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Rotenone-induced Parkinson's Like Disease: Modulating Role of Coenzyme Q_{10}

[1]Hanan M. Abd-El Gawad, [2]Dalaal M. Abdallah and [2]Hanan S. El-Abhar
[1]Department of Biochemistry, [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Abstract: Increasing evidence has suggested an important role for environmental factors such as exposure to pesticides in the pathogenesis of Parkinson's disease (PD). Because of the potential role of mitochondrial dysfunction in striatal neurodegeneration in PD, rotenone, a potent reversible competitive inhibitor of complex I, was chosen as a possible trigger of Parkinson's-like syndrome. The loss of dopaminergic neurons in PD, besides the blockade of mitochondrial complexes, augment the formation of free radicals. Therefore, administration of the mitochondrial enhancer coenzyme Q_{10} (CoQ_{10}), with its known antioxidant activity, may be promising in attenuating the case. Male Wistar albino rats (250-300 g) were allocated into a normal control group, rotenone-induced toxicity group and rotenone + CoQ_{10}-treated group. Rotenone was injected s.c at a dose of 1.5 mg kg^{-1} every other day for a total of six injections. CoQ_{10} was administered orally at a dose of 100 mg kg^{-1} day $^{-1}$ starting from the first day of rotenone injection and continued thereafter for a total period of 11 days. The striatal biochemical parameters were assessed 24 h after the last rotenone injection. Rotenone resulted in significant decrease in the contents of dopamine (DA), glutamate and reduced glutathione (GSH) accompanied by a marked increase in malondialdehyde (MDA) level and lactate dehydrogenase (LDH) activity. However, no change was observed in the levels of superoxide dismutase (SOD) and nitric oxide (nitrite/nitrate). CoQ_{10} reduced the elevated levels of MDA and LDH, while restored that of GSH. However, the activity of SOD enzyme was stimulated. In conclusion, this study indicated the role of CoQ_{10} in ameliorating the oxidative stress associated with PD, but it was not capable of overcoming the whole negative effects of rotenone. Therefore, new approaches besides providing antioxidants that offer neuroprotection of striatal dopaminergic neurons in PD are still in need.

Key words: Rotenone, Parkinson's disease, CoQ_{10}, oxidative stress, rat

INTRODUCTION

Parkinsonism is a clinical syndrome comprising combinations of motor problems, viz., bradykinesia, resting tremor, rigidity and loss of postural reflexes[1].These symptoms are attributed to the progressive and selective loss of dopaminergic neurons in the substantia nigra[2]. Familial Parkinson's disease (PD) is suspected to be linked to mutations in several recently identified genes[3]. Unlike its familial counterpart, the cause of idiopathic PD remains unknown. Multifactorial reasons, involving genetic, environmental, trauma and possibly other factors contribute to its onset[4]. Among these factors, exposure to pesticides appears to correlate strongly with increased incidence of Parkinsonism[5], for example, chronic administration of a common herbicide, rotenone, resulted in a PD-like pathology in rats[6]. It is also implicated as a contributing factor to the increased risk of PD among farmers[7].

Rotenone is a natural product of the *Leguminosae* plant family and has been used as an insecticide, herbicide and fish poison[8] and in routine studies of mitochondrial energy metabolism[9]. It is widely believed as a safe, natural alternative to synthetic pesticides[6].

Rotenone induces toxicity and apoptosis to dopaminergic neurons through its selective inhibition of mitochondrial complex I[9], resulting in energy depletion and increased mitochondrial oxyradical production to which dopaminergic neurons are vulnerable[10]. Moreover, rotenone activates the immune brain cells, microglia, challenging it to produce a variety of pro-inflammatory and neurotoxic factors, including superoxide, nitric oxide (NO), tumor necrosis factor-" (TNF-") and interleukin-1$ (IL-1$)[11]. These pathological characters mimic those of PD since at the cellular level, the pathogenic process in patients with PD likely involves complex I deficiency in the striatum and substantia nigra, mitochondrial dysfunction, increased levels of oxidative stress and

Corresponding Author: Dr. Hanan M. Abd El-Gawad, Department of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt E-mail: hananabdelgawad@hotmail.com

apoptotic cell death[12]. In addition activation of glutamate receptors are believed to play an important role in PD[13], since blockade of N-methyl-D-aspartate (NMDA) receptors provides possible neuroprotection to nigrostriatal dopaminergic neurons[14]. Therefore, studies have validated the usage of rotenone as one of the superior models in inducing a PD-like pathology in rats[6].

