Effect of Ubiquinone and Resveratrol on Experimentally Induced Parkinsonism

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Abstract
Parkinson’s disease is one of the motor system diseases caused by factors that interfere with survival of the dopaminergic neurons in the substantia nigra. Its mechanisms include mitochondrial dysfunction, oxidative stress and neuroinflammation. Ubiquinone is a fat-soluble vitamin found in the inner mitochondrial membrane and is involved in electron transport chain that supplies energy to vital organs. Resveratrol is a natural polyphenolic compound that has been shown to offer protective effects against many cardiovascular, neurodegenerative diseases and cancer. The aim of this work was to study the effect of the combination of L-dopa, ubiquinone and resveratrol in comparison with L-dopa alone on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in mice. 50 albino mice were divided into 5 equal groups: Control untreated group, MPTP group, L-dopa + MPTP group, L-dopa + ubiquinone + MPTP group, L-dopa + resveratrol + MPTP group. Striatal dopamine, tumor necrosis factor alpha (TNF-α), glutathione reductase, malondialdehyde, nitric oxide, mitochondrial adenosine triphosphate (ATP), mitochondrial complex I activity and catalepsy score were measured. The combination between L-dopa and either ubiquinone or resveratrol induced significant increase in the striatal ATP, dopamine, glutathione reductase and mitochondrial complex I activity with significant decrease in striatal TNF-α level, nitric oxide and malondialdehyde level with significant improvement in the catalepsy score better than the group that received L-dopa alone compared to MPTP-treated group. In conclusion, the combination of L-dopa and ubiquinone or L-dopa and resveratrol had a better effect than L-dopa alone on MPTP-induced Parkinsonism in mice.

Keywords: Neuroprotection; Resveratrol; Ubiquinone; Parkinsonism

Introduction
Parkinson’s disease (PD) is the second most common neurodegenerative disorder affecting approximately 1% of the population older than 60 years. Classically, PD is considered to be a motor system disease and its diagnosis is based on the presence of a set of cardinal motor signs that are consequences of death of dopaminergic neurons in the substantia nigra pars compacta [1]. The molecular mechanisms underlying the pathogenesis of PD have not been completely elucidated. However, some progress has been made in identifying factors that compromise survival of the dopaminergic neurons in the substantia nigra (SN) [2-4]. These mechanisms include mitochondrial dysfunction, oxidative stress, excitotoxicity and neuroinflammation [5].

Dopamine is a neurotransmitter that can undergo metabolism either by monoamine oxidase (MAO) or by autoxidation, producing H2O2, superoxide anion and hydroxyl radicals. In addition, nitric oxide (NO), which is produced through inflammation-induced microglia activation or excitotoxic insults, may also play a role in the pathogenesis of PD. Formation of peroxynitrite anions through the combination of ROS with NO may confer additional toxicity to dopaminergic neurons [2].

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is an agent that has been shown to exert selective toxicity in the SN and to produce a parkinsonian-like disease in animals as well as in humans. Consequently, MPTP is an established and popular agent for producing animal models of PD [6]. The pathogenic mechanisms possibly involved in the neurodegeneration induced by MPTP include mitochondrial dysfunction, oxidative stress and activation of apoptosis [4].

Mitochondrial complex I inhibition by MPTP is thought to underlie the neurodegenerative process in PD. Moreover, overproduction of NO due to both cytosolic and mitochondrial inducible nitric oxide synthases (iNOS) causes free radicals generation and oxidative stress, contributing to mitochondrial dysfunction and neuronal cell death caused by MPTP [7].

Ubiquinone (Also known as CoQ10) is a fat-soluble vitamin-like substance found in the inner mitochondrial membrane. It is normally involved in a series of enzymatically catalyzed sequential reactions necessary to carry out oxidative phosphorylation via the electron transport chain which is an essential process that supply energy to vital organs such as the brain, heart, muscles and kidneys [8]. Ubiquinone is the electron acceptor for mitochondrial complexes I and II and a powerful antioxidant. A correlation was reported between mitochondrial ubiquinone levels and activities of complexes I and II/III in PD patients [9].

