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ORIGINAL RESEARCH

Atractylenolide-I Ameliorates Motor Deficits and Reduces Inflammation of the Spinal Cord by SIRT1/PGC-1α Pathway in MPTP Subacute Mouse Model of Parkinson's Disease

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Background: Parkinson's disease (PD) is a neurodegenerative movement disorder that impacts various systems, including the substantia nigra (SN) par compacta (SNpc) and extranigral regions like the spinal cord. The presence of persistent inflammation in the SN and spinal cord is associated with movement difficulties in PD. Atractylenolide-I (ATR-I) is a natural sesquiterpene recognized for its anti-inflammatory and neuroprotective effects. This research aimed to assess the impact of ATR-I treatment on motor function and inflammation in MPTP-induced subacute PD mice, particularly focusing on the role of ATR-I in spinal cord inflammation.

Methods: The motor functions of the mice were assessed using suspension and gait tests. Dopaminergic neuronal loss in the SNpc and microglial activation in both the SNpc and spinal cord were evaluated through immunofluorescence staining. The levels of inflammatory mediators in the spinal cord were measured using RT-qPCR analysis. The expressions of SIRT1 and PGC-1α in the spinal cord were analyzed through Western blotting and RT-qPCR.

Results: ATR-I treatment improved motor deficits in MPTP-induced mice. Moreover, ATR-I reduced the loss of dopamine neurons and microglial activation in the SNpc of MPTP-induced mice. Additionally, ATR-I suppressed spinal cord inflammation by decreasing microglial activation and the mRNA expression of TNF-α, IL-1β, and iNOS in MPTP-induced mice. Interestingly, ATR-I also upregulated SIRT1 and PGC-1α levels in the spinal cord of MPTP-induced mice.

Conclusion: These findings suggest that ATR-I exhibits anti-inflammatory and neuroprotective properties in PD. The attenuation of spinal cord inflammation via the SIRT1/PGC-1α pathway may contribute to enhancing motor function, highlighting ATR-I as a potential therapeutic avenue for PD.

Keywords: Parkinson's disease, atractylenolide-I, spinal cord, inflammation, SIRT1, PGC-1α

Introduction

Parkinson's disease (PD) is a common neurodegenerative condition primarily affects older individuals. It is defined by motor symptoms, including bradykinesia, gait and balance disturbances, and resting tremors, as well as various non-motor symptoms, such as neuropsychiatric disorders (including depression and anxiety), cognitive impairment, autonomic dysfunction, and sleep disorders.[1–3] PD is currently characterized as a multiorgan and multisystemic pathology. The primary pathological manifestation of PD is decreased dopamine levels in the striatum due to the degeneration of dopaminergic neurons (DA) in the substantia nigra (SN) par compacta (SNpc).[4] Recent research indicates that neuropathologic changes in central nervous system regions beyond the SN, including the spinal cord, are also involved in movement disorders during PD pathogenesis.[5–7]

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Despite ongoing research, there is currently no definitive therapy to halt or reverse the advancement of the disease. Emerging evidence have found that chronic inflammation may take an important part in the disease progression of PD.[8] The increase of IL-1β and α-synuclein in plasma of PD patients have been positively correlated with the severity of movement.[9] Furthermore, systemic inflammation and mitochondrial dysfunction triggered by gut dysbiosis are implicated in the advancement of PD.[10] Notably, activation of microglia and subsequent inflammatory responses in the SN and spinal cord of individuals with PD can lead to localized neuronal death.[5,11] Conversely, studies in MPTP-induced mice have demonstrated that reducing inflammation and neurodegeneration in the SN and spinal cord improved gait abnormalities.[12,13] Therefore, targeting inflammation of SN and spinal cord could be a promising strategy for treating PD.

Atractylenolide-I (ATR-I) is a sesquiterpene isolated from the Rhizoma of *Atractylodes macrocephala*, recognized for its wide array of biological properties, including neuroprotective, anti-inflammatory, antioxidant, and anti-tumor activities.[14,15] Studies have demonstrated that ATR-I hinders NLRP3-mediated astrocyte A1 differentiation, reduces tissue inflammation, and ameliorates symptoms of depression in mice.[16] Moreover, ATR-I has been found to suppress acetaminophen-induced elevation of pro-inflammatory mediators like IL-1β, TNF-α, and IL-6, thereby alleviating liver inflammation.[17] ATR-I also inhibits inflammatory signaling pathways and ameliorates intestinal dysbiosis in mice.[18] Furthermore, ATR-I has been shown to reduce MPP^+-induced loss of SH-SY5Y cell viability and decrease apoptosis.[19] Additionally, ATR-I modulates the neurotransmitter levels and improves the behaviors in mice with depression by targeting 5-HT2A.[20] Research has further indicated that ATR-I decreases the astroglial and microglial markers in the striatum, mitigates the loss of DA in the ventral midbrain, and improves motor dysfunction in an MPTP-induced acute mouse model of PD.[21] However, the potential anti-inflammatory properties of ATR-I in the spinal cord and SNpc in a subacute MPTP mouse model remain unexplored.

