Mechanism of the neuroprotective role of coenzyme Q10 with or without L-dopa in rotenone-induced parkinsonism

Amany A. Abdin a,*, Hala E. Hamouda b

a Department of Pharmacology, Faculty of Medicine, Tanta University, AL-Geish Street, Tanta, Egypt
b Department of Medical Biochemistry, Faculty of Medicine, Tanta University, AL-Geish Street, Tanta, Egypt

Abstract

Current treatment options for parkinsonism as a neurodegenerative disease are limited and still mainly symptomatic and lack significant disease-modifying effect. Understanding its molecular pathology and finding the cause of dopaminergic cell loss will lead to exploring therapies that could prevent and cure the disease. Mitochondrial dysfunction was found to stimulate releasing of reactive oxygen species (ROS) with subsequent induction of apoptotic neuronal cell death. The aim of the present study was to throw the light on the role of coenzyme Q10 with or without L-dopa in an experimental model of parkinsonism induced by rotenone in rats. The present work showed that rotenone (2.5 mg/kg/day i.p. for 60 days) induced a model of parkinsonism (group II) resembling the basic findings in human characterized by bradykinesia and rigidity manifested as an increase in catalepsy score (detected after 20 days with bad prognosis after 60 days) with marked decrease in striatal dopamine levels. This model confirmed the implication of mitochondrial-apoptotic pathway in the pathogenesis of parkinsonism as there was a decrease in levels of striatal complex I activity and ATP as well as extreme overexpression of the antiapoptotic protein Bcl-2, and also exhibited the role of coenzyme Q10 where its plasma and striatal levels were found to be decreased in comparison to the normal control rats (group I). This proposed pathogenesis was evidenced by the significant correlation between catalepsy score and the neurochemical parameters obtained in the current work. The treated groups started to receive the drug(s) after 20 days from induction of parkinsonism and continued to complete for 60 days. Oral administration of CoQ10 in a low dose 200 mg/kg/day (group III) or a high dose 600 mg/kg/day (group IV), resulted in amelioration of the mitochondrial induced apoptosis by dose-dependent restoration of striatal complex I activity, ATP levels with temperate increase in expression of Bcl-2 as well as decrease in catalepsy score. Although both low and high doses of Co Q10 resulted in significant increase in its plasma and striatal levels, but only the high dose was shown to reach the recommended therapeutic levels. As a current replacement therapy, oral administration of levodopa 10 mg/kg/day (group V), caused symptomatic improvement in the form of reduction of catalepsy score with restoration of striatal dopamine levels, but it did not show any significant effects on either striatal complex I activity, ATP levels or the expression of Bcl-2, pointing to the lack of its disease-modifying role. On the other hand, its administration with high dose of coenzyme Q10 caused the most marked symptomatic improvement in catalepsy score when compared to its administration with low dose of coenzyme Q10, or when compared to either coenzyme Q10 high dose or L-dopa, respectively. Moreover, administration of high dose coenzyme Q10 with L-dopa provided a significant increase in striatal complex I activity, ATP levels and Bcl-2 expression in comparison to group administered coenzyme Q10 low dose with L-dopa, in addition to the significant restoration of striatal dopamine levels and both plasma and striatal Co Q10 levels. Regarding that L-dopa is viewed as a replacement therapy in parkinsonism, it could be concluded that addition of coenzyme Q10 in a high dose in early parkinson’s disease could be recommended based on its proved disease-modifying role on several levels of the proposed mechanisms, including improvement of respiratory chain activity and intervention with neuronal apoptosis. A further research to investigate other apoptosis-targeted compounds will open a new era in the treatment of parkinsonism.
1. Introduction

