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Protective effect of cyclooxygenase (COX)-inhibitors against drug-induced catatonia and MPTP-induced striatal lesions in rats

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ABSTRACT

The present study explored the involvement of cyclooxygenase (COX) in the pathophysiology of Parkinson's disease (PD). Further, the protective effect of COX-inhibitors against perphenazine-induced catatonia and 1-methyl-4-phenyl-1, 2, 3, 6-tertahydropyridine (MPTP)-induced striatal lesions in rats was evaluated. Administration of perphenazine (5 mg/kg., i.p.) produced severe catatonia (rigid behavior) in rats; the maximum score reached at 4 h (estimated as 100% AUC) and declined within 24 h. An intrastriatal injection of MPTP produced hypolocomotor activity in rats. Both perphenazine and MPTP produced oxidative stress as demonstrated by increased levels of lipid peroxides, nitrite and decreased antioxidant defense system in the whole brain and striatal region, in particular. Pretreatment with various COX-inhibitors *viz.* rofecoxib, celecoxib, nimesulide or naproxen offered protection against perphenazine-induced catatonia, the effect was more pronounced with rofecoxib. Rofecoxib and celecoxib (both selective COX-2 inhibitors) also reversed the perphenazine-induced oxidative stress. Further, prior treatment with rofecoxib (8 mg/kg, p.o.) reversed both the behavioral and biochemical changes induced by MPTP. These results suggest that COX-inhibitors particularly, rofecoxib offers protection against drug-induced catatonia and MPTP-induced striatal lesions possibly by modulating dopaminergic neurotransmission and/or oxidative stress.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the brain and typified by four cardinal features *viz.* bradykinesia (slowness of movement), resting tremor, increased muscular rigidity and impaired postural balance (Montastruc et al., 1996). The most common therapeutic strategy in PD includes combination of levodopa (L-DOPA; dopamine precursor) and carbidopa (a peripheral DOPA-decarboxylase inhibitor). However, the chronic use of this therapy is limited due to the development of "on–off" phenomenon which results in decreased efficacy (Sweet et al., 1975; Fahn, 2005). Other promising drug therapies include treatment with anticholinergics, dopamine receptor (D2/D3) agonists, monoamine oxidase (MAO) inhibitors and catechol-o-methyl transferase (COMT) inhibitors. However, despite the availability of large number of drugs, the relapse rate in PD patients is extremely high which has led the researchers to focus on the search of some alternative therapeutic approaches by which the progressive loss of dopaminergic neurons can be halted.

Perphenazine, a phenothiazine is known to block dopamine D_2 receptors and produces motor disturbance in the form of catatonia (rigidity) (Albin et al., 1989). This exemplifies a very simple and preliminary model for the evaluation of antiparkinson activity of drugs (Khanna and Madan, 1975; Kulkarni et al., 1980; Singh and Kulkarni, 2002; Arzi and Rezaei, 2003; Singh et al., 2003). Although, perphenazine-induced catatonia does not resemble the actual pathophysiological basis of PD; however, it is known to produce symptoms that mimics the disease phenotype, for example, rigidity measured in the form of catatonia (Kulkarni et al., 1980). This model reflects some of the early symptoms of Parkinson disease and can be employed for the preliminary screening of new molecules proposed to be useful for the treatment of the disease (Singh and Kulkarni, 2002; Singh et al., 2003). It is important to mention here that various dopamine D_2 receptor agonists possess antiparkinson properties (Neusch et al., 2000) which justify the importance of this model in antiparkinson drug discovery.

It is hypothesized that neuroinflammation plays an active role in the progression of PD (Hernan et al., 2006; Bartels and Leenders, 2007). Studies have revealed an increase in the expression of various inflammatory molecules within the neurons of PD patients (Teismann et al., 2003; Kim and Joh, 2006). Cyclooxygenase (COX) is a rate-limiting enzyme involved in the production of various prostaglandins and thromboxanes and is known to exist in two isoforms, COX-1 and

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COX-2. COX isoenzymes which are generally expressed only in the peripheral organs (kidneys, stomach, uterus etc.) have been recently found to be up-regulated in the brain following any neuronal insult. Out of these two isoforms, evidences have revealed the involvement of COX-2 isoform in neuropathological conditions (Teismann et al., 2003; Minghetti, 2004). Studies from our laboratory have demonstrated the protective action of COX-inhibitors in various neurological disorders including epilepsy, drug addiction, depression and stress related pathologies (Naidu and Kulkarni, 2002; Dhir et al., 2007; Akula et al., 2008).

