The Beneficial Effect of The Flavonoid Quercetin on Behavioral Changes in Hemi-Parkinsonian Rats

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ABSTRACT

Introduction: A large body of experimental evidence supports a role for oxidative stress as a mediator of nerve cell death in Parkinson's disease (PD). Flavonoids like quercetin have been reported to prevent neuronal degeneration caused by increased oxidative burden, therefore, this study examined whether quercetin administration at a high dose would attenuate behavioral abnormalities in experimental model of PD in rat.

Methods: For this purpose, unilateral intrastriatal 6-hydroxydopamine (6-OHDA)-lesioned rats were pretreated with quercetin (20 mg/kg; i.p.) 1 hour before surgery and treated once a day for one month. After one month, apomorphine-induced rotational behavior was measured postlesion.

Results: Apomorphine-induced rotations were counted after 4 weeks. Quercetin administration could attenuate the rotational behavior in treated lesioned rats as compared to untreated ones.

Discussion: Flavonoid quercetin administration for one month could attenuate behavioral abnormalities in 6-OHDA model of PD.

1. Introduction

Parkinson's disease (PD) is a neuropathological disorder involving the degeneration of dopaminergic neurons in the substantia nigra, with the subsequent loss of their terminals in the striatum. The ensuing loss of dopamine causes most of the debilitating motor disturbances associated with PD (1). Current PD medications treat symptoms without halting or retarding degeneration of dopaminergic neurons (2). In the search for new therapeutic approaches, quercetin, a natural flavonoid, is a strong antioxidant and radical scavenger and a polyphenol component which is abundant in fruits and vegetables (3). It has been reported that quercetin has anti-inflammatory, anti-blood coagulation, anti-ischemic effects, and anti-MMP action (4). Quercetin also has been known to have neuroprotective effect. In in vitro study with PC12 cell line, quercetin showed inhibitory effect against cell damage (5). Quercetin also attenuated neuronal damage following focal brain ischemia in in vivo model. Youdim et al (6) reported quercetin can pass blood-brain barrier. Several studies demonstrated quercetin can inhibit MMP activity. Quercetin treatment has been shown to attenuate UV irradiation-induced increase of MMP-1 in fibroblast (7). In addition, MMP-9 increments in various types of disease model were decreased by quercetin administration (8-10).

Therefore, the beneficial protective effect of querectin was investigated in a model of PD. For this purpose, number of Nissl-stained neurons of the SNC was measured.

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2. Methods

2.1. Animals

Adult male Wistar rats (n = 40) (Pasteur's Institute, Tehran), weighing 200-250 g at the start of the experiment were housed three to four per cage in a temperature-controlled colony room with free access to tap water and standard food. They were held in the colony room for at least one week before being tested. All procedures of this study including animals were approved by the ethical committee on animal experiments of the Research Council of Iran University of Medical Sciences (Tehran, Iran), which is in agreement with the guidelines of the National Institutes of Health for the use of live animals.

2.2. Experimental Procedure

Only rats not showing any biased rotational behavior (net rotations less than 30/hour) following intraperitoneal injection of apomorphine hydrochloride (0.5 mg/kg) were selected for the present study (10). The animals were randomly divided into four groups: sham -operated group (SH), querectin-treated sham-operated group (SH+Q), lesion group (L), and querectin-treated lesion group (L+Q). Since no behavioral and histochemical effects (as compared to SH group) were noted with the querectinin the SH+Q, it was also considered as SH group. The rats were anesthetized with a combination of ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), heads were shaved and placed in a Stoelting stereotaxic apparatus (incisor bar -3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with an iodine solution, incised on the midline and a burr hole was drilled through the skull at the coordinates L –3 mm, AP 9.2 mm, V 4.5 mm from the center of the interaural line, according to the atlas of Paxinos and Watson (11). The injection was made through a 10 µl Hamilton syringe. The L group received a single injection of 5 µl of 0.9% saline containing 2.5 µg/ µl of 6-hydroxydopamine-HCL (6-OHDA, Sigma) and 0.2% ascorbic acid (W/V) at a rate of 1 µl/min. The SH group received an identical volume of ascorbate-saline solution. The L+Q group received the neurotoxin in addition to intraperitoneal injection of quercetin (Sigma) one hour before the injection of the neurotoxin (20 mg/kg), and once a day for a period of 4 weeks. At the end of injection, the needle was left in place for an additional 5 min and then withdrawn at a rate of 1 mm/min.

