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Evaluation of the association between blood homocysteine concentration and the degree of behavioral symptoms in the 6-hydroxydopamine-induced Parkinsonism in rat

Hashem Haghdoost-Yazdi *, Mohammad Sarookhani, Ayda Faraj, Negin Fraidouni, Tahereh Dargahi, Mohammad Hosein Yaghoubidoust, Hassan Azhdari-Zarmehri

Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

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ABSTRACT

Growing evidence indicates that homocysteine (Hcy) may be involved in the pathophysiology of several neurological disorders including Parkinson's disease. In the present study, the association between blood Hcy concentration and the degree of behavioral symptoms in the 6-hydroxydopamine (6-OHDA)-induced Parkinsonism in rat was evaluated. Total serum Hcy (tHcy) was measured before and 6 weeks after the intracerebral injection of 6-OHDA. Apomorphine-induced rotational test was performed at second, third and sixth weeks after 6-OHDA injection. Subsequently, cell replacement therapy was performed on rats with good rotation score. No correlation between tHcy in before 6-OHDA injection and severity of the rotations after 6-OHDA injection was observed. On the other hand, 6-OHDA treatment significantly decreased tHcy level. However, this reduction was only observed in animals with low degree of rotations and in rats with high number of rotations; tHcy did not change significantly. Furthermore, 10 weeks after cell transplantation, tHcy was significantly lower than that found before therapy if the rats showed good improvement in the degree of rotations. We also examined the effect of different supplements of B vitamins on tHcy before and after 6-OHDA injection. In healthy rats, all kinds of B vitamins and also supplement B6 or B12 alone reduced tHcy. Following 6-OHDA injection, B vitamin supplementation failed to cause remarkable effect. Considering the direct correlation between the severity of rotational behavior and the degree of lesion in the substantia nigra (SN), our data indicate that higher tHcy values can predict higher SN dopaminergic neurodegeneration.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting about 1–3% of the population over the age 50. The primary pathological feature of PD is the loss of dopaminergic (DA) neurons within the substantia nigra pars compacta of the midbrain. There is no consensus as to mechanism(s) contributing to DA cell loss; however, growing evidence suggests that oxidative stress and mitochondrial dysfunction play important role (Jenner and Olanow, 1996; Tatton, 2000). Although both genetic and environmental factors are involved in the pathogenesis of PD (Lau et al., 2005; Hancock et al., 2007), specific gene defects have been linked to a very small percentage of the cases and increasing evidence shows that environmental factors such as exposure to toxins (Betarbet et al., 2000) and low antioxidant intake (de Rijk et al., 1997) are important risk factors for the common sporadic forms of PD.

In the last decades, homocysteine (Hcy) has received special attention because of its association with pathogenesis of atherosclerosis and various cerebrovascular and cardiovascular diseases (Kuhn et al., 1998; Diaz-Arrastia, 2000; O'Suilleabhain et al., 2004). Also, elevated plasma Hcy level is a risk factor for cognitive decline and dementia in the general population and has been associated with mild cognitive impairment, Alzheimer's disease (AD), vascular dementia and depression (Bertsch et al., 2001; Prins et al., 2002; Seshadri et al., 2002; Tiemeier et al., 2002; Quadri et al., 2005). There is a rising body of evidence that shows Hcy levels increase in the blood and CSF of patients with PD (Allain et al., 1995; Kuhn et al., 1998; Yasui et al., 2000; dos Santos et al., 2009). High levels of Hcy might accelerate DA cell death through oxidative stress and excitotoxicity (Duan et al., 2002; Sachdev et al., 2002; Obeid and Herrman, 2006). Animal studies have demonstrated that focal infusion of Hcy into either substantia nigra (SN) or striatum exacerbates the symptoms of 6-OHDA and MPTP-induced Parkinsonism (Duan et al., 2002; Xing et al., 2008). The pro-oxidant and pro-apoptotic effects of homocysteine have been also confirmed for *in vitro* models of

* Corresponding author at: Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin 3414951414, Iran. Tel.: +98 281 3366001 6; fax: +98 281 3324970 1.
E-mail addresses: hhaghdoost@yahoo.com, hhaghdoost@gmail.com (H. Haghdoost-Yazdi).

