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ORIGINAL PAPER

Comparative pharmacological study of free radical scavenger, nitric oxide synthase inhibitor, nitric oxide synthase activator and cyclooxygenase inhibitor against MPTP neurotoxicity in mice

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Abstract The biochemical and cellular changes that occur following the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are remarkably similar to that seen in idiopathic Parkinson's disease(PD). There is growing evidence indicating that reactive oxygen species (ROS), reactive nitrogen species (RNS) and inflammation are a major contributor to the pathogenesis and progression of PD. Hence, we investigated whether 7-nitroindazole [neuronal nitric oxide synthase (nNOS) inhibitor], edaravone (free radical scavenger), minocycline [inducible NOS (iNOS) inhibitor], fluvastatin [endothelial NOS (eNOS) activator], pitavastatin (eNOS activator), etodolac [cyclooxygenase-2 (COX-2) inhibitor] and indomethacin (COX inhibitor) can protect against MPTP neurotoxicity in mice under the same conditions. For the evaluation of each drug, the levels of dopamine, DOPAC and HVA were quantified using HPLC with an electrochemical detector. Four administrations of MPTP at 1-h intervals to mice produced marked depletion of dopamine, DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanilic acid) in the striatum after 5days. 7-Nitroindazole prevented dose-dependently a significant reduction in dopamine contents of the striatum 5days after MPTP treatment. In contrast, edaravone, minocycline, fluvastatin, pitavastatin, etodolac and indomethacin did not show the neuroprotective effect on MPTP-induced striatal dopamine, DOPAC and HVA depletions after 5days. The present study demonstrates that the overexpression of nNOS may play a major role in the

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neurotoxic processes of MPTP, as compared with the production of ROS, the overexpression of iNOS, the modulation of eNOS and the involvement of inflammatory response. Thus our pharmacological findings provide further information for progressive neurodegeneration of the nigrostriatal dopaminergic neuronal pathway.

Keywords Parkinson's disease · MPTP · Reactive oxygen species · Reactive nitrogen species · Inflammation · Dopaminergic system · Mice

Introduction

Parkinson's disease (PD) is a progressive and age-related neurodegenerative disease characterized by degeneration of dopaminergic neurons originating in the substantia nigra pars compacta and projecting to the dorsal striatum (Hirsh et al. 1988). It has been proposed that Parkinsonian clinical signs appear at the point when dopaminergic neuronal loss exceeds a critical threshold: 70–80% of striatal nerve terminals and 50–60% of the substantia nigra pars compacta perikaryons (Bernheimer et al. 1973; Agid 1991). Treatment with levodopa, supplying the precursor of dopamine, alleviates major symptom of PD. However, long-term treatment with levodopa is often complicated by the development of adverse effects. There have been additional anti-parkinsonian drugs, such as dopamine agonists, but the available therapies do not protect against dopaminergic neurodegeneration. The patients begin not to respond well to treatment, and start to suffer disabilities that can not be controlled with existing medical therapies. The prevalence of PD is likely to increase in the coming decades as the number of elderly people will increase. Therefore, it is of utmost importance to develop new drugs that show or halt the rate of progression of PD.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that produces a Parkinsonian syndrome in both humans and experimental animals (Dauer and Przedborski 2003). Its neurotoxic effects also appear to involve energy depletion and free radical generation. MPTP is converted to its metabolite MPP^+ (1-methyl-4-phenylpyridium) by MAO-B (monoamine oxidase B). MPP^+ is selectively accumulated by high affinity dopamine transporters and taken up into the mitochondria of dopaminergic neurons, where it disrupts oxidative phosphorylation by inhibiting complex I of the mitochondrial electron transport chain (Tipton and Singer 1993). This leads to impairment of ATP production, elevated intracellular calcium levels, and free radical generation, thereby exhibiting dopaminergic neurotoxicity (Hasegawa et al. 1990; Sriram et al. 1997). However, there are many points systemically where MPTP can affect the dopaminergic system as shown in Fig. 1. Thus, the cause of PD remains unknown, and the mechanisms responsible for nigral dopaminergic neuronal loss is obscure. To examine the role of oxidative stress and inflammation against MPTP neurotoxicity, therefore, we investigated the effects of 7-nitroindazole (nNOS inhibitor), edaravone (free radical scavenger), minocycline (iNOS inhibitor), fluvastatin (eNOS activator), pitavastatin (eNOS activator), etodolac (COX-2 inhibitor) and indomethacin (COX inhibitor) against MPTP neurotoxicity in mice under the same conditions.

