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Research Article

Neuroprotective Effects of Ramelteon on Inflammation via the NF -κB Pathway in a Mouse Model of Parkinson's Disease

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Abstract

Parkinson's Disease (PD) is a serious neurodegenerative disease, especially in the elderly population, with a very high incidence and no effective clinical treatment. Ramelteon is a melatonin receptor agonist that can be used to treat sleep-resistant insomnia, and it also has a definite effect on chronic insomnia and short-term insomnia, and is the first non-addictive insomnia treatment drug that is not listed as a special control. Ramelteon has been shown to alleviate inflammation and oxidative stress by activating the Nrf2 signaling pathway, thereby preventing traumatic brain injury. Ramelteon has also been shown to delay cell senescence in the Parkinson's model of human SH-SY5Y neuronal cells, however, no studies have shown whether Ramelteon can alleviate Parkinson's by inhibiting neuroinflammation. To this end, this study investigated whether Ramelteon can achieve a neuroprotective effect on 6-OHDA-treated human SH-SY5Y neuronal cells and Parkinson's mouse models through the NF-κB pathway. In this study, we found that Ramelteon could potently inhibit ROS release from 6-OHDA-treated SH-SY5Y neuronal cells, improve dyskinesia in Parkinson's mouse models, and effectively inhibit neuroinflammation via the NF-κB pathway. In conclusion, Ramelteon can inhibit neuroinflammation through the NF-κB pathway, thereby achieving neuroprotective effects on Parkinson's mouse models.

2. Keywords: Parkinson; 6-OHDA; Ramelteon; Neuroinflammation; NF-κB pathway

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disease, affecting about 10 million people worldwide [1]. The risk of Parkinson's disease increases with age and is further influenced by genetic and environmental factors [2]. Clinically, the typical symptoms of PD are motor deficits such as bradykinesia, static tremor, muscle rigidity, abnormal posture and gait, and as the disease progresses, other motor and non-motor symptoms such as constipation, loss of smell, anxiety, depression, cognitive impairment, and dementia [3,4]. The cellular hallmarks of PD are the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), and the formation of intracellular Lewy bodies containing α synuclein aggregates, lipid membranes, and amoeba organelles [5,6]. Chronic inflammation is a major neuropathological hallmark of PD, and increased activation of microglia and astrocytes in the substantia nigra promotes the progression of neurodegeneration [7,8]. When signs of injury appear, microglia undergo rapid changes by increasing the expression of surface molecules and releasing pro-inflammatory factors, which can activate astrocytes and stimulate the upregulation of the activated B cytokine kappa-light chain enhancer (NF-κB) signaling pathway, thereby further increasing the inflammatory process [9,10].

Ramelteon is an oral hypnotic drug developed by Takeda and approved by the U.S. Food and Drug Administration in 2005 [11]. It is the first melatonin receptor agonist for the clinical treatment of insomnia, and the first non-addictive insomnia treatment drug that is not listed as a special regulation [12]. Ramelteon regulates cyclic biorhythms over a 24-hour period by specifically targeting receptors in the suprachiasmatic nucleus of the human hypothalamus, which has been shown to penetrate the brain and exert neuroprotective effects [13]. In this study, we used a 6-OHDA constructed human SH-SY5Y neuronal cell PD model and a C57 mouse PD model to evaluate whether Ramelteon treatment can achieve remission in the Parkinson's model by inhibiting neuroinflammation, and investigated the link between its anti-inflammatory mechanism and the NF-κB pathway.

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MATERIALS AND METHODS

Cell Culture and Processing

Human neuroblastoma cells SH-SY5Y (purchased from Hunan Fenghui Biotechnology Co., Ltd.) were cultured in F12 + Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 50 μg/mL penicillin, and 50 μg/mL streptomycin solution. Cells are cultured in a cell culture incubator at 37 °C with 5% CO_2. Cells were treated with 6-OHDA at a concentration of 50 μM with or without 50 or 100 nM Ramelteon for 24 hours.

LDH Release

The cell viability of SH-SY5Y neuronal cells was evaluated by measuring the amount of LDH released. After 45 minutes of incubation with 1% Triton X-100, it was darkly incubated with medium supplemented with lactic acid, NAD+, diaphorase, 0.004% BSA, 0.15% sucrose, and 2-p-iodophenyl-3-nitrophenyl tetrazolium chloride (INT). Subsequently, the absorbance at 490 nm was measured with a microplate reader (Bio-Rad, California, USA).

ROS Content Detection

The ROS scavenging activity of Ramelteon is further quantified by flow cytometry. Briefly, human SH-SY5Y neuronal cells were seeded in 6-well plates, 6-OHDA was used to induce ROS production, then cells were treated with Ramelteon for 24 hours and the medium was removed, cells were collected after staining with DCFH-DA for 30 minutes, and the fluorescence intensity of the cells was detected by flow cytometry after a rapid wash.