The silent information regulator 1 (SIRT1)/peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) axis represents a crucial signaling pathway involved in neuroprotection. Research indicates that NAD+ inhibits mitochondrial damage and ROS production, mitigates cognitive deficits, and suppresses neuroinflammation in models of chronic cerebral hypoperfusion by activating the SIRT1/PGC-1α pathway.[22] Another study demonstrated that resveratrol enhances the cognitive function and reduces neurodegeneration in mouse models of Alzheimer's disease through the upregulation of SIRT1 and the downregulation of the NF-κB/IL-1β/NLRP3 inflammatory pathway. Furthermore, SIRT1 plays a neuroprotective role in the development of PD. SIRT1 ameliorates MPP^+-induced dopaminergic neuronal cell injury by enhancing PGC-1α expression and promoting mitochondrial biogenesis.[23] Some SIRT1 activators, such as alpha-lipoic acid and SRT1720, have exhibited protective effects against various toxins in both in vivo and in vitro PD models.[24,25] Thus, the SIRT1/PGC-1α pathway may serve as a potential target for therapeutic interventions in PD.

The objective of this study is to investigate the impact of ATR-I treatment on motor function and inflammation in the spinal cord and SNpc, with a specific focus on elucidating the underlying mechanisms of ATR-I in spinal cord inflammation.

Materials and Methods

Animals and Drug Administration

Male C57BL6/J mice, aged ten to twelve weeks and weighing 23–27 g, were acquired from Vital River Laboratory Animal Technology Co. Ltd in Beijing, China. All studies were approved by the Ethics Committee of the Second Hospital of Hebei Medical University (2022-AE284). Any procedures for animals were conducted according to the National Institutes of Health Guide for the welfare and utilization of Laboratory Animals. The mice were kept in a controlled environment with a 12-hour light/dark cycle, a temperature of 22 ± 1°C, a humidity of 55% ± 5%, and had access to food and water ad libitum.

Forty mice were randomly divided into four groups, each comprising 10 mice. The groups included a control group that received intraperitoneal (i.p.) injection of a vehicle, a control + ATR-I group that received both the vehicle and ATR-I at 30 mg/kg/day, an MPTP group that received MPTP at 30 mg/kg/day, and an MPTP + ATR-I group that received both MPTP at 30 mg/kg/day and ATR-I at 30 mg/kg/day.

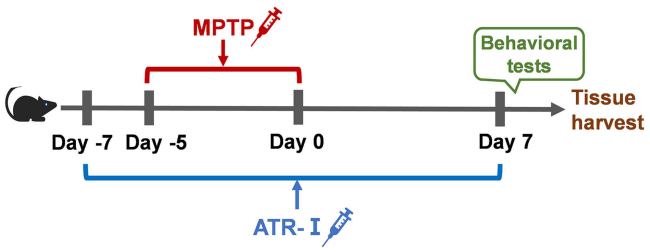

Figure 1 Presents the experimental protocols and treatment to evaluate the effects of ATR-I in the MPTP-induced mouse model.
Abbreviations: ATR-I, atractylenolide-I; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Day −5, the start of MPTP injection; Day 0, the end of MPTP injection; Day −7, the start of ATR-I injection; Day 7, the end of ATR-I injection.

ATR-I, with a purity exceeding 98%, was purchased from Shanghai Yuanye Bio-Technology Co., Ltd in Shanghai, China. MPTP, dimethyl sulfoxide (DMSO), and Tween-20 were acquired from Sigma Biotechnology in MO, USA. ATR-I was dissolved in a solution comprising 1% DMSO and 1% Tween 20 in saline.

The experimental setup, depicted in Figure 1, involved the administration of MPTP to mice in the MPTP group for 5 consecutive days from day −5 to day 0. The MPTP + ATR-I group received ATR-I from day −7 to day 7 for a total of 14 consecutive days, with administration 2 hours prior to MPTP injection from day −5 to day 0. The dose of ATR-I was determined based on previous research.[21]

Behavioral Tests

Behavioral tests were conducted the day after the final administration of ATR-I, and all animals were euthanized on the same day.

The suspension test was carried out according to the literature.[26] In this test, mice were suspended from a horizontal wire with their two front paws and scored based on their ability to grasp the wire with their hind paws. Scoring criteria are as follows: 3 points are awarded if both hind paws can be grasped; 2 points if only one hind paw can be grasped; and 1 point if neither hind paw can be grasped. Each mouse underwent a total of three tests, with the average of these three results calculated, and an interval of three minutes was maintained between each test. The suspension test allows for the assessment of muscle strength, motor coordination, and balance.

The gait test assessed limb coordination by measuring the stride length of mice, following a previously described method.[13] Mice were administered red dye on their forelimbs and blue dye on their hindlimbs, and were guided along a runway into a closed box. Each mouse underwent three training trials. At the end of the training session, a white sheet of paper was placed on the track, and the mice were guided into the box along this sheet, facilitating the recording of consecutive footprints. Step length was calculated as the average distance of three strides.

