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Targeting oxidative stress, mitochondrial dysfunction and neuroinflammatory signaling by selective cyclooxygenase (COX)-2 inhibitors mitigates MPTP-induced neurotoxicity in mice

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ABSTRACT

Several studies have pointed towards the role of oxidative stress, mitochondrial dysfunction and neuroinflammation in Parkinson's disease (PD). The present study was focused on the possible neuroprotective effect of selective cyclooxygenase (COX)-2-inhibitors: valdecoxib and NS-398 in 1-methyl-4-phenyl-1,2,3,6-tertahydropyridine (MPTP)-induced neurotoxicity in mice. MPTP administration in dose of 40 mg/kg, i.p (four injections of 10 mg/kg, i.p. at an interval of 1 h each) significantly induced the Parkinson-like symptoms in mice as indicated by change in locomotor activity, inability to correct posture (bar test), and oxidative stress (increased levels of lipid peroxidation, nitrite concentration, and depletion of antioxidant enzyme). MPTP administration significantly impaired mitochondrial complex-I activity and redox activity, upregulated the caspase-3 and NF-κB levels as compared to vehicle group. Treatment with valdecoxib (5 or 10 mg/kg, p.o.) or NS-398 (5 or 10 mg/kg, p.o.) for 7 days significantly reversed behavioral, biochemical, mitochondrial complex alterations as well as attenuated the induction of proinflammatory mediators in MPTP-treated groups. The findings of the present study substantiate the neuroprotective role of selective COX-2 inhibitors in ameliorating MPTP-induced neurodegeneration in mice and suggest the possible therapeutic potential of these drugs in the management of PD.

1. Introduction

Neuroinflammation an important defense mechanism to pathogenic events and environmental toxins, is the key component in the progression of various neurodegenerative disorders, especially Parkinson's disease (PD) (Monahan et al., 2008; Vroon et al., 2007). Recent research has shown that oxidative stress, mitochondrial dysfunction and neuroinflammation are the major players involved in the pathogenesis of PD along with the dopaminergic neurodegeneration (Bartels and Leenders, 2007; Monahan et al., 2008). Therefore, attenuation of neuroinflammation and oxidative stress represents the prime target so as to halt the progression of the disease. A number of experimental studies have documented the protective effect of non-steroidal anti-inflammatory drugs (NSAIDs) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity model of PD (Aguirre et al., 2008; Gupta et al., 2009, 2010a,b; Reksidler et al., 2007). These drugs exert their anti-inflammatory property by inhibiting cyclooxygenase (COX) isoenzymes which is the rate-limiting enzymes involved in the production of prostaglandins from arachidonic acid (Kulkarni and Dhir, 2009).

Experimental evidence indicates that cellular manifestation of neuroinflammation following MPTP administration takes place in substantia nigra due to microglial activation. Transient activation of microglia precedes the demise of dopaminergic neurons and mimics Parkinson-like symptoms in experimental model of PD (Liu et al., 2006; Smeyne and Jackson-Lewis, 2005). These symptoms are the same as demonstrated in the patients afflicted with Parkinson's disease where increased expression of various inflammatory molecules in the dopaminergic neurons has been observed (Minghetti, 2004). These neuroinflammatory events activate the glial cells *viz* microglia, which is further speculated to upregulate the COX expression (Teismann and Ferger, 2001). One of the key features of PD pathology, long-term gliosis, has been well documented in monkeys and humans exposed to MPTP (Vroon et al., 2007).

Valdecoxib and NS-398 used in the present study belong to the second generation of selective COX-2 inhibitors and have >50 fold selectivity in inhibiting COX-2 isoform (Dannhardt and Kiefer, 2001;

Abbreviations: ANOVA, analysis of variance; COX, cyclooxygenase; HEPES, 5-hydroxytryptamine (5-HT); i.p., intraperitoneal; iNOS, inducible nitric oxide synthase; MAO, monoamine oxidase; MDA, malondialdehyde; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole; NADH, nicotinamide adenine dinucleotide; NF-κB, nuclear factor-κB; NPSH, non-protein thiols; NSAIDS, non-steroidal anti-inflammatory drugs; p.o., per oral; PD, Parkinson's disease; TBARS, thiobarbituric acid reactive substances.

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Rao and Knaus, 2008).Valdecoxib has shown to exert neuroprotection against 3-nitropropionic acid-induced neurotoxicity in rats and chronic stress-induced behavioral and biochemical alterations (Kumar et al., 2007, 2010). Valdecoxib, a diarlyisoxazole class of compound, is metabolized in rodents and dogs to a 5-hydroxymethyl metabolite with additional anti-inflammatory activity (Dannhardt and Kiefer, 2001; Rao and Knaus, 2008). Structural modification of the nimesulide moiety ended up in the development of N-[2-(cyclohexyloxy)-4-nitrophenyl] methanesulfonamide (NS-398), with better COX-2 selectivity and anti-inflammatory activity (Huff et al., 1995). NS-398 has shown to reduce the infarct volume in rats and attenuate prostaglandin E_2 elevation (Huff et al., 1995; Nagayama et al., 1999; Nogawa et al., 1997).

