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Antinociceptive effects of carbidopa levodopa on normal rats and Parkinson's disease mice

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Abstract: Carbidopa levodopa is widely used to ameliorate motor symptoms of Parkinson's disease (PD) patients. Pain is one of common symptoms of PD. The aim of this experiment is to study antinociceptive effects of carbidopa levodopa on normal rats and PD mice. Rats were intragastrically treated with carbidopa levodopa and the hind paw withdrawal latency (HWL) was investigated. PD mouse model was prepared with MPTP and then the antinociceptive effects of carbidopa levodopa on PD mice were evaluated. In normal rats, the HWL to thermal stimulus was augmented after carbidopa levodopa administration ($p<0.05$ or $p<0.01$) and carbidopa levodopa increased the HWL ($p<0.05$ or $p<0.01$) to mechanical stimulus. In PD mice, carbidopa levodopa elevated the HWL of the thermal stimulus in PD mice ($p<0.05$). Furthermore, the HWL in the inflammatory pain of PD mice was also increased by carbidopa levodopa treatmet ($p<0.01$). The current findings indicate that carbidopa levodopa has an antinociceptive effects in normal rats and PD mice. The analgesic effect of carbidopa levodopa on patients with or without PD is worth studying in further research.

Keywords: Carbidopa levodopa, pain, hind paw withdrawal latency, dopamine

INTRODUCTION

Parkinson's disease (PD) is caused by loss of nigrostriatal dopamine neurons. It develops insidiously and progresses slowly in most patients. The common symptoms of PD are tremor, muscle rigidity, gradual loss of spontaneous movement. Levodopa is a precursor of dopamine. Administration of levodopa can increase the dopamine concentration of brain. It is well known that levodopa therapy is effective in ameliorating the motor symptoms of PD and remains the standard drug for the treatment of PD. Levodopa is absorbed in the small intestine. It will be metabolized rapidly by dopa decarboxylase (DDC). DDC (e.g., carbidopa) combining with levodopa will prevent the decarboxylation of levodopa. Therefore, the unchanged levodopa reaches the central nervous system to ameliorate the symptoms of PD patients (Desch *et al.*, 2023; Hsu *et al.*, 2015). Carbidopa levodopa is one of the most widely prescribed medicine for PD.

Pain occurs when tissues are damaged, and it makes people avoid the injury. Pain is one of common symptoms of PD. Pain leads to a negative impact on the quality of life of PD patients (Viseux *et al*., 2023). It is reported that about 25% PD patients who manifests pain as a preceding symptom in an early motor stage (Broen *et al*., 2012). In intermediate stage of PD, the prevalence of pain was about 60% (Rana *et al*., 2017). During the disease course, pain affects up to 80% of PD patients (Mylius *et al*., 2021). Up to date, there were no recommendations to relieve the PD-related pain. Nonsteroidal anti-inflammatory drugs and opioids are widely used for the treatment of pain in PD. However, these drugs are associated with adverse events and individual variation of efficacy (Magni *et al*., 2021).

Recent works demonstrated that dopaminergic system played a key role in modulating pain perception and analgesia (Li *et al*., 2021). Low dopamine production and delivery were likely to contribute to pain. Moreover, abnormalities in dopaminergic system have been observed in pain including fibromyalgia, restless legs syndrome (Yang *et al*., 2020). Although levodopa is not a conventional analgesic, it has been proved that levodopa administration is benefit in relieving pain. A review of treatments for pain demonstrated that levodopa increased thresholds of pain in PD patients (Karnik *et al*., 2020). It was reported that administration of levodopa significantly alleviated the pain in the patients with diabetic neuropathy (Ertas *et al*., 2010). Intrathecal administration of levodopa attenuated the substance P-induced nociceptive behaviors in mice. In a rat model of neuropathic pain, levodopa resulted in a significant decrease in tactile and cold allodynia. These results mentioned above support that levodopa has the antalgic effect in neuropathic pain (Cobacho *et al*., 2015). The aim of this experiment is to

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study antinociceptive effects of carbidopa levodopa on pain in normal rats and PD mice.