Immunofluorescence Staining

Each experimental group's mice were perfused with 0.9% saline and 4% paraformaldehyde via the heart while under deep anesthesia. Subsequently, the lumbar spinal cord and midbrain tissues were dissected, fixed in 4% paraformaldehyde overnight, and shifted to a 20–30% sucrose gradient at 4°C. Then, these tissues were embedded in an OCT compound, frozen, and cut into 10 μm thick slices using a frozen microtome. The slices underwent a series of steps including washing in PBS, treatment with Triton X-100, blocking with BSA, and overnight incubation with anti-tyrosine hydroxylase (TH) (1:150, Warbio, China) and anti-Iba1 (1:500, Wako, Japan) antibodies. The following day, the slices were stained with fluorescent secondary antibodies and fluorescence was examined in three random regions of each section using a Zeiss vert. A1 fluorescence microscope.

Western Blotting

The lumbar spinal cord tissue was homogenized using RIPA buffer with a protease inhibitor cocktail from Sigma (USA) in order to extract all proteins present. Protein concentrations were determined by the BCA assay from Boster Biotechnology (China). Equivalent quantities of protein were segregated via SDS-PAGE from Sigma (USA) and then

Table 1 Primers Used in RT-qPCR

Primers	Forward Sequence (5′-3′)	Reverse Sequence (5′-3′)
TNF-α	TTCCCAAATGGGCTCCCTCT	GTGGGCTACGGGCTTGTCAC
IL-1β	CAGGCAGGCAGTATCACTCA	AGCTCATATGGGTCCGACAG
iNOS	CAGGGAGAACAGTACATGAACAC	TTGGATACACTGCTACAGGGA
SIRT1	ACCAAATCGTTACATATTCC	CAAGGGTTCTTCTAAACTTG
PGC-1α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
GAPDH	GGTCGGTGTGAACGGATTT	GTGGATGCAGGGATGATGTT

transferred onto PVDF membranes originating from Cytiva (USA). Following blocking with 5% skimmed milk, the PVDF membranes were sequentially immunoblotted with primary antibodies against silent information regulator 1 (SIRT1) (1:1000, Millipore, #07-131), peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (1:100, Santa Cruz, sc-518025), and β-actin (1:5000, Bioword, BS6007M) overnight at 4°C. Subsequently, the membranes were subjected to incubation with the secondary antibody (1:10000, Thermo Fisher Scientific, USA).

Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) Assay

RNA from the lumbar spinal cord tissue was extracted using TRIzol reagent. For RT-qPCR, 2.5 μg of total RNA with an OD260/280 ratio ranging between 2.1 and 2.2 was reverse transcribed into cDNA utilizing the SureScript™cDNA Synthesis Kit (GeneCopoeia™). The sequences of qPCR primers can be found in Table 1. Relative mRNA levels were assessed employing the Ct ($2^{-\Delta\Delta Ct}$) algorithm and standardized to GAPDH expression.

Statistical Analysis

All data are expressed as the mean ± SEM. The statistical analyses were performed utilizing SPSS software (version 23.0), employing one-way analysis of variance followed by Tukey's post hoc multiple comparisons test. A P value below 0.05 was deemed statistically significant.

Results

ATR-I Treatment Improved MPTP-Induced Motor Deficits in Mice

The suspension and gait tests were conducted to assess the motor function of mice in each group following the final ATR-I treatment. In the suspension test (Figure 2a), the MPTP group exhibited significantly lower suspension scores compared to the control group (1.76 ± 0.44 in the MPTP group versus 2.71 ± 0.26 in the control group, $P < 0.001$). However,

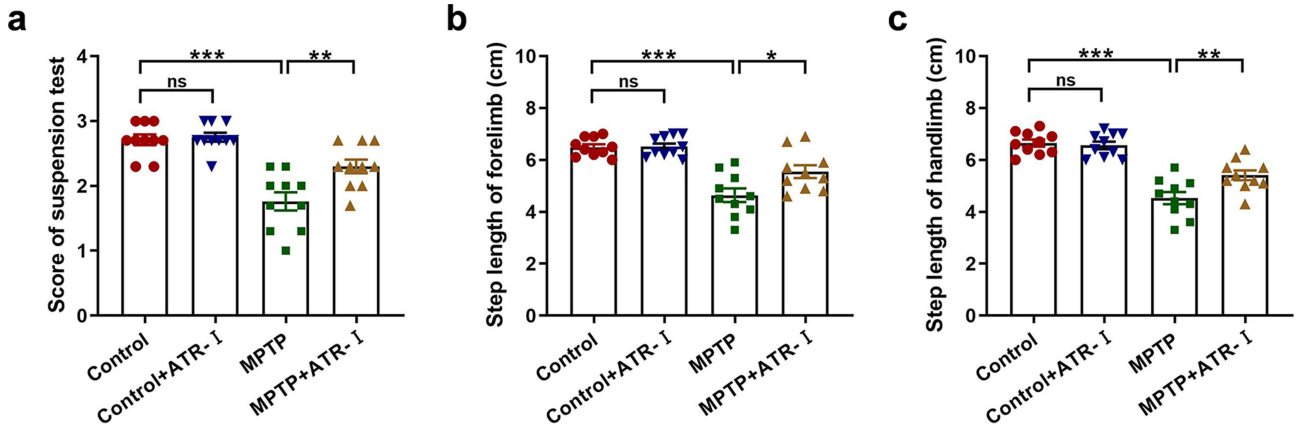

Figure 2 ATR-I treatment improved MPTP-induced motor deficits in mice. (**a**) Suspension score of suspension test. (**b** and **c**) Analysis of the forelimb and hindlimb step lengths in gait test. (mean ± SEM, n=10). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
Abbreviations: ns, not significant. ATR-I, atractylenolide-I; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

suspension scores were notably enhanced in the ATR-I treated mice in comparison to the MPTP group (2.30 ± 0.34 in the MPTP + ATR-I group versus 1.76 ± 0.44 in the MPTP group, $P < 0.01$). There was no significant difference observed between the control group and control + ATR-I groups.

