Neuropharmacology 107 (2016) 432–450

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Ibuprofen or piroxicam protects nigral neurons and delays the development of L-dopa induced dyskinesia in rats with experimental Parkinsonism: Influence on angiogenesis☆

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ARTICLE INFO

Article history:
Received 10 September 2015
Received in revised form
4 March 2016
Accepted 22 March 2016
Available online 23 March 2016

Keywords:
Angiogenesis
Dyskinesia
Ibuprofen
L-dopa
Parkinsonism
Piroxicam
Rat

ABSTRACT

Neuroinflammation and angiogenesis have been involved in the pathogenesis of Parkinson's disease (PD). This study investigated the effect of ibuprofen or piroxicam on the motor response to L-dopa and development of dyskinesia in Parkinsonian rats focusing on the anti-angiogenic role of the two non-steroidal anti-inflammatory drugs (NSAIDs). Rats were divided into nine groups as follows: Group I: the vehicle group, Group II: rotenone group, rats were injected with nine doses of rotenone (1 mg/kg/48 h), group III&IV: rats received rotenone + ibuprofen (10 or 30 mg/kg), Group V-VI: rats received rotenone + piroxicam (1 or 3 mg/kg), Group VII: rats received rotenone + L-dopa/carbidopa (100/10 mg/kg), Group VIII-IX: rats received rotenone + L-dopa/carbidopa + ibuprofen (30 mg/kg) or piroxicam (3 mg/kg). In general, drugs were administered daily for ten weeks. Rotenone-treated rats showed motor dysfunction, lower striatal dopamine, lower staining for nigral tyrosine hydroxylase but higher level of striatal cyclooxygenase-2 (COX-2) and vascular endothelial growth factor (VEGF) compared to vehicle-treated rats ($P < 0.05$). Treatment with L-dopa showed wearing-off over the course of the experiment in addition to development of abnormal involuntary movements and upregulated striatal VEGF level. Treatment with ibuprofen or piroxicam in combination with L-dopa preserved the effect of L-dopa at the end of week 10, delayed the development of dyskinesia and decreased striatal COX-2 and VEGF levels. In conclusion, the current study suggests that ibuprofen and piroxicam are promising candidates for neuroprotection in PD and may have utility in conjunction with L-dopa in order to ensure the longevity of its action and to delay the development of dyskinesia.

1. Introduction

Parkinson's disease is one of the neurodegenerative diseases characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Samii et al., 2004). The symptoms of motor dysfunction in PD include tremors at rest, muscular rigidity, bradykinesia and postural instability (Sian et al., 1999). Some epidemiological studies reported that the exposure to pesticides, metals or solvents increases the risk of development of PD (Di Monte and Lawler, 2001). Rotenone is a pesticide that inhibits complex I of the electron-transport chain and reduces oxidative phosphorylation (Schuler and Casida, 2001). Systemic administration of rotenone has been used as a chronic progressive animal model of PD (Betarbet et al., 2000).

Data from clinical (Mogi et al., 2007; Sawada et al., 2006) and animal studies (Block et al., 2006) provided evidence that neuroinflammation has been involved in the pathogenesis of PD and contributes to the progression of the degenerative process (Hunot and Hirsch, 2003). The substantia nigra is rich in microglia; over-activation of nigral microglia triggers the onset of a cascade of events that leads to the release of toxic factors and a progressive dopaminergic neurodegeneration (Ojha et al., 2015). Activation of the microglia cells induces the synthesis of inducible nitric oxide and COX-2 and activates NADPH oxidase leading to release of free radicals (Liu et al., 2003). Further, the activated microglia release

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http://dx.doi.org/10.1016/j.neuropharm.2016.03.034
0028-3908/

cytokines and pro-angiogenic factors (Naldini and Carraro, 2005). There is evidence that VEGF was upregulated in the substantia nigra of PD patients (Wada et al., 2006; Yasuda et al., 2007). One previous study highlighted greater number of stained nuclei of endothelial cells in the substantia nigra of PD patients with an increase in vessel density (Faucheux et al., 1999). Moreover, an increase in the number of VEGF-expressing neurons and an increase in number of blood vessels were found in the substantia nigra of Parkinsonian non-human primates (Barcia et al., 2005). These data suggest that angiogenic changes may accompany the pathophysiology of PD. Indeed, angiogenesis would be associated with dysfunction of blood brain barrier (BBB) (Barcia et al., 2004). In the brain, immature vessels lack the full characteristics of the BBB. Thus, angiogenesis may contribute to ongoing neuroinflammation by permitting peripheral molecules and immune cells pass to brain parenchyma (Carvey et al., 2005).

L-dopa is the cornerstone of the treatment of PD, however, chronic L-dopa therapy has many clinical hazards as dyskinesias, drug-induced involuntary movements (AIMs) as well as motor fluctuations (Buck and Ferger, 2010; Pham and Nogid, 2008), which limit its usefulness. Previous studies demonstrated the reasons for such long-term problems and suggested that L-dopa generates oxidative damage which perpetuates the cell death (Milusheva et al., 2010). Another study suggested that motor complications accompanied with sustained L-dopa therapy are linked to irregular and discontinuous delivery of L-dopa to the brain, resulting in non-physiologic pulsatile stimulation of striatal L-dopa receptors (Poewe et al., 2010). Medical therapy that prolongs the action of L-dopa without motor complications would cause major advance in PD therapy (Olanow et al., 2006).