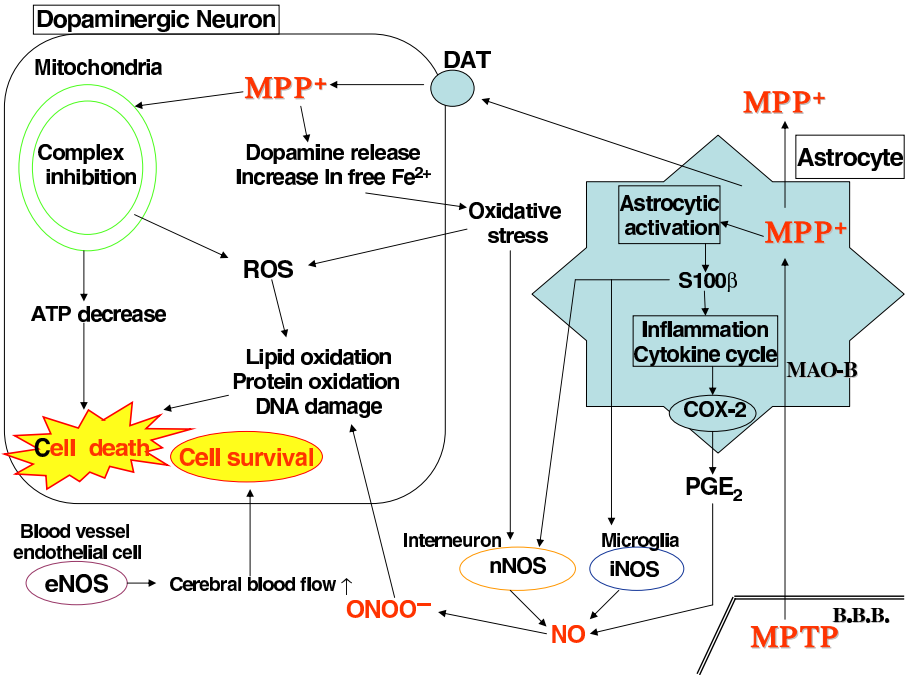

Fig. 1 Schematic representation of the mechanisms of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (*MPTP*) action in the nigrostriatal system. MPTP crosses easily the blood brain-barrier (*B.B.B.*) and is converted to its metabolites 1-methyl-4-phenylpyridium (MPP^+) by glial monoamine oxidase-B (*MAO-B*). MPP^+ is selectively taken in dopamine neurons throughout dopamine transporters (*DAT*) and accumulated in the mitochondria, where it inhibits complex I of the mitochondrial electron transport chain. The mitochondrial inhibition leads to reductions of oxygen consumption and ATP production. Oxidative stress and inflammation generated by MPP^+ and ATP decrease lead to neuronal cell damage. *NO* nitric oxide, *NOS* NO synthase, *nNOS* neuronal NOS, *iNOS* inducible NOS, *eNOS* endothelial NOS, *ROS* reactive oxygen species, $ONOO^-$ peroxynitrite, *COX-2* cyclooxygenase-2

Materials and methods

Experimental animals

Male C57BL/6 mice (Nihon SLC Co., Shizuoka, Japan), 8weeks of age, were used in this study. The animals were housed in a controlled environment (23 ± 1°C, 50 ± 5% humidity) and were allowed food and tap water ad libitum. The room lights were on between 8:00 and 20:00. The mice were injected intraperitoneally (i.p.) four times with MPTP (10mg/kg) at 1-h intervals, the total dose per mouse being 40mg/kg, as described previously (Araki et al. 2001; Muramatsu et al. 2003). Control animals received i.p. four injections of physiological saline. All experiments were performed in accordance with the Guidelines for Animal Experiments of the Tokushima University School of Medicine.

Effect of 7-nitroindazole

The animals were divided into five groups: (1) peanut oil (vehicle)-treated group; (2) 7-nitroindazole (50mg/kg)-treated group; (3) MPTP + peanut oil-treated group; (4)

MPTP + 7-nitroindazole (30mg/kg)-treated group; (5) MPTP + 7-nitroindazole (50mg/kg)-treated group. The mice were injected i.p. with 7-nitoindazole or peanut oil 30min before and 90min after the first administration of MPTP (groups 3–5). For groups 1 and 2, the peanut oil-treated and 7-nitroindazole-treated animals were injected in the same manner with saline instead of MPTP. 7-Nitroindazole (Sigma-Aldrich Co., St. Louis, USA) was suspended in peanut oil.

Effect of edaravone

The animals were divided into five groups: (1) saline (vehicle)-treated group; (2) edaravone (10mg/kg)-treated group; (3) MPTP + saline-treated group; (4) MPTP + edaravone (3mg/kg)-treated group; (5) MPTP + edaravone (10mg/kg)-treated group. The mice were injected i.p. with edaravone or saline 30min before and 90min after the first administration of MPTP (groups 3–5). For groups 1 and 2, the saline-treated and edaravone-treated animals were injected in the same manner with saline instead of MPTP. Edaravone was generously provided by Mitsubishi Wellpharma Co. Ltd. Tokyo, Japan, and was dissolved in saline.

Effect of minocycline

The animals were divided into five groups: (1) saline (vehicle)-treated group; (2) minocycline (50mg/kg)-treated group; (3) MPTP + saline-treated group; (4) MPTP + minocycline (30mg/kg)-treated group; (5) MPTP + minocycline (50mg/kg)-treated group. The mice were injected i.p. with minocycline or saline 30min before and 90min after the first administration of MPTP (groups 3–5). For groups 1 and 2, the saline-treated and minocycline-treated animals were injected in the same manner with saline instead of MPTP. In addition, minocycline hydrochloride (Sigma-Aldrich Co., St. Louis, USA) was dissolved in saline.