The gait test results indicated that MPTP mice exhibited shorter stride lengths compared to control mice (see Figure 2b and c). Specifically, the step length of the forelimb was 4.64 ± 0.83 cm in the MPTP group and 6.49 ± 0.35 cm in the control group ($P < 0.001$), while the step length of the hindlimb was 4.53 ± 0.74 cm in the MPTP group and 6.65 ± 0.42 cm in the control group ($P < 0.001$). However, in ATR-I-treated MPTP mice, there was a notable improvement in the shortened stride length induced by MPTP (see Figure 2b and c). Specifically, the step length of the forelimb was 5.55 ± 0.77 cm in the MPTP + ATR-I group versus 4.64 ± 0.83 cm in the MPTP group ($P < 0.05$), and the step length of the hindlimb was 5.41 ± 0.60 cm in the MPTP + ATR-I group versus 4.53 ± 0.74 cm in the MPTP group ($P < 0.01$). Treatment with ATR-I alone did not significantly impact the step lengths of either the forelimb or hindlimb compared to control mice.

ATR-I Treatment Attenuates MPTP-Induced DA Neurons Loss and Microglia Activation in the SNpc

The survival of TH^+ neurons and activation of $Iba1^+$ microglia were assessed through an immunofluorescence assay in the SNpc. Figure 3a and b show that the fluorescence intensity of TH in the SNpc of MPTP mice was notably lower compared to the control group (0.74 ± 0.06 in the MPTP group versus 1.00 ± 0.09 in the control group, $P < 0.001$).