Immunofluorescence

The treated human SH-SY5Y neuronal cells are fixed with 4% paraformaldehyde for 30 minutes, then permeabilized in 0.5% Triton X-100 for 30 minutes, blocked in PBS containing 1% BSA for 1 hour, and incubated with the primary antibody for 12 hours at 4°C. After washing three times, incubate with the corresponding secondary antibody for 1 hour in the dark. Cells were transferred to DAPI and fixed on glass slides, imaged and analyzed using an FV3000 confocal laser scanning microscope (Olympus), and images were analyzed with Image J.

Experimental Design and Ethical Statement

All surgeries performed on mice in this study were performed under the guidance of the Animal Research Council of Guangxi University. 12-weeks-old male C57 mice were selected to establish the model. Mice were anesthetized with a 2,2,2-tribromoethanol solution before injection with 6-hydroxydopamine (3.3 μg/μL) using a stereotaxic apparatus. Injections were performed at the following two coordinates: anteroposterior (AP), 0.3 mm; mediolateral (ML), −2.2 mm; dorsoventral (DV), −3 mm; AP, 1.1 mm; ML, −1.7 mm; DV, −2.9 mm. The 6-hydroxydopamine solution (2 μL) was injected at each point and the needle was left in place for 5 minutes to promote drug absorption and prevent reflux. Then, 20,000 IU penicillin was injected for the first 3 days after the operation to prevent surgical infection. One week after surgery, mice received an i.p. injection of apomorphine (0.5 mg/kg). After 5 minutes of acclimatization, rotational data were continuously recorded for 30 minutes and the number of revolutions of more than seven circles per minute was considered to be successful model [14]. Subsequently, mice in the Ramelteon-treated group were administered continuously for 28 days by oral gavage at a concentration of 10 mg/kg, and the dosing was based on the previous study [15]. The body weight changes of mice are observed during dosing. After dosing, mice were again intraperitoneally injected with apomorphine (0.5 mg/kg) to verify whether Ramelteon had a behavioral improvement in their dyskinesia before euthanizing the mice and collecting brain tissue.

Antibody and Chemical Testing

Ramelteon (CAS: 196597-26-9) with a purity exceeding 99% was purchased from Aladdin (China). 6-OHDA (CAS: 636-00-0) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (China). The LDH Cytotoxicity Assay Kit (C0016) was purchased from Beyotime (China). Anti-TH (1:5000; 25859-1-AP), anti-GAPDH (1:10000; 60004-1-Ig) and anti-IL-18 (1:1000; 10663-1-AP) was purchased from Proteintech (China). Anti-NF-κB p65 (1:1000; bs-0465R), anti-β-actin (1:5000; bs-0061R), anti-IL-1β (1:1000; bs-0812R), anti-Iba1 (1:1000; bs-1363R), and anti-TNF-α (1:1000; bs-10802R) were obtained from Bioss (China).

Western Blotting

Fresh brain tissues were homogenized at 4°C in a mixture of PMSF and RIPA lysis buffer (in a 1:100 ratio). The supernatant was collected following centrifugation. Quantification was done using the Bradford Coomassie Brilliant Blue (BCA) assay kit. 5 × buffer was added to the supernatant, followed by vortexing and centrifugation. Subsequently, the mixture underwent a constant temperature water bath at 95 °C for 10 minutes. The samples were separated using 10% PAGE gels. Proteins were then transferred to PVDF membranes using a transfer solution. After blocking the membrane with a rapid blocking solution for 20 minutes, the PVDF membrane and the primary antibody were incubated at 4°C overnight. The membrane was washed three times with TBST. Finally, it was incubated with secondary antibody at room temperature for one hour. Protein bands were detected using an ECL chemiluminescence detection kit. Band intensity was analyzed using Image J to measure grayscale values.

Immunohistochemistry

The brain tissue washed by PBS was immediately fixed in 4% paraformaldehyde, and placed in an embedding box for dehydration and embedding after fixation. The tissue was continuously divided into sections, deparaffinized with xylene for 20 minutes, and then hydrated with fractionated alcohol. After three PBS washes, they were blocked with 10% BSA. Diluted the primary antibody with PBS in the correct ratio and incubated with the corresponding sections. PBS washed three times and then incubated with secondary antibody. Observed the sections under a microscope and analyzed the pictures with Image J.