Resveratrol is a natural polyphenol found in grapes and red wine. It has been shown to offer protective effects against many cardiovascular and neurodegenerative diseases and cancer. Although the mechanisms of action of resveratrol have not yet been clearly elucidated, many studies have attributed this to its antioxidant, anti-inflammatory and anti-apoptotic effects [10-12]. Resveratrol protects dopaminergic neurons through inhibition of activation and release of...
pro-inflammatory factors and decreased production of reactive oxygen species [13].

Materials and Methods

Chemicals and drugs

Resveratrol, MPTP and other chemicals were obtained from Sigma–Aldrich Chemical Co. Ubiquinone (Coenzyme Q10 capsule, 30 mg) was obtained from MEPACO, Egypt and L-dopa (levocar tablet, levodopa 250 mg plus carbidopa 25 mg) was obtained from ACAPI Co., Egypt.

The present study was carried out on 50 BALB/c mice weighing 20-25 grams collected from local source with free access to food and tap water ad libitum through the whole period of the work. All the experiments were conducted according to the National Research Council's guidelines. Animal handling was followed according to Helsinki declaration of animal ethics. They were divided into five equal groups each of 10 mice as follows:

Group I: Control group received intraperitoneal injection of normal saline for 28 consecutive days.

Group II: Received intraperitoneal injection of MPTP dissolved in normal saline in a dose of 30 mg/kg body weight at 24 h intervals for 28 consecutive days [14].

Group III: Received L-dopa in a dose of 10 mg/kg/day orally [15].

Group IV: Received L-dopa in a dose of 10 mg/kg/day orally concomitantly with ubiquinone in a dose of 200 mg/kg/day orally [16].

Group V: Received L-dopa in a dose of 10 mg/kg/day orally concomitantly with resveratrol in a dose of 50 mg/kg/day orally [11].

The treatment with either L-dopa, ubiquinone or resveratrol was started 2 weeks before and continued during administration of MPTP. The development of parkinsonism was detected at 28 days from starting induction with MPTP, by occurrence of tremors and observation of bradykinesia and rigidity in mice that further quantified by catalepsy score. The first part was the grid test where the mouse was hung by its paws on a vertical grid (25.5 cm wide and 44 cm high with a space of 1 cm between each wire), and the time for the mouse to move its paws or any sort of first movement was recorded. The second part was the bar test where the mouse was placed with both forepaws on a bar (9 cm above and parallel from the base), and the time of removal of the paw was recorded [15].

At the end of the work, all mice were killed. Brain of each mouse was immediately excised, washed with ice-cold saline and freeze at −70°C. Then, striata of the two hemispheres were isolated and weighed. One striatum was processed for assay of striatal dopamine levels and the other for the preparation of mitochondria. Mitochondria were obtained from the striatal homogenate. Mitochondrial pellets that were washed once with the previous collecting buffer to remove microsomal and cellular contamination. Finally the mitochondria were resuspended in 9 volumes of the collecting buffer, pH 7.8 [17]. Mitochondrial protein was determined using Lowry method [18].

Fluorometric assay of striatal dopamine levels: The striatum part of each mouse was homogenized in ice-cold n-butanol as 10% (W/V) using Teflon pestle homogenizer, centrifuged at 1000 × g and supernatant stored at −70 °C for further assay of striatal dopamine levels according to method described by Ciarolone [19].

Spectrophotometric assay of mitochondrial complex I activity (NADH: Coenzyme Q oxido-reductase enzyme activity): It was measured according to method of Birch-Machin et al. [20], by following the decrease in the absorbance due the oxidation of NADH at 340 nm with the use of extinction coefficient = 6.81 l/mmol/cm.

Spectrophotometric assay of mitochondrial complex II activity: It was performed using adenine 5'-triphosphate (ATP) determination kit, according to the principal based on the reaction between 3-phosphoglycerate and ATP catalyzed by phosphoglycerate kinase. The reaction was coupled with a dephosphorylation reaction involving the enzyme glyceraldehyde phosphate dehydrogenase (GAPD) that involved the oxidation of NADH. Formation of NAD was then quantitated by measuring the decrease in the absorbance at 340 nm a measure of the amount of ATP originally present is obtained [21].