MATERIALS AND METHODS

Animals

Male SD rats (200-220 g) and male Swiss mice (20-24g) were from the company (Jinan Pengyue laboratory animal breeding Co. Ltd). The rats and mice were housed in cages (42.5×27×19 cm). The condition of feeding room is 12h light/dark cycle and the room temperature is at 22±1^{o}C. The animals were allowed to get water and food *ad libitum*. The animals acclimatize to the environment for 3 days. The experiments were approved by Institutional Animal Care and Use Committee of Yantai University (the authorization number is YTDX20190619). The study followed the NIH Guide for the Care and Use of Laboratory Animals.

Drug and chemical agents

Carbidopa levodopa (containing 25 mg of carbidopa and 100 mg of levodopa) was from Merck & Co., Inc. (St. Whitehouse, NJ, USA). Anti-tyrosine hydroxylase (TH) antibody was from Millipore (Billerica, Massachusetts, USA). Carrageenan and MPTP were from Sigma-Aldrich (St Louis Missouri, USA).

Inflammatory pain model

According to precious reports (Winter *et al*., 2015), The inflammatory pain model was induced by intraplantar injection with carrageenan (dispersed in saline, 2% m/v). In brief, the left hind paw of rats or mice was injected with carrageenan (0.1ml or 20μl, respectively). The rats or mice of control were intraplantarly given with the same volume of saline.

Experimental procedure

Rats were assigned into six groups (n=8): control, model, carbidopa levodopa (6.25, 12.5, 25, and 50 mg/kg). The tablets of carbidopa levodopa were suspended in 2% sodium carboxymethyl cellulose. At 2 h after carrageenan injection, the rats in carbidopa levodopa groups were intragastrically treated with carbidopa levodopa (6.25, 12.5, 25, or 50 mg/kg). The animals in control and model were intragastrically given 2% sodium carboxymethyl cellulose. Nociceptive tests were conducted at 0.5, 1.0, 1.5, 2.0, 3.0h after carbidopa levodopa administration (fig. 2A, 4A).

The trained mice were assigned into three groups (n=8): control, MPTP, and carbidopa levodopa. To prepare PD model, the mice were injected intraperitoneally with MPTP (30 mg/kg, once a day) for 5 days. Pole test was carried out at the end of MPTP exposure. The mice were treated with carbidopa levodopa (50 mg/kg) at 48 h after the last MPTP challenge. The mic of control and model were administered with 2% sodium carboxymethyl cellulose. Nociceptive tests were performed at 1.0h after treatment. Previous study showed that levodopa and carbidopa were completely removed at 12 h after administration (Antonini *et al*., 2018). Therefore, 2 d later, the mice in MPTP and carbidopa levodopa groups were subjected with carrageenan injection. Two hours later, the mice were treated with carbidopa levodopa (50 mg/kg). Hot plate test was conducted at 1.0h after carbidopa levodopa treatment (fig. 5A).

Nociceptive tests

The nociceptive response thresholds to mechanical and thermal stimuli were investigated with the Randall-Selitto and hot plate tests. Hind paw withdrawal latency (HWL) was recorded as the time from placing hind paw to hind paw withdrawal. Mechanical allodynia was evaluated using the Randall-Selitto test. The apparatus with cone-shaped tip (Ugo Basile, Type 7200, Italy) produced an ever-increasing pressure (30g/s) on the hind paw of rats. The animals were placed on a 52±0.2°C hot plate (Intelligent Heat Panel Instrument, China). The animals were trained with the Randall-Selitto and hot plate tests for 3 days before the experiment (fig. 1A, 3A).

Pole test

The animals were placed on the top of pole. The time that mice climbed down to the floor was recorded during a 60 s trial. The time was recorded as 60s if the climbing time exceed 60 s. Average climbing time of three trials was calculated.