Statistical Analysis

GraphPad Prism8.0 software was used to statistically analyze the experimental results, the difference between the two groups was t-test, and the one-way ANOVA was used for more than two groups, and the data were expressed by mean ± SEM, and the difference was considered significant when $P < 0.05$. All experiments were performed in at least 3 replicates.

Results

Ramelteon Ameliorates 6-OHDA-Induced Decreased Viability of Human SH-SY5Y Neuronal Cells and Slows Down Intracellular ROS Production

It has been shown that the appropriate dosing concentration of Ramelteon for human SH-SY5Y neuronal cell experiments is 50-100 nM, for which the cells are divided into four groups, cultured with 50 μM 6-OHDA alone, with 100 nM Ramelteon alone, simultaneously with 50 μM 6-OHDA, 50 nM Ramelteon and simultaneously with 50 μM 6-OHDA, 100 nM Ramelteon for 24 hours at 37 °C, 5% CO_2. It can be observed that the cell group administered 6-OHDA alone had an extremely high mortality rate, while the cell group administered Ramelteon alone had a significant difference in mortality (Figure 1A), implying that Ramelteon was less cytotoxic. At the same time, according to the two sets of experiments with the simultaneous administration of 6-OHDA and Ramelteon (Figure 1A), it can be seen that Ramelteon improves the decrease in cell activity caused by 6-OHDA, and the concentration of 100 nM is better than the concentration of 50 nM, so the concentration of 100 nM is selected for subsequent cell experiments. Based on the results (Figure 1B), Ramelteon was effective in alleviating the increased ROS release caused by 6-OHDA, indicating that Ramelteon alleviated inflammation and oxidative stress in the cell model.

Ramelteon Potently Inhibits Neuroinflammation and Achieves Neuroprotective Effects on Human SH-SY5Y Neuronal Cells through the NF-κB Pathway

Immunofluorescence experiments were performed on normal group, model group and Ramelteon-treated group cells to verify the effect of Ramelteon. According to the experimental results, it can be seen that the fluorescence intensity of Tyrosine Hydroxylase (TH) was significantly reduced by 6-OHDA, while the Ramelteon-treated group significantly improved this change (Figure 2A), and there was a significant difference of the fluorescence intensity between the model group and Ramelteon-treated group (Figure 2B), which not only proved the feasibility of 6-OHDA for modeling, but also proved that Ramelteon had a significant therapeutic effect on Parkinson's cell model. The activation of microglia is closely related to neuroinflammation, so we explored the effect of Ramelteon on microglia activation and found that 6-OHDA also had a significant effect on microglia activity, Iba1 is a microglia marker, and the induction of 6-OHDA significantly increased the fluorescence intensity of Iba1, while the treatment of Ramelteon significantly improved this effect (Figure 2C), and there was a significant difference in the fluorescence intensity with the model group (Figure 2D). Based on these results,

Ramelteon can significantly inhibit neuroinflammation in Parkinson's model. On this basis, we further verified whether the improvement effect of Ramelteon on neuroinflammation in the Parkinson's model is achieved through the NF-κB pathway, and the results showed that Ramelteon can effectively alleviate the significant increase in NF-κB induced by 6-OHDA (Figure 2E), and there are significant differences between the treatment group and the model group (Figure 2F), which proves that Ramelteon's neuroinflammation alleviation is closely related to the NF-κB pathway.

Weight Change and Behavioral Validation in a Mouse Model of Parkinson's Disease

We used a stereotaxic device to inject 6-OHDA into the striatum of normal C57 male mice to construct a mouse Parkinson's model, and one week later administered an intraperitoneal injection of apomorphine to verify the success of the model construction, and the results obtained by continuously recording rotation data for 30 minutes were analyzed

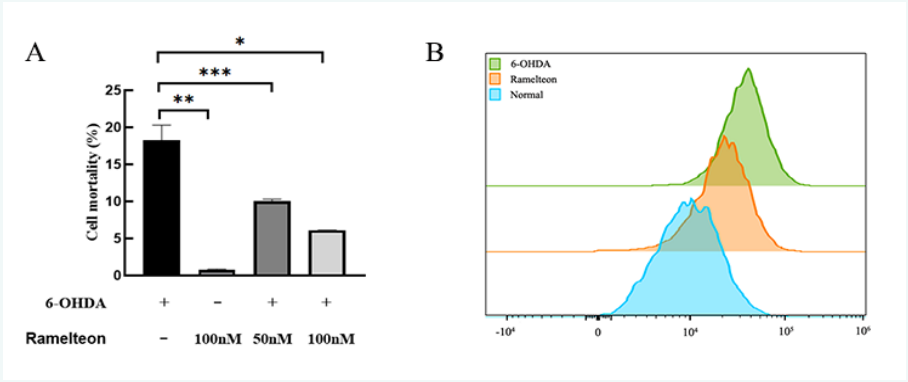

Figure 1 Ramelteon ameliorates decreased cell viability and reduces cellular ROS production due to 6-OHDA (A) Cell mortality with 6-OHDA alone versus Ramelteon alone. Comparison of the effect of the session at Ramelteon concentrations of 50 and 100 nM, respectively (B) Comparison of ROS release between normal group, model group and treatment group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

