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The effect of paeonol on motor deficits and depressive and anxiety-like behavior in cuprizone-induced model of multiple sclerosis

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Abstract

Background and Objective: Patients with multiple sclerosis (MS) exhibit varying degrees of motor deficits, anxiety, and depression. Chronic oral cuprizone through evoking demyelination process is generally used to induce a model of MS to assess potential efficacy of promising therapeutics. Paeonol is a phenol compound with beneficial anti-inflammatory and protective effects. This study was conducted to investigate the effect of paeonol on motor deficits and depressive and anxiety-like behavior using open field, elevated plus maze, and tail suspension test in C57Bl/6 mice.

Materials and Methods: Mice received oral cuprizone at a dose of 400 mg/kg for 6 weeks and paeonol was given orally at doses of 25 or 100 mg/kg/day from the second week for next five weeks. To assess motor function and depressive and anxiety-like behavior, open field task, tail suspension test (TST), and elevated-plus maze (EPM) test were used, respectively. Data were analyzed by one-way ANOVA and Tukey post-test with significance level < 0.05.

Results: Paeonol administration to cuprizone group at the higher dose of 100 mg/kg improved locomotor activity and motor coordination as was evident by higher line crossing and an increase in the spent time in the center. In addition, paeonol treatment of cuprizone group significantly reduced spent time in the open arms of elevated-plus maze and also decreased immobility time in tail suspension task.

Conclusion: This study showed reversal effects of paeonol regarding motor deficits and depressive and anxiety-like behavior in cuprizone-induced model of MS.

Keywords: Cuprizone, Paeonol, Motor deficits, Anxiety, Depression

1. Introduction

Multiple sclerosis (MS) is a chronic and disabling disease of the central nervous system (CNS) typified by inflammatory demyelinating and axonal damage processes (1). Patients with MS exhibit varying degrees of motor deficits. About 75% of MS patients experience walking disturbances including lower cadence and shorter step length resulting in a reduced walking speed (2). MS is also usually associated with psychological problems such as depression and anxiety. Anxiety is common in chronic forms of MS which is somewhat correlated with level of disability. The role of stress in the pathogenesis of MS has been known and stress level is correlated with worsening of the neurological symptoms of MS. Fear is the key part of anxious thoughts in patients with MS and patients experience more fear particularly in relapse phases of the disease, according to unpredictable nature of relapses. Fear of the progression of disabilities is disturbing and MS patients need psychological and medical treatment support (3). In addition, it is estimated that about half of people with MS will finally develop a major depression condition during the course of the disease. Furthermore, development

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of depression and anxiety usually affects the patients' employment and social interaction. Even, more than 50% of patients have reported moderate to severe forms of depression which negatively correlates with their life quality and living style (4). Besides, factors associated to development of depression and anxiety in MS patients also contribute to the worsening of neurodegeneration in this disease (5). One of the factors influencing the adherence to recommended treatments is an MS patient's emotional state. It has been demonstrated that both pharmacological treatments and psychological interventions are supportive and beneficial in the management of depression and anxiety in MS patients. Therefore, it is highly important to undertake and apply an effective treatment to reduce mood-related complications of MS including anxiety and depression (6).

Paeonol (2′-hydroxy-4′-methoxyacetophenone) is regarded a major phenolic compound obtained from the root of Moutan Cortex and can serve as an active ingredient with pharmacological effects in herbal medicine. It has been shown that paeonol possess beneficial pharmacological effects consisting of sedation, analgesia, immunoregulation, anti-tumor, and also anti-inflammatory properties. It has also been reported that paeonol can exert neuroprotective effect in ischemic conditions and to alleviate neurological impairment and neuronal loss. In addition, paeonol can slow down pathogenic processes related to Alzheimer's disease due to its potential anti-oxidant activity. In addition, paeonol has shown therapeutic effects in Parkinson's disease through decreasing oxidative stress (7-10). It has been demonstrated that paeonol can exert anti-depressant activity and also reduce neuronal injury with concomitant changes of BDNF during chronic unpredictable mild stress (CUMS) in rats (11) and is able to attenuate lipopolysaccharide (LPS)-induced depressive-like behaviors in mice (12). To the best of our knowledge, there have been no reports demonstrating the effects of Pae on RPs damage. Meanwhile, paeonol has shown its ability to alleviate aggressive and anxiety-like behaviors in rat model of premenstrual dysphoric disorder (13) and has been presented as a potential anxiolytic agent in mice (14). Therefore, this study was conducted to investigate the effect of paeonol on motor deficits and depressive and anxiety-like behavior in cuprizone-induced model of MS in C57Bl/6 mice.

