Research Report

Genetic Susceptibility Model of Parkinson’s Disease Resulting from Exposure of DJ-1 Deficient Mice to MPTP: Evaluation of Neuroprotection by Ubisol-Q_{10}

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Abstract

Introduction: Parkinson’s disease arises from a combination of environmental and genetic risk factors. At present neither the curative nor preventative therapies are available; hence, there is an urgent need to develop reliable animal models to facilitate their development. Water soluble nanomiceller formulation of CoQ10 (Ubisol-Q_{10}) has shown neuroprotection against neurotoxin on human neuronal cells. We have combined the genetic deficiency of DJ-1/PARK7 mice with MPTP exposure and developed a genetic susceptibility model of PD and evaluated the neuroprotective efficacy of (Ubisol-Q_{10}).

Methods: Transgenic mice with DJ-1 deficiency (DJ-1/PARK7) were given either water or Ubisol-Q_{10} prophylactically at a dose of 6 mg/kg/day added directly to a drinking water for one month followed challenged with MPTP injections while keeping the same drinking water regimen. Four weeks after the last injection we evaluated neuroprotective efficacy of Ubisol-Q_{10} in DJ-1/MPTP model of PD using histochemical and behavioral readouts.

Results: We confirmed genetic susceptibility to MPTP and showed that prophylactic oral treatment with Ubisol-Q_{10} significantly offset the neurotoxicity and ameliorated motor dysfunction, otherwise correlated with the MPTP injury.

Conclusion: Ubisol-Q_{10} protects against MPTP-induced neurodegeneration and motor dysfunction in DJ-1 deficient mice. Ubisol-Q_{10} might be a treatment prospect for people genetically predisposed to PD as well as with sporadic PD.

Keywords: PD, DJ-1, MPTP, genetic susceptibility, coenzyme Q_{10}, mitochondrial dysfunction

INTRODUCTION

Parkinson’s disease (PD) is one of the most common progressive neurodegenerative disorders, affecting 1–2% of all individuals over the age of 60 and this number steadily increases as the population ages [1]. The clinical diagnosis is based on the decline in motor functions (bradykinesia, rigidity and postural instability), which stem from the loss of dopaminergic (DA) neurons of the substantia nigra pars compactus (SNpc) region of the brain [2]. These symptoms develop after 60–70% of the DA neurons are already eliminated [2], highlighting the urgent need to develop more effective treatments. The disease etiology is still unknown and the majority of cases are considered sporadic. Over

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the years several environmental toxins (i.e., paraquat, MPTP, rotenone) are shown to selectively kill DA neurons in the brain. The widely used herbicide paraquat selectively induces death of the SNpc neurons in animal models and several studies indicate a greater risk of PD among subjects exposed to paraquat [3, 4]. The selective killing of DA neurons by MPTP remains one of the most commonly used PD models in research today. All these toxins are the inhibitors of complex I of the mitochondrial electron transport chain, causing neuronal death by ATP depletion and generation of oxidative stress [5]. Even under normal conditions, the DA neurons are exposed to high levels of oxidative stress due to the production of reactive oxygen species during dopamine metabolism [5]. The hypothesis for oxidative stress as a primary trigger of neuronal death has been supported by postmortem studies of PD brains [6].

In the past decade over 18 genetic loci have been linked to the familial forms of PD revealing that approx. 10% of all PD cases exhibit Mendelian inheritance. Of the many genes implicated is PARK7/DJ-1. DJ-1 is a member of C56 family of peptidases, shown in vitro to protect neurons against oxidative stress and cell death due to its putative function as a redox-sensitive chaperone and sensor for oxidative stress [7]. A homozygocity in DJ-1 loss-of-function mutation accounts for 1-2% of early-onset of PD cases [8]. Although this loss-of-function mutation predisposes humans to EOPD, no nigrostriatal degeneration was found in DJ-1 deficient mice [9]. Therefore, an animal model recapitulating the characteristics of Parkinsonian neurodegeneration needs to be developed in order to study this form of hereditary PD [10]. Here we described such a model by exposing the DJ-1 deficient transgenic mice to MPTP neurotoxicity. We used this model to test the utility of Ubisol-Q10 as a neuroprotectant.