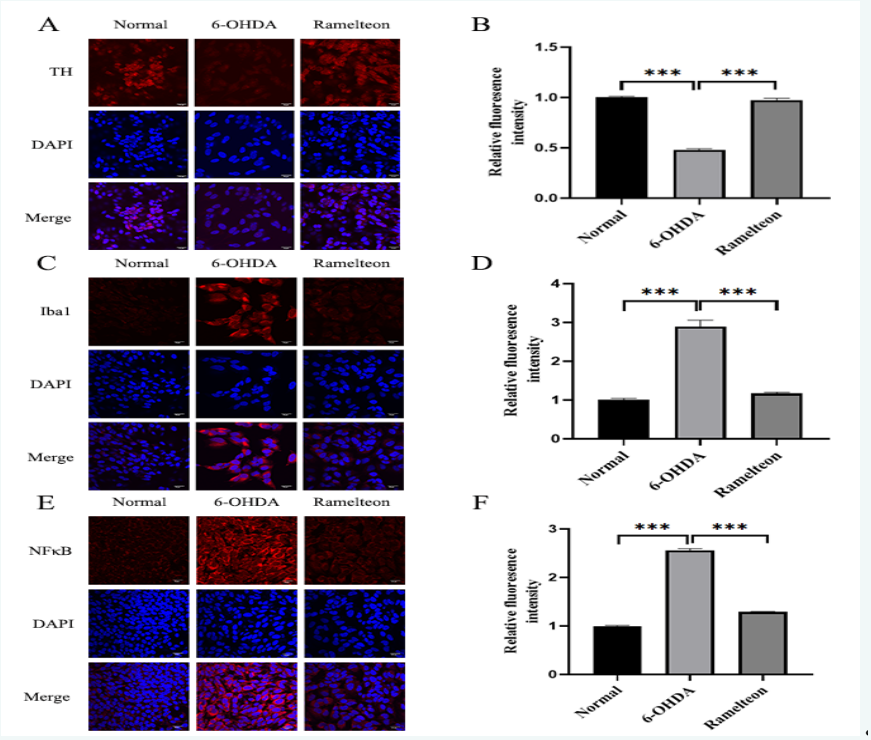

Figure 2 Ramelteon effectively inhibits neuroinflammation and achieves neuroprotective effects on human SH-SY5Y neuronal cells through the NF-κB pathway (A) Comparison of TH fluorescence intensity between the three groups of cell models (B) Data analysis of TH fluorescence intensity, it can be seen that there are significant differences between the treatment group and the building module. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (C) Comparison of Iba1 fluorescence intensity between the three groups of cell models (D) Data analysis of Iba1 fluorescence intensity, It can be seen that there are significant differences between the treatment group and the manufacturing module. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (E) Comparison of the fluorescence intensity of NF-κB between the three groups of cell models (F) The data analysis of the fluorescence intensity of NF-κB shows that there is a significant difference between the treatment group and the manufacturing module. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(Figure 3A), and the number of revolutions exceeding 7 revolutions per minute was considered to be successful. After 28 days of continuous administration of Ramelteon, the mice were injected intraperitoneally with apomorphine, and the results were analyzed (Figure 3A), and it was seen that the number of turns in the treatment group was lower than the 7 revolutions per minute required for successful modeling, and there was a significant difference between the treatment groups before and after administration, and the above results proved that Ramelteon improved the dyskinesia of Parkinson's mouse model. Subsequently, the mice in the treatment group were administered by oral gavage at a concentration of 10 mg/kg for 28 days, and the body weight was recorded, and the body weight change curve was plotted (Figure 3B), according to which it was seen that the continuous application of Ramelteon had no significant effect on the body weight of the mice, and the Ramelteon treatment improved the weight loss of mice caused by 6-OHDA surgical modeling.

Effect of Ramelteon on TH Reactivity in a Mouse Model of Parkinson's Disease

According to the obtained whole brain sagittal section and 20x local magnification (Figure 4A), it can be seen that the induction of 6-OHDA significantly reduced the positive signal of TH index in the striatum region of the mouse model, while the treatment of Ramelteon significantly improved this change. The results of the analysis of the relative positive area of the TH index obtained by immunohistochemistry (Figure 4B) also confirmed that there was a significant difference between the model group and Ramelteon-treated group. We further performed WB experiments to verify the expression of TH protein in the striatum of the mouse model, and the resulting bands (Figure 4C) showed that the use of Ramelteon had a significant improvement on the reduction of TH indicators by molding, and data analysis of the gray values of the resulting TH bands (Figure 4D) further verified that there was a significant difference between the treatment group and the model group.

