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Oxidative stress and mitochondrial dysfunction are the underlying events of dopaminergic neurodegeneration in homocysteine rat model of Parkinson's disease

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ABSTRACT

Homocysteine (Hcy) when injected intranigrally in rat caused parkinsonian behavioural phenotypes and loss of nigral dopaminergic neurons but the underlying mechanisms of neurotoxicity remains elusive. In the present study, we focused on oxidative stress as one of the mechanisms of neurotoxicity in Hcy-induced hemiparkinsonian rat model. Unilateral intranigral infusion of Hcy (1.0 μmol in 2 μl) caused inhibition of mitochondrial complex-I activity, decrease in the level of striatal dopamine, loss of midbrain dopaminergic neurons, and motor abnormalities. Hcy caused oxidative stress in the nigrostriatal pathway, with increase in generation of hydroxyl radicals, depletion in the level of reduced glutathione and enhanced activity of antioxidant enzymes (superoxide dismutase and catalase). Our results provided the evidence of critical involvement of oxidative stress as one of the mechanisms underlying Hcy-induced dopaminergic neurotoxicity in nigrostriatal pathway. As oxidative stress is one of the prime mechanisms of neurodegeneration in different animal models of Parkinson's disease, and since Hcy caused equivalent parkinsonian pathologies in rat model, the present study proclaims Hcy-induced rat model as a viable rodent model of Parkinson's disease.

1. Introduction

Parkinson's disease (PD), an age related neurodegenerative disorder, arises due to the death of dopamine containing neurons in the *substantia nigra* (SN) *pars compacta* area of midbrain resulting in four cardinal motor abnormalities: tremor, rigidity, bradykinesia and postural instability (Kempster et al., 2007; Kalia and Lang, 2015). The exact mechanism of nigral neurodegeneration is not yet known, however the formation of reactive oxygen species and the resulting oxidative stress has been postulated as the foremost event in PD pathogenesis (Dawson and Dawson, 2003; Blesa et al., 2015). Other mechanisms that contribute to PD pathogenesis are mitochondrial defects, inflammation, proteolytic stress and excitotoxicity (Dawson and Dawson, 2003; Dexter and Jenner, 2013; Di Maio et al., 2016). Human postmortem studies have indicated downregulation of antioxidant protective mechanisms in PD brain, which includes reduced glutathione (GSH) level, and decrease in the activity of superoxide dismutase (SOD), catalase (CAT) and peroxidases (Ambani et al., 1975; Nagatsu and Sawada, 2007).

Apart from environmental and genetic factors, several endogenous molecules (Borah and Mohanakumar, 2012; Borah et al., 2013; Paul et al., 2015), including homocysteine (Hcy; Doherty, 2013; Paul and Borah, 2016), have been implicated in the pathogenesis of PD. Hcy, a non-proteogenic sulphur containing amino acid synthesized during the metabolism of methionine, is an independent risk factor for neurodegenerative diseases, including PD (Doherty, 2013; Ansari et al., 2014; Paul and Borah, 2015, 2016). Several studies have provided the evidence of association of elevated levels of Hcy in plasma of PD patients (Allain et al., 1995; Kuhn et al., 1998; Louis et al., 2007). L-3,4-dihydroxyphenylalanine (L-DOPA) therapy, the gold-standard drug for PD, is known to increase the level of Hcy in plasma (Lamberti et al., 2005; Zoccolella et al., 2010; Hu et al., 2013; Paul and Borah, 2016) as well as in dopamine rich region of brain (Bhattacharjee et al., 2016a). In animal model of PD, Hcy exaggerates dopaminergic neurodegeneration (Duan et al., 2002; Xing et al., 2008) and intranigral infusion of it in rat has been