2.3. Behavioral Observations

The animals were tested for rotational behavior by apomorphine hydrochloride (2 mg/kg, i.p.) one week before (baseline) and two weeks after the surgery. The rotations were measured according to a method as described previously by Fujita et al [15]. Briefly, the animals were allowed to habituate for 10 min and then 1 min after the injection of drugs, full rotations were counted in a cylindrical container (a diameter of 33 cm and a height of 35 cm) at 10-min intervals for the first 60 min in a quiet isolated room. Net number of rotations was defined as the positive scores minus the negative scores.

2.4. Statistical Analysis

All data were expressed as mean ± S.E.M. For the drug-induced rotational behavior, one-way ANOVA followed by Tukey post-hoc test was performed. In all analyses, the null hypothesis was rejected at the 0.05 level

3. Results

All animals well tolerated surgical operations and there was no mortality due to treatments.

The protective and beneficial effect of quercetin was evaluated on apomorphine-induced rotations for a period of 1 hour (Table 1). There were no significant differences among the groups at baseline (before surgery). Statistical analysis of the total net number of rotations made over a 60-min period 4 weeks after the surgery showed that apomorphine caused a very significant contralateral turning in the rats of the L group ($P<0.001$) and induced less significant rotations in the L+Q group ($P<0.005$) in comparison with the SH group. Moreover, the group L+Q showed a significant reduction of rotations ($P<0.01$) when compared to the L group.

Table 1. Total Apomorphine-induced Rotation Behavior

	SH	L	L + Q
One Week Presurgery	-3.7 ± 2.8	-4.1 ± 3.7	-0.2 ± 3.6
Four Weeks Postsurgery	-2.8 ± 3.4	148.1 ± 18.5**	76.7 ± 10.8*

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Total net number of rotations (mean ± SEM) induced by apomorphine (2 mg/Kg, i.p) over a period of 60 min in sham-operated (SH), lesioned (L), and quereetin-treated lesioned (L+Q groups. Note that the positive values indicate turns contralateral to the side of lesion.
* $P<0.005$, ** $P<0.001$ (Compared to the SH group), †$P<0.01$ (L+Q versus L)

4. Discussion

In this study, the protective effect of quercetin was investigated in a model of PD. For this purpose, number of apomorphine-induced rotations for 1 h was counted. There are two major conclusions to be drawn from the obtained results. First, 6-OHDA caused a significant increase in apomorphine-induced rotations as compared to

sham-operated group. Previous studies have demonstrated that the unilateral damage of the nigrostriatal dopaminergic system through intrastriatal injection of 6-OHDA is followed by a reduction in the striatal dopamine level and an up-regulation of dopaminergic postsynaptic receptors at the same side (13). These changes produce a prominent functional and motor asymmetry that can be evaluated by dopaminergic agonists like apomorphine (13). These rotations are considered as reliable indicators of nigrostriatal dopamine depletion (13).