Effect of fluvastatin

The animals were divided into five groups: (1) distilled water (vehicle)-treated group; (2) fluvastatin (80mg/kg)-treated group; (3) MPTP + distilled water-treated group; (4) MPTP + fluvastatin (40mg/kg)-treated group; (5) MPTP + fluvastatin (80mg/kg)-treated group. The mice were injected orally with fluvastatin or distilled one a day for 5days before MPTP treatment. MPTP was administered in mice 1h after the last treatment with fluvastatin or distilled water (groups 3–5). For groups 1 and 2, the distilled water-treated and fluvastatin-treated animals were injected in the same manner with saline instead of MPTP. Fluvastatin sodium was generously provided by Tanabe Company Ltd. Osaka, Japan, and was dissolved in distilled water.

Effect of pitavastatin

The animals were divided into five groups: (1) 0.5% carboxymethylcelluose (0.5% CMC, vehicle)-treated group; (2) pitavastain (30mg/kg)-treated group; (3) MPTP + 0.5% CMC-treated group; (4) MPTP + pitavastatin (10mg/kg)-treated group; (5)

MPTP + pitavastatin (30mg/kg)-treated group. The mice were injected orally with pitavastatin or 0.5% CMC twice a day for 5days before MPTP treatment. MPTP was administered in mice 1h after the last treatment with pitavastatin or 0.5% CMC (groups 3–5). For groups 1 and 2, the vehicle-treated and pitavastatin-treated animals were injected in the same manner with saline instead of MPTP. Pitavastatin was generously provided by Kowa Company Ltd. Tokyo, Japan, and was suspended in 0.5% CMC.

Effect of etodolac

The animals were divided into five groups: (1) 0.5% CMC (vehicle)-treated group; (2) etodolac (10mg/kg)-treated group; (3) MPTP + 0.5% CMC-treated group; (4) MPTP + etodolac (3mg/kg)-treated group; (5) MPTP + etodolac (10mg/kg)-treated group. The mice were injected i.p. with etodolac or 0.5% CMC 30min before and 90min after the first administration of MPTP (groups 3–5). For groups 1 and 2, the saline-treated and etodolac -treated animals were injected in the same manner with saline instead of MPTP. Etodolac was generously provided by Nippon Shinyaku Company Ltd. Kyoto, Japan, and was suspended in 0.5% CMC.

Effect of indomethacin

The animals were divided into five groups: (1) saline (vehicle)-treated group; (2) indomethacin (0.5mg/kg)-treated group; (3) MPTP + saline-treated group; (4) MPTP + indomethacin (0.3mg/kg)-treated group; (5) MPTP + indomethacin (0.5mg/kg)-treated group. The mice were injected i.p. with indomethacin or saline 30min before and 90min after the first administration of MPTP (groups 3–5). For groups 1 and 2, the saline-treated and indomethacin-treated animals were injected in the same manner with saline instead of MPTP. In addition, indomethacin (Sigma-Aldrich Co., St. Louis, USA) was dissolved in saline.

Measurement of dopamine, DOPAC and HVA levels

The mice were killed by cervical dislocation 5days after MPTP treatment. After cervical dislocation, the striatum were rapidly dissected out and sonicated in ice-cold 0.2M perchloric acid containing 100ng/ml isoproterenol as an internal standard. Dopamine, DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanillic acid) were quantified by HPLC with an electrochemical detector (ECD; Eicom, Kyoto, Japan). Concentrations of dopamine and its metabolites are expressed as microgram per gram tissue weight, as described previously (Araki et al. 2001; Kurosaki et al. 2003).

Statistical analysis

For measurement of dopamine, DOPAC and HVA levels, all values were expressed as means ± SD. and statistical significance was evaluated by one-way ANOVA followed by Fisher's PSLD multiple comparison tests. Each group consisted of four to six mice.

Results

Effect of 7-nitroindazole

Four administrations of MPTP at 1-h intervals to mice produced marked depletion of dopamine, DOPAC and HVA in the striatum after 5days. 7-Nitroindazole dose-dependently prevented a significant decrease in dopamine contents of the striatum 5days after MPTP treatment. Furthermore, 7-nitroindazole at a dose of 30mg/kg prevented a significant decrease in DOPAC and HVA levels of the striatum after MPTP treatment. In addition, 7-nitroindazole alone showed no significant alterations in the dopamine, DOPAC and HVA in the striatum, as compared with vehicle-treated group (Fig. 2).

Effect of edaravone

Edaravone showed no significant changes in dopamine, DOPAC and HVA levels in the striatum 5days after MPTP treatment. Furthermore, edaravone only showed no significant alterations in the dopamine, DOPAC and HVA contents in the striatum, as compared with vehicle-treated group (Fig. 3).

