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Antagonism of quercetin against tremor induced by unilateral striatal lesion of 6-OHDA in rats

Xin Mu\textsuperscript{a1}, Xia Yuan\textsuperscript{b1}, Li-Da Du\textsuperscript{c}, Guo-Rong He\textsuperscript{a} and Guan-Hua Du\textsuperscript{a*}

\textsuperscript{a}Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China; \textsuperscript{b}State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China; \textsuperscript{c}The Chinese University of Hong Kong, Hongkong, China

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Quercetin, a flavonoid present in many plants, is reported to be effective in models of neurodegenerative diseases. The aim of the present study was to evaluate the anti-tremor effects of quercetin in 6-hydroxydopamine (6-OHDA)-induced rat model of Parkinson’s disease. In rats, quercetin had no effect on apomorphine-induced rotations, but it could significantly attenuate muscle tremor of 6-OHDA lesioned rats. Interestingly, quercetin could decrease the burst frequency in a dose- and time-dependent manner. These results suggest that quercetin may have a protective effect on models to mimic muscle tremors of Parkinson’s disease. This effect of quercetin may be associated with serotonergic system, but further study is needed.

Keywords: Parkinson’s disease; quercetin; tremor; 6-hydroxydopamine

1. Introduction

Tremor is one of the core symptoms of Parkinson’s disease. The tremor of Parkinson’s disease behaves in a more complex and heterogeneous manner than do the other cardinal signs of bradykinesia and rigidity [1]. As yet, the pathophysiological mechanisms of tremor generation are not clearly understood. A more complex view of tremor has emerged including a network of brain regions and several neurotransmitter systems thought to play a role [2]. Few drugs currently used for Parkinson’s disease therapy are clearly effective for tremor, and most of them do not have a consistent benefit in therapeutic efficacy and side effects are often observed with long-term treatment. Therefore, development of new anti-tremor drugs, with better curative effects and fewer side effects for Parkinson’s disease therapy, is urgently needed.

Neurotoxin to dopaminergic neurons such as 6-hydroxydopamine (6-OHDA), is usually applied to create experimental parkinsonism. 6-OHDA is a hydroxylated analogue of the natural neurotransmitter dopamine (DA) [3], which can be uptaken into catecholaminergic nerve endings and induce death of dopaminergic neurons. 6-OHDA has been reported to produce some of the behavioural, biochemical, and pathological changes that were encountered in Parkinson’s disease [4], and has thus become a research tool in experimental studies aimed at determining more effective antiparkinsonian drugs.

Quercetin is a flavonoid that has been isolated from many plants, including Quercus mongolica Fish, Apocynum venelin Linu, and Hypericum ascyron L. Various studies have shown that quercetin has wide-ranging pharmacological actions as a cardioprotective, anticarcinogenic, antioxidant,
and antiapoptotic agent [5–8]. Recent reports have demonstrated that quercetin protects against the dopaminergic neurotoxin 1-methyl-4-phenyl pyridinium (MPP\(^+\)) in a midbrain slice culture [9] and reduces neuronal death caused by kainate plus N-methyl-d-aspartate [10]. It is proposed that MPP\(^+\) causes mitochondrial dysfunction and oxidative stress, and kainate plus N-methyl-d-aspartate causes neuronal excitotoxicity. These factors may play an substantial role in the pathogenesis of Parkinson’s disease. Therefore, we speculate that quercetin may exert therapeutical effects in experimental parkinsonism. The aim of the present study was to evaluate the effects of quercetin in 6-OHDA-induced rat model of Parkinson’s disease.

2. Results

2.1 Effect of quercetin on apomorphine-induced rotation in 6-OHDA unilaterally lesioned rats

Rats exhibited rotational behavior in the direction opposite to the lesion (contralateral rotation) following apomorphine challenge 2 weeks after unilateral administration of 6-OHDA (Figure 1). Significant increases in the number of apomorphine-induced rotations were seen in 6-OHDA-lesioned rats compared with the sham-operated rats (P < 0.05). However, quercetin treatment has no effect on apomorphine-induced rotations (P > 0.05).

2.2 Effect of quercetin on tremor in 6-OHDA unilaterally lesioned rats

The recordings revealed characteristic muscle activity in sham-operated rats, but 6-OHDA unilaterally lesioned rats showed a high burst activity (Figure 2(a)). 6-OHDA could increase the burst frequency compared with controls (P < 0.05). However, quercetin could decrease the burst frequency in a dose- and time-dependent manner (P < 0.05). The inhibition rate of burst frequency was increased with increasing concentrations of quercetin. The peak occurred at 120 min and it was almost reduced to control levels within 300 min after intragastric administration (Figure 2(b)).