Estimation of striatal TNF-α: It was performed using mouse TNF-α ELISA kits supplied by RayBiotech, Inc.

Estimation of striatal NO: Tissue nitrite and nitrate were estimated as an index of NO production [22].

Estimation of striatal MDA: According to Uchiyama and Mihrara [23].

Estimation of striatal GR: According to the method of Manso and Wroblewski [24].

Statistical analysis

Values of the measured parameters were expressed as mean ± SD. One way-ANOVA was used to test significance of the difference among more than two arithmetic means followed by Schefe test to test the difference between each two means. The significance was considered at p values less than 0.05.

Results

Effect of different treatments on catalepsy score (Table 1)

Administration of MPTP to mice induced significant increase in catalepsy score of either grid test or bar test compared to the normal control group. Administration of L-dopa induced significant decrease in catalepsy score of either grid test or bar test compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in catalepsy score compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in catalepsy score compared to the group that received L-dopa alone.

Effect of different treatments on striatal dopamine (Table 2)

Administration of MPTP to mice induced significant decrease in striatal dopamine level compared to the normal control group.
Administration of L-dopa induced significant increase in striatal dopamine compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in striatal dopamine compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in striatal dopamine compared to the group that received L-dopa alone.

Effect of different treatments on striatal TNF-α level (Table 2)

Administration of MPTP to mice induced significant increase in striatal TNF-α level compared to the normal control group. Administration of L-dopa produced non-significant effect on striatal TNF-α level compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in striatal TNF-α level compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in striatal TNF-α level compared to the group that received L-dopa alone.

Effect of different treatments on striatal NO level (Table 2)

Administration of MPTP to mice induced significant increase in striatal NO level compared to the normal control group. Administration of L-dopa produced non-significant effect on striatal NO level compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in striatal NO level compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in striatal NO level compared to the group that received L-dopa alone.

Effect of different treatments on mitochondrial complex I activity (Table 2)

Administration of MPTP to mice induced significant decrease in mitochondrial complex I activity compared to the normal control group. Administration of L-dopa produced non-significant effect on mitochondrial complex I activity compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in mitochondrial complex I activity compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in mitochondrial complex I activity compared to the group that received L-dopa alone.

Effect of different treatments on mitochondrial ATP level (Table 2)

Administration of MPTP to mice induced significant decrease in mitochondrial ATP level compared to the normal control group. Administration of L-dopa produced non-significant effect on mitochondrial ATP level compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in mitochondrial ATP level compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in mitochondrial ATP level compared to the group that received L-dopa alone.
Effect of different treatments on the antioxidant status (Table 2)

Administration of MPTP to mice induced significant decrease in tissue GR with significant increase in tissue MDA compared to the normal control group. Administration of L-dopa produced non-significant effect on tissue MDA and GR compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in tissue GR with significant decrease in tissue MDA compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in tissue GR with significant decrease in tissue MDA compared to the group that received L-dopa alone.

Discussion

Parkinson's disease (PD) is a movement disorder characterized by progressive degeneration of nigrostriatal dopaminergic (DA) neurons [25]. Lines of treatment of PD are limited and mainly affect the symptoms without significant disease-modifying effect. For this reason, understanding the molecular pathology and the cause of dopaminergic cell loss will lead to new lines of treatment that may prevent and cure the disease [26].

MPTP is an agent that has been shown to produce a parkinsonian-like disease in animals as well as in humans. In the present study, the administration of MPTP to mice induced a model of parkinsonism resembling the basic findings in human where bradykinesia and rigidity were manifested as an increase in catalepsy score with significant decrease in striatal ATP, dopamine levels, GR and mitochondrial complex I activity with significant increase in striatal TNF-α level, MDA and striatal NO level compared to the normal control group. These results are in agreement with other studies that indicated that exposure to MPTP causes nigrostriatal dopaminergic degeneration associated with neurochemical and behavioural features of PD [4,6,11,27].