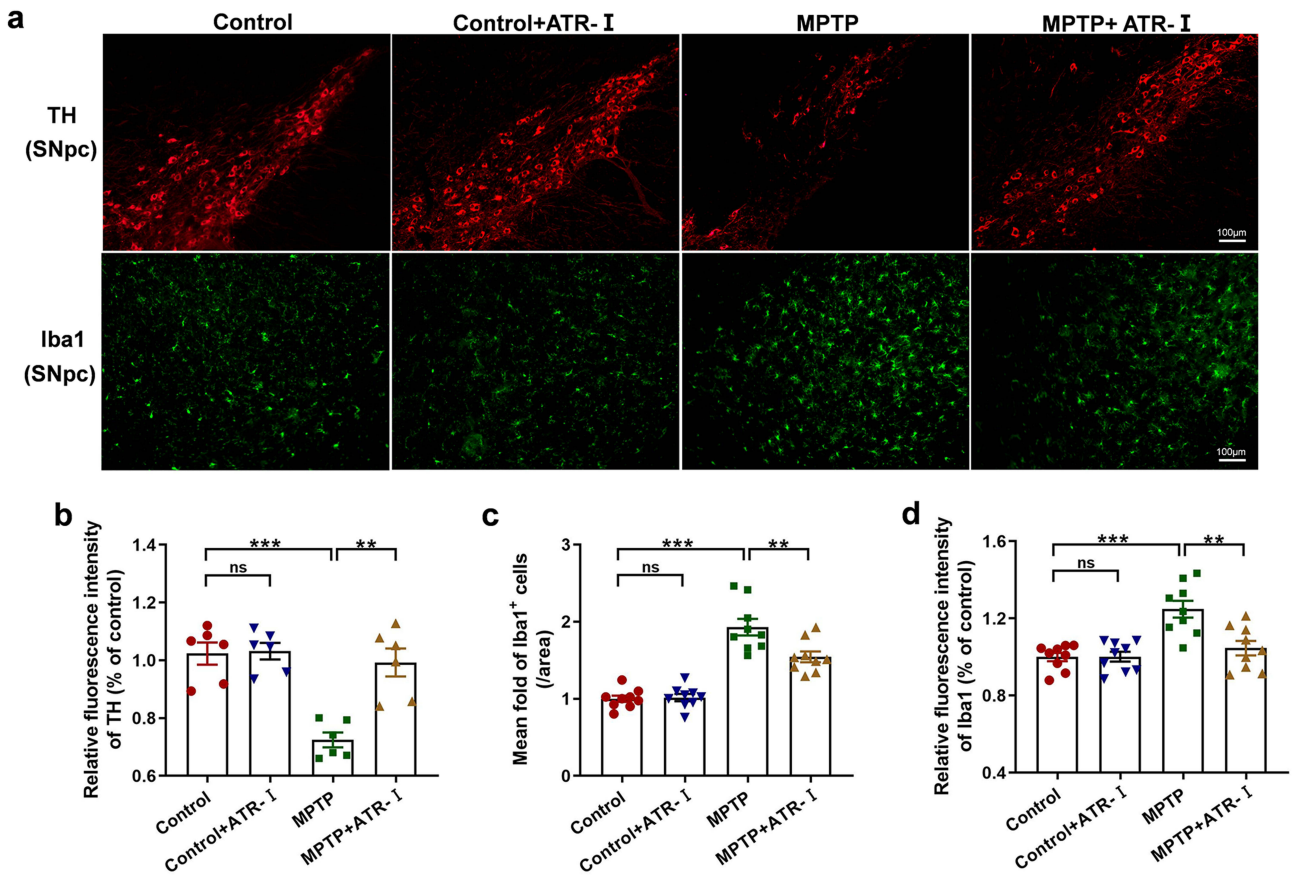

Figure 3 ATR-I treatment attenuates MPTP-induced loss of DA neurons and activation of microglia in the SNpc. (**a**) Immunofluorescence staining of TH and Iba1 in the SNpc. (**b**) Relative fluorescence intensity of TH in the SNpc. (**c**) Relative numbers of $Iba1^+$ cell in the SNpc. (**d**) Relative fluorescence intensity of Iba1 in the SNpc. Scale bar: 100 μm. (mean ± SEM, n=3). $^{**}P < 0.01$ and $^{***}P < 0.001$.

Abbreviations: ns, not significant. ATR-I, atractylenolide-I; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TH, tyrosine hydroxylase; Iba1, ionized calcium binding adaptor molecule 1.

Treatment with ATR-I resulted in a significant recovery of TH^+ neuronal loss in MPTP-treated mice (0.98 ± 0.11 in the MPTP + ATR-I group versus 0.74 ± 0.06 in the MPTP group, $P < 0.01$). There was no significant difference observed between the control group and the control + ATR-I groups.

In addition, the results of the Iba1 assay revealed significant microgliosis in the SNpc of MPTP mice in comparison to control mice, as indicated by the $Iba1^+$ microglia numbers ($P < 0.001$) and fluorescence intensity ($P < 0.001$) of Iba1 (Figure 3a, c and d). Treatment with ATR-I resulted in lower $Iba1^+$ microglial numbers and fluorescence intensity in MPTP mice, suggesting attenuation of microgliosis (Figure 3a, c and d, $P < 0.01$, $P < 0.01$, respectively). The changes in microglia numbers and fluorescence intensity of Iba1 were as follows: control group (1.00 ± 0.12), control + ATR-I group (1.02 ± 0.14), MPTP group (1.92 ± 0.31) and MPTP + ATR-I group (1.54 ± 0.21) for $Iba1^+$ microglial numbers and control group (1.00 ± 0.07), control + ATR-I group (1.00 ± 0.08), MPTP group (1.25 ± 0.13) and MPTP + ATR-I group (1.06 ± 0.11) for fluorescence intensity of Iba1. There was no statistically significant difference between the control group and control + ATR-I groups.

ATR-I Treatment Alleviates MPTP-Induced Microglia Activation in the Spinal Cord

Next, the impact of ATR-I treatment on microglia activation in the spinal cord was assessed by measuring Iba1 expression. In Figure 4a–c, it is evident that MPTP-treated mice exhibited a significant increase in $Iba1^+$ microglia numbers and fluorescence intensity compared to the control group ($P < 0.01$ for both). Treatment with ATR-I notably improved the microgliosis status, leading to lower $Iba1^+$ microglia numbers ($P < 0.05$) and fluorescence intensity ($P < 0.01$). The changes in microglia numbers and fluorescence intensity of Iba1 were as follows: control group (1.00 ± 0.14), control + ATR-I group (0.96 ± 0.15), MPTP group (1.41 ± 0.28) and MPTP + ATR-I group (1.09 ± 0.20) for $Iba1^+$ microglia numbers and control group (1.00 ± 0.09), control + ATR-I group (0.98 ± 0.11), MPTP group (1.30 ± 0.20) and MPTP + ATR-I group (1.05 ± 0.16) for fluorescence intensity of Iba1. No significant difference was observed between the control group and control + ATR-I groups.

