h, 72 h), the cell viability was detected as above.

**Detection of Cell Apoptosis and Necrosis by Hoechst/PI Staining**

Cells were seeded into 6-well plates at a density of 1 × 10⁵ cells/well, and then pretreated with melatonin for 2 h; 1mM ropivacaine was added and co-incubated for 24 h. The medium was removed, and 1 ml of phosphate buffer saline (PBS) containing 5 μl of Hoechst33342 and 5 μl of propidium iodide (PI) was added to each well, followed by incubation for 15 min at 4 °C. Therefore, cells were washed with PBS, and observed under a fluorescence microscope (Olympus, IX73, Japan). Representative photographs were captured. The red fluorescence positive cells were counted and the percentage of necrotic cells was calculated.

**Determination of Caspase-3 Activity**

Cells were seeded in a 6-well plate at a density of 1 × 10⁵ cells/well, and when the density reached 60~80%, various concentrations of melatonin (5μM, 10μM, 20μM) were added; after cultured for 2 h, those cells were treated with 1mM ropivacaine. Then, the caspase-3 activity of PC12 and HT22 cells was detected by caspase-3 spectro-photometric detection kit ( Wanleibio, Shenyang, China) according to the manufacturer’s instructions.

**Determination of the Mitochondrial Reactive Oxygen Species**

Cells were seed in a 12-well plate at a density of 1 × 10⁴ cells / well, and when the density reached 60~80%, various concentrations of melatonin (5μM, 10μM, 20μM) were added; after cultured for 2 h, those cells were treated with 1mM ropivacaine. After the incubation of 24 h, the mitochondrial reactive oxygen species was measured by the H₂DCFDA ROS assay and the MitoTracker Red CMXRos according to the manufacturer’s instructions. At last, the Hoechst 33,258 was added for 5 min to label the nucleus.

**Determination of the Mitochondrial Membrane Potential Assay**

The mitochondrial membrane potential (ΔΨm) assay was based on JC-1 staining (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide). JC-1 has two forms including monomers and polymers, and the emission spectra of which are different. When the mitochondrial membrane potential reduces, the JC-1 polymer with red fluorescence decreases, and the JC-1 monomer with green fluorescence was in the cytoplasm. Following the instructions of JC-1 assay kits, the flow cytometry was to measure ΔΨm.

**Immunofluorescence Staining**

The cell’s mitochondria were labeled by MitoTracker Red CMXRos according to the manufacturer’s instructions. Cells were fixed in 4% PFA and permeabilized with 0.2% Tween X-100. After being blocked with normal goat serum, cells were incubated with rabbit anti-rat p-STAT3 (1:200) overnight at 4 °C, and the second antibody (1:1000) was incubated for 1 h at 37°C in dark. Hoechst33258 was added followed by incubation for 5 min to label nuclei. The fluorescence microscope (Olympus, Japan) was used to observe the cells. The fluorescence intensity was analyzed with ImageJ software.

**Western Blotting**

Cells were lysed with pre-cold RIPA lysis buffer. After centrifugation (12,000 rpm/min) for 15 min at 4°C, the protein concentration was measured with a bicinchoninic acid (BCA) protein assay kit (Beyotime, Shanghai, China).
A total of 40 μg proteins were subjected to protein separation by the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were incubated with 5% non-fat skim milk for 1 h. The membranes were incubated with primary antibodies (Bax, 1:1000; Bcl-2, 1:1000; cleaved-caspase-3 (1:500), LC3, 1:1000; p62, 1:1000; Beclin1, 1:1000; PINK1, 1:1000; Parkin, 1:1000) at 4°C overnight, and then with HRP-linked secondary antibody for 1 h at room temperature. Specific proteins were detected by enhanced chemiluminescence assay (Bio-rad, USA), and the protein bands were quantified with Image Lab software (Bio-rad, USA).

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 software. The quantitative data were expressed as mean ± standard deviation (SD). The comparisons between two groups were done with Student’s t-test. The comparison among groups was performed by one-way ANOVA. A value of $P < 0.05$ was considered statistically different.