Effect of minocycline

Minocycline showed no significant changes in dopamine, DOPAC and HVA levels in the striatum 5days after MPTP treatment. Also, this compound alone showed no

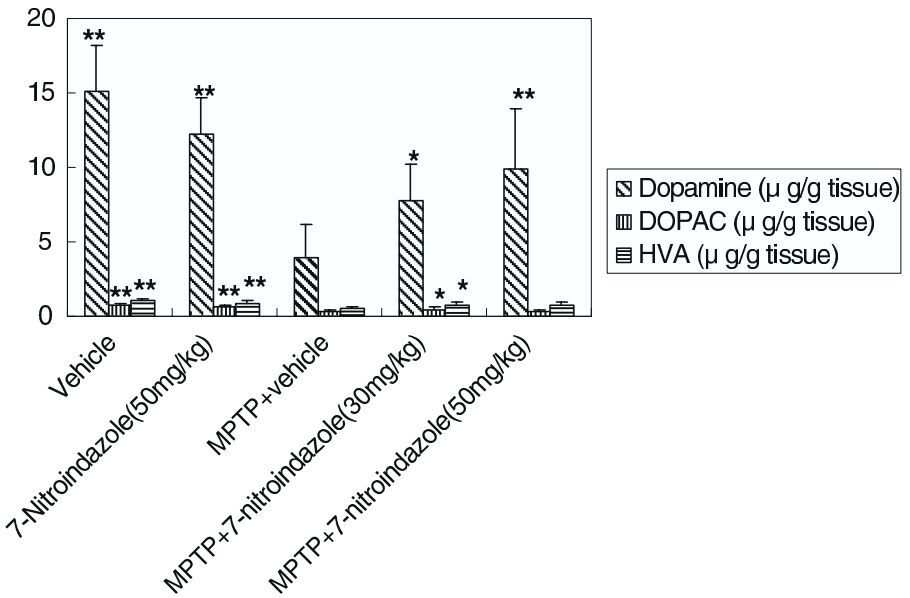

Fig. 2 Neuroprotective effects of 7-nitroindazole on the striatal dopamine, DOPAC and HVA contents in mice 5 days after MPTP treatment. Values were expressed as means ± SD. *$p<0.05$, **$p<0.01$ compared with MPTP + vehicle group (Fisher's PLSD multiple comparison test). n=5–6

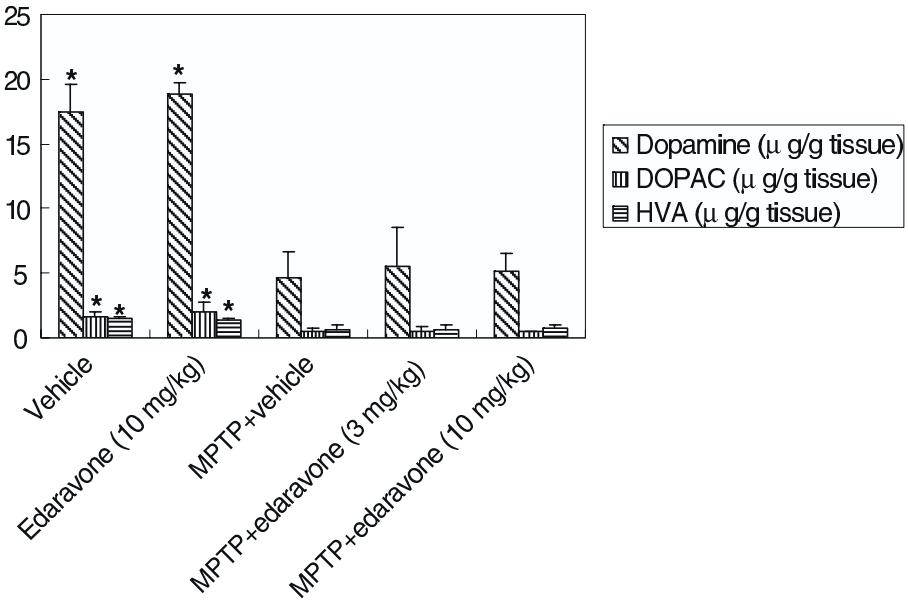

Fig. 3 Neuroprotective effects of edaravone on the striatal dopamine, DOPAC and HVA contents in mice 5 days after MPTP treatment. Values were expressed as means ± SD. *$p<0.01$ compared with MPTP + vehicle group (Fisher's PLSD multiple comparison test). $n=5–6$

significant alterations in the dopamine, DOPAC and HVA contents in the striatum, as compared with vehicle-treated group (Fig. 4).

Effect of pitavastatin and fluvastatin

Pitavastatin showed no significant effects in dopamine, DOPAC and HVA levels in the striatum 5days after MPTP treatment. Furthermore, fluvastatin showed no significant effects in dopamine, DOPAC and HVA levels in the striatum after MPTP treatment. Both drugs alone showed no significant alterations in the dopamine, DOPAC and HVA contents in the striatum, as compared with vehicle-treated group (Figs. 5 and 6). In addition, these drugs exhibited a tendency to increase the striatal dopamine contents in the striatum, as compared with vehicle-treated group.