The rotenone-induced midbrain-injured area is related to both dose and route of administration, where a selective damage to the striatum area occurs only after systemic administration of rotenone[15].

Coenzyme Q_{10} (CoQ_{10}) is an essential component of the electron transport chain where it serves as an electron donor and acceptor and is able to bridge a defect in the electron transport chain. It has been reported to have a powerful antioxidant efficacy[16] and to produce effectively both clinical and biochemical improvement in some patients with mitochondrial encephalopathy[17] and PD patients[12]. Moreover, CoQ_{10} protected against the striatal lesions induced by the mitochondrial toxin malonate[18] and MPTP-induced loss of striatal dopamine and dopaminergic axons[19].

Therefore, the goal of the present study was to characterize and investigate the potential usefulness of coenzyme Q_{10} in animal models of PD, using the striata of rotenone treated rats. This model was chosen to understand the impact of reduced complex I activity and CoQ_{10} on the regulation of vital cellular processes such as production of free radicals (FRs), excitotoxic neurotransmitter changes, NO production and protection of dopaminergic neurons. This aim was achieved through the measurement of the neurotransmitters, viz., dopamine, glutamate and NO as well as assessing the redox balance through determination of malondialdehyde, index of lipid peroxidation (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and lactate dehydrogenase (LDH) content/activity.

MATERIALS AND METHODS

Chemicals: All chemicals, unless specified otherwise, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Animals: Male Wistar albino rats (250-300 g) were obtained from the National Research Center Laboratory, Cairo, Egypt. They were housed in the animal facility of Faculty of Pharmacy, Cairo University, Cairo, Egypt. Rats were kept under standard conditions, fed standard chow diet and provided with water *ad libitum* along the period of the study.

Experimental protocol: Animals were divided into two sets, each of three groups. The first group served as normal control (n =8) receiving appropriate volume of dimethylsulfoxide (DMSO) and the other two groups (n=12 each) received 6 injections of a subchronic dose of rotenone dissolved in DMSO (1.5 mg kg^{-1}, s.c. every other day)[20]. One group was kept without further treatment while the other received a daily oral dose of coenzyme Q_{10} (100 mg kg^{-1})[18] starting from the first day of rotenone injection and continued thereafter for a total period of 11 days. The first set of animals was used to determine dopamine content, whereas other parameters were assessed in the second set. The rotenone-treated animals showed clinical illness after 11 days, marked by akinesia and rigidity.

Tissue sampling: Twenty four hours following the last rotenone injection, animals were sacrificed by decapitation. Brains were quickly removed, washed with ice-cold saline and the striata were isolated, weighed and homogenized using an ice-cold Teflon homogenizer (Potter Elvehjem type) for 1 min. Homogenization was carried out as 10% (w/v) either in acidified n-butanol (dopamine assay) or saline (rest of parameters).

Biochemical analysis: An aliquot of each homogenate was mixed with an ice-cold medium appropriate to the parameter being measured.

Dopamine: Homogenate was centrifuged at 600 g at 4°C for 15 min using a Dupont Sorvall Combiplus ultra-centrifuge. The supernatant was subjected to spectroflourometric assay using the principle of Ciarlone and Juras[21].

Glutamate: Equal volumes of homogenate and 1.2 M perchloric acid were mixed, centrifuged at 600 g at 4°C for 15 min and the supernatant was used according to Lund[22].

No_x (NO_2^-/NO_3^-): They were determined as described previously[23,24] in the supernatant resulting from centrifugation of homogenate at 20,000 g at 4°C for 10 min. The assay is based on the reduction of NO_3^- into NO_2^- using nitrate reductase (Roche, Mannheim, Germany) then diazotization of sulfanilic acid with the total NO_2^- in the medium with subsequent coupling with N- (1-naphthyl)-ethylene diamine. The azo dye produced was measured calorimetrically at 540 nm.