RESULTS

Melatonin Pretreatment Rebounded Ropivacaine-Induced Decrease in Cell Viability

Cell viability was firstly assessed by CCK-8 assay when exposed to different concentrations of melatonin (5–40 μM), and the results showed that cell viability of PC12 and HT22 cells did not change after melatonin treatment (Fig. 1A). Our previous study has indicated that 1mM ropivacaine induced a significant decrease in cell viability. In this research, we demonstrated that melatonin pretreatment rebounded a ropivacaine-induced decrease in PC12 cells and HT22 cells in a dose-dependent manner. Meanwhile, we compared the cell viability of drug combinational treatment at different duration, and the protective effect of melatonin gradually diminished with the extension of exposure times (Fig. 1B, C). Furthermore, cells exposed to ropivacaine showed apoptotic bodies and were fewer in number compared with control, but melatonin pretreatment prevented the morphological changes, increased living cell numbers, and reduced the apoptotic cells (Fig. 1D); those results further validated the protective potential of melatonin against ropivacaine-induced neurotoxicity.

Melatonin Pretreatment Attenuated Ropivacaine-Induced Apoptosis

To investigate the impact of melatonin on ropivacaine-induced apoptosis, Western blot and PI/Hoechst staining were used to detect the apoptotic levels. As expected, ropivacaine treatment increased the apoptotic levels of PC12 and HT22 cells compared with the control group, including upregulating the expression of pro-apoptotic proteins Bax and cleaved-caspase-3 and decreasing the expression of anti-apoptotic protein Bcl-2 (Fig. 2A, B). In addition, as shown in Fig. 2C, the numbers of PI-positive cells in PC12 and HT22 cells were significantly increased by ropivacaine. Furthermore, the caspase-3 cleavage and activity were also detected. Compared to the control group, the caspase-3 activity and cleavage were notably elevated after ropivacaine treatment (Fig. 2F, G). Although ropivacaine increased the apoptotic rate of PC12 and HT22 cells, pretreatment of the cells with melatonin reduced the apoptotic rate, including reducing the ratio of Bax/Bcl-2, PI-positive cell amounts, and caspase-3 activity and cleavage. The above results confirmed that melatonin pretreatment suppressed ropivacaine-induced apoptosis in the endogenous apoptotic pathway.

Melatonin Pretreatment Improved Ropivacaine-Induced Mitochondrial Dysfunction

To further demonstrate the protective effect of melatonin on ropivacaine-induced neurotoxicity, the mitochondrial function of PC12 and HT22 cells was accessed by the detection of mitochondrial reactive oxygen (mitoROS) and mitochondrial membrane potential ($\Delta \Psi_m$). As shown in Fig. 3A and B, the co-localization of ROS and MitoTracker-labeled mitochondria was increased after ropivacaine treatment, but melatonin decreased the generation of mitoROS in a dose-dependent manner. Furthermore, the $\Delta \Psi_m$ detection kit (JC-1) was measured by flow cytometry. Compared with the control group, ropivacaine decreased the cell numbers in the red fluorescent channel, so that reduced the $\Delta \Psi_m$ of PC12 and HT22 cells, but melatonin pretreatment improved this effect and increased the $\Delta \Psi_m$ in a dose-dependent manner (Fig. 3C, D). Those results indicated that melatonin pretreatment improved ropivacaine-induced mitochondrial dysfunction.
Melatonin Pretreatment Inhibited Ropivacaine-Induced Autophagy

To explore the effects of melatonin and ropivacaine on the autophagy levels, the expression of molecules involved in autophagy was determined by Western blot. As shown in Fig. 4, ropivacaine elevated LC3-II/LC3-I ratio and Beclin1 levels, and decreased the expression of p62 in PC12 and HT22 cells, but pretreatment with melatonin followed by ropivacaine inhibited the activation of autophagy, including reduced the LC3-II/LC3-I ratio and Beclin1 levels, but increased the expression of p62. In addition, to further demonstrate the protective effects of melatonin on autophagy, the co-localization of the autophagy-associated protein LC3 with MitoTracker-labeled mitochondrial was also detected by the fluorescence microscope before and after the treatment with ropivacaine and melatonin. The results from immunofluorescence were consistent with Western blot. Melatonin decreased the numbers of
autophagic vacuoles engulfing mitochondria in PC12 and HT22 cells, but this effect was inhibited by melatonin pretreatment (Fig. 4C, D).