L’Epsico and al. [28] reported that selective degeneration of DA neurons in the substantia nigra (SN) is a pathological hallmark of both PD and MPTP animal model of PD. The decline of dopamine in the striatum is associated clinically with progressive bradykinesia, tremors, rigidity and postural instability. Soliman et al. [27] reported that MPTP mediates dopaminergic degeneration by inhibiting electron transport chain activity at complex I where decreased production of ATP through the electron transport chain can bring about rapid neuronal depolarization and a calcium mediated cascade of cell death. Also, MPTP induces key enzymes involved in the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and contributes to DA neuronal death. Additionally, Kao et al. [29] found that MPTP increases production of proinflammatory cytokines, such as TNF-α and IL-1β that contribute to DA neuronal death. Other studies demonstrated that MPTP activates the brain-resident immune cells which lead to increased production of inflammatory cytokines. Moreover, it was found that if TNF-α receptor expression is genetically suppressed, microglial activation is absent and MPTP-induced neurotoxicity is significantly blunted [11]. Gao and Hong [30] reported that MPTP directly led to dopaminergic neuronal damage initially and then the damaged neurons release toxic soluble factors such as α-synuclein, which in turn induced microglial activation and production of proinflammatory factors and contributed to additional neuronal damage. This proposed pathogenesis was evidenced by the significant correlation between the catalepsy score and the neurochemical parameters obtained in the current study.

L-dopa is the precursor of dopamine that is used clinically in treatment of PD and dopamine-responsive dystonia in combination with a peripheral decarboxylase inhibitor (e.g. carbidopa) to reduce nausea and vomiting associated with L-dopa therapy and allow a greater proportion of L-dopa to enter the brain [26]. Although its administration in the present work caused symptomatic improvement in the form of reduction of catalepsy score with restoration of striatal dopamine levels that was in concordance with Alam and Schmidt [15], but it did not show any significant effects on striatal complex I activity, ATP levels, TNF-α, GR, MDA or NO level. These results are in accordance with Mogi et al. [31] who reported that L-dopa didn’t increase the level of TNF-α in the brain in PD. In addition, there is a concern that L-dopa might be toxic to dopamine neurons as it undergoes oxidative metabolism and has the potential to generate cytotoxic free radicals by their two free hydroxyl residues on their benzene ring [32]. The effects of L-dopa on NO production in the DA neurons and striatal DA terminals, in which degenerated DA neurons have been observed in PD, remain unclear. However, few reports have suggested that dopamine modulates NO release and/or production. Kashihara et al. [33] reported that systemic treatment with L-dopa did not enhance NO production in the striatum of rats. In contrast, Itokawa et al. [34] reported that L-dopa may increase NO production in the striatum of mice. Melis et al. [35] showed that a dopamine agonist increased NO production by activating D2 receptors in the paraventricular nucleus of the hypothalamus. Ben-Shachar et al. [36] reported that treatment with L-dopa was associated with elevated dopamine concentrations in the brain with significant reduction in the activity of complex I of the mitochondrial respiratory chain. Additionally, a significant decrease in striatal ATP concentrations was detected which was associated with dopamine-derived neurotoxic effects. This was attributed to the inhibitory effect of dopamine on pyruvate- and succinate-dependent electron transport.

Ubiquinone is an essential biological cofactor of the electron transport chain that serves as an important antioxidant in mitochondrial and lipid membranes [37]. Ubiquinone is able to penetrate blood brain barrier and can modulate the mitochondrial electron transport chain, modulate mitochondrial apoptosis and generally reduce oxidative stress in mitochondria [38]. Because of these functions, ubiquinone has attracted attention as a neuroprotective agent in neurodegenerative disorders linked to mitochondrial defects or oxidative stress, such as PD [39].