Parkinson disease (PD) is an age-related neurodegenerative disease, occurs worldwide with an equal incidence in both sexes, and its prevalence is predicted to increase dramatically in the coming decades due to the aging of the population (Schapira and Olanow, 2004). It has increased relative risk of mortality ranges from 1.6 to 3.0 compared with matched control populations (Clarke and Moore, 2007). The cardinal clinical features of PD are resting tremor, rigidity, and bradykinesia. Pathologically, the disease is characterized by loss of dopamine neurons in the substantia nigra pars compacta (SNc), with intracellular proteinaceous inclusions or Lewy bodies and a reduction in striatal dopamine. PD is multifactorial in terms of both etiology and pathogenesis. Several biochemical factors appear to be involved in the pathogenetic cascade of events leading to cell dysfunction and death in PD, including free radicals, a mitochondrial complex I deficiency, excitotoxicity, and inflammation (Schapira and Olanow, 2004). Mitochondrial dysfunction can result in excessive production of reactive oxygen species, triggering the apoptotic death of dopaminergic cells in Parkinson’s disease. Neurotoxins that induce Parkinsonian neuropathology, such as rotenone, has been reported to stimulate superoxide production at complex I of the electron transport chain (Fiskum et al., 2003). Evidence implicating apoptosis in PD is controversial (Anglade et al., 1997; Kosel et al., 1997). The specific factors involved in apoptosis have been investigated, demonstrating a role for the antiapoptotic protein Bcl-2 (Yang et al., 1998), that most of its protective effects against cell death have been attributed to its mitochondrial location (Kowaltowski et al., 2004).

Despite advances in modern therapy, patients with PD continue to experience unacceptable disability. Currently, medical therapy continues to be levodopa mixed with a peripheral decarboxylase inhibitor, carbidopa (Savitt et al., 2006). Although in the early stages of the disease, symptoms are relatively well controlled with levodopa and other dopaminergic agents, but they lack significant disease-modifying effect and over time the majority of patients experience levodopa-related motor complications and the disease progresses with development of features such as freezing, falling, autonomic dysfunction, and dementia that do not adequately respond to dopamine replacement therapies (Schapira and Olanow, 2004; Savitt et al., 2006). These disorders do not have cures because the neurons of central nervous system cannot regenerate on their own after cell death or damage (Rachakonda et al., 2004). Thus, the main challenge is to develop a neuroprotective therapy that can be administered early in the course of the disease and slow, stop, or reverse disease progression (Schapira and Olanow, 2004). Coenzyme Q10 is a fat-soluble vitamin-like substance, also known as ubiquinone, used in treatment of a variety of disorders primarily related to suboptimal cellular energy metabolism and oxidative injury. It is an ubiquitous compound, found in highest concentrations in tissues with high energy turnover such as the brain, heart, liver, and kidney. Coenzyme Q10 has wide-ranging cellular properties implicate it for the potential treatment of numerous conditions that may improve with mitochondrial and antioxidant support (Bonakdar and Guarneri, 2005). Recently, coenzyme Q10 was investigated as a promising neuroprotective agent for neurodegenerative disorders such as Parkinson’s disease. Joining that there is both a complex I defect and oxidative damage in Parkinsonism, to the fact that coenzyme Q10 is an intrinsic component of the mitochondrial respiratory chain that functions both as an enhancer of adenosine triphosphate (ATP) production and as an antioxidant, this compound was a logical candidate for study (Schapira and Olanow, 2004). Moreover, coenzyme Q10 is involved in prevention of oxidative stress-induced apoptosis in neuronal cells, however the mechanism of this protection has not been fully elucidated (Naderi et al., 2006). So, the aim of this study is to investigate the role of coenzyme Q10 with or without L-dopa in an experimental model of Parkinsonism induced by rotenone in rats.  

2. Material and Methods

2.1. Chemicals and drugs

Rotenone and the other chemicals used were obtained from Sigma–Aldrich Chemical Co. Coenzyme Q10 0.01% solution and Q10 capsules 200 mg obtained from MEGACO, Egypt and L-dopa (levocar tablet, levodopa 250 mg plus carbidopa 25 mg) was obtained from ACAPi Co., Egypt. The present work was conducted on 70 albino rats weighing 125–150 g, allowed food and water ad libitum during a whole period of the work (60 days). They were divided into 7 groups (each of 10 rats).