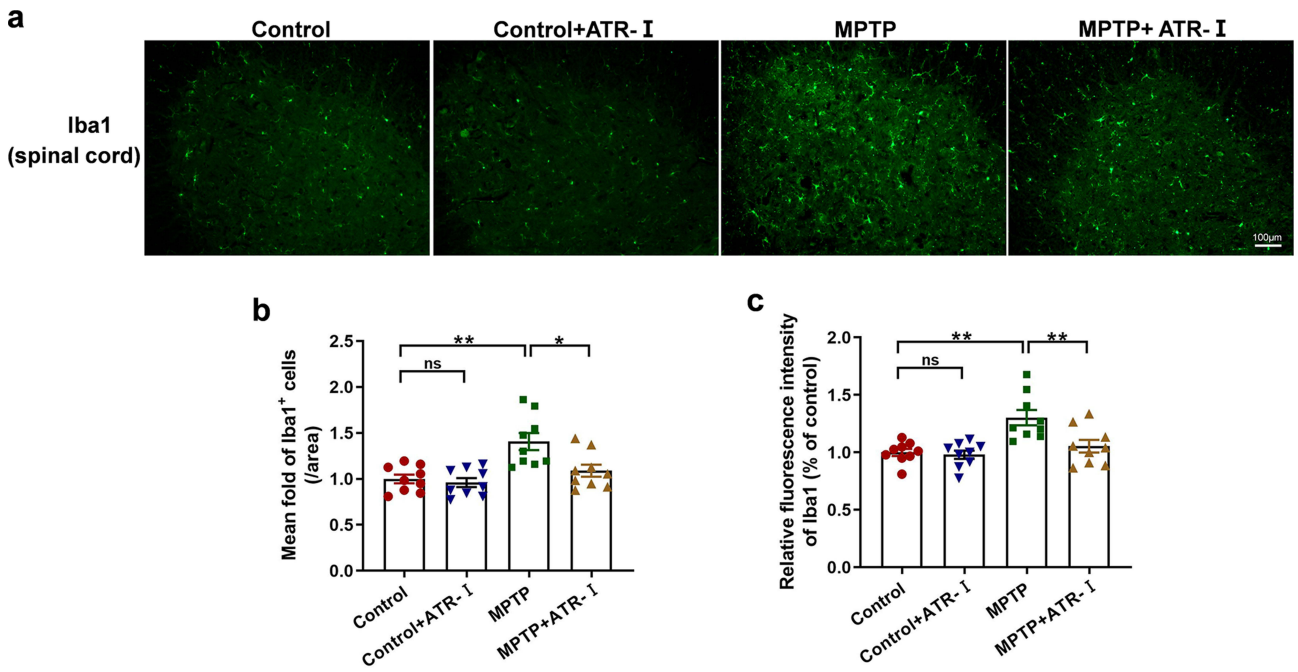

Figure 4 ATR-I treatment alleviates MPTP-induced microglia activation in the spinal cord. (**a**) Immunofluorescence staining of Iba1 in the spinal cord. (**b**) Relative numbers of $Iba1^+$ cell in the spinal cord. (**c**) Relative fluorescence intensity of Iba1 in the spinal cord. Scale bar: 100 μm. (mean ± SEM, n=3). *$P < 0.05$ and **$P < 0.01$.
Abbreviations: ns, not significant. ATR-I, atractylenolide-I; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Iba1, ionized calcium binding adaptor molecule 1.

ATR-I Treatment Decreased the Expression of the Inflammatory Mediator Genes in the Spinal Cord of MPTP-Induced Mice

Furthermore, we analyzed the expression of inflammatory mediator genes in the spinal cord of MPTP mice by RT-qPCR to evaluate the impact of ATR-I treatment on inflammatory regulation. The results presented in Figure 5a–c demonstrate that the mRNA levels of TNF-α (2.67 ± 0.64 versus 1.00 ± 0.09, $P < 0.01$), IL-1β (2.97 ± 0.54 versus 1.02 ± 0.23, $P < 0.01$), iNOS (2.16 ± 0.28 versus 1.13 ± 0.07, $P < 0.01$) were elevated in the spinal cord of MPTP mice compared to control mice. Following ATR-I treatment in MPTP mice, a significant decrease in the expression of TNF-α (1.38 ± 0.30 versus 2.67 ± 0.64, $P < 0.01$), IL-1β (1.74 ± 0.30 versus 2.97 ± 0.54, $P < 0.05$), iNOS (1.35 ± 0.31 versus 2.16 ± 0.28, $P < 0.01$) was observed in the spinal cord when compared to the MPTP treatment group.

ATR-I Treatment Mediated SIRT1/PGC-1α Pathway in the Spinal Cord of MPTP-Induced Mice

The results depicted in Figure 6a–c reveal a decrease in protein levels of SIRT1 and PGC-1α in the spinal cord of MPTP mice compared to control mice (0.61 ± 0.18 versus 1.05 ± 0.10, $P < 0.01$; 0.22 ± 0.07 versus 0.49 ± 0.07, $P < 0.05$, respectively), indicating inhibition of the SIRT1/PGC-1α pathway. Treatment with ATR-I in MPTP mice led to a significant increase in SIRT1 and PGC-1α protein levels in the spinal cord (0.95 ± 0.10 versus 0.61 ± 0.18, $P < 0.05$; 0.49 ± 0.15 versus 0.22 ± 0.07, $P < 0.05$, respectively). RT-qPCR analysis confirmed these results (Figure 6d and e), showing downregulation of SIRT1 and PGC-1α mRNA expression in MPTP-induced mice and upregulation of these genes with ATR-I treatment (0.91 ± 0.22 versus 0.47 ± 0.06, $P < 0.05$; 0.95 ± 0.14 versus 0.62 ± 0.12, $P < 0.05$, respectively). These results suggest a potential therapeutic effect of ATR-I in modulating the SIRT1/PGC-1α pathway.

Discussion

The study's main findings reveal that ATR-I alleviates motor deficits, prevents the loss of DA neurons and microglia activation in SNpc, and attenuates inflammation in the spinal cord in a subacute PD mouse model induced by MPTP. These results indicate that ATR-I is a promising candidate in the development of PD therapy. Previous studies have showed that ATR-I possesses a high safety margin and is not associated with any apparent toxic effect.[27] Our findings revealed that the control group treated with ATR-I exhibited outcomes similar to those of the control group, with no significant differences observed in either behavioral tests or subsequent pathological and biochemical analyses. These results suggest that ATR-I has a certain safety profile at the animal level, and further clinical studies are needed to confirm its efficacy and safety.