Tyrosine hydroxylase immunohistochemistry

After nociceptive tests, the mice (n=3) were anesthetized with isoflurane. Then, they were perfused with PBS and 4% paraformaldehyde. The brains of mice were collected and fixed in 4% paraformaldehyde at 4°C overnight. Then, the brains were transferred to 20% sucrose at 4°C for 24 h. Coronal sections of brain (10 μm) were cut. The sections were rinsed in PBS and Triton X-100. Quenched with 3% H_2O_2, the brain sections were incubated in blocking solution. Incubatied with anti-TH antibody (1: 200. 4°C, 24h) and biotinylated secondary antibody (37°C, 1h). TH positive cells were counted by an experimenter blinded to the design using a microscope (IX-70; Olympus Corp., Tokyo, Japan).

STATISTICAL ANALYSIS

Statistical analysis was performed using the IBM SPSS Statistics program (version 20.0; IBM Corp., Armonk, NY, USA). The data were shown as mean ± standard deviation (S.D.). One-way ANOVA and then Tukey's post hoc test was performed to compare multiple groups. Significance was defined if $p<0.05$.

RESULTS

The HWL to thermal stimulus before the carbidopa levodopa treatment

To make sure that there were no differences in the HWL to thermal stimulus among the groups before the carbidopa levodopa treatment, each rat was conducted

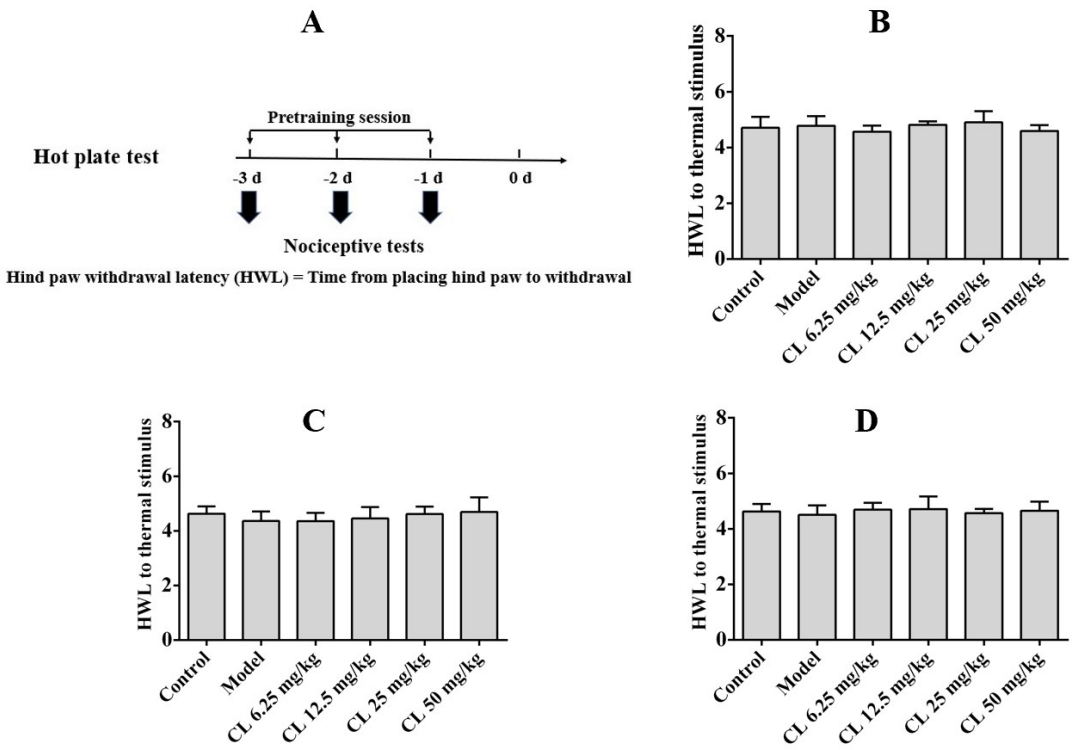

Fig. 1: The HWL of pretraining to thermal stimulus. A, Schematic diagram of pretraining; B, Day 1; C, Day 2; and D, Day 3. Data were represented as mean ± S.D. (n = 8 in each group). CL, carbidopa levodopa.