**Autophagy Activation by Rapamycin Inhibits the Neuroprotective Effect of Melatonin**

To further illustrate the impact of autophagy on the neuroprotective effect of melatonin, rapamycin (an autophagy activator), 3-methyladenine (3-MA, an early autophagy inhibitor), and Bafilomycin A1 (Baf, a late autophagy inhibitor) were added to the medium before the treatment with ropivacaine and melatonin, and the expressions of apoptotic and autophagy index proteins were detected by Western blot. As shown in Fig. 5A and B, compared to the control group, ropivacaine alone treatment increased LC3-II/LC3-I ratio and decreased p62 protein level in PC12 and HT22 cells, but this effect was reversed by melatonin. However, after the pretreatment with rapamycin, the inhibition
of melatonin on ropivacaine-induced autophagy was downregulated, but 3-MA pretreatment enhanced the inhibition of melatonin on ropivacaine-induced autophagy. Moreover, we detected the expression of apoptosis-related proteins Bax and Bcl-2 after the autophagy activation or inhibition. Compared to the melatonin + ropivacaine group, rapamycin pretreatment enhanced ropivacaine-induced apoptosis, including upregulating Bax/Bcl-2 ratio, so that decreasing the neuroprotective effect of melatonin. On the contrary, 3-MA decreased apoptosis levels and strengthened the neuroprotective effect of melatonin. In addition, we used Baf to inhibit the late autophagy and then to analyze all the products including autophagosomes and mitochondria that are being degraded by autophagy, as shown in supplementary Fig. 1, compared to the melatonin + ropivacaine group, Baf pretreatment increased LC3-II/LC3-I ratio and the amounts of LC3-labeled autophagosomes, and reduced p62 protein expression. And the results from supplementary Fig. 2 demonstrated that the inhibition of autophagy by Baf strengthened the protective effects of melatonin on
ropivacaine-induced apoptosis. Those results indicated that the protective effect of melatonin on ropivacaine-induced apoptosis was dependent on the inhibition of excessive autophagy. Moreover, the co-localization of LC3-labeled autophagosome and MitoTracker-labeled mitochondrial in HT22 cells was detected by the fluorescence microscope, the results from Fig. 5C were consistent with Western blot, rapamycin rescued the downregulation of melatonin on ropivacaine-induced autophagy, but 3-MA strengthened the effect of melatonin. Finally, we detected the cleaved-caspase-3 levels before and after the treatment with rapamycin or 3-MA, and our results once demonstrated autophagy activation by rapamycin inhibited the neuroprotective effect of melatonin (Fig. 5D, F).

Fig. 4 Melatonin pretreatment inhibited ropivacaine-induced autophagy. PC12 and HT22 cells were pretreated with 10 μM melatonin for 2 h, then adding 1mM ropivacaine and co-culturing for 24 h. (A) (B) The expressions of molecules involved in autophagy were detected by Western blot, and quantifications of the gray values were measured by Image Lab. (C) (D) The co-localization of LC3 distribution and MitoTracker-labeled mitochondrial in HT22 cells were detected by the fluorescence microscope. Data are shown as the means ± SD of 3 independent experiments. *P < 0.05, **P < 0.01 vs control group, *P < 0.05 vs Rop group.

Melatonin Pretreatment Inhibited the Activation of Parkin/PINK1 Pathway

The result from Fig. 5C indicated that melatonin could inhibit ropivacaine-induced mitophagy, and to further verify the results, the expression of mitophagy index protein including PINK1 and Parkin, and mitochondrial marker protein including Tomm20 and COXIV was detected by Western blot, as shown in Fig. 6A and B, compared to the control group, ropivacaine downregulated Tom20 and COXIV levels and upregulated the expression of PINK1 and Parkin in PC12 and HT22 cells. In addition, compared to the ropivacaine group, melatonin pretreatment decreased the expression of PINK1 and Parkin and increased Tom20.
and COXIV levels. Next, we further detected the ratio of autophagic lysosome-engulfed mitochondria, with Mito and LysoTracker staining to label mitochondria and lysosomes. The results from Fig. 6C shows that ropivacaine increased the ratio of cells double-positive for both Mito and Lyso staining, and melatonin pretreatment reversed this effect of ropivacaine, suggesting melatonin inhibited ropivacaine-induced mitophagy.