Coenzyme Q10 has been under investigation as a potential therapy for PD for over a decade. CoQ10 levels in the brain, and other tissues, decrease with age in human and animal tissues [11]. The SNpc region has the lowest CoQ10 content in the brain [12]. The highly hydrophobic CoQ10 in oil-soluble formulations has not been successful in clinical trials, and the preclinical efficacy for neuroprotection requires extremely high doses [13, 14]. An apparently water-soluble nanomicellar formulation of CoQ10, Ubisol-Q10, was developed by the National Research Council of Canada (US Patent No. 6,045,826) by exploiting the self-emulsifying properties of polyoxyethylene-l-a-tocopherol succinate (PTS). Ubisol-Q10 showed significantly enhanced bioavailability and, consequently, neuroprotection of the SNpc neurons when given either prophylactically or therapeutically at low doses to rats exposed to paraquat and mice treated with MPTP [3, 15, 16].

In the present study we evaluated neuroprotective efficacy of Ubisol-Q10 in DJ-1/MPTP model of PD using histochemical and behavioral readouts. We confirmed genetic susceptibility to MPTP and showed that prophylactic oral treatment with Ubisol-Q10 significantly offset the neurotoxicity and ameliorated motor dysfunction, otherwise correlated with the MPTP injury. These results offer some hope for finding a preventative treatment for humans genetically predisposed to PD.

MATERIALS AND METHODS

Animal care and experimental treatments

Male DJ-1 deficient and C57BL/6 wild type mice (Jackson Laboratory) were divided into four groups: (i) control (saline-injected and drinking regular water); (ii) unprotected (MPTP-injected and drinking regular water); (iii) protected (MPTP-injected and drinking Ubisol-Q10 supplemented water containing 50 μg/ml of CoQ10 and 150 μg of PTS/ml); (iv) placebo-PTS (MPTP-injected and drinking PTS supplemented water containing 150 μg PTS/ml). Ubisol-Q10 and PTS were provided by Zymes LLC (Hasbrouck, NJ). The MPTP-injected mice received intraperitoneal (i.p.) injections of MPTP at 20 mg/kg once a day for six consecutive days [17]. Control group was injected with saline in a similar way. Animals were housed under standard conditions: constant temperature of 20°C, 12 hour dark-light cycle and free access to food and drinking solutions. The experiments were carried out for total of 8 weeks with Ubisol-Q10 and PTS supplementations starting 4 weeks prior to the MPTP injection and continued until the final evaluations 4 weeks after the last injection.

All procedures and protocols were approved by the University of Windsor Animal Care Committee (AUPP#11-07) and were carried out in accordance with the EU Directive 2010/63/EU.

Immunohistochemistry and stereological data analysis

The mice were anaesthetized and perfused with a minimum of 10 mL of Tyrode solution containing heparin. Brains were collected and fixed with PBS.
buffered 10% formaldehyde and stored at 4°C as previously described [15]. Coronal midbrain sections at 30 μm were cut on a Leica CM3050S cryostat and were immunostained with anti-tyrosine hydroxylase (TH) antibody as described before [3, 16]. The TH-positive neurons in the SNpc were counted (on one side) in every fourth brain section (total 12 sections per brain) using a Stereologer 2000 software (Stereology Resource Center Inc., Chester, MD) as described previously [15]. Statistical significance of the data was calculated using GraphPad Prism 5.