Effect of etodolac

Etodolac showed no significant changes in dopamine, DOPAC and HVA levels in the striatum 5days after MPTP treatment. Also, this compound alone showed no significant alterations in the dopamine, DOPAC and HVA contents in the striatum, as compared with vehicle-treated group (Fig. 7).

Effect of indomethacin

Indomethacin showed no significant changes in dopamine, DOPAC and HVA levels in the striatum 5days after MPTP treatment. Also, this compound alone showed a

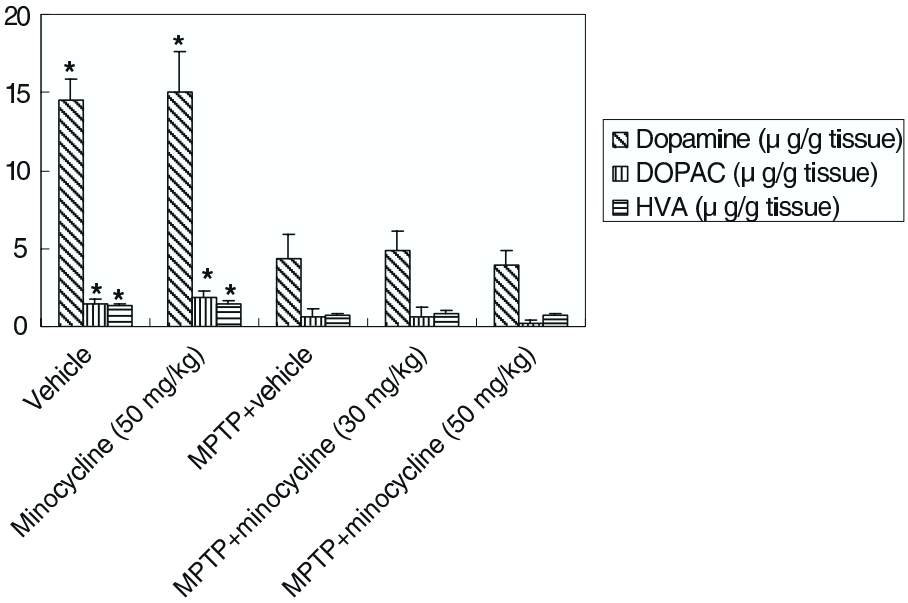

Fig. 4 Neuroprotective effects of minocycline on the striatal dopamine, DOPAC and HVA contents in mice 5 days after MPTP treatment. Values were expressed as means ± SD. *$p<0.01$ compared with MPTP + vehicle group (Fisher's PLSD multiple comparison test). n=4–6

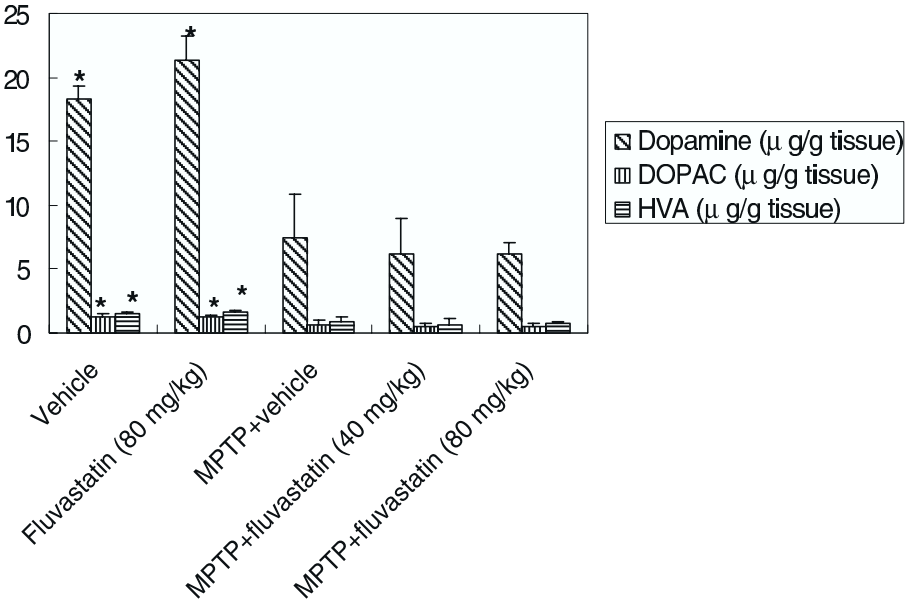

Fig. 5 Neuroprotective effects of fluvastatin on the striatal dopamine, DOPAC and HVA contents in mice 5 days after MPTP treatment. Values were expressed as means ± SD. *$p<0.01$ compared with MPTP + vehicle group (Fisher's PLSD multiple comparison test). n=5–6