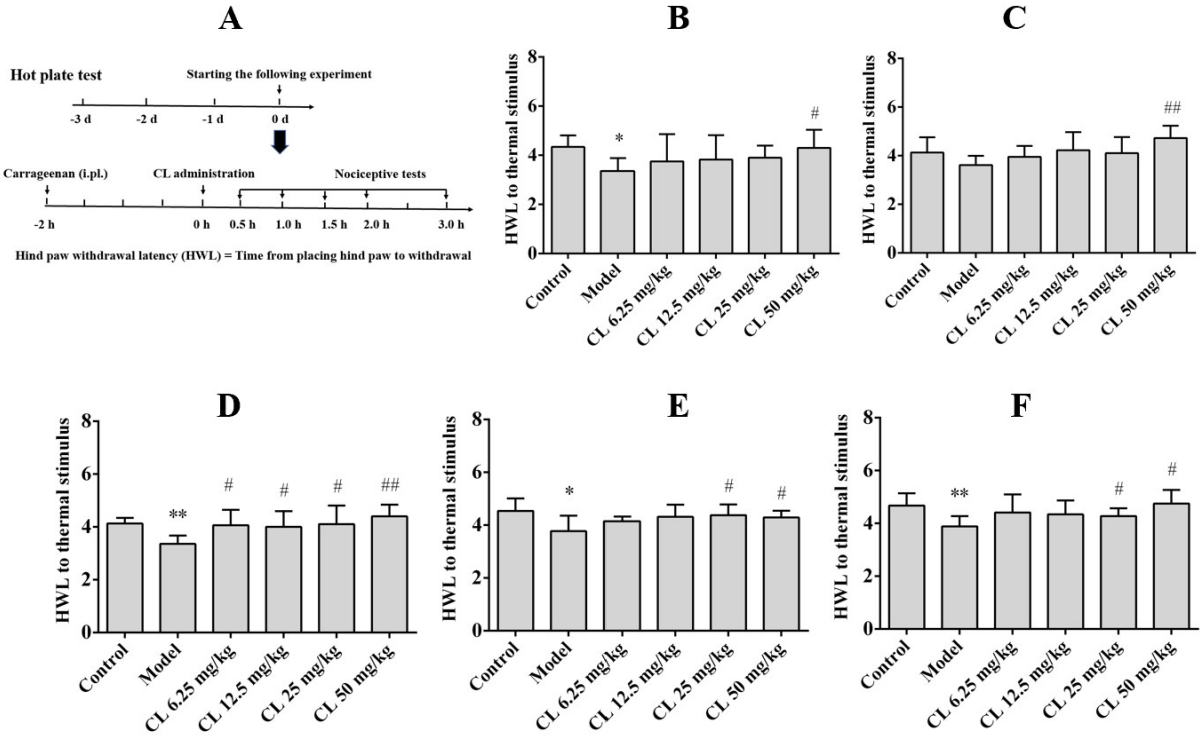

Fig. 2: Effect of CL on HWL to thermal stimulus. A, Schematic diagram of the experimental timeline in hot plate test; B, At 0.5 h after CL treatment; C, At 1.0 h after CL treatment; D, At 1.5 h after CL treatment; E, At 2.0 h after CL treatment; F, At 3.0 h after CL treatment. Data were represented as mean ± S.D. (n = 8 in each group). $^{*}p<0.05$, $^{**}p<0.01$ versus the control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ versus the model group. CL, carbidopa levodopa.

