ABSTRACT: Recent studies provide evidence that phospholipase $A_2$ (PLA$_2$) may play a role in the development of experimental parkinsonism. In this investigation an attempt was made to determine a possible protective effect of quinacrine (QNC), a PLA$_2$ inhibitor on MPTP as well as 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in rodents. For MPTP studies, adult male mice (C57 BL) were treated with MPTP (30 mg/kg, i.p.) daily for 5 days. QNC was injected i.p. in the doses of 0, 10, 30 and 60 mg/kg daily 30 min before MPTP in four different groups. Two other groups of mice received either vehicle (control) or a high dose of QNC (60 mg/kg). Two hours after the last injection of MPTP, striata were collected for the analysis of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and glutathione (GSH). For the 6-OHDA study, male Wistar rats were infused with 6-OHDA (60 μg) in the right striatum under chloral hydrate anesthesia. The rats in different groups were treated with 0, 5, 15 and 30 mg/kg of QNC (i.p.) for 4 days, while first injection was given 30 min before 6-OHDA. On day 5, rats were sacrificed and striata were stored at -80°C. Administration of MPTP or 6-OHDA significantly reduced striatal DA, which was significantly attenuated by QNC. Concomitant treatment with QNC also protected animals against 6-OHDA-induced depletion of striatal GSH. Our findings clearly suggest the role of PLA$_2$ in MPTP and 6-OHDA-induced neurotoxicity and oxidative stress. However, further studies are warranted to explore the therapeutic potential of PLA$_2$ inhibitors for the treatment of Parkinson’s disease.

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KEY WORDS: Parkinsonism, Phospholipase, 6-OHDA, MPTP, Oxidative stress, Dopamine, Glutathione.

INTRODUCTION

Parkinson’s disease (PD) is a commonly occurring neurodegenerative disorder that produces muscular rigidity, bradykinesia, tremor of resting limbs and loss of postural balance [44]. The basic neuropathology of PD involves degeneration of pigmented neurons in substantia nigra resulting in depletion of dopamine (DA) and its metabolites [23,32]. The discovery of the two neurotoxins, 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), with the ability to destroy DA-producing cells in substantia nigra in animals has overwhelmingly accelerated the pace of research in the field of PD. Peripheral administration of MPTP in C57 black mouse and intrastriatal injection of 6-OHDA in rat have been widely used as convenient and acceptable models for the induction of experimental parkinsonism [3,12,13,29,57].

The agents with the ability to interfere with the synthesis, release, uptake, metabolism, or drug-receptor interaction of DA have been shown to alter the course of experimental parkinsonism [4,11,24,46,72,76]. Recently, a direct role of phospholipase A$_2$ (PLA$_2$) in regulation of DA release from neuronal cells has been shown [36]. Intracerebral injection of PLA$_2$ enzyme has been shown to significantly reduce DA-mediated rotational behavior in rats indicating the long-lasting inhibition of nigrostriatal dopaminergic pathways [9,10]. Furthermore, Kivinen et al. [34] observed that mice deficient in cytosolic PLA$_2$ are resistant to MPTP neurotoxicity suggesting the role of this enzyme in the etiopathology of Parkinson’s disease. Enhanced activity of PLA$_2$ has also been observed in several neurological conditions including cerebral ischemia [6,15], stroke [21,56], neurotrauma [5,22], epileptic seizures [18], schizophrenia [25,60] and Alzheimer’s disease [19,68]. These studies point towards a possible beneficial effect of PLA$_2$ inhibitors for the treatment of neurodegenerative disorders.

Quinacrine (QNC, also known as mepacrine) is an acridine derivative, which was widely used during World War II as an antimalarial agent [59]. It is a cell membrane stabilizer and a potent inhibitor of PLA$_2$ [75]. Recently, QNC has been shown to predominantly block the activity of PLA$_2$ in neurons [71]. Peripheral injection of QNC is considered to be one of the most suitable methods for inhibiting PLA$_2$ in the central nervous system [58]. Besides preventing PLA$_2$-related pathophysiological mechanisms, QNC has also been shown to downregulate production of oxygen-derived free radicals (ODFR) and reduce oxidative stress-mediated cellular toxicity [1,70]. The present investigation was conducted to study the effect of QNC on 6-OHDA- and MPTP-induced neurotoxicities in rodents.
METHODS

Animals and Treatment

MPTP studies were undertaken in C57 BL male mice (30 ± 2 g). The mice were divided into six groups of eight animals each. One group served as control and received vehicle only, whereas another group was treated with high dose (60 mg/kg) of quinacrine (ICN, Costa Mesa, CA, USA) and served as QNC alone group (without MPTP). The remaining four groups were treated with MPTP (30 mg/kg, i.p.) daily for 5 days; three of these groups also received i.p. injections of QNC in the doses of 10, 30 and 60 mg/kg, 30 min before MPTP (RBI, Natick, MA, USA). The animals were sacrificed 2 h after the last injection of MPTP. The striata were carefully isolated from the cerebrum and immediately frozen in liquid nitrogen and then stored at −80°C until analyzed for DA, DOPAC, HVA and GSH.