**DISCUSSION**

Ropivacaine, as an amide local anesthetic (LA), is widely used in the clinic for peripheral nerve blocks, epidural anesthesia, spinal anesthesia, and pain management [18]. The epidemiological reports demonstrated that the exposure of ropivacaine could provoke neurotoxicity at high dosage and long duration in the intravertebral anesthesia, as manifested by the transient neurological syndrome (TNS) and cauda equine syndrome (CES) (Fig. 7). Although there is a study that ropivacaine has the least neurotoxicity among the other LAs including lidocaine and bupivacaine [19, 20], the permanent nerve damage from ropivacaine is concerned by many doctors and there lack some effective preventive and therapeutic measures. Up to now, the molecular mechanism of ropivacaine-induced neurotoxicity remains unclear, and many potential mechanisms have been proposed, including intercellular calcium overload [21–23], neuronal mitochondrial dysfunction [24, 25], neuronal apoptosis [26, 27], and
Fig. 6 Melatonin pretreatment inhibited the activation of Parkin/PINK1-mediated mitophagy. PC12 and HT22 cells were pretreated with 10 μM melatonin for 2 h, then adding 1mM ropivacaine and co-culturing for 24 h. (A) (B) The expression of mitophagy index proteins was detected by Western blot, and quantification of the gray values was measured by Image Lab. (C) Mito and LysoTracker co-staining analysis in PC12 and HT22 cells treated with ropivacaine and melatonin. Data are shown as the means ± SD of 3 independent experiments. *P < 0.05, **P < 0.01 vs control group, #P < 0.05 vs Rop group.
Some studies have reported that T-type calcium channels and their regulatory proteins were markedly upregulating in ropivacaine-induced neurotoxicity, and the inhibition of them could improve the neurotoxicity from ropivacaine [28].

Apart from the intercellular calcium overload, Niu et al. found that ropivacaine damaged the mitochondrial biogenesis of neuronal cells by reducing the mitochondrial mass and impairing the mitochondrial respiratory rate [24]. Our results also demonstrated that ropivacaine induced mitochondrial dysfunction of PC12 and HT22 cells by upregulating mitoROS levels and reduced the ΔΨm, but this effect was reversed by melatonin pretreatment. Moreover, other studies revealed that neuronal apoptosis was involved in ropivacaine-induced neurotoxicity, including our previous studies; we found that ropivacaine upregulated the expression of Fas and Fasl during the process of neuronal apoptosis [17], and the current results were consistent with the above, ropivacaine induced the apoptosis of PC12 and HT22 cells via increasing Bax/Bcl-2 ratio and cleaved-caspase-3 levels, but melatonin pretreatment could decrease it.

Autophagy is a dynamic process in which macromolecules and damaged organelles in the cytoplasm are degraded. It stabilizes the intercellular environment in most cells including neurons [29, 30]. During the process, the cytosolic LC3 (LC3-I) is converted to the autophagosomal membrane type (LC3-II). The presence of LC3 in autophagosomes and the conversion of LC3-I to LC3-II have thus been used as indicators of autophagy. Therefore, the increase of LC3-II/LC3-I after ropivacaine treatment indicated ropivacaine-induced autophagy; however, this effect was reversed by melatonin pretreatment. Sometimes the moderate autophagy sustains cell survival, but excessive autophagy promotes cell death [31]. In our study, we demonstrated that ropivacaine induced the apoptosis of PC12 and HT22 cells via the upregulation of excessive autophagy, but melatonin pretreatment decreased the apoptotic levels by downregulating the autophagy. In addition, we found that following ropivacaine treatment, the mitochondrial dysfunction of PC12 and HT22 was observed, as manifested by the

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**Fig. 7** Schematic diagram illustrating melatonin attenuates ropivacaine-induced apoptosis. Ropivacaine induces the generation of ROS and the decrease of mitochondrial membrane potential, while damage to mitochondrial function. The dysfunctional mitochondria with lysosomes form autolysosomes, causing aberrant mitophagy. Finally, ropivacaine induced the apoptosis of PC12 and HT22 cells. Melatonin pretreatment inhibits ROS production and protects the dysfunctional mitochondria, as well as inhibiting autolysosomal formation and mitophagy, thereby downregulating the levels of apoptosis.
downregulation of mitochondrial marker Tom20 and COXIV and excessive ROS production and decreased the ΔΨm. As we know, the damaged mitochondria are eliminated through mitophagy to maintain the cellular homeostasis.