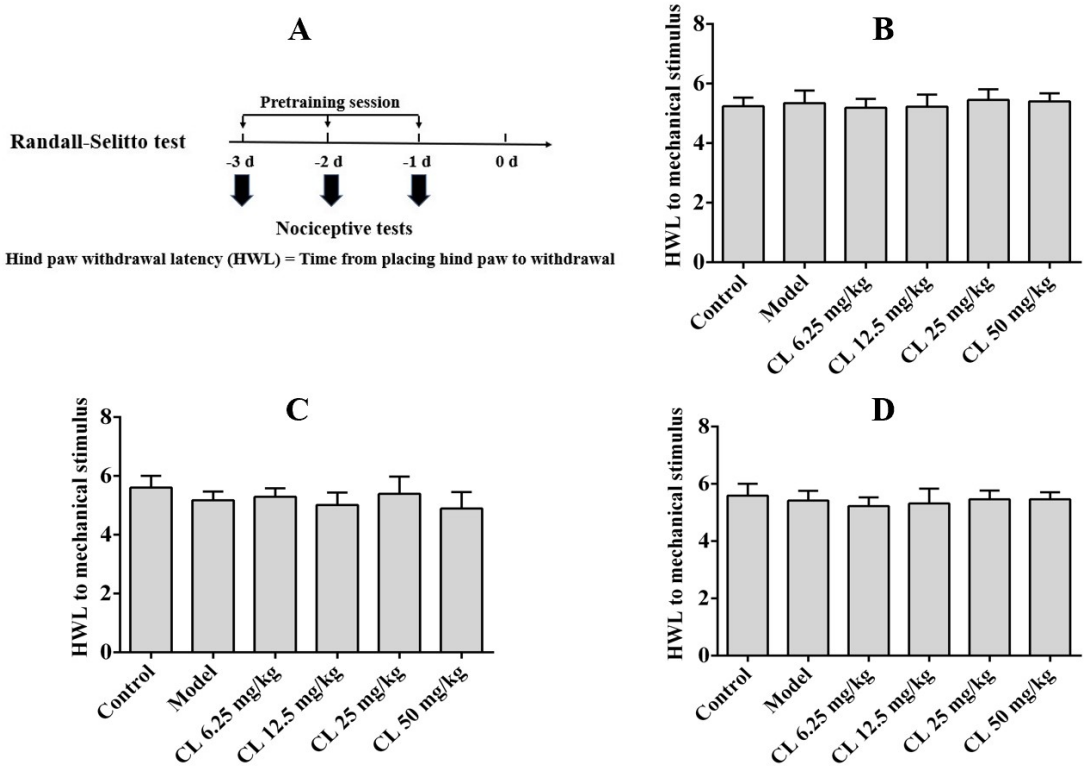

Fig. 3: The HWL of pretraining to mechanical stimulus. A, Schematic diagram of pretraining; B, Day 1; C, Day 2; and D, Day 3. Data were represented as mean ± S.D. (n = 8 in each group). CL, carbidopa levodopa.