For 6-OHDA studies, Wistar male rats (300 ± 20 g) were divided into four groups of five animals each. Rats were anesthetized with chloral hydrate (450 mg/kg, s.c.) and positioned in a stereotaxic instrument (Stoelting, Wood Dale, IL, USA). 6-OHDA (60 µg in 4 µl of normal saline containing 0.02% ascorbic acid) was injected (1 µl/min) over a period of 4 min into the right striatum at coordinates: AP 0.7, ML 2.6 and DV 4.5 from Bregma [53] as described by earlier investigators [12,54,63]. The needle was left in place for another 4 min to allow the complete diffusion of the drug and to prevent the back flow. The rats in different groups received intraperitoneal injections of QNC in the doses of 0, 5, 15 and 30 mg/kg respectively, daily for a period of 4 days; first QNC injection was given 30 min before 6-OHDA (ICN) infusion. On day 5, the rats were sacrificed and striata were isolated and stored similarly as mentioned above in MPTP study. The animals were housed in a temperature-controlled room maintained at 12 h light/dark cycles. The standard laboratory animal food and tap water were freely available ad libitum. The experimental protocol of this study was approved by the Research and Ethical Committee of Armed Forces Hospital, Riyadh.

Analysis of DA, DOPAC and HVA

The analysis of DA and its metabolites in striatum was done according to the procedure of Patrick et al. [52]. The striata were weighed and homogenized for 10 s in 300 µl (for mice) or 500 µl (for rats) of 0.1 M perchloric acid containing 0.05% EDTA, using teflon homogenizer. The homogenates were immediately centrifuged at 10,000 rpm at 4°C for 10 min. The supernatants were filtered using 0.45 µm pore filters and analyzed by high performance liquid chromatography (HPLC). The HPLC system consisted of electrochemical detector from Metrohm (Model 65, Herisani, Switzerland), autoinjector (Model 712, Waters Associate Inc., Milford, MA, USA), solvent delivery pump (Waters Model 510), integrator (Waters Model 745) and C-18 µBondapak (3.9 x 150 mm) column (Waters). The mobile phase consisted of a mixture of 0.1 M citric acid monohydrate, 0.1 M sodium acetate, 7% methanol, 100 µM EDTA and 0.01% sodium octane sulfonic acid. The flow rate of mobile phase was maintained at 1 ml/min and the injection volume was 20 µl.

Analysis of GSH

GSH was measured enzymatically by the method described by Owen [51]. The striata were homogenized in ice-cold perchloric acid (0.2 M) containing 0.01% EDTA. The homogenate was centrifuged at 10,000 rpm at 4°C for 10 min. The enzymatic reaction was started by adding 200 µl of clear supernatant in a spectrophotometric cuvette containing 500 µl of 0.3 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 100 µl of 6 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and 10 µl of 25 units/ml glutathione reductase (all the above three reagents were freshly prepared in phosphate buffer at pH 7.5). The absorbance was measured over a period of 3 min at 412 nm at 30°C. The GSH level was determined by comparing the change of absorbance (ΔA) of test solution with the ΔA of standard GSH.

Statistics

The data were analysed by one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison post hoc test. A value of p < 0.05 was considered as statistically significant.

RESULTS

Administration of MPTP (30 mg/kg, i.p. for 5 days) produced significant depletion of striatal DA (ANOVA F = 18.63, p < 0.001), DOPAC (ANOVA F = 23.15, p < 0.001) and HVA (ANOVA F = 14.82, p < 0.001) in mice, whereas the animals treated with QNC alone (60 mg/kg) showed no significant change in striatal DA, DOPAC and HVA levels. Co-treatment with QNC significantly and dose-dependently attenuated MPTP-induced striatal DA depletion in mice, whereas it failed to produce any significant change in the levels of DA metabolites (Fig. 1). Intrastriatal infusion of 6-OHDA (60 µg) in rats produced significant depletion of DA (ANOVA F = 14.32, p < 0.001), DOPAC (ANOVA F = 4.54, p < 0.01) and HVA (ANOVA F = 2.97, p < 0.05) in the ipsilateral striatum (Fig. 1). Treatment of animals with QNC significantly and dose-dependently attenuated 6-OHDA-induced depletion of striatal DA, whereas QNC failed to modify the effect of 6-OHDA on striatal DOPAC and HVA levels. QNC alone (30 mg/kg, i.p.) did not affect the levels of DA and its metabolites in contralateral striatum.