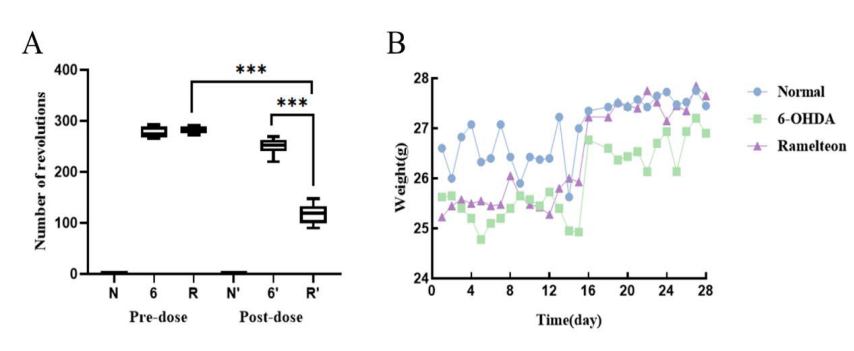

Figure 3 Weight changes of animal samples during administration and behavioral verification before and after administration (A) Mice were intraperitoneally injected with apomorphine before and after administration and the number of rotations for 30 minutes was recorded, and the results were collated (among them, N, 6, and R groups were pre-dose data, N', 6', R' The three groups are post-dose data), and there were significant differences between the post-dose Ramelteon group, the pre-dose Ramelteon group, and the post-dose model. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (B) During the administration period, the body weight of mice was monitored daily and the body weight change curve was plotted.

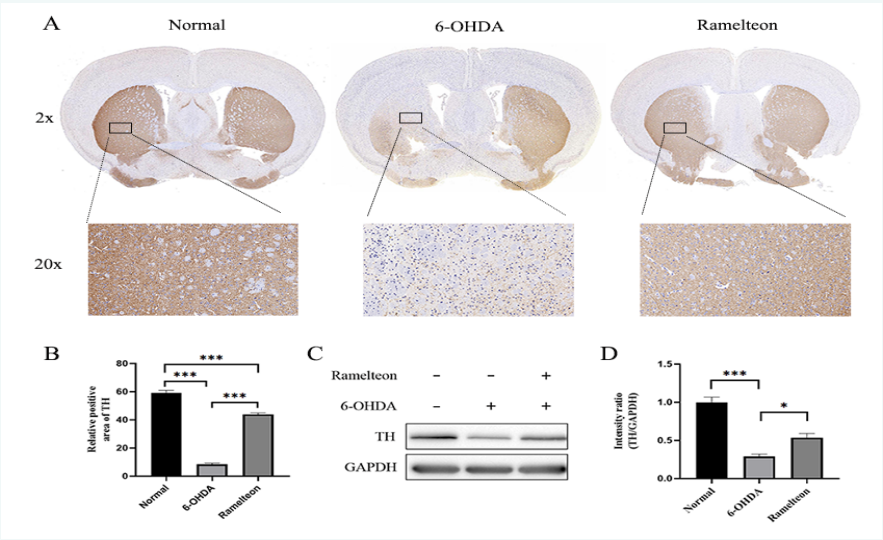

Figure 4 Effect of Ramelteon on tyrosine hydroxylase reactivity in a mouse model of Parkinson's disease (A) Immunohistochemical picture of TH expression in brain slices of three groups of mouse models, including 2x image and 20x magnification image (B) Data analysis of the relative positive area of TH index in 20x magnification of brain slices of three groups of mouse models, It can be seen that there are significant differences between the treatment group and the model. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (C)The results of the TH index obtained by the brain tissue of the three groups of mouse models for WB experiments (D)The data comparison results obtained from the analysis of the grey values of the TH bands obtained by the three groups of mouse models show that there are significant differences between the treatment group and the model. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Effect of Ramelteon on Microglia Activation in a Mouse Model of Parkinson's Disease

Similarly, in order to verify the effect of Ramelteon on microglia activation, we also performed immunohistochemical experiments on brain tissue sections of three groups of mouse models, and according to the obtained 20x local magnification and 40x magnification of brain tissue (Figure 5A), it can be seen that the induction of 6-OHDA significantly increased the positive signal of Iba1 index in the striatum region of the mouse model, and the treatment of Ramelteon significantly improved this change. The results of the analysis of the relative positive area of the Iba1 index obtained by immunohistochemistry (Figure 5B) also confirmed that there was a significant difference between the model group and Ramelteon-treated group. We also conducted further WB experiments to verify the expression of Iba1 protein in the striatum of the mouse model, and the resulting bands (Figure 5C) showed that the use of Ramelteon had a significant improvement on the increase of Iba1 indicators by molding, and the analysis of the gray values of the resulting Iba1 bands (Figure 5D) further verified that there was a significant difference between the model group and Ramelteon-treated group.