GSH: A portion of homogenate was mixed with an equal volume of ice-cold 5% sulfosalicylic acid, centrifuged at

1800 g at 4°C for 15 min. The GSH content was measured according to Beutler *et al*.[25].

MDA: Another portion of homogenate was mixed with an ice-cold 2.3% KCL (1:1), centrifuged at 600 g at 4°C for 15 min and the level of TBA-reactive substance was determined[26].

Preparation of cytosolic fraction: An aliquot of the tissue homogenate was ultracentrifuged at 105,000 g at 4°C for 45 min. The supernatant was used for the determination of SOD and LDH activities.

SOD: It was assayed using the principle of Marklund and Marklund[27] by following the inhibition of pyrogallol autoxidation at 420 nm. SOD activity was determined of a standard curve of percent inhibition of pyrogallol autoxidation with known SOD activity.

LDH: It was determined using commercially available kits (Stanbio, San Antonio, TX, U.S.A.) LDH activity was measured by monitoring the rate of increase in absorbance at 340 nm for 3 min as a result of NAD reduction into NADH[28].

Protein: It was estimated in the cytosolic fraction by the method of Lowry *et al*.[29].

Statistical analysis: The data were expressed as the mean±SE and compared using one way analysis of variance (ANOVA). Comparisons among groups were made according to Dunnett test. The significance levels were tested at p# 0.05.

RESULTS AND DISCUSSION

Subchronic treatment with rotenone led to a significant decrease in striatal dopamine and glutamate contents by 20 and 23%, respectively as compared to normal control values (Table 1). However, rotenone treated rats showed no alteration in NO level (Table 1). In addition, a marked disturbance in the oxidative status was observed as reflected by 1.5 fold elevation in the level of both MDA and LDH in comparison to the basal one (Table 2). This was accompanied by a significant decrease in GSH mounted to 67% of the normal control (Table 2). Regarding SOD, its activity was not affected by this dose regimen of rotenone (Table 2). Coenzyme Q_{10} improved the disturbed oxidative status. The content of MDA was leveled off by 54%, associated with a significant increase in GSH level (32%) (Table 2) and the LDH activity was almost normalized. On the other hand, a significant elevation in SOD activity was observed as compared to normal and rotenone-treated animals (Table 2). Conversely, both glutamate and dopamine responded negatively to that treatment (Table 1).

Rotenone is considered an excellent model for Parkinson's disease since experimental animals injected with rotenone developed rigidity and unsteady movements, reproducing the key features of Parkinson's diseased humans. Moreover, in the rotenone-treated rat's brains, the dopamine-producing circuits deteriorated and surviving cells had cellular deposits that looked a lot like Levy bodies, another hallmark of Parkinson's disease[30].

Rotenone, unlike MPP^+, can slip through any cell's membranes, causing inhibition of mitochondrial complex I activity throughout the brain, with degeneration of only the dopaminergic cells[30], production of striatal lesions, as well as lesions of the dopaminergic nigrostriatal pathway[31]. This supports the present findings, since rotenone-treated rats showed low intracellular level of dopamine in the striatum area. This effect could be explained by the vulnerability of dopaminergic neurons, more than other cells, to oxidative damage induced by the released Frs caused by rotenone disruption of complex I[30]. This vulnerability can be referred to the high concentration of dopamine that is prone to oxidative modification and to the low antioxidant capacity of these neurons[5].

This is resembled by the low density of glutathione positive cells surrounding these neurons[14], which along with glutathione peroxidase form the primary defense system of dopaminergic neurons against the reactive oxygen species (ROS). Moreover, Gao *et al*.[5] found recently that rotenone-induced activation of microglia and their release of superoxide further overwhelm these neurons. This mechanism is of great importance since the midbrain is the most microglia-enriched area in the brain[32]. Microglia, the resident immune cells in the brain, are responsible for host defense and tissue repair and their induction with rotenone produces a wide array of immunomodulatory and cytotoxic factors, including TNF-", IL-1$, eicosanoids, NO and superoxide free radical. Although the majority of these factors can participate in neurodegeneration, release of ROS remain the dominant degenerative factor for the dopaminergic neurons[5]. Moreover, impairment of energy-dependent reuptake process of dopamine may cause further decrease in its level[20].