Administration of MPTP alone in mice significantly reduced striatal GSH levels, whereas QNC alone had no effect on striatal GSH in mice (Fig. 2). Concomitant treatment with high dose (60 mg/kg) of QNC significantly protected mice against MPTP-induced depletion of striatal GSH (ANOVA F = 3.73, p < 0.01), whereas low and medium doses of QNC showed insignificant recovery in MPTP-induced GSH depletion (Fig. 2).

There was a significant depletion of striatal GSH following 6-OHDA lesion in striatum (ANOVA F = 2.794, p < 0.05). Although intraperitoneal administration of QNC did not affect the GSH level in unlesioned striatum, it dose-dependently attenuated the depletion of striatal GSH in 6-OHDA lesioned side (Fig. 2).

DISCUSSION

The results of this study clearly demonstrated the ability of QNC to attenuate MPTP and 6-OHDA-induced depletion of striatal DA in a dose dependent manner (Fig. 1). Beneficial effect of QNC has been observed against a variety of neuropathological conditions including experimental stroke [21] and ischemic neuronal injury [56]. The mechanism of QNC-induced protection against these neurotoxins is far from clear. Although both 6-OHDA and MPTP have been shown to produce neurodegeneration, they act in different manners [20,77]. While MPTP exerts its neurotoxicity by selectively depleting dopaminergic neurons [40], 6-OHDA produces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7]. In brain, MPTP is converted to its toxic metabolite MPP⁺ in presence of enzyme monoamine oxidase B (MAO-B). MPP⁺ is actively taken up into nigrostriatal neurons [40], 6-OHDA induces broad-spectrum degeneration of catecholaminergic pathways [7].
tivity to MPTP, suggesting that conversion of MPTP to MPP⁺ by MAO-B is not the only rate-limiting factor for MPTP neurotoxicity [2]. On the other hand, MAO-B inhibitor Ro 19-6327 failed to protect rats against 6-OHDA neurotoxicity, excluding the role of MAO-B in 6-OHDA-induced progressive loss of DA neurons [28]. Recent findings indicate that PLA₂ activation may play an important role in neurodegenerative process [5,18,22,60,68]. Increased PLA₂ activity may lead to the generation of potentially noxious platelet aggregating factor (PAF), lysophospholipids, arachidonic acid (AA) and its metabolites, including prostaglandins, thromboxanes and leukotrienes [17,30,41]. AA metabolites have been shown to increase glutamate release, decrease glutamate reuptake and potentiate N-methyl-D-aspartate (NMDA) receptor activity [5,42,48]. Enhanced glutamatergic activity has been associated with degeneration of dopaminergic neurons and the development of symptoms of Parkinson’s disease [35,45,50,65]. Support to this hypothesis is provided by the studies demonstrating the protection of rodents against MPTP- [8,73,74] and 6-OHDA- [43,66] induced neurotoxicities by NMDA antagonists. In addition, disturbance of intracellular Ca²⁺ homeostasis by excessive cellular entry of Ca²⁺ has been implicated in neurotoxin-mediated cell death [27,79]. MPTP [14] and 6-OHDA [37] have been shown to enhance cellular influx of Ca²⁺, whereas Ca²⁺ channel inhibitors prevented MPTP-induced neurotoxicity in mice [26,38]. The cytoprotective effect of QNC against MPTP- and 6-OHDA-induced neurotoxicities may be attributed to its ability to inhibit cellular Ca²⁺ influx by binding to L-type Ca²⁺ channels [67,69], to reduce glutamate release [49] and to block PLA₂ in brain [21,61,71].

The results of this study also showed a significant decrease in

FIG. 1. Effect of quinacrine (QNC) on MPTP or 6-OHDA-induced changes in striatal DA, DOPAC and HVA levels in mice and rats, respectively. *p < 0.05 and **p < 0.01 vs. control,
*p < 0.05 vs. MPTP alone and @p < 0.05 vs. 6-OHDA alone group using Dunnett’s multiple comparison test.
striatal GSH in the animals treated with MPTP or 6-OHDA (Fig. 2). This finding is in agreement with earlier workers who also reported a significant depletion of striatal GSH in MPTP- [39,47] and 6-OHDA- [37,54,55] treated rodents. Depletion of GSH by buthionine sulfoximine has been shown to potentiate both 6-OHDA [62] and MPTP [78] neurotoxicities, whereas increased GSH levels protect rodents against 6-OHDA [31] or MPTP toxicity [47]. QNC dose-dependently protected animals against MPTP- and 6-OHDA-induced depletion of striatal GSH, clearly suggesting the antioxidant property of QNC (Fig. 2). These findings are in agreement with earlier reports showing protective effect of QNC against free radical-mediated oxidative damage of tissue [1,70].

In conclusion, the results of this preliminary study clearly demonstrate the protective effect of QNC against 6-OHDA and MPTP-induced dopaminergic neurotoxicity, suggesting a role of PLA2 in drug-mediated neurodegeneration. Further studies are warranted to determine the possible role of PLA2 inhibitors in neurodegenerative process.

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