Neuroprotective Effect of Quercetin in a Model of Parkinson's Disease in Rat: A histochemical Analysis

Mehdi Mehdizadeh*, Mohammad Taghi Joghataei, Maliheh Nobakht, Roya Aryanpour

Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

Article info:
Received: 10 July 2009
First Revision: 15 August 2009
Accepted: 7 September 2009

Key Words:
Quercetin,
6-Hydroxydopamine,
Parkinson's disease,
Rat

ABSTRACT

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. Quercetin, a natural flavonoid, is a strong antioxidant and radical scavenger. Therefore, its neuroprotective effect in a model of Parkinson's disease in rat was evaluated.

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. Nissl-stained neurons of substantia nigra pars compacta (SNC) were counted.

Results: Number of Nissl-stained neurons in left side of SNC of lesion group was lower relative to sham-operated group ($p<0.005$) and it was higher in quercetin-treated lesion group as compared to untreated lesion group ($p<0.01$).

Discussion: Flavonoid quercetin administration for one month could protect the neurons of SNC against 6-OHDA toxicity.

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.

**** Corresponding Author:***
Dr. Mehdi Mehdizadeh,
Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran
E-mail: mmehdizadeh@hotmail.com

2. Methods

2.1. Animals

Adult male Wistar rats (n = 80) (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 µ l Hamilton syringe. The L group received a single injection of 5 µl of 0.9% saline containing 2.5 µ g/µl of 6-hydroxydopamine-HCL (6-OHDA, Sigma) and 0.2% ascorbic acid (W/V) at a rate of 1 µ l/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. Histological Study

At the end of behavioral experiments, the rats were deeply anesthetized with a high dose of ketamine (150 mg/kg) and perfused through the ascending aorta with 200 ml of 0.9% saline followed by 500 ml of fixative solution containing 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) followed by 100 ml of 0.1 M PB containing 10% sucrose. Following perfusion, the brains were removed from the skull, the blocks of forebrain and brainstem were prepared, embedded in paraffin, and then sections were cut at a thickness of 30 µm on a freezing microtome and collected in PB (0.1 M). Sections were Nissl-stained with 0.1% cresyl violet (Sigma).

2.4. Neuronal Counting

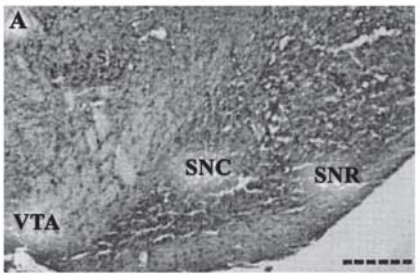
For each animal, mesencephalic sections (Interaural 2.9-4.2 mm) were examined by a method as described previously (12). Briefly, Nissl-stained neurons of the SNC were counted manually (Light microscopy; X400) using a superimposed grid to facilitate the procedure. At least two sections representative of each of four Paxinos-Watson planes (4.2, 3.8, 3.2, 2.97; Interaural) were examined by scanning the entire extent on each side. Counting was done blind to the treatments received. Number of SNC neurons was expressed as the total counts obtained from the representative sections.

2.5. 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. For each group, the values of Nissl-stained cells for the injected and non-injected sides were compared using two-tailed student's t-test for paired samples. In all analyses, the null hypothesis was rejected at the 0.05 level.

3. Results

The results of histological studies (Table 1 and Fig. 1) showed that although there was no significant difference for the number of Nissl-stained neurons on the right and left sides of SNC in SH group, a significant reduction was observed for L ($P<0.001$) and L+Q ($P<0.01$) groups. Meanwhile, there was no significant difference between SH and L+Q groups when comparing number of Nissl-stained neurons on the left side of SNC.



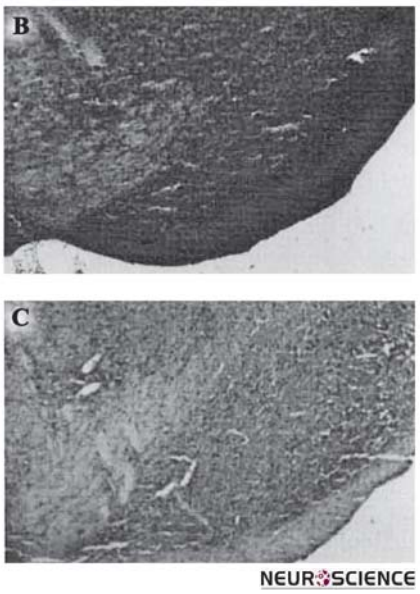


Fig. 1. Photomicrographs of typical coronal sections through the midbrain showing Nissl-stained neurons in SH (A), L (B) and L+Q (C) groups. A severe reduction in the number of neurons in SNC was observed in the L group, but no such marked decrease was noted in the L+Q group in comparison with the SH group. Scale bar = 450 μ m. (Abbreviations: SNC=Substantia nigra pars compacta, SNR=Substantia nigra pars reticulata, VTA= Ventral tegmental area)

4. Discussion

In this study, the protective effect of quercetin was investigated in a model of PD. For this purpose, number of Nissl-stained neurons of the SNC was quantified. There are two major conclusions to be drawn from the obtained results. First, 6-OHDA caused a significant reduction in Nissl-stained neurons of SNC region 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 upregulation 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, nigrostriatal neurons within SNC were mainly preserved in the presence of querecetin against neurodegenerative effects induced by the neurotoxin 6-OHDA. 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 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.

Table 1. Total number of Niss1-stained neurons on the left and right sides of SNC counted on its 4 levels

	SH (n=8)	L (n=8)	L + Q (n=8)
SNC			
Left	574.1 ± 5.6	376.5 ± 6.74***†	487 ± 6.1 *
Right	605.5 ± 12.39	582.9 ±12.8	602.1 ± 11

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Total number of Niss1-stained neurons sham-operated (SH), lesioned (L), and quercetin-treated lesioned (L+Q) groups one month after the experiment.

*P<0.01, *** P<0.001 (in comparison with the right side)

† P<0.01 (in comparison with SH group up on the left side)

Acknowledgement

This work was supported in part by a grant from the Research Council of the Iran University of Medical Sciences (Tehran, Iran).

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