2.3 Effects of quercetin on the levels of DA, 5-hydroxytryptamine (5-HT) and their metabolites in the striatum

The present study confirmed that stereotaxic injection of 6-OHDA induced a marked decrease in the levels of DA and

![Figure 1. Effect of quercetin on rotational behavior in 6-OHDA-lesioned rats. Significant increases in the number of apomorphine-induced rotations were seen in 6-OHDA-lesioned rats compared with the sham-operated rats. Quercetin treatment has no effect on apomorphine-induced rotations. Data are expressed as mean ± SEM (n = 10). *P < 0.05 compared with control group.](image-url)
its metabolites compared with the control group \( (P < 0.05) \). However, quercetin treatment did not affect any of these values \( (P > 0.05) \) (Figure 3).

The present study showed that the striatal injection of 6-OHDA induced a marked decrease in the levels of 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA) compared with the control group \( (P < 0.05) \). Treatment with quercetin attenuated the decrease in the level of 5-HT \( (P < 0.05) \), but had no significant effect on the level of 5-HIAA \( (P > 0.05) \). In addition, the rate of the 5-HT catabolism measured as the 5-HIAA:5-HT ratio was enhanced by 6-OHDA injection and the degree of enhancement of the ratio was decreased with increasing concentrations of quercetin (Figure 4).

3. Discussion
Tremor in Parkinson’s disease is generated by an oscillatory neuronal network consisting of cortex, basal ganglia, and thalamus. Multiple brain locations and multiple neurotransmitter systems, including serotonergic and cholinergic systems, contribute to parkinsonian tremor [1]. Our results first demonstrated that quercetin could attenuate muscle tremor in 6-OHDA lesioned rats. Moreover, it was revealed that this effect of quercetin was closely associated with serotonergic systems.

Recent studies have shown that 5-HT acts at the posttranscriptional level to decrease striatal \( \gamma \)-aminobutyric acid (GABA) synthesis [11]. Therefore, a decrease in striatal 5-HT transmission in Parkinson’s disease may increase striatal
GABAergic neuronal activity. This alteration affects the lateral globus pallidus, which become hypoactive, ultimately leading to a decrease in the inhibitory control exerted over the subthalamic nucleus. In recent years, much attention in tremor research has been focused on one particular part of the basal ganglia, the subthalamic nucleus [12–15]. Significant coherence in the tremor frequency was

Figure 3. Effects of quercetin treatment on the levels of DA (a), DOPAC (b) and HVA (c) in the striatum. Data are expressed as mean ± SEM (n = 10). *P < 0.05 compared with control group.
detected between muscular activity and neuronal subthalamic nucleus activity [16].

In the present study, the decrease in 5-HT turnover induced by quercetin administration was observed and demonstrated by the 5-HIAA:5-HT ratio. These results suggested that quercetin could increase the level of 5-HT in striatum, and this may trigger a cascade of functional changes affecting the subthalamic nucleus activity. The anti-tremor effects of quercetin may act partly through this.

In this study, quercetin treatment appears to have no effect on apomorphine-induced rotations. In rats, amphetamine-induced rotation has been shown to correlate with dopaminergic systems [17]. Our results suggested that quercetin treatment did not have any significant effects on striatal DA, 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) levels, which were in accordance with the earlier studies [18]. Tremor is more poorly correlated with a dopaminergic deficit when compared with bradykinesia (e.g. rotational behavior). Parkinson’s disease tremor might be modulated by the activity of other central neuronal systems (e.g. serotonergic) [19]. Therefore, we concluded that quercetin treatment may be useful in tremor dominant parkinsonian patients, but had no effects against dyskinesia (e.g. rotational behavior).

In conclusion, the present study confirmed the anti-tremor effects of quercetin in 6-OHDA-induced rat model of Parkinson’s disease. The mechanism is most likely related to its pro-serotonin action. These results suggested that quercetin might be a promising candidate for the treatment of tremor-dominant Parkinson’s disease.