There is evidence that angiogenesis and BBB impairment takes place in an experimental model of L-dopa-induced dyskinesia (Westin et al., 2006). Substantial preclinical evidence showed that NSAIDs and selective COX-2 inhibitors have anti-angiogenic properties (Gallo et al., 2001). This information opens the prospect of using NSAIDs for treating of angiogenesis-dependent diseases. In addition, there is evidence that NSAIDs provide protective effects in dopaminergic neurons in animal models of PD (Maharaj et al., 2006).

Based on the neuroinflammatory hypothesis that describe the pathology of PD, the current study aimed to examine the effect of treatment with two NSAIDs, ibuprofen and piroxicam, on rotenone-induced parkinsonism in rats focusing on their anti-angiogenic capability. In addition, the current study monitored the decline in the therapeutic effect of L-dopa and the development of L-dopa-induced AIMs. Furthermore, this study tested the importance of adding ibuprofen or piroxicam to L-dopa therapy and whether they may reduce and/or delay the development of L-dopa-induced dyskinesia in Parkinsonian rats.

2. Materials and methods

2.1. Drugs and chemicals

Rotenone (Sigma-Aldrich, MO, USA) was dissolved in 1:1 (v/v) dimethylsulfoxide (DMSO, Sigma-Aldrich, MO, USA) and polyethylene glycol-400 (PEG-400; Sigma-Aldrich, MO, USA). Ibuprofen, piroxicam and L-dopa/carbidopa were dissolved in distilled water. Enzyme linked immunosorbent assay (ELISA) kits for dopamine and VEGF were purchased from Sun Red Bio. (Shanghai, China). ELISA kit for COX-2 was purchased from Glory Science Co. (TX, USA). Polyclonal rabbit tyrosine hydroxylase (TH) antibodies were purchased from Biorbyt (Cambridge, UK). Vectastain Elite ABC Kit and peroxidase substrate kit (3,3′-diaminobenzidine) were purchased from Vector Lab (Burlingame, USA).

2.2. Animals

Female albino rats with body weight range 100–130 g were used in the present study. Rats were placed in stainless steel cages under hygienic controlled laboratory conditions and normal light/dark cycle. Water and food were given *ad libitum*. All the experimental protocols were approved by the institutional research ethics committee at the Faculty of Pharmacy, Suez Canal University and carried out in accordance with guide for the care and use of laboratory animals published by the National Institutes of Health (NIH Publications No. 8023, revised 1978). Further, all animal studies complied with the ARRIVE guidelines.

2.3. Induction of experimental Parkinsonism using rotenone

Rotenone was employed to induce experimental Parkinsonism in rats and was administered following a previously reported schedule (Zaitone et al., 2012a) with some modifications. The previously reported schedule consisted of 6 injections of rotenone (1.5 mg/kg/48 h, s.c.) for induction of Parkinsonism in rats. In the current experiment, the same cumulative dose of rotenone (9 mg/kg) was divided over 9 injections rather than 6 injections; this schedule reduced the mortality percent to zero at the end of the rotenone model. The validation of the experimental rat model was important, since alternatives to the widely used 6-hydroxy-dopamine (6-OHDA) model are needed in experimental studies. The idea to move towards a bilateral model of Parkinsonism is important since so far most studies have been performed in hemiparkinsonian rodents.

2.4. Experimental design

This study consisted of 2 experiments, the first one (experiment I) aimed to study the protective effect of the two NSAIDs on the integrity of nigral neurons and preserving the action of L-dopa and the second one (experiment II) aimed at determining the role of the two NSAIDs in delaying the development of L-dopa-induced dyskinesia.

Ninety female rats were randomly divided into nine groups, ten rats each, and assigned as follows:

Group I (vehicle group): rats received nine injections of the vehicle (DMSO/PEG-400) in a volume equals 1 ml/kg/48 h, in a schedule similar to that adapted for rotenone injections.

Group II (rotenone group): rats were injected with nine doses of rotenone (1 mg/kg/48 h, s.c.) in a volume equals 1 ml/kg/48 h. Further, rats were given distilled water (5 ml/kg/day, p.o.) in a schedule similar to that designed for the drug therapies.

Group III and IV: rats were injected with rotenone (1 mg/kg/48 h, s.c.) and were given ibuprofen (10 or 30 mg/kg/day, p.o.), respectively, in a volume equals 5 ml/kg.

Group V and VI: rats were injected with rotenone (1 mg/kg/48 h, s.c.) and were given piroxicam (1 or 3 mg/kg/day, p.o.), respectively, in a volume equals 5 ml/kg.

Group VII: rats were injected with rotenone (1 mg/kg/48 h, s.c.) and treated with L-dopa/carbidopa (100/10 mg/kg/day, p.o) (Padovan-Neto et al., 2009; Zaitone et al., 2013) in a volume equals 5 ml/kg.

Group VIII and IX: rats were injected with rotenone (1 mg/kg/48 h, s.c.) and treated with a combination of L-dopa/carbidopa (100/10 mg/kg/day, p.o) plus ibuprofen (30 mg/kg/day, p.o.) or piroxicam (3 mg/kg/day, p.o.), respectively.

The current dose of L-dopa/carbidopa is the greatest dose that reported to induce dyskinesia when administered orally in rats (Padovan-Neto et al., 2009); the development of dyskinesia upon use of this dose was confirmed in a preliminary study before conducting the experiments. In general, ibuprofen or piroxicam was