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PD; e.g. homocysteine aggravated the neurotoxic effects of pesticide rotenone in human dopaminergic cells (Todorovic et al., 2006). On the other hand, there is a considerable body of evidence indicating that high level of Hcy in patients with PD is induced by treatment with levodopa (L-DOPA) (Miller et al., 1997; Kuhn et al., 1998; Rogers et al., 2003; Religa et al., 2006). A possible mechanism for the L-DOPA-induced hyper-Hcy is the biotransformation of L-DOPA to dopamine which leads to a depletion of S-adenosylmethionine required for Hcy conversion to methionine (Miller et al., 1997; dos Santos et al., 2009). This transformation needs O-methylation which is catalyzed by COMT (Zoccolella et al., 2005). Several studies have shown that treatment of PD with a combination of L-DOPA and COMT inhibitors decreases Hcy level (Siniscalchi et al., 2006).

6-Hydroxydopamine (6-OHDA)-induced Parkinsonism is one of the most common animal models of PD. 6-OHDA is a hydroxylated analogue of natural dopamine that selectively destroys catecholamine neurons. In addition to production of reactive oxygen species (ROS) which damage proteins, lipids and DNA, 6-OHDA through inhibition of mitochondrial complexes I and IV leads to mitochondrial impairment and ATP deficiency (Kumar et al., 1995; Soto-Otero et al., 2000; Blum et al., 2001; Rodriquez et al., 2002; Dauer and Przedborski, 2003). In the present study, we investigated the association between blood Hcy and the degree of behavioral symptoms of 6-OHDA-induced Parkinsonism. We also evaluated the effect of cell replacement therapy and B vitamin supplementation on the serum level of Hcy in 6-OHDA-treated rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Razi Institute, Karaj, Iran), weighing 220–250 g at the beginning of study were housed in large cages (38 × 59 × 20 cm, 10–12 rats in each) at a temperature-controlled colony room maintained at 21 ± 3 °C under 12:12 h light/dark cycle with lights on at 6:00 a.m. They were given free access to tap water and standard rat chow. All procedures carried out throughout this study were according to the guidelines for animal experiments approved by the Research Council of Qazvin University of Medical Sciences.

2.2. Surgical procedures

Rats were anesthetized with intraperitoneal injection of a solution containing ketamine (100 mg/kg) and xylazine (10 mg/kg). 6-OHDA (10–15 μg, dissolved in saline containing 0.2% ascorbic acid) was injected unilaterally into 2 sites in the right medial forebrain bundle (MFB) with coordinates of 1: AP: −4.4, L: −1.2, and DV: −7.8 with the tooth bar (TB) positioned below the interaural line: −2.3 and 2: AP: −4, L: −0.8, and DV: −8 with TB: +3.4 using stereotaxic apparatus (Stoelting, USA) and through a 10-μl Hamilton syringe. For the experiments evaluating B vitamin supplementation, 6-OHDA was unilaterally injected into the right striatum with coordinates of AP: 0.2 and L: −3.5 and also DV: −8 with TB: −3.3. AP and L were measured from bregma and DV was measured from the surface of skull. All coordinates were calculated according to the atlas of Paxinos and Watson (2007). 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. Apomorphine-induced rotational test

Apomorphine-induced rotational test was performed according to the method previously described by Fujita et al. (1996). Briefly, animals were initially given a 5-min habituation time followed by injection of apomorphine hydrochloride (0.5 mg/kg, i.p., dissolved in saline, sigma). A minute later, the number of full rotations was counted at 10-min intervals for 1 h in a cylindrical container (at a diameter and height of 28 and 38 cm, respectively). Contralateral and ipsilateral rotations (far away and toward the lesion side, respectively) were counted as positive and negative scores and the net number of rotations was defined as the positive scores minus the negative ones. All tests were carried out between 01.00 and 04.00 p.m.