4. Materials and methods

4.1 Reagents

Quercetin was purchased from Shannxi Huike Botanical Development Co., Ltd. and repurified and its β crystal form was prepared by Prof. Lu Yang in Institute of Materia Medica. The purity of quercetin is 98% tested by high-performance liquid chromatography (HPLC) method. 6-OHDA hydrobromide, apomorphine hydrochloride, DA, HVA, DOPAC, 5-HT, and 5-HIAA were purchased from Sigma-Aldrich (St. Louis, MO, USA).
4.2 Animals
Adult male Sprague-Dawley rats (Beijing Vital River Laboratory Animal Technology Co., Ltd; weight 180–200 g; licence: SCXK (JING) 2007-0001) were used in this study. They were housed at 22°C, under 12-h light/12-h dark conditions with *ad libitum* access to food and water. All experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by our local Animal Ethics Committee.

4.3 Surgery
Rats were anesthetized with 3% sodium pentobarbital (45 mg/kg ip) and received unilateral lesions of the left striatum made by stereotaxic injection of 6-OHDA. 6-OHDA was dissolved in sterile 0.02% ascorbate saline at a concentration of 2 g/l. The volume of the 6-OHDA-infusion was 5 μl and it was dispensed over a 10-min period. 6-OHDA was injected unilaterally in two deposits at the following coordinates according to the atlas of Paxinos and Watson [20] (in mm relative to bregma and the surface of the dura mater): anterior (A) = −0.7, lateral (L) = 2.5, ventral (V) = −5.5, tooth bar at −3.3; and A = −0.3, L = 3.5, V = −5.5, tooth bar at −3.3, respectively. The sham-lesioned rats (*n* = 10) received only vehicle at the same coordinates.

4.4 Apomorphine-induced rotation
The rotation of all rats induced by apomorphine (0.5 mg/kg, sc) was tested at 2 weeks after 6-OHDA lesion. Rats were placed in individual transparent cylinders with a diameter of 20 cm. They were allowed to habituate to their environment for 10 min before the administration of apomorphine. Full 360° turns in the direction contralateral to the lesion were counted, and rotational behaviors were assessed for 30 min. Results were expressed as contralateral turns/30 min.

4.5 Drug administration procedures
Forty rats which showed more than 210 rotations per 30 min (turns contralateral to the lesion subtracted from ipsilateral turns) were used for further investigation. The selected rats were divided into four groups randomly according to rotation number: group B (model, saline, ig); group C (quercetin 100 mg/kg, ig); group D (quercetin 200 mg/kg, ig); and group E (quercetin 400 mg/kg, ig). The sham-lesioned rats were as group A (control, saline, ig). All the rats were treated as described above since the 15th day post-lesion, and at day 21, 28 post-lesion, the rotational behaviors were tested according to the same procedure.

4.6 Monitoring of 6-OHDA-induced tremors
The effect of quercetin on 6-OHDA-induced tremors was determined using tremor recording apparatus (BL-420S, Tme, Chengdu, China), as described previously [21]. The dose-dependent effect of quercetin was determined at the doses of 100, 200, and 400 mg/kg. The time-dependent effect of quercetin was determined at 0, 10, 30, 60, 90, 120, 180, 240, and 300 min after intragastric administration. Results were expressed as inhibition rate of burst frequency compared with that before treatment.

4.7 Measurement of DA, 5-HT and their metabolites in the striatum by HPLC with electrochemical detection
Rats were decapitated 3 days after the last behavioral assessment and the brains were removed immediately. The lesioned-side striatum was dissected and frozen in liquid nitrogen, followed by homogenized in 200 μl of 0.02 mol/L perchloric acid (HClO₄) and centrifuged at 12,000g at 4°C for 30 min to precipitate proteins. Supernatant was used to determine concentration of DA and its metabolites DOPAC and HVA, 5-HT and its metabolite 5-HIAA by
HPLC with electrochemical detection. The mobile phase consisted of 0.1 mol/L NaH$_2$PO$_4$ buffer, 0.85 mol/L octane sulfonic acid, methanol 11%, and 0.5 mmol/L EDTA-Na$_2$, and the pH of the mobile phase was set to 3.25. The column temperature was set at 35°C and the flow rate was 1.2 ml/min.

4.8 Statistical analysis

Data were expressed as means ± SEM. To analyze the differences between groups, statistical analysis was conducted with one-way ANOVA tests followed by Dunnett’s test. A $P < 0.05$ was considered significant.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Note

1. Both contributed equally to this work.

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