Ameliorating Effect of Ramelteon on Neuroinflammation in a Mouse Model of Parkinson's Disease

Finally, in order to verify the effect of Ramelteon on neuroinflammation in mouse models, we conducted immunohistochemical experiments on brain tissue sections of three groups of mouse models, and according to the obtained 20x local magnification and 40x magnification of brain tissue (Figure 6A), it can be seen that the induction of 6-OHDA significantly increased the positive signal of IL-18 indicators in the striatum region of the mouse model, and the treatment of Ramelteon significantly improved this change. The results of the analysis of the relative positive area of the IL-18 index obtained by immunohistochemistry (Figure 6C) also confirmed that there was a significant difference between the model group and Ramelteon-treated group. We also conducted further WB experiments to verify the protein expression of IL-1β, TNF-α and IL-18, which are closely related to neuroinflammation, in the striatum of the mouse model, and the resulting bands (Figure 6B) showed that Ramelteon treatment had a significant improvement in the increase of the three pro-inflammatory indexes induced by 6-OHDA, and the data analysis of the obtained bands (Figure 6D-F) further verified that there were significant differences between the treatment group and the model group.

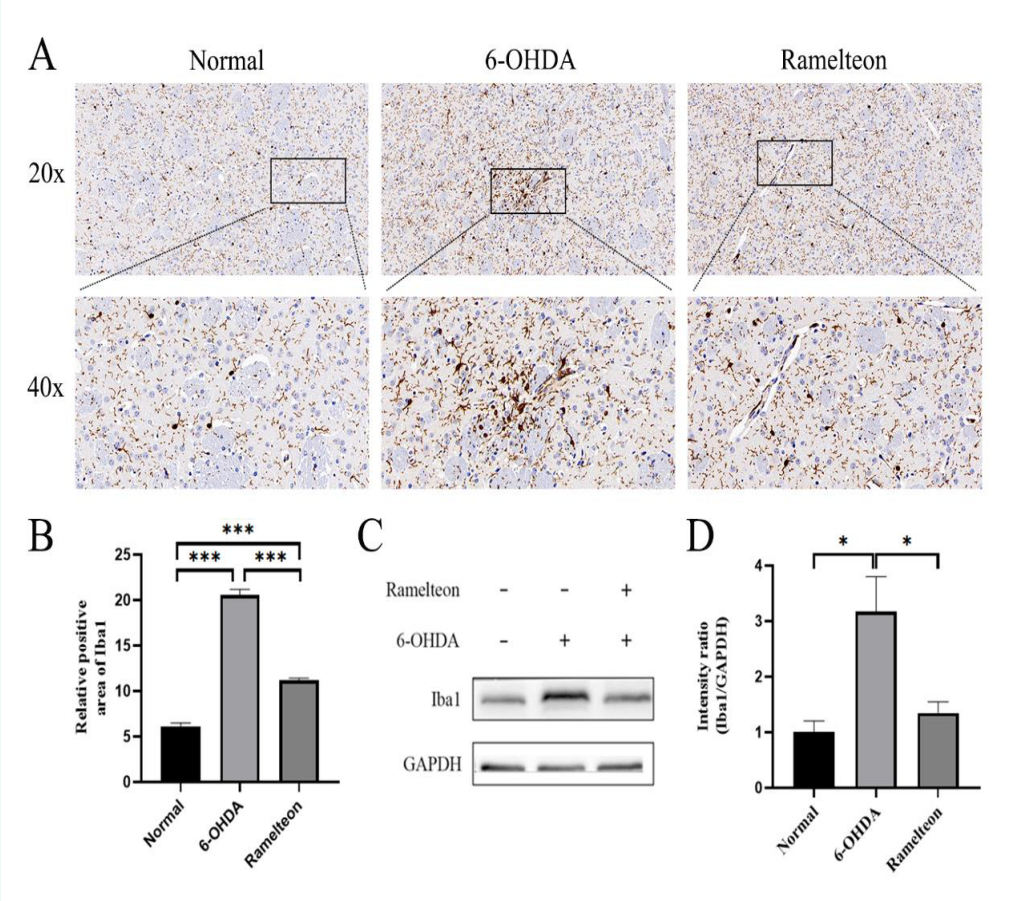

Figure 5 Effect of Ramelteon on tyrosine hydroxylase reactivity in a mouse model of Parkinson's disease (A) Immunohistochemical picture of TH expression in brain slices of three groups of mouse models, including 2x image and 20x magnification image (B) Data analysis of the relative positive area of TH index in 20x magnification of brain slices of three groups of mouse models, It can be seen that there are significant differences between the treatment group and the model. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (C)The results of the TH index obtained by the brain tissue of the three groups of mouse models for WB experiments (D)The data comparison results obtained from the analysis of the grey values of the TH bands obtained by the three groups of mouse models show that there are significant differences between the treatment group and the model. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