PD is also associated with increased free iron and decreased GSH levels[33]. Rotenone also may have a possible direct effect on free iron, since in reticulocytes treated with rotenone, free iron accumulates in cytosolic proteins and mitochondria, rather than in the normal low MW pool[34].

Iron catalyzed the generation of FRs from catecholamines, with subsequent depletion of glutathione and oxidant-induced destruction of neurons. When

Table 1: Effect of coenzyme Q_{10} (100 mg kg^{-1} dayG^1, p.o.) on striatal dopamine (DA), glutamate (Glut) and nitric oxide (NO_x) contents in rotenone-induced parkinsonism in rats, (mean of six to eight animals±SEM)

	Parameters		
Groups	DA (µg gG^1 tissue)	Glut (µmol gG^1 tissue)	No_x (nmol gG^1 tissue)
Normal control	85.047±4.605	11.834±0.409	165.93±10.9
Rotenone	68.33±2.305 *	9.11±0.425 *	171. 342±12.203
Rotenone +coenzyme Q_{10}	69.61±2.334 *	9.796±0.862 *	180.366±16.051

As compared with normal control (*) group (one-way ANOVA followed by Dunnett's test), $p<0.05$.

Table 2: Effect of coenzyme Q_{10} (100 mg kg^{-1} dayG^1, p.o.) on striatal lipid peroxides (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and lactate dehydrogenase (LDH) content/activity in rotenone-induced parkinsonism in rats, (mean of six to eight animals±SEM)

	Parameters			
Groups	MDA (nmol gG^1 tissue)	GSH (µmol gG^1 tissue)	SOD (U mgG^1 protein)	LDH (µmol NADH,H+$minG^1$ mgG^1 protein)
Normal control	101.796±12.117	2.246±0.119	14.197±1.399	0.942±0.072
Rotenone	257.743±20.92*	1.513±0.046*	13. 285±0.833	1.432±0.105*
Rotenone+coenzyme Q_{10}	119.1±6.074@	1.988±0.055*@	19.213±1.038*@	0.966±0.015@

As compared with normal control (*) and rotenone-treated (@) groups (one-way ANOVA followed by Dunnett's test), $p< 0.05$.

dopamine reacts with superoxide FR, the catecholamine oxidation products can bind to non-protein sulfhydryl groups and cause rapid loss of intracellular glutathione. Moreover, these sulfoconjugation products correlate with and possibly cause, selective degeneration of dopaminergic nerve terminals[33]. These findings confirm the present results, where rotenone caused a decrease in glutathione content and add another possible explanation for the loss of striatal dopamine.

It was reported that tissue acidosis increases the level of free iron through mobilization of iron from proteins (e.g. transferrin, ferritin)[33]. Acidosis was evidenced in this study by the elevation of the cytosolic LDH activity after treatment with rotenone that correlates with the rotenone-induced increase in lactate level proven by Thiffault *et al.*[20]. In oxidative stressors, that deplete GSH and induce mitochondrial dysfunction, glucose is mainly metabolized glycolytically in astrocytes, causing the release of lactate as a metabolic substrate for neurons.

In this study, rotenone-treated rats showed a decrease in the intracellular content of glutamate. Relevant to this data that were reported in Wilson' review[33] on cytosolic glutamate content. It was stated that this decrease is an inevitable effect of energy depletion, caused by mitochondrial failure. This will cause glutamate to pass from the cytosol of neurons and astrocytes to the extracellular fluid, similar to the effect of ischemia. Moreover, acidification of the extracellular fluid by lactate stimulates astrocytic release of glutamate and slows its reuptake. In addition, ROS interact with glutamate transporters, thus slowing its clearance and further elevating its extracellular concentration. Moreover, it can be assumed that mitochondria-induced release of ROS occurs when extracellular glutamate rises to pathological level. Glutamate affects also GSH content, where the high extracellular concentrations of glutamate block uptake of cysteine into astrocytes, an amino acid that is essential for sustained glutathione synthesis. Therefore, this blockade eventually decreases glutathione concentration.

This extensive production of FRs from activated microglia, increased free iron and inhibited mitochondrial function is counterbalanced with only low amount of glutathione as a defense system, creating an imbalance in the redox system. This will elevate lipid peroxidation products, as reported in this study and as supported by Waterfall *et al.*[35]. This finding mimics that in PD, where studies of postmortem brain tissue from PD patients demonstrated increased membrane lipid peroxidation[36].