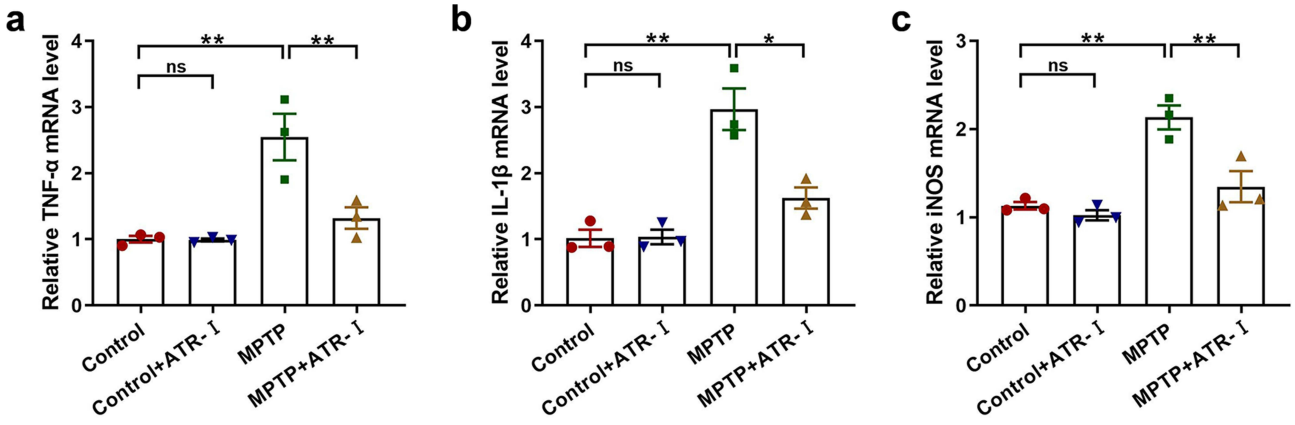

Figure 5 ATR-I treatment decreased the mRNA expression of inflammatory factors in the spinal cord of MPTP-induced mice. (**a–c**) Analysis of mRNA level of the inflammatory factors TNF-α, IL-1β, and iNOS using RT-qPCR. (mean ± SEM, n=3). *$P < 0.05$ and **$P < 0.01$.

Abbreviations: ns, not significant. ATR-I, atractylenolide-I; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TNF-α, tumour necrosis factor alpha; IL-1β, interleukin-1beta; iNOS, inducible nitric oxide synthase.