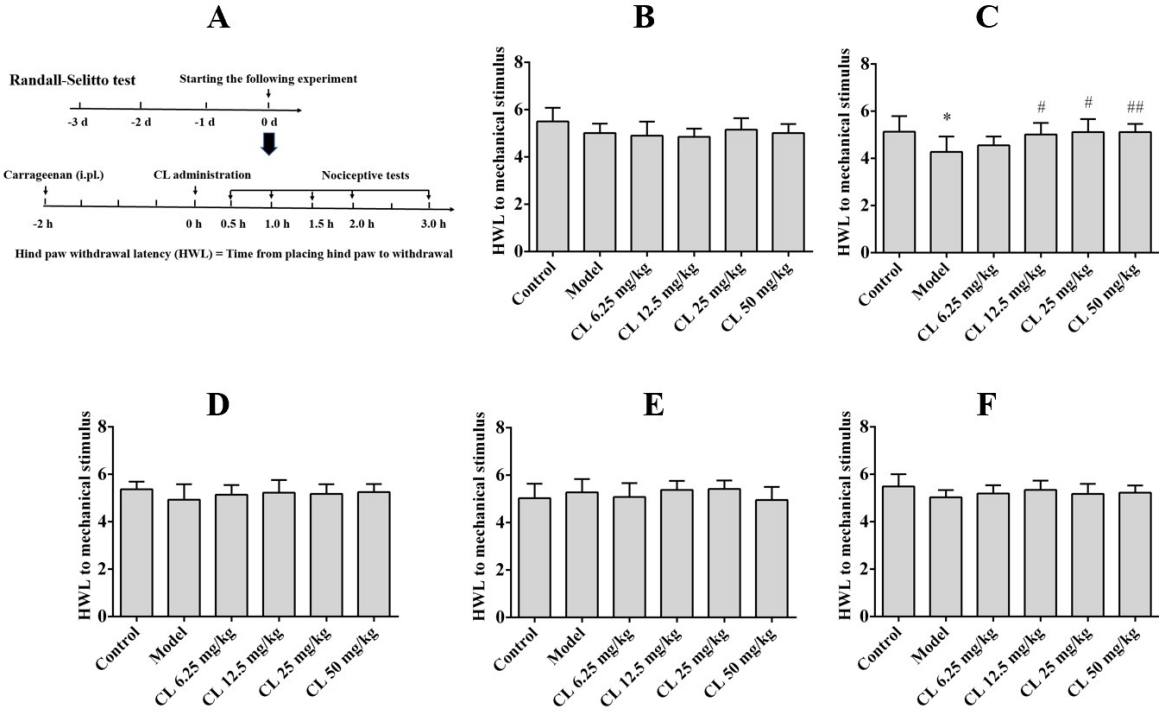

Fig. 4: Effect of CL on HWL to mechanical stimulus. A, Schematic diagram of the experimental timeline in Randall-Selitto test. B, At 0.5 h after CL treatment; C, At 1.0 h after CL treatment; D, At 1.5 h after CL treatment; E, At 2.0 h after CL treatment; F, At 3.0 h after CL treatment. Data were represented as mean ± S.D. (n = 8 in each group). $^{*}p<0.05$ versus the control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ versus the model group. CL, carbidopa levodopa.

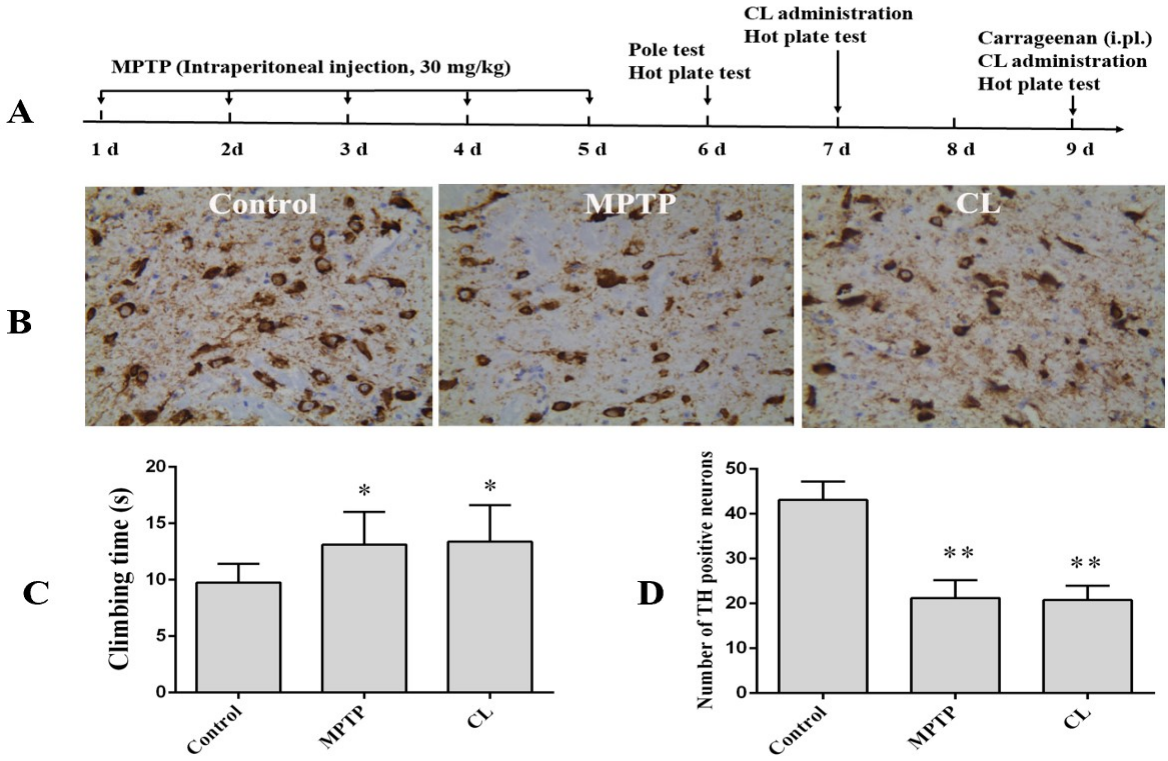

Fig. 5: MPTP-induced PD mice model. A, Schematic diagram of the experimental timeline in Parkinson's disease mice; B, The representative pictures of TH immunohistochemistry; C, Climbing time; D, The number of TH positive neurons. Data were represented as mean ± S.D. (n = 8 in each group). *p<0.05, **p<0.01 versus the control group. CL, carbidopa levodopa.