Group I: served as normal control group, received vehicle of sunflower oil 1.0 ml/kg/day i.p. for 60 days.

Group II: parkinsonism was induced by rotenone (emulsified in sunflower oil to a concentration of 2.5 mg/ml), given i.p. in a dose of 2.5 mg/kg once daily for 60 days (Alam and Schmidt, 2002).

Group III: rotenone-induced parkinsonism treated with low dose of coenzyme Q10 200 mg/kg/day orally (Beal and Matthews, 1997).

Group IV: rotenone-induced parkinsonism treated with high dose of coenzyme Q10 600 mg/kg/day orally (Honda et al., 2007).

Group V: rotenone-induced parkinsonism treated with coenzyme Q10 l-dopa in a dose of 10 mg/kg/day orally (Alam and Schmidt, 2004).

Group VI: rotenone-induced parkinsonism treated with coenzyme Q10 low dose and L-dopa.

Group VII: rotenone-induced parkinsonism treated with coenzyme Q10 high dose and L-dopa.

The treatment with either coenzyme Q10 or L-dopa was started after 20 days from induction of parkinsonism and continued to complete for 60 days. The development of parkinsonism was detected after 20 days from induction with rotenone, by occurrence of tremors and observation of bradykinesia and rigidity in rats that further quantified by “Catalepsy test”. The first part was the grid test where the rat 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 rats to move its paws or any sort of first movement was recorded. The second part was the bar test where the rat 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 (Alam and Schmidt, 2004). The test was repeated once again at the end of the experiment for further evaluation of the disease progress. At the end of the work (after 60 days), all rats were sacrificed; blood samples were obtained, transferred to tubes containing EDTA-fluoride, immediately centrifuged at 3000 × g and the collected plasma stored at −20 °C for determination of plasma levels of Coenzyme Q10. Brain of each rat was immediately excised, washed with ice-cold saline and freeze-dried at −70 °C. Then, striata of the two hemispheres were isolated and weighed. One striatum was processed for assay of striatal dopamine levels, and striatal mitochondria were isolated for estimation of mitochondrial levels of Coenzyme Q10, mitochondrial complex I activity and mitochondrial levels of ATP. The other striatum was processed for detection of Bcl-2 expression.

2.2. Preparation of rat brain mitochondria

The striatum of each rat was collected in the following medium (10 mM Tris–HCl, 1 mM EGTA, 0.32 M sucrose) obtained by dissolving 10.94 g sucrose, 1.21 g Tris–HCl, and 0.38 g EGTA in 100 ml distilled water and adjusted to pH 7.8. Homogenization was done in 9 volumes of this cold medium with three or four strokes using Teflon pestle homogenizer. Then the homogenate was centrifuged at 700 × g for 10 min at 4 °C, the supernatant was centrifuged for 20 min at 10000 × g to obtain mitochondria 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 (Tarpenning et al., 1988). Mitochondrial protein was determined using Lowry method (Lowry et al., 1951).
assay of striatal dopamine levels according to method described by Ciarolone (1978).

(3) Spectrophotometric assay of mitochondrial complex I activity (NADH: coenzyme Q oxidoreductase enzyme activity): It was measured according to method of Birch-Machin et al. (1994), by following the decrease in the absorbance due to the oxidation of NADH at 340 nm with the use of extinction coefficient – 6.81 l/(mmol/cm).

(4) Spectrophotometric assay of mitochondrial ATP level: Using Adenosine 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 using the enzyme glyceraldehyde phosphate dehydrogenase (GAPD) that involved the oxidation of NADH. Formation of NADH was then quantitated by measuring the decrease in the absorbance at 340 nm a measure of the amount of ATP originally present is obtained (Adams, 1963).