2.4. Cell replacement therapy

2.4.1. Cell preparation

Embryonic day 14 (E14) ventral mesencephalic (VM) cells obtained from pregnant female Wistar rats were used for cell replacement therapy. E14 VM tissues were dissected and processed as previously described (Dunnett and Björklund, 1997; Nikkhah et al., 2009). Briefly, fetuses from both uterine horns were removed from timed-pregnant female and collected into saline-glucose (4 and 0.6%, respectively) solution (GS) at room temperature. Then, using sterile dissection instruments, the VM tissue pieces were divided into 4 segments and pooled in GS. To dissociate the tissue pieces into a single cell suspension, the VM segments were incubated in 0.1% trypsin (Worthington), 0.05% DNase (Sigma DN 25) and DMEM (Dulbecco's modified Eagle's medium, Gibco) at 37 °C for 30 min followed by four rinses with 0.05% DNase/DMEM. Later, trituration process was performed using 1-ml and 200-μl Eppendorf pipette tips (about 15 strokes each) in a solution with 50% cell culture medium and 50% trituration solution. Cell culture medium contained 77% DMEM, 20% fetal calf serum, 2% B-27 and 1% Pen-Strep and fungizone (sigma). Trituration solution contained 0.001% DNase and 1% bovine serum albumin (sigma) in Hank's balanced salt solution (sigma). Cell suspensions were pelleted by centrifugation at 600 rpm for 5 min and the remaining was resuspended with 0.05% DNase/DMEM at a final volume of 5 μl per VM. The final cell suspension contained 45,000 cells/μl and the viability was more than 85%, as determined by trypan blue exclusion assay on a hemocytometer. Before transplantation, the cell suspensions were incubated overnight at 0 °C in a hibernation medium containing KCl, glucose, MgCl2, NaH_2PO_4, Na_2HPO_4 and 30% lactic acid at pH = 7.2.

2.4.2. Cell transplantation

The cell suspension was transplanted into the right striatum of 6-OHDA-treated rats with good rotational score using stereotaxic surgery and 2-μl Hamilton syringe. A volume of 2 μl of suspension at the rate of 0.5 μl/min was implanted in each rat at 4 sites with coordinates of (in relation to bregma) AP: +0.5, L: +2.3, and DV: −5 or −4 mm with TB: −3.3 and also AP: +0.5, L: +3.3, and DV: −5 or −4 mm with TB: −3.3. There was 1 min stop after injection into each site and 3 min stop after the last injection. Then, the needle was slowly (1 mm/min) withdrawn.

2.4.3. B vitamin supplementation experiments

All kinds of B vitamins were purchased from the Sigma-Aldrich Company. Feeding with B vitamin supplements was begun 1 month before the injection of 6-OHDA and continued for 6 weeks afterwards. Animals were divided into eight experimental groups as follows: (1) control, which received B vitamins equal to that in normal MEM (minimum essential medium); (2) complex, which received a combination of all kinds of B vitamins (Table 1) 5-folds of that in normal MEM; (3–5) FA 2X, FA 5X and FA 10X which received folic acid 2, 5 and 10-folds of that in normal MEM, respectively; (6) FA + B6 + B12 which received a combination of folic acid, vitamin B6 and vitamin B12, 5-folds of that in normal MEM; (7 and 8) B6 and B12 which received vitamin B6 and B12, respectively 5-folds of that in normal MEM. The number of animals (n) was 12 for each group. Considering normal dietary regime contains B vitamins equal to normal MEM, additional B vitamins to provide required supplements were added to drinking water. Two 500 ml bottles of water were placed in each cage to ensure that each rat receives enough amounts of water and B vitamins. Drinking water was replaced every 2 days.

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