Analysis of tissue MPP+ contents

Mice received either regular drinking water or Ubisol-Q10 supplemented drinking water (30 mg CoQ10/kg body weight, ad libitum) for 2 weeks and then were challenged with a single intraperitoneal injection of MPTP (25 mg/kg body weight). They were sacrificed either 90 minutes or 4 hours post-MPTP injection. Tissues, striatum and liver, were collected and analyzed for the content of MPP+ using the HPLC method [16]. Briefly, tissues were homogenized in 10 volumes of ice-cold 0.1 M perchloric acid and 0.1 mM EDTA containing 10 μM 4-phenylpiridine (Sigma-Aldrich, Oakville) as an internal standard. Clear supernatants were injected onto a reverse-phase C18 HPLC column (4.6 × 150 mm; TSK-GEL ODS-100 S, 7 μm particle size; Tosoh Biosep LLC, Montgomeryville) equipped with a 1 mm C18 OPTI-GUARD column (Optimize Technologies, Oregon City, OR). The mobile phase, consisting of 0.02 M NaH2PO4, 3 mM tetrabutylammonium bisulfate (Sigma-Aldrich), 0.5 mM 1-heptanesulfonic acid sodium salt (Sigma-Aldrich) and 10% isopropanol adjusted to pH 2.5 with orthophosphoric acid, was delivered at a flow rate of 1.0 mL/min at ambient temperature. Eluting peaks were detected by UV at 293 nm (System Gold® HPLC, model 166 Programmable UV Detector Module; Beckman-Coulter Canada Inc., Mississauga, ON) and were analyzed using Beckman-Coulter’s System Gold 32 Karat™ software. The data is reported as nmoles of MPP+ per g of tissue and is plotted using GraphPad Prism 6.0.

Horizontal beam test: Behavioral assessment

All mice were assessed for performance on a horizontal beam-walking test for motor skills deficits by measuring the leg slips according to the previously described protocol [18]. The animal had to traverse a 1.03 m long and 6 mm by 20 mm aluminum beam to enter a ‘safe’ 20 cm³ black chamber. This apparatus was placed 0.5 m above a rectangular box containing saw dust to cushion any fall or prevent any escape. A mirror on the wall extended along the length of the beam allowed an unobstructed view of the animal as it moved towards the covered chamber. The locomotor activities of each mouse were recorded with a standard digital video camera (JVC) located 2.15 m away from the center of beam and 0.23 m above it. Video clips for each beam test session were recorded and the numbers of leg slips were counted. These data were analyzed by comparing least significant differences and by multiple post-hoc comparisons. Effects were considered significant at p ≤ 0.05.

RESULTS AND DISCUSSION

Effects of MPTP and Ubisol-Q10 on DJ-1 deficient mice

Mice were given 6 daily injections of MPTP and the surviving TH+ neurons in the SNpc region were counted 4 weeks after the last injection. This termination time point was selected based on the recent study showing that in mice subjected to this sub-chronic MPTP model the neuronal death processes are completed by 4 weeks post the last MPTP injection [16]. Consistent with the later study, we have established here that following such a sub-chronic exposure to MPTP, approx. 50% of the SNpc DA neurons in the DJ-1 deficient mice (Fig. 1B) and approx. 44% in the wild type mice (Fig. 2B) were lost at 4 weeks post-treatment. This was clearly evident on TH-stained photomicrographs of the tissue sections (Figs. 1A and 2A) as well as on stereological counts of TH-positive cells (Figs. 1B and 2B). Although, the difference in cell loss between these two strains of mice was not very big, it confirmed previously published work [17] indicating that DJ-1 deficiency rendered these mice more sensitive to the MPTP toxicity.