(5) Estimation of Bcl-2 mRNA expression by a quantified RT-PCR: Total RNA of each striatum was extracted immediately using MagNa Pure Compact RNA Isolation Kit (Cat. No. 54029503001) supplied by Roche, Germany according to the manufacturer’s instructions using the MagNa Pure Compact Instrument (Roche, Germany) which is fully automated system. The yield of total RNA obtained was determined spectrophotometrically. Then, cDNA was synthesized immediately using Prime RT Premix (2 l) supplied by Genet Bio Korea (Cat. No. R-2000). The kit contains Prime MMLV RTase, reaction buffer, dNTPs mixture, RNase inhibitor and protein stabilizer. 2 l of the total RNA was mixed with 0.5 g of the oligo dt primer in RNase free (DEPC-treated) water so that their total volume is 10 l, incubated at 70 °C for 5 min and then chilled on ice then 10 l of Prime RT Premix was added to have a total reaction volume of 20 l and mixed by pipetting gently up and down. The mixture is incubated for 60 min at 37 °C. The reaction was stopped by heating at 70 °C for 10 min then chilling on ice.

The sequences of internal standard, primer and probe are:

(Internal standard)

GAPDH forward primer: GAAGGTGAAGGTCGGTGGATCC
GAPDH reverse primer: GGACTTAACGTGACGAAACCCC
GAPDH probe: VIC-GAAGGTGAAGGTCGGTGGATCC-TAMRA

(Primer and probe)

Bcl-2 forward primer: TCTCGTGAGTCTGAGTCTGGATCC
(Antisense) Bcl-2 reverse primer: AGAGACAGCCAGGAGAAATCAAAC
Bcl-2 probe: Fam-CCCTGCTGAAACATATGCCCACAG-TAMRA

A final reaction volume of 20 l was prepared using Light Cycler-DNA Master SYBR Green I kit (Cat. No. 2 015 099) – Roche diagnostics, Germany. Each mix is formulated of 1 l each primer (0.5 M), 2 l Light Cycler-DNA Master SYBR Green I (1 l), 2.4 l MgCl2 stock solution (4 mM), 11.6 l H2O sterile PCR grade and 2 l of cDNA formed of 1 l each primer (0.5 M), 2 l Light Cycler-DNA Master SYBR Green I (1 l), 2.4 l MgCl2 stock solution (4 mM), 11.6 l H2O sterile PCR grade and 2 l of cDNA. The samples were mixed by pipetting gently up and down. The mixture is incubated for 60 min at 37 °C. The reaction was stopped by heating at 70 °C for 10 min then chilling on ice.

When the catalepsy score after 60 days (after treatment) of either grid test or bar test, respectively, was compared to that after 20 days (before treatment) (Table 3), it showed significant increase in both group II (untreated rotenone-induced parkinsonism) and group III (treated with low dose Co Q10), with non-significant change in group IV (treated with high dose Co Q10), group V (treated with L-dopa), group VI (treated with low dose Co Q10 and L-dopa) and group VII (treated with high dose Co Q10 and L-dopa), while group treated by L-dopa (group V) showed non-significant change in Bcl-2 expression, when compared to the control group (group I).

In comparison to rats with untreated rotenone-induced parkinsonism (group II), the treated groups by either low dose Co Q10 (group III), high dose Co Q10 (group IV), low dose Co Q10 with L-dopa (group VI), or high dose Co Q10 with L-dopa (group VII); exhibited significant elevation of plasma and striatal coenzyme Q10 levels, striatal mitochondrial complex I activity and ATP levels as well as significant reduction in catalepsy score of grid test and bar test. The present study also showed that administration of high dose Co Q10 either without (group IV) or with L-dopa (group VII), respectively, caused significant elevation of plasma and striatal coenzyme Q10 levels, striatal mitochondrial complex I activity and ATP levels as well as significant increase in Bcl-2 expression with significant reduction in catalepsy score of grid test and bar test, when compared to low dose Co Q10 either without (group III) or with L-dopa (group VI).

When group VII (treated with high dose Co Q10 and L-dopa) was compared to either group IV (treated with high dose Co Q10) or group V (treated with L-dopa), it showed significant reduction in catalepsy score of grid test and bar test.