Secondly, a lower rotational behavior was observed in querectin-treated lesion group as compared to untreated lesion group. In this respect, it has been reported that reactive oxygen radicals are involved in the toxicity of 6-OHDA-induced nigrostriatal lesions that is used as an experimental model of unilateral Parkinsonism (13). Neuroprotective effect of quercetin against neurotoxin-induced damage has already been reported in central nervous system (14). In addition, its systemic administration could protect hippocampal neurons against global ischemic consequences (15). There is also some evidence that following lesions and repetitive electrical stimulation of neuronal circuits, expression of matrix metalloproteinase (MMP) increases (14-15). This pathway may be one candidate for beneficial effect of quercetin in the present study and in this way the flavonoid could reduce neuroplastic changes in neural circuits and augmented excitability in certain sited involved in epilepsy. On the other hand, quercetin and its derivatives in the body can selectively inhibit NMDA receptor functionality (in some ways acting as an antagonist) (16) and in this way exert their beneficial effect in some animal model of neural diseases like PD. The demonstration of the neuroprotective effect of quercetin in 6-OHDA model of PD in this study establishes a potential neural basis for the epidemiological association between querectin consumption and a reduced risk of PD in future.

To conclude, these data establish a potential basis for the inverse association between quercetin administration and the development of PD and this may put forward flavonoids like quercetin as a novel treatment for this neurodegenerative disease.

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References

1. Sauer H, Oertel WH. Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neurosci. 1994; 59: 401-415.

2. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003; 39:889-909.

3. Choi DW. Ischemia-induced neuronal apoptosis. Curr Opin Neurobiol. 1996; 6: 667-672.

4. Moon SK, Cho GO, Jung SY, Gal SW, Kwon TK, Lee YC, et al. Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK1/2 cell-cycle regulation, and matrix metalloproteinase-9. Biochim Biophys Res Commun. 2003; 301: 1069-1078.

5. Gelinas S, Martinoli MG. Neuroprotective effect of estradiol and phytoestrogens on MPP+-induced cytotoxicity in neuronal PC12 cells. J Neurosci Res. 2002; 70: 90–96.

6. Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA, Abbott NJ. Flavonoid permeability across an in situ model of the blood-brain barrier. Free Radic Biol Med. 2004; 36: 592-604.

7. Moon HI, Lee J, Zee OP, Chung JH. The effect of flavonoid glycoside on the expressions of matrix metalloproteinase-1 in ultraviolet-irradiated cultured human skin fibroblasts. J Ethnopharmacol. 2005; 101: 176–179.

8. Huang YT, Hwang JJ, Lee PP, Ke FC, Huang JH, Huang CJ, et al. Effects of luteolin and quercetin,

inhibitors of tyrosine kinase on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. Br J Pharmacol. 1999; 128: 999–1010.

9. Moon SK, Cho GO, Jung SY, Gal SW, Kwon TK, Lee YC, et al. Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK1/2 cell-cycle regulation, and matrix metalloproteinase-9. Biochim Biophys Res Commun. 2003; 301: 1069–1078.

10. Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology 2003; 189: 1-20.

11. Paxinos G, Watson C. The rat brain in stereotaxic coordinates, 2nd ed., Academic Press, San Diego, 1986.

12. Roghani M, Behzadi G. Neuroprotective effect of vitamin E on the early model of Parkinson's disease in rat: behavioral and histochemical evidence. Brain Res. 2001; 892: 211-217.

13. Schwarting RKW, Huston JP. The unilateral 6-hydroxydopamine lesion model in behavioral brain research: analysis of functional deficits, recovery and treatments. Prog Neurobiol. 1996; 50: 275-331.

14. Zbarsky V, Datla KP, Parkar S, Rai DK, Aruoma OI, Dexter DT. Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. Free Radic Res. 2005; 39: 1119-25.

15. Cho J.Y., Kim I.S., Jang Y.H., Kim A.R., Lee S.R. Protective effect of quercetin, a natural flavonoid against neuronal damage after transient global cerebral ischemia. Neuroscience Lett. 2006; 404: 330-335.

16. Wagner C, Fachinetto R, Dalla Corte CL, Brito VB, Severo D, de Oliveira Costa Dias G, et al. Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation in vitro. Brain Res. 2006; 1107: 192-8.