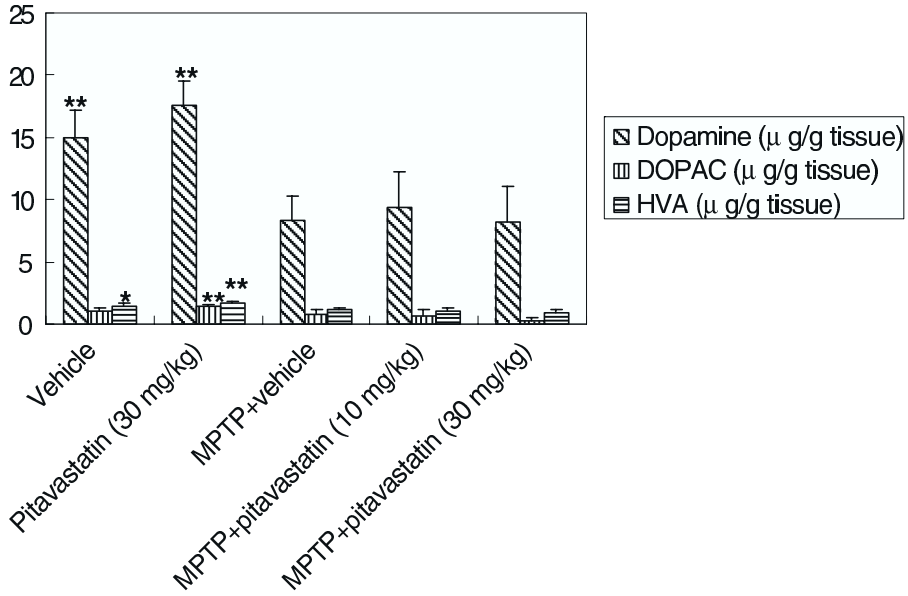

Fig. 6 Neuroprotective effects of pitavastatin on the striatal dopamine, DOPAC and HVA contents in mice 5 days after MPTP treatment. Values were expressed as means ± SD. *$p<0.05$, **$p<0.01$ compared with MPTP + vehicle group (Fisher's PLSD multiple comparison test). $n=5$

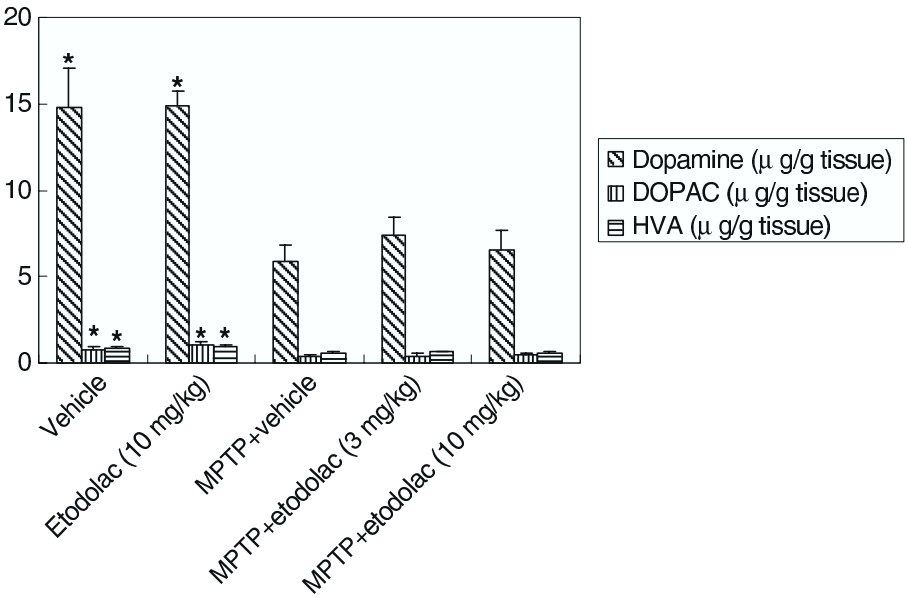

Fig. 7 Neuroprotective effects of etodolac on the striatal dopamine, DOPAC and HVA contents in mice 5 days after MPTP treatment. Values were expressed as means ± SD. *$p<0.01$ compared with MPTP + vehicle group (Fisher's PLSD multiple comparison test). $n=5$

significant increase in the dopamine and HVA contents in the striatum, as compared with vehicle-treated group (Fig. 8).

Discussion

Reactive oxygen species (ROS) have been implicated in the progression of many neurodegenerative diseases. ROS, also referred to as free radicals, are molecules that are short-lived but are able to donate unpaired electrons that act in part by damaging lipid membranes and ultimately affect cellular integrity (Rice-Evans 1994). Free radical damage has been shown to have a significant impact in the pathogenesis of a number of neurodegenerative diseases including PD (Beal 2003).