When the catalepsy score after 60 days (after treatment) of either grid test or bar test, respectively, was compared to that after 20 days (before treatment) (Table 3), it showed significant increase in both group II (untreated rotenone-induced parkinsonism) and group III (treated with low dose Co Q10), with non-significant change in group IV (treated with high dose Co Q10), group V (treated with L-dopa), group VI (treated with low dose Co Q10 and L-dopa) and group VII (treated with high dose Co Q10 and L-dopa), while group treated by L-dopa (group V) showed significant reduction in it.

In rats with untreated rotenone-induced parkinsonism (group II), there was significant negative correlation of catalepsy score

3. Results

Table 2 showed that rotenone-induced parkinsonism (group II) caused a significant reduction in both plasma and striatal coenzyme Q10 levels with significant reduction in striatal levels of dopamine, mitochondrial complex I activity and ATP levels, as well as significant increase in Bcl-2 expression when compared to the control group (group I). This model of rotenone-induced parkinsonism exhibited a significant increase in catalepsy score (Table 1) of either grid test or bar test after 20 days in comparison to the control group (group I). The Bcl-2 expression was significantly increased in the groups treated with either low dose Co Q10 (group III), high dose Co Q10 (group IV), low dose Co Q10 with L-dopa (group VI), or high dose Co Q10 with L-dopa (group VII); while the group treated by L-dopa (group V) showed non-significant change in Bcl-2 expression, when compared to the control group (group I).

When group VII (treated with high dose Co Q10 and L-dopa) was compared to either group IV (treated with high dose Co Q10) or group V (treated with L-dopa), it showed significant reduction in catalepsy score of grid test and bar test.

When the catalepsy score after 60 days (after treatment) of either grid test or bar test, respectively, was compared to that after 20 days (before treatment) (Table 3), it showed significant increase in both group II (untreated rotenone-induced parkinsonism) and group III (treated with low dose Co Q10), with non-significant change in group IV (treated with high dose Co Q10), group V (treated with L-dopa), group VI (treated with low dose Co Q10 and L-dopa) and group VII (treated with high dose Co Q10 and L-dopa), while group treated by L-dopa (group V) showed significant reduction in it.

In rats with untreated rotenone-induced parkinsonism (group II), there was significant negative correlation of catalepsy score

\[
\text{Bcl-2 amplification curve. Semiaquantitation of genes expression was calculated as a ratio between target gene and internal control (IC).}
\]
after 60 days (either grid test or bar test, respectively) with the measured neurochemical parameters (plasma Co Q10, ... VI (treated with low dose Co Q10 and L-dopa).

### 4. Discussion

Current treatment options for neurodegenerative diseases including parkinsonism are limited and mainly affect only the symptoms of disease and lack significant disease-modifying effect. For this reason, understanding its molecular pathology and finding the cause of dopaminergic cell loss will lead to exploring therapies that prevent and cure the disease (Waldmeier and Tatton, 2004; Savitt et al., 2006). For such understanding, the present work showed that rotenone induced a model of parkinsonism resembling the basic findings in human where bradykinesia and rigidity were manifested as an increase in catalepsy score with evident decrease in striatal dopamine levels. Many authors indicated that chronic exposure to rotenone, causes highly selective nigrostriatal dopaminergic degeneration that is associated with neurochemical, behavioral and neuropathological features of PD (Betarbet et al., 2000; Alam and Schmidt, 2002; Sherer et al., 2002). As shown in the present study, rotenone develops slow onset of degeneration that makes it suitable to study neuroprotective strategies (Sherer et al., 2003; Schmidt and Alam, 2006). Mitochondria was found to play essential roles in regulation of key steps in both apoptotic and necrotic cell death by affecting energy metabolism, participating in intracellular Ca\(^{2+}\) homeostasis, regulating the activation of caspases, and releasing reactive oxygen species (ROS) (Kowaltowski et al., 2004). This model confirmed the implication of mitochondrial dysfunction in the pathogenesis of parkinsonism.