2. Materials and Methods

2.1. Animals

Adult male C57BL/6 mice (19-24 g; 9-11 weeks old) were purchased from Baqiyatallah University of Medical Sciences and housed under normal laboratory conditions (temperature set to about 22□C, humidity set to about 50% and with 12-h lighting cycle). They were freely fed with food and water. Used methods were approved by Ethics committee of Shahed University (Ethics no. IR.SHAHED.REC.1399.041).

2.2. Experimental Design

After adaptation, mice (n = 50) were randomly divided into 5 equal groups, i.e., control, control with paeonol treatment at a dose of 100 mg/kg, cuprizone, and cuprizone groups under treatment with paeonol at doses of 25 or 100 mg/kg. Cuprizone (MerckMillipore, Germany) was given by gavage needle and it was daily administered at a dose of 400 mg/kg (15, 16) for 6 weeks. Paeonol was administered p.o. one week after starting cuprizone administration till the end of the study on a daily basis.

2.3. Behavioral tests

2.3.1. Open field test

To analyze spontaneous locomotor pattern of animals, number of line crossing and rearing and the spent time in the central arena were measured and recorded for 5 min using a video camera. Used protocol for this test was in accordance to an earlier report (17). The apparatus consisted of a cylinder (diameter = 60 cm, height = 50 cm). The floor of the platform was divided into 19 areas by drawing 9 radial lines and also with two internal circles. The platform was illuminated by a 60 W lamp located 70 cm above the surface. To conduct the test, each mouse was placed in the innermost circle and allowed to explore and navigate the apparatus. After each testing session, the surface of the area was cleaned by 70% ethanol to remove odor cues.

2.3.2. Elevated-plus maze (EPM) task

This task was conducted to evaluate anxiety-like behavior. For this test, mice were put in the center of the equipment with their head toward an open arm. The animal activity was observed for five minutes. The animal's entry into any of the four arms was counted when all four paws were crossed from the central region of the apparatus into an arm. The time spent in the open arms was recorded and results were brought as a percentage of the total spent time. Also, number of total arm entries were recorded to have an overall assessment of locomotor activity (18).

2.3.3. Tail suspension test (TST)

This task was performed to evaluate depressive-like behavior. Mouse tail was clamped and suspended so that the animal was hanging upside down while their head was about 12 cm far from the bottom surface, facing the observer. Each experiment took six minutes and immobility time was the time when the animal gave up its struggle and showed disappointment. This time can also reflect the emotional changes of the animal.

2.4. Statistical tests

Presented data are brought as mean ± standard error of mean (SEM) and all analyses were done by the statistical software GraphPad Prism 8.4 (GraphPad Software Inc., USA). To verify normal distribution, Shapiro-Wilk test was used. To find out significance levels, one-way analysis of variance (ANOVA) and Tukey's tests were applied. Level of statistical significance was p-value lower than 0.05.

3. Results

The animals in all groups had normal drinking and eating. In addition, there were no deaths in any of the groups during tests and procedures.

In this study, to assess motor function of animals in different groups, we used open field test to find out line crossings, number of rearing, and spent time in the central area (Figures 1A-C). Our one-way ANOVA test showed a significant difference between the groups ($F(4,45)=6.51$, $p<0.01$). In this respect, animals in cuprizone group have less locomotor activity and motor coordination, as shown by lower number of line crossing ($p<0.01$) and number of rearing ($p>0.05$) when compared to comparable data of control group. Besides, the time in the center of open field was significantly lower in cuprizone group ($p<0.01$). Paeonol administration at a dose of 100 mg/kg to cuprizone group significantly improved locomotor activity and also motor coordination and properly attenuated anxiety-like behavior, as is evident by higher line crossing ($p<0.05$) and an increase in the time in center ($p<0.05$) when it was compared to relevant data of cuprizone group. Such significant effect was not observed for animals in cuprizone group receiving paeonol at a dose of 25 mg/kg. In addition, no significant effect was observed in the control group under treatment with paeonol at the higher dose (100 mg/kg) regarding these motor indices.

To assess anxiety-like behavior in different groups, we employed elevated plus maze task (Figure 2). One-way ANOVA test showed a marked and significant difference between the groups ($F(4,45)=9.53$, $p<0.001$). In this regard, cuprizone administration for 6 weeks significantly and unexpectedly increased time spent in open arms in elevated plus maze when compared to control group ($p<0.01$). Such significant increase to a less extent was also observed in cuprizone group treated with paeonol at a dose of 25 mg/kg ($p<0.01$). In contrast, cuprizone group treated with paeonol at a dose of 100 mg/kg showed a significantly lower time spent in open arms as compared with vehicle-treated cuprizone group ($p<0.05$). In other words, cuprizone dose-dependently was capable to reverse behavioral deficits in elevated plus maze following cuprizone.