Nitric oxide (NO) has emerged as a key endogenous modulator of neuronal function. NO is an intracellular and short-lasting second molecule that is synthesized from L-arginine in the several regions by a reaction catalyzed by NOS (Ignarro 1990; Dawson et al. 1991). NOS is not only located in the endothelium, but also in neurons, perivascular nerves, and glial cells (Bredt and Snyder 1990; Murphy et al. 1993). The family of NOS, the enzymes that induce NO, consists of two different classes: the inducible and constitutive forms (Dawson and Snyder 1994; Marletta 1994). The inducible NOS (iNOS) is not regulated by calcium concentration but is regulated transcriptionally, and is primarily expressed in astrocytes, microglia, and inflammatory cells (Nathan and Xie 1994; Di Monte et al. 1997). In contrast, there are two distinct constitutive calcium-dependent NOS (cNOS) isoforms, i.e. neuronal

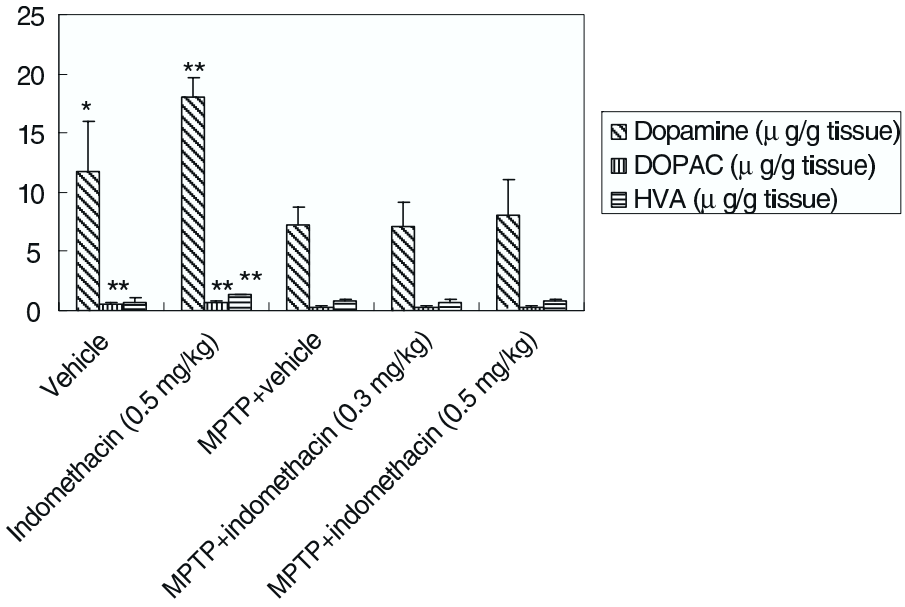

Fig. 8 Neuroprotective effects of indomethacin on the striatal dopamine, DOPAC and HVA contents in mice 5 days after MPTP treatment. Values were expressed as means ± SD. *$p<0.05$, **$p<0.01$ compared with MPTP + vehicle group (Fisher's PLSD multiple comparison test). $n=5$

NOS (nNOS) form in neurons and endothelial NOS (eNOS) form in pyramidal dells and endothelial cells (Moncada et al. 1991). Thus, NOS is well known to be abundant in brain tissues (Marletta 1994; Araki et al. 1999; Muramatsu et al. 2003).

Much attention has been focused the possible role of NO as a retrograde intracellular messenger mediating cell-to-cell interactions in the brain including the cell-mediated immune system, cerebral smooth muscle relaxation, inhibition of platelet aggregation, learning, and synaptic plasticity (Bredt and Snyder 1994; Schulz et al. 1995). A number of experimental studies have also demonstrated that NO and NO donors can enhance the basal release of several neurotransmitters in the mammalian brain, including dopamine, glutamate, and acetylcholine (Lonart and Johanson 1992; Nathan and Xie 1994). These observations are of interest in regard to the role of NO as an intracellular messenger in the CNS. Several lines of evidence have implicated both oxygen free radical and NO for neurodegeneration. The entry of calcium through NMDA (*N*-methyl-D-asparatate) receptors into cells stimulates NOS activity by binding to calmodulin, a cofactor for NOS (Bredt and Snyder 1990). A previous study with cell cultures demonstrated that NOS inhibitors can block NMDA-induced cell damage (Dawson and Snyder 1994). Furthermore, NO may react with superoxide to generate peroxynitrite (Beckman et al. 1990), which may promote nitration to tyrosine (Beckman et al. 1992; Ischiropoulos et al. 1992) and produce hydroxyl radicals (Beckman et al. 1992; Tipton and Singer 1993). The association of NO pathways with PD is strengthened by studies showing: firstly, that induction of iNOS in glial cells contributes to degeneration of dopamine-containing neurons in a mice model of PD (Liberatore et al. 1999); secondly, that inhibition of nNOS prevents Parkinsonism in animal models (Hantraye et al. 1996; Dehmer et al. 2000). Thus, the generation of the NO followed by production of peroxynitrite may be implicated in neuronal cell death. Interestingly, a recent study demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, simvastatin, can prevent in a model of PD (Selley 2005). Furthermore, a previous study suggested that HMG-CoA reductase inhibitors can reduce cerebral ischemia and infarct size by up-regulating eNOS expression in normocholesterolemic mice (Endres et al. 1998). Therefore, the up-regulation of eNOS may ameliorate neurological deficits caused by MPTP treatment.