Ongoing research using various animal models has demonstrated the neuroprotective potential of COX-2 inhibitors in this disease (Aubin et al., 1998; Reksidler et al., 2007; Sanchez-Pernaute et al., 2004). In one of the experimental studies, parecoxib, a selective COX-2 inhibitor exhibited neuroprotection against 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson-like symptoms in rats (Reksidler et al., 2007). Similarly, aspirin, a non-selective COX-inhibitor has demonstrated neuroprotective effect against MPTP-induced dopamine depletion in mice (Aubin et al., 1998). The mechanism of antiparkinson-like effect of these COX-inhibitors is not clear. Some of the proposed hypothesis includes: i) inhibition of nitric oxide free radicals formation, ii) agonistic action for peroxisome proliferator-activated receptor gamma, and/or iii) possible suppressive effects against dopamine quinone formation (Asanuma et al., 2003). Contrary to this, one of the clinical studies has demonstrated the ineffectiveness of COX-inhibitors in the treatment of PD (Bornebroek et al., 2007). Although the experimental and epidemiological studies suggest the beneficial role of COX-inhibitors in the treatment of PD (Aubin et al., 1998; Esposito et al., 2007; Etminan et al., 2008), still the exact mechanism of their protective action has not been properly explored.

With this background, the present study was designed to evaluate the effect of various selective and non-selective COX-inhibitors *viz.* rofecoxib, celecoxib (both selective COX-2 inhibitors), nimesulide (preferential COX-2 inhibitor) and naproxen (non-selective COX-inhibitor) against perphenazine-induced catatonia in rats. Further, the protective effect of rofecoxib, a selective COX-2-inhibitor against MPTP-induced striatal lesions was studied in rats. The possible role of oxidative stress and its reversal by COX-2 inhibitors in its neuroprotective action was also explored.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 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. 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. Drug treatment and schedule

Perphenazine (PPZ) (5 mg/kg, i.p.) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (32 μmol in 2 μl) (Sigma, St. Louis, MO, USA), rofecoxib (ROF) (2–8 mg/kg, p.o.), celecoxib (CEL) (10–40 mg/kg, p.o.), nimesulide (NIM) (2.5–10 mg/kg, p.o.), naproxen (NPX) (7–20 mg/kg, p.o.) (Panacea Biotec Ltd., Lalru, India) were used in the present study. All the drugs except perphenazine or MPTP were suspended in 0.25% w/v carboxymethyl cellulose (CMC). Perphenazine was dissolved with the aid of diluted hydrochloric acid, pH adjusted to neutral and volume was made up with distilled water. MPTP was dissolved in normal saline.

All the drugs except MPTP were administered in a constant volume of 0.5 ml/100 g body weight of rat. MPTP was administered by intrastriatal injection (32 μmol in 2 μl). Perphenazine was administered by intraperitoneal injection. The present study was carried out in two stages:

Study 1 — Perphenazine-induced catatonia: COX-inhibitors were administered per orally 60 min before challenging with perphenazine. All the doses were selected based on the previous studies reported from our laboratory (Naidu and Kulkarni, 2002; Dhir et al., 2007; Akula et al., 2008).

Study 2 — MPTP-induced striatal lesions: Rofecoxib treatment was started 7 days before the administration of MPTP. On the 7th day, 60 min after the rofecoxib treatment, MPTP was administered by intrastriatal injection (32 μmol in 2 μl) and rofecoxib treatment was continued further for 5 days.

2.3. Sterotaxic surgery

Animals were anesthetized with thiopental sodium (45 mg/kg, i.p.), MPTP was infused as a single intrastriatal (coordinates: anterior +1.7 mm; lateral ±2.7 mm; ventral—4.8 mm from Bregma and Dura) injection (32 μmol in 2 μl) using the Hamilton microsyringe (Paxinos et al., 1985).

2.4. Experimental protocols and procedures

2.4.1. Study 1 — perphenazine-induced catatonia

Two tests were employed in the present study to assess the severity of catatonia following the perphenazine administration (5 mg/kg, i.p.) and the assessment of catatonic response was done at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 12 and 24 h in both the tests as per the protocols described below. Six to eight animals were included in each group.

2.4.1.1. Bar test. This test was conducted as per the procedure previously validated in our laboratory (Singh and Kulkarni, 2002; Singh et al., 2003). In brief, front paws of the rat were gently placed on a horizontal metal bar with 5–6 mm diameter and placed 10 cm above ground level and the length of time, the rats 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 (Singh et al., 2003). Area under the curve (AUC) (0 to 24 h) was calculated graphically using trapezoidal rule.

2.4.1.2. Block test. In block test, the development and severity of the four stages of catatonia were observed and scored as follows: Stage 1, rat moves when placed on the table, score = 0; Stage 2, rat moves only when touched or pushed, score = 0.5; Stage 3, rat placed on the table with front paws set alternately on a 3 cm high block fails to correct the posture in 10 s, score = 0.5 for each paw with a total of 1 for this stage; State 4, rat fails to move when the front paws are placed alternately on a 9 cm high block, score = 1 for each paw with a total score 2 for this stage. Thus, the maximum possible score would be 3.5 reflecting full catatonia. Lesser score would mean an apparently lesser degree of catatonia (Kulkarni et al., 1980). Area under the curve (AUC) (0 to 24 h) was calculated graphically using trapezoidal rule.

2.4.2. Study 2 — MPTP-induced striatal lesions

Seven groups were employed in the present study, each comprising of 6–8 animals. Group I comprised of control group and