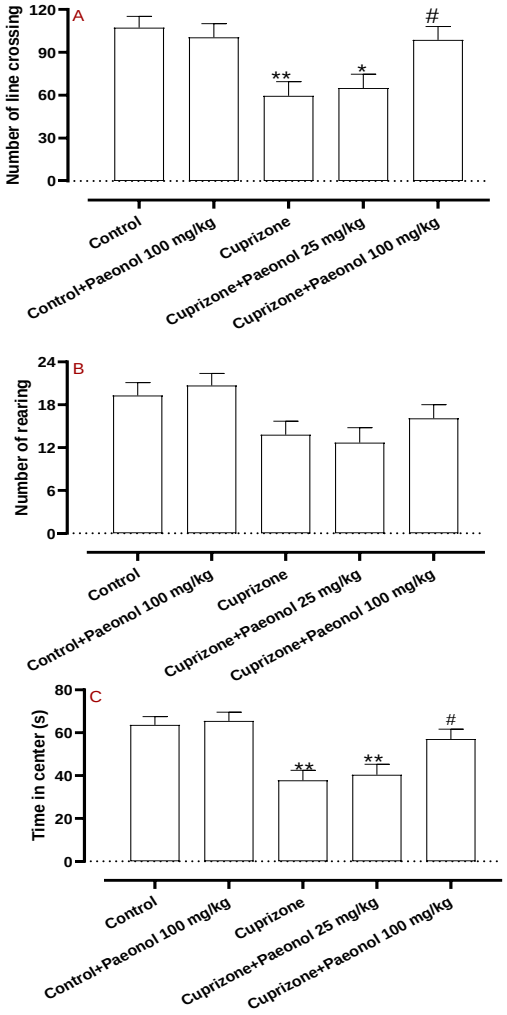

Figure 1. Performance of animals in open field test including line crossings (A), number of rearing (B), and time in the central area (C). Cuprizone was given by gavage needle and it was daily administered at a dose of 400 mg/kg for 6 weeks. Paeonol was administered *p.o.* one week after starting cuprizone administration till the end of the study on a daily basis. Data are shown as Mean±SEM. * and ** indicate $p<0.05$ and $p<0.01$, respectively (as compared to control); # indicates $p<05$ (when compared to cuprizone).

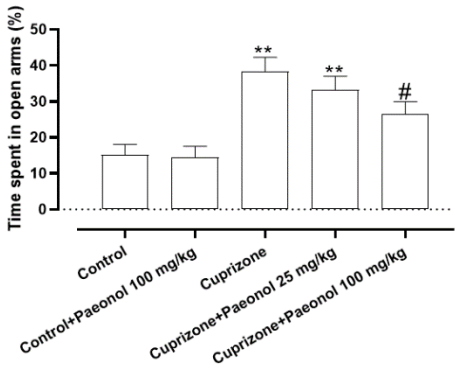

Figure 2: Performance of mice in elevated plus maze in different groups as an anxiety evaluation task. ** $p<0.01$ (relative to control); # $p<0.05$ (relative to cuprizone)

To evaluate the effect of treatments on depressive behavior, we used tail suspension test (Figure 3). Performing one-way ANOVA showed a marked and significant difference between the groups ($F(4,45)=8.91$, $p<0.001$). In this regard, cuprizone administration for 6 weeks significantly increased immobility time in this task when comparing it with related data of the control group ($p<0.01$). Such significant increase to a lower degree was also obtained in cuprizone group treated with paeonol at a dose of 25 mg/kg ($p<0.05$). On the contrary, cuprizone group treated with paeonol at the higher dose of 100 mg/kg showed a significantly lower time as compared to vehicle-treated cuprizone group ($p<0.05$). In other words, cuprizone dose-dependently was capable to reverse behavioral deficits in tail suspension test following cuprizone and also to exert an anti-depressant activity.