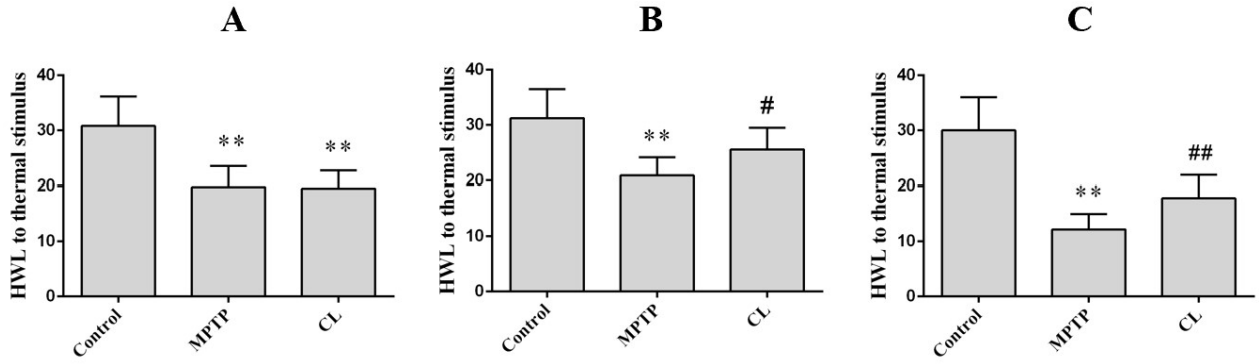

Fig. 6: Antinociceptive effects of CL in Parkinson's disease mice. A, The HWL to thermal stimulus before CL administration; B, The HWL of PD mice to thermal stimulus after CL administration; C, The HWL of PD mice to thermal stimulus after CL administration in inflammatory pain. Data were represented as mean ± S.D. (n = 8 in each group). **p<0.01 versus the control group; $^{\#}$p<0.05 versus the MPTP group. CL, carbidopa levodopa.

with a pretraining session of the hot plate test once a day for 3 days. No difference was observed in the HWL of rats in each group (p>0.05), (fig. 1B, C, D).

Effect of carbidopa levodopa on the HWL to thermal stimulus

Compared with control, the HWL to the thermal stimulus of animal in model group was significantly decreased (p<0.05 or p<0.01). Compared with model, carbidopa levodopa at dose of 50mg/kg increased the HWL to the thermal stimulus at 0.5, 1.0h after carbidopa levodopa treatment (p<0.05 or p<0.01); The HWL was augmented at 1.5h after carbidopa levodopa treatment (p<0.05 or p<0.01); The HWL was enhanced at 2.0, 3.0h after carbidopa levodopa (25 or 50mg/kg) administration (p<0.05), (fig. 2B, C, D, E, F).

The HWL to mechanical stimulus before carbidopa levodopa treatment

To prove that there were no differences in the HWL to mechanical stimulus among the groups before carbidopa levodopa treatment, each rat was conducted with a pretraining session of a Randall-Selitto test once a day for 3 days. No difference was observed in the HWL to mechanical stimulus in each group ($p>0.05$), (fig. 3B, C, D).

Effect of carbidopa levodopa on the HWL to mechanical stimulus

Compared with control, the HWL to the mechanical stimulus of animal in model group was significantly reduced ($p<0.05$ or $p<0.01$). Compared with model, carbidopa levodopa at dose of 12.5, 25, or 50mg/kg elevated the HWL at 1.0 h after treatment ($p<0.05$ or $p<0.01$), (fig. 4B, C, D, E, F).

The climbing time and number of TH positive neurons

Compared with control, the climbing time of mice in MPTP and carbidopa levodopa groups was elevated ($p<0.05$), while TH positive neurons of mice in MPTP and carbidopa levodopa groups were reduced ($p<0.01$). These results confirmed the validity of PD model (fig. 5B, C, D).