Increased inflammation as well as oxidative stress has been implicated in dopaminergic neuronal death as elevated levels of COX-2 and reactive microglia has been observed in brains of PD (McGeer et al. 1988; Teismann et al. 2003). COX, present as COX-1 and COX-2 isoforms, is the rate-limiting enzyme in arachidonic acid-derived prostaglandin production (Samuelsson 1991; Wenzel 1997). COX-1 is expressed constitutively in many cell types, whereas COX-2 expression is generally induced by cytokines and other stress-induced stimuli (Smith et al. 2000). In brain, COX-2 is present in selected neurons and its expression is up-regulated in numerous pathological conditions, including Alzheimer's disease (Pasinetti 1998). Especially, microglia are one of the major cell type expressing COX-2 in the brain (Bauer et al. 1997; Minghetti et al. 1999). It is believed that COX-2 is primary importance in the inflammatory response (O'Banion 1999). Several studies have shown that the induction of COX-2 expression parallels the appearance of neuronal apoptosis in cell types affected by kainic acid and excitotoxin-induced neuronal death in vitro is accompanied by a selective elevation in COX-2 mRNA. Theses observations

indicate that the expression of COX-2 may be involved in the pathway leading to neuronal death.

Therapeutically, the use of anti-inflammatory drugs to prevent dopaminergic degeneration in PD has not yet been formally tested in patients. The concept that anti-inflammatory agents may be beneficial in PD thus relies on pre-clinical studies of in vitro and in vivo models of the disease. In vivo experiments, various compounds such as non-steroidal anti-inflammatory drugs (NSAIDs) with targets COX, NF-kB (nuclear factor kappa B) and others, steroids, immunophilins, thalidomide, and phosphodiesterase IV inhibitors, have been studied with variable results (Hirsch et al. 2003). Furthermore, several studies have shown that deletion of pro-inflammatory proteins such as COX-2 and iNOS significantly protects against MPTP neurotoxicity (Liberatore et al. 1999; Teismann et al. 2003). Deletion of COX-2 in mice resulted in protection against MPTP-induced dopaminergic cell loss (Feng et al. 2002). However, a recent report claimed that the neuroprotective effect of COX-2 inhibition against MPTP in vivo was not due to decreased microglial activation but was more likely related to the blockade of COX-2-mediated dopamine oxidation by COX-2 inhibitors (Teismann et al. 2003). On the other hand, the most promising therapeutic substances tested in vivo so far, in good agreement with the hypothesis that activated microglia play a key role dopaminergic neuronal degeneration, are inhibitors of microglial activation such as minocycline and pioglitazone. Both agents have been shown to be highly protective in animal models of PD (Breidert et al. 2002; Wu et al. 2002). Minocycline, which has been used as a tetracycline antibiotic for decades and whose toxicological profile is well known, is now entering phase II and III trials in PD (Ravina et al. 2003). The experimental work quoted on the role of microglial- and cytokine-mediated death of dopaminergic neurons in experimental PD models has been conducted exclusively in rodents and no primate data on inflammation in PD have been published. A previous study showed findings in several monkeys rendered parkinsonian by slow injections of MPTP (Hurley et al. 2003). Although dopaminergic cell loss in the substantia nigra and behavioral symptoms were variable, microglial activation was robust and identical in experimental animals. Furthermore, there was little evidence of microglial activation in the striatum despite evidence of axonal degeneration in this target structure for nigral dopamine neurons. These observations indicate that nigral dopaminergic neuronal loss and inflammation are not causally linked (Hurley et al. 2003). Thus there is no consensus regarding the significance of the role of inflammation in dopaminergic neuronal cell loss.

In the present study, we confirmed that 7-nitroindazole can protect dose-dependently against the striatal dopamine depletions in mice after MPTP treatment, as shown in Fig. 2. In contrast, edaravone, minocycline, fluvastatin, pitavastatin, etodolac and indomethacin did not show the neuroprotective effect on MPTP-induced striatal dopamine depletion, as shown in Figs. 3, 4, 5, 6, 7 and 8. These results demonstrate that the overexpression of nNOS may play a major role in the neurotoxic processes of MPTP, as compared with the production of ROS, the overexpression of iNOS, the modulation of eNOS and the involvement of inflammatory response. The findings by our pharmacological study strongly supports our previous report that nNOS inhibitor can protect dopaminergic neurons against MPTP neurotoxicity (Watanabe et al. 2008).

In conclusion, the present study demonstrates that the overexpression of nNOS may play a key role in the neurotoxic processes of MPTP, as compared with the production of ROS, the overexpression of iNOS, the modulation of eNOS and the involvement of inflammatory response. Thus our findings provide strong evidence for neuroprotective properties of nNOS inhibitor in animal model of PD. Therefore, further studies with the exact relationship among oxidative stress, inflammatory reactions and overexpression of nNOS may lead to a better understanding of PD as well as provide clues to novel target for therapeutic interventions. Thus our pharmacological study provides further information for progressive neurodegeneration of the nigrostriatal dopaminergic neurons after MPTP neurotoxicity.

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