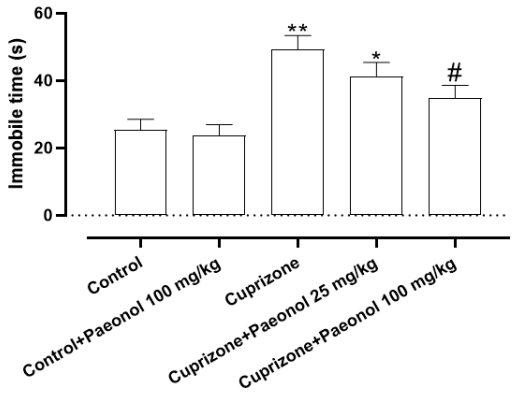

Figure 3. Performance of mice in tail suspension test in different groups as a depression evaluation task. * $p<0.05$, ** $p<0.01$ (relative to control); # $p<0.05$ (relative to cuprizone)

4. Discussion

In this study, we fed cuprizone at a dose of 400 mg/kg to mice for 6 weeks to induce demyelinating phenotype of MS. Cuprizone is a copper ion-chelating chemical which its consumption leads to oligodendrocyte damage and loss with final demyelination process in the CNS of rodents (19, 20). The myelin sheath is vital for provision of nutritional support for the axons and increasing notably the conduction velocity of evoked action potentials, which is very pivotal for physiological functioning of the CNS (18). Disruption of myelin development may interfere with communication between neurons (21). The severity of demyelination due to cuprizone is somewhat different in several schizophrenia-associated regions of the brain (19). In this respect, myelin sheath shows notable damage and loss in hippocampus, cerebral cortex, and olfactory bulb and the effects of cuprizone on thalamus and hypothalamus is milder (22). Patients with multiple sclerosis show various degrees of sensorimotor deficits. Such conditions are also observed in its animal models (23, 24). To show development of motor deficits in this study after cuprizone challenge, we used open field task. Our results showed that cuprizone group has significantly lower number of line crossing, indicating occurrence of motor deficits (25, 26). Such finding has also been reported before Recent investigations have also reported that cuprizone exposure, especially at lower doses, can cause behavioral abnormalities that are similar to those symptoms in human schizophrenia including reduced anxiety-like responses, deficits in memory and learning abilities, and decrease of social activity (20, 27). Therefore, cuprizone-induced model of MS in mice can also be used for assessment of schizophrenia in addition to explore the potential efficacy of promising agents. In our study, cuprizone administration for 6 weeks led to higher level of depression as evaluated by tail suspension test with a higher duration of immobility time and lower anxiety as shown by higher duration of stay in open arms in elevated plus maze. The latter parameter may also be seen as a schizophrenia-related phenotype following cuprizone exposure and this is consistent with clinical findings from patients with schizophrenia (19).

In our study, paeonol administration for 5 weeks in a dose-dependent manner significantly reversed behavioral changes due to cuprizone including improvement of performance in open field task and amelioration of open arms stay and immobility time durations in elevated-plus maze and tail suspension tests, respectively, partly indicating that paeonol can effectively alleviate part of behavioral derangements in our model of MS. A relevant study has shown that paeonol can improve performance of mice in rotarod and open‑field tests in methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)/probenecid-induced model of Parkinson's disease (28). Also, according to a related study, paeonol through alleviating neuronal injury and also through concomitant alterations of brain-derived neurotrophic factor (BDNF), Rac1 and RhoA levels besides increasing dendritic length and complexity and also density of dendritic spines in the hippocampal CA1 and in the dentate gyrus (DG) can exert an antidepressant effect in a model of chronic unpredictable mild stress (CUMS) in rats (11). Furthermore, paeonol can attenuate lipopolysaccharide (LPS)-evoked depressive-like behavior in mice as shown by improving open-field activity as well as decreasing immobility duration in forced swim (FST) and tail suspension tests (TST) through reversal of the concentrations of 5-HT, NE and reduction of inflammatory factory such as IL-6 and TNF-α levels besides its effective downregulation of BDNF, tropomyosin-related kinase B (TrkB) and nuclear factor-κB (NF-κB) in the hippocampal tissue (12). In some animal models that are mechanistically different

from our cuprizone model of MS, paeonol has been effective to alleviate aggressive and anxiety-like behaviors in a model of premenstrual dysphoric disorder in rats, as evaluated in open-field test (OFT), elevated plus maze (EPM), and light dark box (LDB) (13). In addition, anxiolytic effect of paeonol has been shown previously in mice (14).

To conclude, these findings demonstrated reversal effects of paeonol regarding motor deficits and depressive and anxiety-like behavior in cuprizone-induced model of MS and this compound may have potential clinical advantage in demyelinating conditions such as MS.

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Declaration of interest

The authors hereby declare that there is no conflict of interest.

Author contribution

S.P. conducted experiments, helped in data analysis and manuscript preparation. Z.K. and M.R. designed the study, supervised the experiments and wrote the manuscript. M.R. analyzed data and M.Kh. helped in designing experiments and preparation of the manuscript.

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