### Table 1
Comparison between the different studied groups for catalepsy score

<table>
<thead>
<tr>
<th>Catalepsy score</th>
<th>Group I (control)</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
<th>Group VII</th>
<th>F value (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grid test (s)</td>
<td>7.5 ± 0.83</td>
<td>8.0 ± 0.82</td>
<td>After 20 days</td>
<td>After 60 days</td>
<td>32.0 ± 2.13, p&lt;0.001</td>
<td>34.5 ± 2.29, p&lt;0.001</td>
<td>29.0 ± 2.33, p&lt;0.001</td>
<td>26.5 ± 2.24, p&lt;0.001</td>
</tr>
<tr>
<td>Bar test (s)</td>
<td>8.0 ± 0.82</td>
<td></td>
<td></td>
<td></td>
<td>0.19, p&lt;0.001</td>
<td>0.15 ± 0.26, p&lt;0.001</td>
<td>0.20 ± 0.26, p&lt;0.001</td>
<td>0.20 ± 0.26, p&lt;0.001</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM.

Scheffe test: p<0.05 vs group II (untreated parkinsonism), group III (treated with low dose Co Q10), group IV (treated with high dose Co Q10), group V (treated L-dopa), group VI (treated with low dose Co Q10 and L-dopa), group VII (treated with high dose Co Q10 and L-dopa); p<0.05 vs group VI (treated with low dose Co Q10 and L-dopa).

### Table 2
Comparison between the different studied groups for the measured parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (control)</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
<th>Group VII</th>
<th>F value (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma coenzyme Q10 (nmol/ml)</td>
<td>0.85 ± 0.03</td>
<td>0.54 ± 0.03</td>
<td>1.37 ± 0.07</td>
<td>2.14 ± 0.06, p&lt;0.001</td>
<td>0.52 ± 0.03, p&lt;0.001</td>
<td>1.18 ± 0.04, p&lt;0.001</td>
<td>2.25 ± 0.07, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Striatal coenzyme Q10 (nmol/mg tissue protein)</td>
<td>4.20 ± 0.22</td>
<td>2.62 ± 0.19, p&lt;0.001</td>
<td>4.43 ± 0.17, p&lt;0.001</td>
<td>6.06 ± 0.25, p&lt;0.001</td>
<td>2.97 ± 0.24, p&lt;0.001</td>
<td>4.45 ± 0.01, p&lt;0.001</td>
<td>5.75 ± 0.32, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Striatal dopamine (ng/mg tissue protein)</td>
<td>78.85 ± 2.75</td>
<td>58.17 ± 2.45, p&lt;0.001</td>
<td>58.30 ± 2.63, p&lt;0.001</td>
<td>57.49 ± 3.04, p&lt;0.001</td>
<td>78.38 ± 2.64, p&lt;0.001</td>
<td>78.30 ± 2.06, p&lt;0.001</td>
<td>80.68 ± 2.44, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Striatal complex I activity (nmol/min/mg tissue protein)</td>
<td>24.89 ± 1.69</td>
<td>15.11 ± 0.92, p&lt;0.001</td>
<td>21.37 ± 0.95, p&lt;0.001</td>
<td>27.52 ± 0.98, p&lt;0.001</td>
<td>15.69 ± 0.91, p&lt;0.001</td>
<td>21.08 ± 0.59, p&lt;0.001</td>
<td>27.71 ± 1.13, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Striatal ATP (nmol/mg tissue protein)</td>
<td>11.42 ± 0.27</td>
<td>5.60 ± 0.34, p&lt;0.001</td>
<td>8.03 ± 0.31, p&lt;0.001</td>
<td>10.55 ± 0.25, p&lt;0.001</td>
<td>5.86 ± 0.46, p&lt;0.001</td>
<td>9.30 ± 0.31, p&lt;0.001</td>
<td>11.17 ± 0.26, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Striatal Bcl-2 expression</td>
<td>0.02 ± 0.002</td>
<td>3.69 ± 0.17, p&lt;0.001</td>
<td>0.06 ± 0.02, p&lt;0.001</td>
<td>0.85 ± 0.02, p&lt;0.001</td>
<td>0.03 ± 0.02, p&lt;0.001</td>
<td>0.05 ± 0.03, p&lt;0.001</td>
<td>0.91 ± 0.01, p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM.