During the last few decades, efforts have been made to develop novel anti-inflammatory drugs so as to arrest the progression of PD. With the increasing knowledge about the detrimental effects of neuroinflammatory cascade in the pathogenesis of PD, therapeutic agents that suppress or interfere this pathway represent the probable candidates for the neuroprotection in PD. Based on these observation, we designed the study to explore the neuromodulatory role of valdecoxib and NS-398 in experimental model of PD. The present study is an attempt to unravel the possible cellular mechanisms and to gain more insight into the neuroprotective effect produced by COX-2 inhibitors against MPTP-induced neuronal toxicity in mice.

2.1. Materials and methods

2.1.1. Animals

Male Laca mice (25–30 g), bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 h and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy (INSA) Guidelines for the use and care of experimental animals.

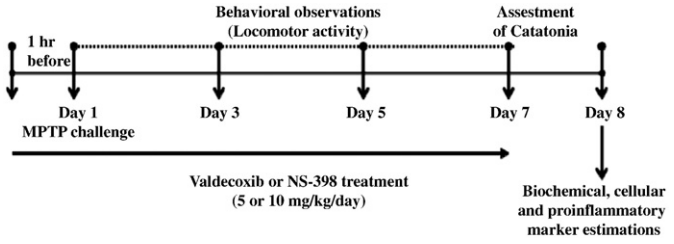
2.2. Drugs and treatment schedule

The following drugs were used: MPTP (Sigma, St.Louis, MO, USA); valdecoxib and NS-398 (Panacea Biotec Ltd., New Delhi, India). Valdecoxib and NS-398 were suspended in 0.25% w/v sodium carboxymethyl cellulose (Na-CMC) solution. MPTP was dissolved in distilled water. All the drugs except MPTP were administered per orally (p.o.). MPTP was administered intraperitoneally (i.p.) in a total dose of 40 mg/kg (four injections of 10 mg/kg at an interval of 1 h).

2.3. Experimental protocol and procedure

Eight groups were employed in the present study, each comprising of 12–15 animals. Group I: vehicle group — received equivalent volume of vehicle for 7 days. Group II: MPTP-treated group — received four injections of MPTP in a dose of 10 mg/kg, i.p. at an interval of 1 h (a total dose of MPTP = 40 mg/kg). Group III and IV animals received valdecoxib *per se* (10 mg/kg, p.o.) and NS-398 *per se* (10 mg/kg, p.o.) respectively for 7 days. Group V, VI, VII and Group VIII animals received valdecoxib (5 mg/kg, p.o.), valdecoxib (10 mg/kg, p.o.), NS-398 (5 mg/kg, p.o.) and NS-398 (10 mg/kg, p.o.), respectively 1 h before the first MPTP injection followed by three more injections of MPTP as per Group II and each drug treatment continued for 7 days.

MPTP was administered only on day 1 in the entire groups except vehicle-treated group while valdecoxib or NS-398 was administered 60 min before the first MPTP injection on day 1 and drug treatments continued daily for total seven days as per the scheme mentioned below:



2.4. Behavioral parameters

2.4.1. Total locomotor activity

Total locomotor activity (ambulatory and rearing) was monitored using activity meter (IMCORP, India). Animals were individually placed in activity meter and total activity count was registered for 5 min after first 2 min of acclimatization. The locomotor activity was expressed in terms of total photo beam counts/5 min per animal (Gupta et al, 2010b).

2.4.2. Assessment of catatonia

The severity of catatonia was measured by employing the bar test as per the procedure previously validated in our laboratory (Gupta et al 2009, 2010b). In brief, front paws of the mice were gently placed on a horizontal metal bar with 5–6 mm diameter and placed 6 cm above ground level and the length of time, the mice maintained in this abnormal posture with at least one paw was recorded. The test was terminated when the animal withdrew its paw and attained the normal posture or 180 s had passed. The total time till which animals stayed on the bar was recorded. If the animal did not hold on to the bar after three attempts, it received zero score (Gupta et al., 2010b).

2.5. Biochemical estimations

2.5.1. Post-mitochondrial supernatant preparation (PMS)

On the 8th day, following the behavioral assessment, the animals were randomized into two groups: one group was used for biochemical analyses and the other group was used for the estimation of mitochondrial dysfunction. For biochemical analyses, animals were sacrificed by decapitation. The brains were quickly removed, put on ice and the striatum was separated and weighed. A 10% (w/v) tissue homogenate was prepared in chilled 0.1 M phosphate buffer (pH 7.4) using a Potter Elvenhjem homogenizer. The homogenate was centrifuged at 12,000 *g* for 20 min, 4 °C to obtain the post mitochondrial supernatant (PMS), which was used for further enzymatic analysis.

2.5.2. Estimation of lipid peroxidation levels

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) (Ohkawa et al., 1979). In brief, the reaction mixture consisted of 0.2 ml of 8.1% w/v sodium lauryl sulfate, 1.5 ml of 20% v/v acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% w/v aqueous solution of thiobarbituric acid was added to 0.2 ml of 10% (w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95 °C for one hour. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) were added, shaken well and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm. TBARS were quantified using extinction coefficient of $1.56\times10^5\,M^{-1}\,cm^{-1}$ and expressed as nmoles of MDA/mg protein.