Neuroscience 231 (2013) 420–431

ANTI-INFLAMMATORY PROPERTIES RATHER THAN ANTI-OXIDANT CAPABILITY IS THE MAJOR MECHANISM OF NEUROPROTECTION BY SODIUM SALICYLATE IN A CHRONIC ROTENONE MODEL OF PARKINSON'S DISEASE

P. THAKUR AND B. NEHRU *

Department of Biophysics, Panjab University, Chandigarh 160014, India

Abstract—Parkinson's disease (PD) is the second most common neurodegenerative disorder manifesting in motor, cognitive and behavioral anomalies. Loss of dopaminergic neurons in the substantia nigra region of the brain is the hallmark feature of PD, which is attributed to oxidative and inflammatory stress besides other diverse factors and hence drugs targeting these pathways hold promise as neuro-therapeutics.

The anti-oxidative as well as anti-inflammatory properties of sodium salicylate (SS), suggest its neuroprotective potentials in PD. Since PD is a progressive neurodegenerative disorder, the mechanistic basis for utilizing SS as a neuroprotectant in PD could be better understood in the chronic models. The present study utilizes a rotenone-based model of PD to evaluate the neuro-modulatory efficacy of SS. Subcutaneous injection of rotenone (2 mg/kg body weight) was given to male SD rats every day, for a period of 5 weeks, which developed all the essential features of PD in these animals. Simultaneously, another group was injected SS intraperitoneally at the dose of 100 mg/kg body weight, in addition to the rotenone. In the animals receiving rotenone + SS, significant improvement was observed in the various characteristic hallmarks of PD such as dopamine and tyrosine hydroxylase levels as well as the motor dysfunction symptoms. It attenuated the reactive oxygen species levels significantly but failed to reduce the levels of protein carbonylation and lipid peroxidation. However, SS effectively abridged the levels of inflammatory mediators like cyclooxygenase-2 (COX-2), nuclear factor kappa B and inducible nitric oxide synthase. Correspondingly, a significant decrease in the levels of pro-inflammatory cytokines interleukin-6, interleukin-1β and tumor necrosis factor-α was also observed following SS co-treatment. Thus, neuroprotective efficacy of SS in this chronic model of PD can be largely attributed to its anti-inflammatory effects rather than its free radical-scavenging properties.

Key words: Parkinson's disease, neuroinflammation, rotenone, sodium salicylate, oxidative stress, apoptosis.

INTRODUCTION

Parkinson's disease (PD) is a chronically progressing neurodegenerative disease that primarily affects the dopaminergic neurons of the substantia nigra in the midbrain region (Moore et al., 2005). Several diverse factors such as oxidative stress, mitochondrial defects, ATP depletion, apoptosis, excitotoxicity, and neuroinflammation have been implicated in the progression of the disease (Yacoubian and Standaert, 2009). Studies have suggested that the process of neurodegeneration in the substantia nigra may not be purely neuronal, but could also involve the glial cells, the innate immune component of the brain (Hald and Lotharius, 2005). The substantia nigra is extremely susceptible to cytotoxicity by activated microglia since it has lower amounts of natural anti-oxidant defenses and higher levels of free radicals due to the presence of iron and dopamine metabolism. Further, it is the region of the brain where the relative proportion of glial cells vis-a-vis the neuronal cells is more (Kim et al., 2000).

Activation of glial cells contributes to the disease pathology via production of cytokines, prostaglandins, reactive oxygen species (ROS), complement activation, etc. (Chen and Tansey, 2011). Stimulation of microglial-inducible nitric oxide synthase (iNOS), NADPH oxidase and myeloperoxidase (Witte et al., 2010) augments ROS production. Free radicals such as peroxynitrite and hydroxyl radicals are important mediators in the inflammation-mediated apoptosis. Another factor contributing to oxidative stress is the drastic increase in COX-2 levels observed in the PD-affected brain (Hald and Lotharius, 2005). COX enzyme mediates the potentiation of cytotoxic effects via production of pro-inflammatory prostaglandins as well as through the ROS generated in conversion of prostaglandin-G to

*Corresponding author. Tel: +91-0172-2534128.
E-mail addresses: poonamthkur@gmail.com (P. Thakur), bnehru@pu.ac.in (B. Nehru).

Abbreviations: ANOVA, analysis of variance; COX-2, cyclooxygenase-2; DCFH-DA, 2′,7′-dichlorfluorescein-diacetate; DNPH, 2,4-Dinitrophenylhydrazine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediamine tetraacetic acid; 4-HNE, 4-hydroxynonenal; HVA, homovanillic acid; H_2O_2, hydrogen peroxide; IL, interleukin; iNOS, inducible nitric oxide synthase; LPO, lipid peroxidation; LPS, lipopolysaccharides; MAO, monoamine oxidase; MDA, malonaldehyde; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NADPH, nicotinamide adenine dinucleotide phosphate; NBT, nitro blue tetrazolium; NF-κB, nuclear factor kappa B; NO, nitric oxide; NSAIDs, non-steroidal anti-inflammatory drugs; PD, Parkinson's disease; ROS, reactive oxygen species; SOD, superoxide dismutase; SS, sodium salicylate; TCA, tricarboxylic acid; TH, tyrosine hydroxylase; TNF, tumor necrosis factor.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.11.006

prostaglandin-H (Rogers and Kovelowski, 2003). These heightened ROS levels result in the oxidation of various cellular biomolecules (Malkus et al., 2009). This is confirmed by the increased levels of protein carbonylation and lipid peroxidation (LPO) markers like malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), etc. in the PD brain (Anderson, 2004). Thus, there seems to be an interplay between neuroinflammation and oxidative stress, which could perpetuate the phenomenon of neurodegeneration through activation of apoptosis (Lee et al., 2009).