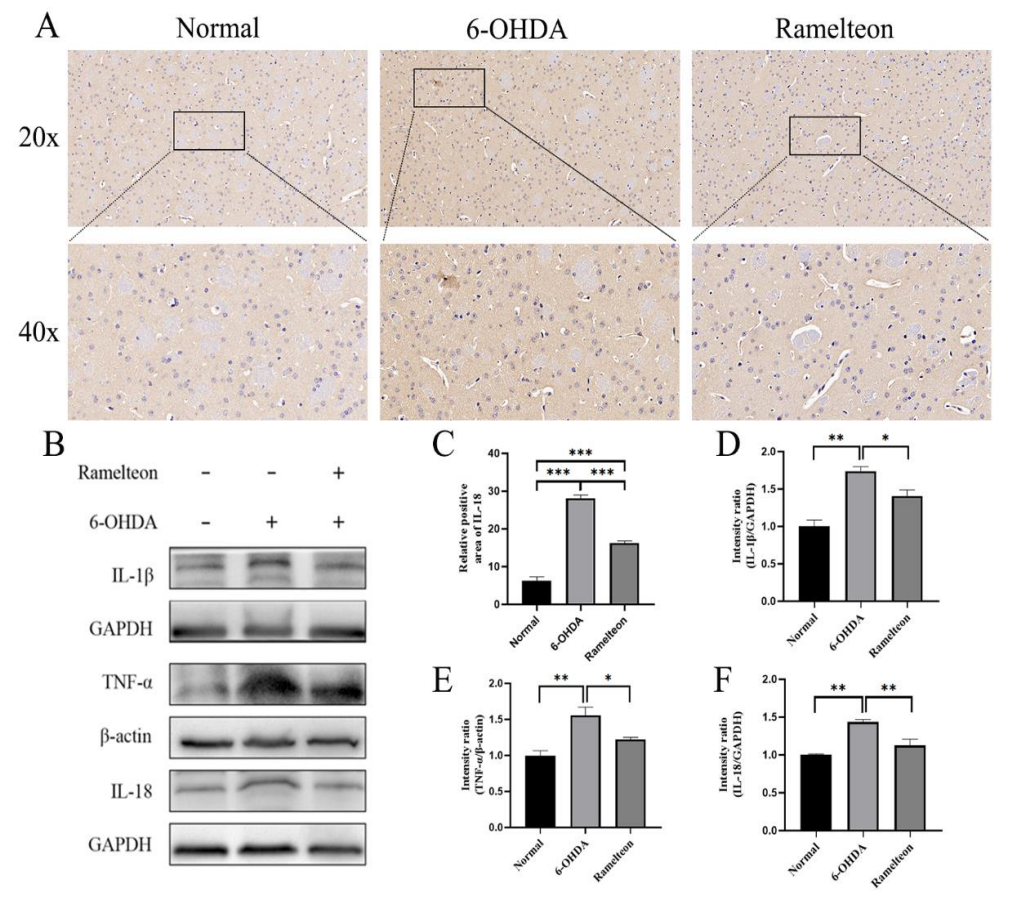

Figure 6 Ameliorating effect of Ramelteon on neuroinflammation in a mouse model of Parkinson's disease (A) Immunohistochemical images of IL-18 expression in brain sections of three groups of mouse models, including 20x magnified images and 40x magnified images (B) The results of IL-1β, TNF-α and IL-18 related to neuroinflammation were obtained from the brain tissues of the three groups of mouse models for WB experiments (C) Data analysis of the relative positive area of IL-18 index in the 40x magnified image of the brain section of the three groups of mouse models, showing that there are significant differences between the treatment group and the model group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (D)The comparison of the data obtained from the analysis of the grey value of the IL-1β bands obtained from the analysis of the three groups of mouse models shows that there is a significant difference between the treatment group and the model group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (E)The data comparison results obtained from the analysis of the grey value of the TNF-α bands obtained from the analysis of the three groups of mouse models, it can be seen that there is a significant difference between the treatment group and the model group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (F) The data comparison results obtained from the analysis of the grey value of the IL-18 bands obtained by the three groups of mouse models show that there is a significant difference between the treatment group and the model group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