The present study demonstrated the role of administration of ubiquinone in combination with L-dopa in amelioration of PD where it caused increase of the striatal dopamine, complex I activity, GR and ATP levels as well as decrease in the catalepsy score, striatal TNF-α, MDA and NO level compared to MPTP treated group. These results are in agreement with Yang et al. [40] who reported that treatment with ubiquinone significantly blocked MPTP-induced oxidative stress and reduced pathological changes which contribute to a cascade of pathological changes in striatal neurons. Also, it was suggested that ubiquinone reduced α-synuclein aggregates and decreased brain oxidative stress. Ubiquinone is a particularly important antioxidant in the inner mitochondrial membrane, where it transfers electrons from complexes I/II to complex III with final synthesis of ATP and can directly scavange free radicals through interactions with α-tocopherol [37]. It also prevents apoptotic cell death by blocking Bax binding to mitochondria, and by inhibiting activation of the mitochondrial permeability transition. Real [41] suggested that ubiquinone may activate mitochondrial uncoupling proteins leading to reduction in mitochondrial-free radical generation which offers marked...
neuroprotection against the MPTP toxicity. Bessler et al. [42] reported that ubiquinone can modulate the immune function and decreases TNF-α production. Schmelzer et al. [43] showed that ubiquinone decreases TNF-α secretion in human and murine mononuclear cell lines by its anti-inflammatory effects. Moreover, Abd El-Gawad and Khalifa [44] reported that ubiquinone protects against lipid peroxidation and NO generation in rat brain by its antioxidant and radical scavenging effect. The present study showed that combined administration of ubiquinone and L-dopa induced significant improvement in the biochemical parameters with significant decrease in catecholase activity compared to L-dopa treated group. This can be explained by the antioxidant and anti-inflammatory effects of ubiquinone with its ability to prevent depletion of and regenerate endogenous antioxidants, decrease production of inflammatory cytokines and restore brain level of dopamine with increased ATP production through electron transport chain.

Resveratrol is a type of natural phenols whose effects are currently a topic of numerous animal and human studies [45]. In the present study, the combination of resveratrol with L-dopa induced significant increase in striatal ATP, dopamine, GR and mitochondrial complex I activity with significant decrease in catecholase activity, striatal TNF-α level, MDA and striatal NO level compared to MPTP-treated group. These results are in accordance with recent studies reporting the protective effects of resveratrol against MPTP-induced motor coordination impairment, hydroxyl radical overloading and neuronal loss [12,46].

Anandhan et al. [46] showed that pretreatment of resveratrol significantly reversed toxic effects of MPTP by increasing the levels of striatal dopamine, reduced glutathione and activities of glutathione peroxidase, catalase and superoxide dismutase together with reducing levels of MDA and enhanced behavior performance. Bi et al. [10] and Zhang et al. [13] reported that resveratrol inhibits NO and TNF-α production by lipopolysaccharide-activated microglia and protects dopamine neurons against lipopolysaccharide-induced neurotoxicity through its anti-inflammatory and antioxidant properties.

Ryan et al. [47] reported that resveratrol suppresses oxidative stress, restores mitochondrial function and increases complex I activity. Zini et al. [48] suggested that resveratrol-induced limitation of dysfunction of mitochondria isolated from rat brain in an anoxia-reoxygenation model with at least three mechanisms: antioxidant properties, action on complex III and a membrane stabilizing effect. Zhang et al. [13] reported that microglias were the targets of resveratrol action and resveratrol exerted neuroprotection through the inhibition of microglial activation. Microglial activation leads to reaction of superoxide anion with NO to form highly reactive intermediates which have an important role in neurodegeneration. Recent studies suggested that NO serve as secondary messengers to recent studies demonstrated that resveratrol significantly decreased the levels of COX-2, TNF-α, mRNA and COX-2 protein expression in the substantia nigra and hence its neuroprotective effect [12,49,50].

The present study showed that the combination of resveratrol and L-dopa induced significant improvement in the biochemical parameters with significant decrease in catecholase score compared to L-dopa treated group. This can be attributed to the antioxidant and anti-inflammatory properties of resveratrol with its ability to inhibit NO and TNF-α production by microglia and improve the functions of electron transport chain in the striatal mitochondria with increased production of ATP. In conclusion, the combination of L-dopa and ubiquinone or L-dopa and resveratrol has better effect than L-dopa alone on MPTP-induced Parkinsonism in mice.

References