Due to the involvement of neuroinflammation, there has been huge interest in the possible neuroprotective role of non-steroidal anti-inflammatory drugs (NSAIDs) in the management of PD. Many epidemiological studies indicate that prolonged use of NSAIDs is associated with a lower risk of PD (Moore et al., 2010). Both, COX-dependent as well as COX-independent mechanisms have been implicated in the neuroprotective effects exerted by NSAIDs. Acetyl salicylic acid (ASA) popularly known as aspirin, is the most widely used analgesic worldwide. Its major metabolite is salicylic acid that lacks the acetyl group of aspirin (Amann and Peskar, 2002). There is no clear consensus about its effect on COX activity. It does not seem to inhibit COX-1 and COX-2 in *in vitro* systems (Mitchell et al., 1994) but in the *in vivo* system it shows a weak inhibition of the COX enzyme (Patrignani et al., 1997; Giuliano and Warner, 1999). It has been postulated that its COX inhibitory activity is influenced by the amount of arachidonic acid present in the cells (Giuliano et al., 2001). Independent of its COX activity, sodium salicylate (SS) still possesses anti-inflammatory properties, which are thought to act via its inhibitory effects on intracellular signaling molecules like nuclear factor kappa B (NF-κB) (Bayon et al., 1999), mitogen-activated protein kinase (MAPK) (Esposito et al., 2007) or activator protein-1 (AP-1) (Tegeder et al., 2001).

Of late SS has also been proposed as a neuroprotective agent especially with regard to PD wherein its prophylactic actions have been attributed to its free radical-scavenger properties (Mohanakumar et al., 2000; Esposito et al., 2007). These studies are based on acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models (Mohanakumar et al., 2000; Sairam et al., 2003) of PD and involve short-term administration of SS. However, in humans, PD is a chronically progressing disease and any neuroprotective agent will need to be administrated for a long term. In this context, a PD model based on the chronic administration of rotenone has been utilized. The underlying neuroinflammatory mechanisms, which could be the major pathways by which SS exerts its neuroprotective effects, have been explored in addition to the oxidative stress.

RESULTS

Body weight changes

Animals in the control group showed a significant ($p \leqslant 0.001$) weight gain over the period of 5 weeks. Rotenone-treated animals showed a 14% decrease ($p \leqslant 0.001$) in the body weight at the end of the study. However, with co-treatment of SS the animals showed only 6% decline in the body weight. Animals in the SS alone treatment group showed a weight gain pattern similar to that of control (Fig. 1).

Behavioral changes

Catalepsy bar test. Catalepsy is defined as the inability of animals to correct an externally imposed posture such as placement of forelimbs on a bar in half-rearing position (Sanberg et al., 1988) and is an important behavioral indicator of motor dysfunction occurring due to neurochemical changes in the basal ganglia and mid brain region. There was a significant increase in catalepsy after the rotenone treatment, which increased around eightfold over time ($p \leqslant 0.001$). During the 1st week, there was no significant difference between control animals and rotenone-treated animals. However, after 2 weeks of rotenone treatment, the increase in catalepsy was significantly higher as compared to control ($p \leqslant 0.001$). Co-treatment with SS caused a significant reduction ($p \leqslant 0.001$) in the cataleptic behavior as compared to rotenone-treated animals at all the time points. A multivariate analysis of variance (ANOVA) with groups as between-subject factor was also found to be significant ($F = 18.955$, $p \leqslant 0.001$) for all the time points (Fig. 2A).

General movement analysis. Treatment with rotenone alone led to the onset of waning in movement within 1 week, which continued to increase throughout the study (Fig. 2B). However, co-treatment with SS prevented the deterioration in general movement until 3 weeks and deterioration was less pronounced than in the rotenone-treated group in subsequent weeks. The impairment in general movement quantitated with the Ludolph scale was found to be significant over time with rotenone treatment ($p \leqslant 0.001$) while the changes in the SS co-treatment group were non-significant ($p \leqslant 0.178$). The score for control and SS alone groups was zero for all the time points.

Actophotometer test. Actophotometer measures the locomotor activity and exploratory behavior of animals. Counts were represented as a sum of rearing and ambulation movements. A multivariate ANOVA with groups as between-subject factor was found to be significant ($F = 44.570$, $p \leqslant 0.001$). There was a significant progressive decline in the total locomotor activity of the rotenone-treated animals over the period of 5 weeks ($p \leqslant 0.001$). There was no change in the total locomotion of the control group over time ($p \leqslant 0.120$). However, the SS co-treatment groups ($p \leqslant 0.001$) also registered a significant decline in the locomotor activity over time. The decline in total locomotor activity of the rotenone-treated group was significant when compared to control ($p \leqslant 0.01$) at all the time points. SS prevented the decline in locomotor activity as compared to the rotenone group at the 1st week ($p \leqslant 0.01$). However, there was no significant