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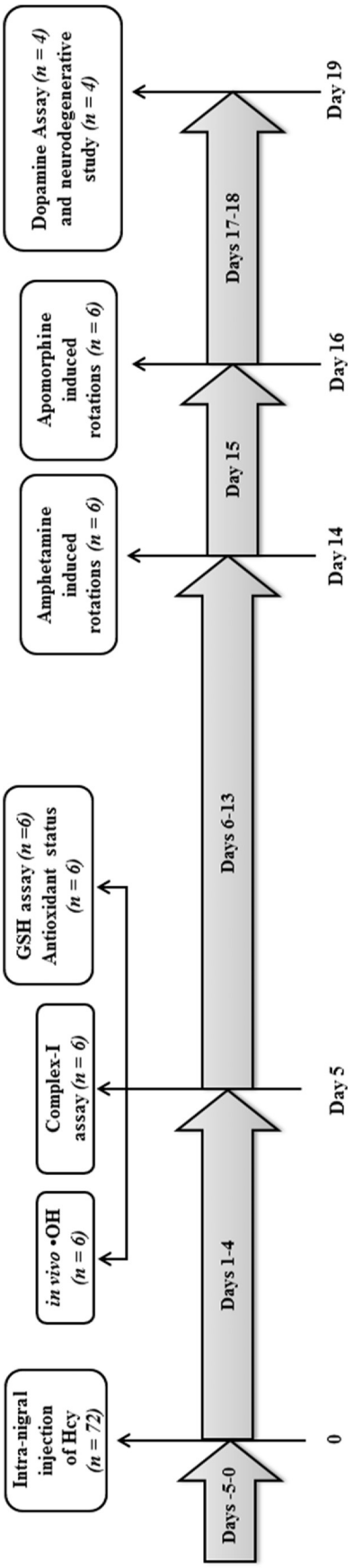

Fig. 1. Schematic representation of the experimental design.

demonstrated to reduce the striatal dopamine level as well as cause midbrain dopaminergic neurodegeneration (Chandra et al., 2006). Hcy itself can serve as a pro-oxidant and contribute to oxidative stress in neuronal cells (Perna et al., 2003; Hoffman, 2011). However, the mechanism of Hcy-induced dopaminergic neurotoxicity in *in vitro* or *in vivo* model of PD remains elusive. The present study put forward the involvement of oxidative stress as one of the mechanisms of Hcy-induced dopaminergic neurotoxicity in rats.

2. Experimental procedure

2.1. Animals

Male Sprague-Dawley rats (275–300 g), used in the present study, were maintained under standard conditions and the experimental protocols met the National and intuitional guidelines.

2.2. Drugs, chemicals and others

Homocysteine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 2,3-dihydroxybenzoic acid (2,3-DHBA), 2,5-dihydroxybenzoic acid (2,5-DHBA), coenzyme Q_0, NADH, glutathione (GSH), heptane sulfonic acid, triethylamine, orthophosphoric acid, chloral hydrate, poly-L-lysine, D-amphetamine, apomorphine, ethylenediaminetetraacetic acid disodium salt (EDTA) and 3, 3-diaminobenzidine (DAB) liquid substrate system were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Orthophosphoric acid, triethylamine, perchloric acid, acetonitrile, Triton X-100, and deionized water for high-performance liquid chromatography (HPLC) study were procured from SISCO Research Laboratories (Mumbai, India). Rabbit anti-tyrosine hydroxylase (TH) antibody and anti-rabbit goat secondary antibody tagged with horseradish peroxidase (HRP) were purchased respectively from Abcam (Cambridge, UK) and Millipore Co. (USA) respectively.

2.3. Experimental design

In the present study we chose the dose of 1 μmol of Hcy which caused about 60–70% striatal dopamine depletion on the 19th day after intranigral administration of Hcy (Chandra et al., 2006). Hcy (1.0 μmol in 2 μl) or vehicle was infused into the right substantia nigra by employing a micro-infusion apparatus. A group of animals (n = 6) were sacrificed after 2 h of administration of salicylic acid (100 mg/kg, i.p.) on 5th day post-infusion of Hcy. For the analysis of activity of mitochondrial complex, level of reduced glutathione and antioxidant enzymes, the Hcy infused rats were sacrificed on 5th day following surgery (n = 6/assay). Amphetamine- and apomorphine-induced rotational bias was performed on 14th and 16th day respectively following surgery (n = 6/test). Dopamine (n = 4) and its metabolites and TH-immunohistochemistry (n = 4) were assayed on the 19th day after intranigral administration of Hcy (Fig. 1). The vehicle injected side (left side) or the contralateral SN or NCP serves as control while Hcy was injected into the other side (right side) or ipsilateral.

2.4. Stereotaxic surgery

Rats were anaesthetized with chloral hydrate (350 mg/kg; i.p.) and held over a stereotaxic frame (Stoelting, USA) with incisor bar kept at 3.5 mm below the interaural line. Hcy (dissolved in saline; 1.0 μmol in 2 μl) or vehicle, was infused into the right SN at the flow rate of 0.5 μl/min by employing a micro-infusion apparatus. After infusion, the probe was kept for further 5 min to allow proper diffusion and then slowly retracted. The coordinates used for SN were anterio-posterior - 0.58, lateral - 0.22 and dorso-ventral - 0.85