Effect of carbidopa levodopa on the HWL to thermal stimulus in PD model

To clarify whether the threshold of pain of PD mice was different from that of the mice in control, the HWL of thermal stimulus was investigated before carbidopa levodopa treatment. Compared with control, the HWL of thermal stimulus in MPTP and carbidopa levodopa groups was reduced ($p<0.01$). Without carbidopa levodopa administration, there was not a significant difference in the HWL of thermal stimulus observed between MPTP group and carbidopa levodopa group. Then, the mice were treated with carbidopa levodopa (50mg/kg). Compared with control, the HWL of thermal stimulus in MPTP group was decreased ($p<0.01$). However, the HWL of thermal stimulus in carbidopa levodopa group was significantly augmented when compared with MPTP group ($p<0.05$), (fig. 6A, B). Compared with control, the HWL of PD mice in the inflammatory pain was significantly decreased ($p<0.01$). Compared with MPTP, treatment with carbidopa levodopa increased the HWL of PD mice to the thermal stimulus in the inflammatory pain ($p<0.01$), (fig. 6C).

DISCUSSION

In this experiment, we prepared an inflammatory pain model in rat and found that the HWL to thermal stimulus was decreased in model group. However, the HWL to thermal stimulus was increased after carbidopa levodopa administration. carbidopa levodopa did not augment significantly the HWL to mechanical stimulus except at 1.0h after administration. These findings indicate that carbidopa levodopa can relieve inflammatory pain in normal rats. And the analgesic effect of carbidopa levodopa on mechanical injury is not as good as thermal injury. It should be noted that the HWL of rats in the model group to mechanical stimulus was not significantly decreased. And this factor may be the reason that carbidopa levodopa did not show an analgesic effect in mechanical injury.

The results mentioned above suggests that carbidopa levodopa exerts the analgesic effect in normal rats. Next, we tried to evaluate whether carbidopa levodopa *al*so can relieve the pain in PD model. According to previous method (Li *et al*., 2022), a mouse model of PD was induced by MPTP injection. The dopaminergic neurons of mice were damaged by MPTP exposure. The mice in MPTP group also showed an impairment in motor function. These data demonstrated that the damage of dopaminergic nerve cannot be repaired by carbidopa levodopa. But carbidopa levodopa attenuated the motor impairments of PD mice, showing its antiparkinsonian action. These findings validated that the model was suitable to study the effect of carbidopa levodopa on the pain in PD. Hot plate test was employed to investigate the HWL to thermal stimulus in PD mice. MPTP-induced PD mice showed a decrease of the HWL, which is consistent with the previous report (Park *et al*., 2015; Chen Y *et al*., 2022). Before the carbidopa levodopa treatment, there was not a significant difference in HWL to thermal stimulus between the MPTP group and carbidopa levodopa group. However, the mice showed the longer HWL to thermal stimulus after carbidopa levodopa treatment. This result demonstrated that carbidopa levodopa partially ameliorated the pain hypersensitivity of PD model. Therefore, it is reasonable to speculate that Sinem *et al* so has an effect for relieving the pain in PD.

There are limitations in this study. Firstly, sedation and motor function may influence the HWL evaluation. However, we did not design a series of experiments to study the effect of carbidopa levodopa on motor function and sedation. Secondly, although the present findings demonstrate that carbidopa levodopa has an analgesic effect in rats and mice, the mechanism of action of carbidopa levodopa was not studied; and whether carbidopa levodopa can relieve the pain of patients should be confirmed by the future clinical trials. Thirdly, carbidopa levodopa containing carbidopa and levodopa, the present study cannot clarify whether carbidopa is involved in the analgesic effect of carbidopa levodopa.

CONCLUSION

In conclusion, this study demonstrates that carbidopa levodopa has an antinociceptive effects in normal rats and

PD mice. The present findings indicate that carbidopa levodopa may have potentials to relieve the pain of patients with or without PD. The results of this study also provide evidence supporting further clinical research to evaluate the analgesic effect of carbidopa levodopa in patients.

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