In the present study, neither NO level, nor SOD activity was altered by the administration of rotenone. In previous report, Gao *et al.*[5] stated that although rotenone-induced activated microglia produced several cytotoxic substances and NO, yet a significant accumulation of NO was not observed and the inhibition of NO production did not afford neuroprotection, pointing to the predominance of superoxide FR over NO in dopaminergic neurodegeneration. Moreover, it was found that in the striatum of MPTP treated mice, expression of mRNA for inducible NO synthase (iNOS) peaked at 24 h and started decreasing on the 3rd day[37]. Cutillas *et al.*[38] stated that rotenone did not significantly alter nitric oxide production.

Regarding SOD activity, previous works reported contradictory results. While Thiffault *et al.*[39] showed a decrease in the activity of SOD in the substantia nigra (SN) and an increase in the striatum of mice treated with MPTP, others stated no change in the cytosolic SOD in autopsied human brains with PD[40], patients with idiopathic PD[41]. These findings go in harmony with our results, reflecting the possible resistance or the high threshold of the enzyme against the evolved FRs.

Coenzyme Q_{10} (CoQ_{10}) is an essential cofactor of the electron transport chain, as well as an important

antioxidant, which is particularly effective within mitochondria[43]. Prior studies in several patients with mitochondrial disorders suggested that CoQ_{10} therapy results in both biochemical and clinical improvement[12,17,18]. It protects striatal neurons against MPTP-induced Parkinson's-like disease[44], prevents malonate-induced striatal lesions[45] and has a neuroprotective effect against glutamate neurotoxicity in cultured cerebral neurons[46]. In addition, it is considered as a putative enhancer of mitochondrial function[44] and a blocker for ATP depletion[18].

In this study, the neuroprotective efficacy of CoQ_{10} against rotenone-induced striatal lesions was reflected by a correction in the level of the endogenous antioxidant GSH, the content of lipid peroxides and the activity of the cytotoxic indicator LDH. CoQ10-induced restoration of ATP, prevention of its depletion[18] and enhancement of mitochondrial function[44] may result in decreasing the release of ROS[16]. As a result the level of both GSH and MDA was regained. Moreover, CoQ_{10} by itself has an antioxidant effect that aids in the recovery of both parameters.

Decreased activity of LDH enzyme upon administration of CoQ_{10} mirrored the recovery of mitochondrial function and energy metabolism. This finding was supported by other studies[18,47], they found a decrease in brain lactate level of patients with mitochondrial cytopathy treated with CoQ_{10}.

On the other hand, CoQ_{10}, in this study, did not normalize the level of intracellular glutamate. Although it was reported previously to protect against glutamate neuro-toxicity[46], the possible effect of CoQ_{10} against glutamate, in this study, may be related indirectly with glutamate receptors, rather than inhibiting its release or improving its uptake. This assumption may be possible since neurodegenerative diseases that involve energy impairment are followed by activation of NMDA receptors, leading to FRs regeneration[44]. In addition, CoQ_{10} offered an additive effect when combined with NMDA receptor antagonists in Huntington's disease model[43].

Dopamine content was not reinstated by this regimen of CoQ_{10}, an effect that may be influenced with the dose level used or points to the involvement of other factors besides the ROS and mitochondrial dysfunction. Although Brouillet *et al.*[48] showed that CoQ_{10} reduced significantly dopamine depletion in a striatal lesioned model, yet its level was still significantly less than the control value.

CoQ_{10} elevated SOD activity in this study, which may indicate that CoQ_{10} can increase the activity of free radical scavenging enzymes in striatum, resembling in this the action of deprenyl, the antiparkinsonian drug, on striatal SOD in rats[49].

This study concluded the efficacy of the rotenone in inducing a PD-like model, as it represents most of the hallmarks in Parkinson's disease. Moreover, these data suggest that CoQ_{10} may play a role in cellular dysfunction found in PD, where it ameliorates the oxidative stress associated with PD, however its correcting effect did not involve all cellular defects accompanied with rotenone. Therefore, although it is useful to test various combined agents, as a new therapeutic strategy in the treatment of neurodegenerative disease, yet further studies are still in need.

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