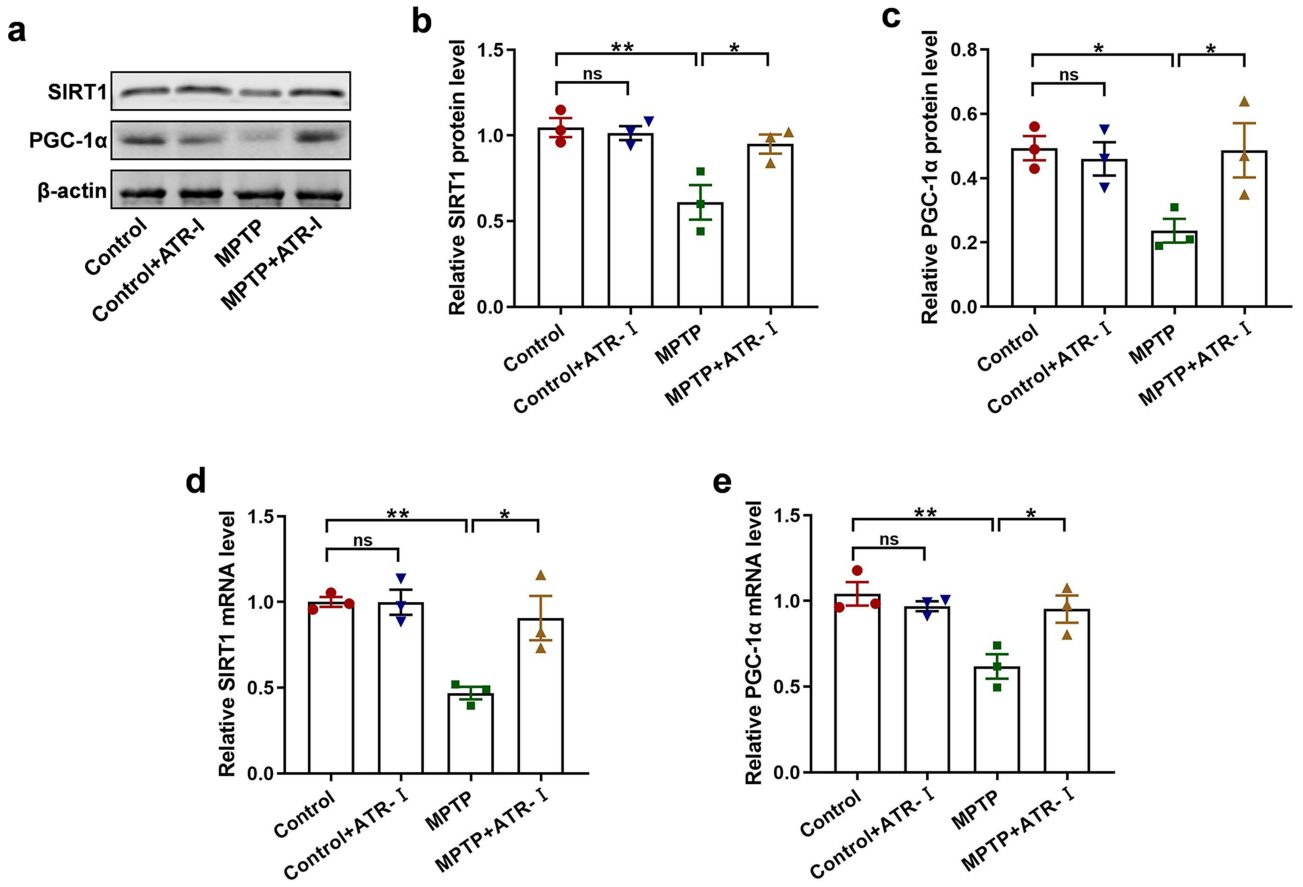

Figure 6 ATR-I treatment mediated SIRT1/PGC-1α pathway in the spinal cord of MPTP-induced mice. (**a**) Representative images of SIRT1 and PGC-1α protein expression using Western blotting. (**b** and **c**) Quantitative analysis of SIRT1 and PGC-1α protein levels. (**d** and **e**) Analysis of the mRNA levels of SIRT1 and PGC-1α using RT-qPCR. (mean ± SEM, n=3). *$P < 0.05$ and **$P < 0.01$.

Abbreviations: ns, not significant; ATR-I, atractylenolide-I; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SIRT1, silent information regulator 1; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α.

PD is a complex disease involving various nervous system, such as the SN, striatum, spinal cord, etc. Although the mechanisms of neurodegeneration in PD are not fully understood, inflammation has been implicated in the development of PD.[28,29] Activation of microglia and neuroinflammatory responses have been observed in the SN and spinal cord of both PD patients and animal models.[5,13,30–32] The study reveals that ATR-I significantly reduces microglia activation in the SNpc and spinal cord of MPTP-induced mice, suggesting that ATR-I may offer neuroprotection against PD by exerting anti-inflammatory effects.

Previous research has linked spinal cord lesions to clinical symptoms of PD, such as gait abnormalities, balance issues, pain, constipation, and dysuria.[33] Autopsy study reveals spinal cord pathology in PD patients exhibiting the presence of alpha-synuclein, neuron death, microglial activation, and T-cell infiltration.[5,34] Inhibition of spinal cord inflammatory has been shown to improve motor deficits in MPTP-induced mice.[12,13] Importantly, in our study, we found that ATR-I treatment was able to significantly attenuate the inflammatory response in the spinal cord, including decreased microglia activation and levels of inflammatory factors and improve motor function such as increased suspension scores and stride length in MPTP-induced mice. These results suggest that the anti-inflammatory effects of ATR-I in the spinal cord may also be involved in the improvement of movement disorders in PD.

To gain a deeper understanding of the anti-inflammatory signaling pathways in spinal cord modulated by ATR-I in PD, we performed biochemical analyses and discovered that ATR-I activated SIRT1/PGC-1α pathways in MPTP-induced mice. It has been demonstrated that SIRT1 activation can reduce inflammatory responses in microglia induced by LPS.[35,36] Additionally, SIRT1 agonists have the potential to enhance functional recovery and neuronal survival by

decreasing the number of macrophages/microglia and levels of inflammatory cytokines after spinal cord injury.[37] Recent reports have shown that SIRT1/PGC-1α pathway suppresses the proinflammatory responses of microglia and attenuates pathology in PD.[38,39] These results imply that targeting SIRT1 could be a promising approach for anti-inflammatory therapy in PD. Our study revealed that ATR-I notably decreased inflammatory factor levels and increased SIRT1 and PGC-1α protein and gene expression in the spinal cord of MPTP-induced mice, indicating that the SIRT1/PGC-1α pathway may mediate the anti-inflammatory effects of ATR-I in the spinal cord of PD.

In this study, we obtained preliminary and exploratory results; however, several limitations remain. First, our findings indicate a short-term therapeutic benefit of ATR-I, necessitating further research to investigate the potential long-term effects and sustainability of ATR-I treatment. Second, the mechanisms by which ATR-I regulates the SIRT1/PGC-1α pathway, including the upstream and downstream key effectors, remain unknown. Additional in vitro and in vivo studies are required to comprehensively analyze the role of ATR-I in PD therapy. Third, to validate the protective effect of ATR-I, further investigations should be conducted in a dose-dependent manner in both animal models and cell cultures, thereby providing an experimental foundation for clinical applications.

Conclusion

Our research shows that ATR-I ameliorates motor impairments, mitigates the loss of DA neurons and microgliosis in the SNpc, and suppresses spinal cord inflammation via the SIRT1/PGC-1α signaling pathway. These results indicate that ATR-I possesses a broad spectrum of neuroprotective properties, positioning it as a promising candidate for treating PD and potentially enhancing our understanding of PD pathogenesis.

Data Sharing Statement

The datasets used in this study may be obtained from the corresponding author upon reasonable request.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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