Scheffe test: p<0.05 vs group II (untreated parkinsonism), group III (treated with low dose Co Q10), group IV (treated with high dose Co Q10), group V (treated L-dopa), group VI (treated with low dose Co Q10 and L-dopa), group VII (treated with high dose Co Q10 and L-dopa); p<0.05 vs group VI (treated with low dose Co Q10 and L-dopa).

parkinsonism as there was a decrease in striatal complex I activity and ATP levels; those are considered as potential biomarkers for diagnosis of Parkinson disease (Savitt et al., 2006). Moreover, this proposed pathogenesis was evidenced by the significant correlation between the catalepsy score and the neurochemical parameters obtained in the current work. Rotenone is highly lipophilic and thus readily gains access to all organs including the brain, where its distribution paralleling regional differences in oxidative metabolism and accumulates in subcellular organelles such as mitochondria (Talpade et al., 2000). In mitochondria, rotenone impairs oxidative phosphorylation by inhibiting reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase activity of the multipolypeptide enzyme complex I of the electron transport chain (Schuler and Casida, 2001). The mechanism by which inhibition of complex I lead to degeneration of dopaminergic neurons, involves activation of mitochondria-dependent apoptotic molecular pathways (Waldmeier and Tatton, 2004; Ekstrand et al., 2007). It has been reported that neurotoxins inducing parkinsonian neuropathology, such as rotenone, stimulate superoxide production at complex I of the electron transport chain and also stimulate free radical production at proximal redox sites including mitochondrial matrix dehydrogenases (Fiskum et al., 2003). This oxidative stress promotes the expression and/or intracellular distribution of proapoptotic proteins to the mitochondrial outer membrane (Savitt et al., 2006). In the current study, the antiapoptotic protein Bcl-2 was found to be extremely overexpressed in the striata of rats with rotenone-induced parkinsonism. Bcl-2 overexpression was reported to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptotic process, and that neurons with lower Bcl-2 expression tended to be a compensatory response of neurons to overcome such apoptosis such as preventing release of proapoptotic proteins to the mitochondrial outer membrane (Savitt et al., 2006). In the current study (4 patients). Most of Bcl-2 protective effects against cell death have been attributed to its mitochondrial location (Kowaltowski et al., 2004). Bcl-2 temperate expression has been previously shown to act at multiple steps of mitochondrial mediated apoptosis such as preventing release of proapoptotic mitochondria.

### Table 3

<table>
<thead>
<tr>
<th>Catalepsy score</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
<th>Group VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grid test (s)</td>
<td>After 20 days (before treatment) 32.0 ± 2.13</td>
<td>34.5 ± 2.29</td>
<td>29.0 ± 2.33</td>
<td>26.5 ± 2.24</td>
<td>28.5 ± 2.48</td>
<td>29.0 ± 2.21</td>
</tr>
<tr>
<td></td>
<td>After 60 days (after treatment) 61.0 ± 3.86</td>
<td>47.0 ± 2.13***</td>
<td>32.5 ± 2.50 NS</td>
<td>28.0 ± 2.00 NS</td>
<td>23.0 ± 1.70 NS</td>
<td>10.5 ± 1.77***</td>
</tr>
<tr>
<td>Bar test (s)</td>
<td>After 20 days (before treatment) 27.5 ± 2.14</td>
<td>28.5 ± 2.24</td>
<td>27.0 ± 2.91</td>
<td>27.5 ± 2.61</td>
<td>26.5 ± 2.24</td>
<td>26.0 ± 2.08</td>
</tr>
<tr>
<td></td>
<td>After 60 days (after treatment) 52.5 ± 3.10***</td>
<td>41.5 ± 2.66***</td>
<td>28.5 ± 1.83 NS</td>
<td>24.5 ± 2.29 NS</td>
<td>22.5 ± 1.34 NS</td>
<td>10.0 ± 1.29***</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM. NS: non-significant. **: Significant at p < 0.01. ***: Significant at p < 0.001.