Mitophagy is a special type of selective autophagy, which is dependent on the mediation of some receptors, and the phosphatase and tensin homolog (PTEN)—induced kinase 1 (PINK1)/Parkin pathway is a well-known route. Here, we found that the exposure of ropivacaine upregulated the expression of PINK1 and Parkin, and melatonin pretreatment inhibited the activation of PINK1 and Parkin, suggesting melatonin inhibited ropivacaine-induced PINK1/Parkin-mediated mitophagy. Although melatonin plays a critical role in the selective degradation of damaged or redundant mitochondria, the dysfunction of mitophagy might be responsible for many neuronal disorders, including neurodegenerative diseases, cerebrovascular diseases, and some neurotoxicity diseases [32, 33]. Increasing evidence revealed that excessive mitophagy could lead to the death of neurons [34, 35]. However, there is no report on the effect of mitophagy in local anesthetic–induced neurotoxicity.

Melatonin, as a chief hormone secreted from the pineal gland, regulates numerous biological functions in a variety of cells including neurons [2]. The neuroprotective effects of melatonin have been reported [36, 37]. Melatonin regulates the neurogenesis in both in vivo and in vitro models, including maintaining the neuronal characteristic of different neuronal stem cells, and increasing the structural plasticity of nerve fibers in the 8-week-old mice [3, 38]. In addition, melatonin can decelerate the progress of many neuronal disorders such as depression[39], aging[40], and neurodegenerative diseases[8]. It reduces the depression-like behavior in male mice and enhances the efficacy of antidepressant citalopram [38, 41]. Similarly, melatonin can improve the spatial memory deficits in aging mouse models and attenuate neuronal impairment in PD animal models [42, 43]. Apart from protecting the brain neurons, it was reported that melatonin promoted nerve regeneration after spinal cord injury and peripheral nerve impairment [44, 45]. In recent years, many clinical drugs have been reported to induce neurotoxicity, including scopolamine (a prescription drug used for the prevention of nausea and vomiting), but melatonin pretreatment reversed the behavioral deficits and reduced the neurotoxicity of scopolamine [46, 47]. In our study, we demonstrate that melatonin pretreatment has a protective effect on ropivacaine-induced neurotoxicity, including increasing cell viability, decreasing apoptosis, improving mitochondrial dysfunction, and reducing mitophagy levels. Although the inhibition of melatonin on mitophagy have been reported by other studies [48–50], our study was the first to illustrate that melatonin inhibited ropivacaine-induced mitophagy, which may a potential treatment for ropivacaine-induced neurotoxicity.

In conclusion, melatonin was beneficial at increasing cell viability, reducing neuronal apoptosis, dysfunctional mitochondria, and ROS generation, and downregulating the autophagy and mitophagy levels under ropivacaine-induced neurotoxicity. And we also demonstrated that the protective effects of melatonin against ropivacaine-induced apoptosis were modulated by suppressing excessive mitophagy via the inhibition of Parkin/PINK1 pathway. The above results indicated that melatonin could be a candidate treatment in LA-induced neurotoxicity, and Parkin/PINK1 mediated mitophagy presents a potential target as well.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

LHY and DXD designed the studies, ZL and HJF undertook the cell experiments and the construction of animal experiments, LCG and ZFY undertook the molecular biology testing, and ZL, ZFY, and ZZ analyzed data and wrote the draft of the manuscript.

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AVAILABILITY OF DATA AND MATERIALS

Data that support the study findings are available from the corresponding author upon reasonable request.

DECLARATIONS

Consent for Publication The authors declare the consent for publication.
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Competing Interests The authors declare no competing interests.

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