Currently, neurotoxin-induced permanent dopamine depletion models are commonly used in vitro experiments to mimic the pathogenesis of PD, including 6-Hydroxydopamine (6-OHDA), Tetrahydropyridine (MPTP), and Methamphetamine (MA) [16]. 6-OHDA is a hydroxylated derivative of dopamine, which is similar to DA in its chemical structure, so it can compete with DA for the uptake site and then be taken into the cell. After entering the cell, 6-OHDA can be oxidized and decomposed to produce reactive oxygen species, which can further produce oxygen radicals through MAO (monoamine oxidase), or directly cause mitochondrial dysfunction, resulting in the death of dopaminergic neuron, which causes biochemical and neurochemical damage similar to PD in animals, so 6-OHDA was used to induce PD in animal PD models [17].

Tyrosine hydroxylase is an enzyme responsible for catalyzing the conversion of the amino acid L-tyrosine to Dihydroxyphenylalanine (dopa), a precursor of dopamine, which is encoded by the TH gene in humans [18]. Because the motor symptoms of Parkinson's disease are caused by the death of dopamine cells in the SNc of the midbrain, so the expression of TH can directly reflect the severity of Parkinson's disease [19]. In this study, Ramelteon's treatment improved the reduction of TH expression caused by 6-OHDA, whether it was WB, immunohistochemistry or immunofluorescence assays, indicating that Ramelteon successfully achieved the alleviating effect of Parkinson's disease.

Chronic inflammation is closely related to the occurrence and development of Parkinson's disease, and the increased activation of microglia and astrocytes in the substantia nigra promotes the progression of neurodegeneration [20]. Microglia have the characteristics of polysynaptic and plastic, are inherently immune effector cells in the central nervous system, and play an extremely important role in the physiological

processes of the central nervous system [21]. When the central nervous system is affected, the microglial phenotype is altered and inflammatory mediators are released, mainly cytokines and chemokines [22]. Studies have shown that microglia-mediated chronic inflammation is involved in the pathological process of a variety of chronic neurodegenerative diseases, including Parkinson's disease [23,24]. In this study, the expression of microglia marker Iba1 increased after 6-OHDA modeling, but decreased significantly after Ramelteon treatment, indicating that Ramelteon significantly inhibited microglial activation.

The inflammatory mediators released after microglia activation include various inflammatory factors [25,26], among which TNF-α actively participates in various inflammatory responses and plays a central role in inflammatory responses, and IL-18 is a pro-inflammatory cytokine that is a member of the IL-1 family along with IL-1β [27]. The expressions of TNF-α, IL-18 and IL-1β in mouse brain tissues increased after 6-OHDA modeling, indicating that there was inflammation in brain tissues, and the expressions of all three decreased significantly after Ramelteon treatment, indicating that Ramelteon can effectively inhibit neuroinflammation in brain tissues.

Alterations in microglia phenotype and the release of inflammatory mediators can also activate astrocytes, which stimulates the upregulation of the activated NF-κB signaling pathway, further increasing the inflammatory process [28,29]. In the immunofluorescence experiment on human SH-SY5Y neuronal cells, the fluorescence intensity of NF-κB in the cells increased significantly after 6-OHDA modeling, but decreased significantly after Ramelteon treatment, which showed that Ramelteon not only effectively inhibited neuroinflammation, but also closely related to the NF-κB pathway.

Conclusion

We both established a human SH-SY5Y neuronal cell model of Parkinson's disease and an animal model of C57 mice. Our study showed that Ramelteon attenuated dyskinesia in C57 mice with 6-OHDA injury. It protects dopaminergic neurons in SNc from neuronal death, reduces microglial activation, and simultaneously decreases NF-κB activation. The results of our multiple studies prove that Ramelteon has potent neuroprotective effects on various mechanisms of neurodegenerative progression in Parkinson's disease. The current results suggest that Ramelteon has the potential for clinical application as an adjuvant treatment for Parkinson's disease [30].

Conflict of Interest

There are no conflicts of interest to declare.

Statement of Author Contributions

Zhong-Quan Qi and Yun-Jin Lin designed the study and applied for Research Ethics Board approval. Yun-Jin Lin and Si-Yao Cheng conducted the experiment. Can-Can Wang and Xin-Yue Zhang assisted with analysis tools of data. Yun-Jin Lin and Xiao-Min Liu discussed the results and manuscript. Zhong-Quan Qi Yun-Jin Lin and Fei Yu wrote the manuscript. All authors approved the final manuscript.

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