### Table 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grid test, r1</th>
<th>Bar test, r2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma coenzyme Q10</td>
<td>-0.826*</td>
<td>-0.690*</td>
</tr>
<tr>
<td>Striatal coenzyme Q10</td>
<td>-0.856**</td>
<td>-0.687*</td>
</tr>
<tr>
<td>Striatal dopamine</td>
<td>-0.933**</td>
<td>-0.787**</td>
</tr>
<tr>
<td>Striatal complex I activity</td>
<td>-0.724*</td>
<td>-0.736*</td>
</tr>
<tr>
<td>Striatal ATP</td>
<td>-0.834**</td>
<td>-0.845*</td>
</tr>
<tr>
<td>Striatal Bcl-2 expression</td>
<td>0.666</td>
<td>0.915**</td>
</tr>
</tbody>
</table>

*Significant at p < 0.05; **significant at p < 0.01.
It was indicated that the dosage and the resulting plasma and presumably brain levels of Co Q10 might be important determinants of its effectiveness (Storch et al., 2007). In contrast to the current work, Ibrahim et al. (2000) found that Co Q10 levels were unaltered in brain and serum of rats, but this could be contributed to the use of a lower dose for shorter duration (500 mg for 14 or 28 days) relative to that used in the present work (600 mg for 40 days), highlighting that treatment with high doses of Co Q10 for longer periods may enable its uptake into other organs including the brain. In spite of the limited uptake of Co Q10, a functional improvement in organs upon its administration was observed (28 days) relative to that used in the present work (600 mg). ICH and serum of rats, but this could be contributed to the use of a lower dose for shorter duration (500 mg for 14 or 28 days) relative to that used in the present work (600 mg for 40 days), highlighting that treatment with high doses of Co Q10 for longer periods may enable its uptake into other organs including the brain. In spite of the limited uptake of Co Q10, a functional improvement in organs upon its administration was proposed. This limitation, however, does not exclude the possibility that Co Q10 modifies organ functions, where its interaction with cell surface receptors can activate intracellular signaling mechanisms such as G-proteins, which could explain the positive effects observed (Turunen et al., 1999). Coenzyme Q10 high dose used in the current work has been proved previously to be safe in a study conducted by Honda et al. (2007), where neither death nor any toxicological signs were observed in the rats during administration period for up to 13 weeks.

Currently, medical therapy of parkinsonism continues to be levodopa mixed with a peripheral decarboxylase inhibitor, carbidopa. Combining levodopa with a peripheral decarboxylase inhibitor, such as carbidopa or benserazide significantly reduced the nausea and vomiting associated with levodopa therapy and allowed a greater proportion of levodopa to enter the brain (Savitt et al., 2006). Although its administration in the present work caused symptomatic improvement in the form of reduction of cataleptic score with restoration of striatal dopamine levels that was in concordance with other studies (Alam and Schmidt, 2004; Schmidt and Alam, 2006), but it did not show any significant effects on either striatal complex I activity, ATP levels or the expression of Bcl-2, pointing to the lack of its disease-modifying role. Despite the landmark symptomatic benefit of levodopa as a monotherapy, but this vanes with time due to the development of motor complications including wearing-off in the form of return of PD symptoms too soon after a given levodopa dose, the presence of involuntary abnormal movements, and the emergence of treatment-resistant symptoms such as gait impairment, cognitive decline, autonomic symptoms such as gait impairment, cognitive decline, autonomic abnormal movements, and the emergence of treatment-resistant symptoms such as gait impairment, cognitive decline, autonomic morbidity for the development of motor complications including wearing-off in the form of return of PD symptoms too soon after a given levodopa dose, the presence of involuntary abnormal movements, and the emergence of treatment-resistant symptoms such as gait impairment, cognitive decline, autonomic

References