We have previously reported that Ubisol-Q10 protects DA neurons against paraquat toxicity and prevents degeneration of SN in rat model of PD [3]. Subsequently, we have established that Ubisol-Q10 acts as very effective neuroprotectant when given therapeutically, i.e., delivered post-neurotoxin exposure as a supplement in drinking water [15, 16]. This is true for both paraquat in rats and MPTP in mice and it is effective for as long as the supplementation continues. Upon discontinuation of the supplementation the neurodegeneration commences again and
neuronal cell death processes are reactivated [15, 16]. In the present study we addressed a question whether this formulation could prevent neurodegeneration in genetically predisposed individuals where the link between mutation in the gene and the risk of developing PD has already been established. Therefore, we tested its efficacy in a genetic susceptibility model of PD. In these experiments mice were given prophylactically Ubisol-Q10 supplemented drinking water, starting 4 weeks prior to the MPTP injections and continued for 4 more weeks after the last injection (Figs. 1 and 2). The results revealed a significant neuroprotection by Ubisol-Q10 in the MPTP-treated DJ-1 mice, with less than 20% of DA neuronal loss in the...
Fig. 2. Effects of Ubisol-Q10 on the survival of TH-positive neurons in the SNpc of MPTP-treated C57BL/6 wild type mice. The experimental treatments of the wild type mice (i.e., Ubisol-Q10 supplementation, MPTP injection) were the same as the DJ-1 deficient transgene. Three groups of mice were examined: (i) saline/H2O (saline injected and drinking regular water, control); (ii) MPTP/H2O (MPTP injected drinking regular water, unprotected); (iii) MPTP/Ubisol-Q10 (MPTP injected receiving Ubisol-Q10 supplementation, protected). (A) Representative photomicrographs of anti-tyrosine hydroxylase stained brain sections showing normal distribution of TH-positive neurons in control brains of wild type mice (saline/H2O), reduced TH-immunostaining and the loss of DA neurons in unprotected brains (MPTP/H2O) and the preservation of TH-positive cell bodies and neuronal fibers in the Ubisol-Q10 protected brains (MPTP/Ubisol-Q10). All mice were dissected one month after the last injection. The bars are: 200 μm in the upper (low magnification) panel and 20 μm in the lower (high magnification) panel. (B) Survival of TH-positive neurons in the SNpc calculated using the Stereologer 2000 software. Every 4th section of the midbrain (sectioned at 30 microns thickness) was analysed, in total 12 sections per each mice. The number of TH positive neurons in each experimental group was established and the results are expressed as percentage of neurons found in the SNpc of control brains (saline/H2O). There was a significant decrease in the number of TH - positive neurons in the unprotected group (MPTP/H2O) as compared to the saline injected control group (44% cell loss; *p<0.05, unprotected vs. control). The Ubisol-Q10 supplementation brought about nearly complete neuroprotection (only 6% neurons were lost; **p<0.05, protected vs. unprotected).

protected group (MPTP/Ubisol-Q10) as compared to nearly 50% in the unprotected (MPTP/H2O) and 40% in the placebo (MPTP/PTS) groups (Fig. 1). Even more robust neuroprotection by Ubisol-Q10 was found in the wild type mice (Fig. 2). Here, in the protected group (MPTP/Ubisol-Q10) only a negligible percentage of DA neurons were lost (approx. 6%) in comparison to 44% in the unprotected group (MPTP/H2O). In these experiments, mice received on average 6 mg CoQ10/kg/day, similar to the concentrations previously used in rats and mice [15, 16]. In the later studies we show that the neuroprotection correlated with the brain penetration of CoQ10 [15, 16]. We have also ruled out a possibility that this neuroprotection was due to an interaction between CoQ10 and MPTP preventing the generation of the neurotoxic MPP+. As shown in Fig. 3, the same levels of MPP+ were measured in tissues of control mice and mice drinking for 2 weeks Ubisol-Q10 supplemented water, even when Ubisol-Q10 was applied at a concentration 5 times higher (30 mg/kg CoQ10) than that used to achieve the described above neuroprotection (6 mg/kg). The data revealed higher contents of MPP+ at 90 minutes than at 4 hours post-injection indicating further its unaffected metabolism and showing that the presence of CoQ10 did not interfere with the brain penetration of MPP+. The neuroprotective effectiveness of Ubisol-Q10 at such a low dose is in a sharp contrast with previous preclinical data obtained on the mouse MPTP model using an oil-soluble formulation ‘Tishcon CoQ10’ [14].
The reported neuroprotection required extremely high doses of CoQ10, i.e., 400–1600 mg/kg/day, which could not be applicable in the patients’ care. On the other hand, the effective Ubisol-Q10 dose would translate to 420 mg CoQ10 per day for a 70 kg patient signifying the need for its further clinical evaluation.

Although the mechanism of neuroprotection by Ubisol-Q10 is not fully understood, evidence points toward its role in mitochondrial stabilization. The DJ-1 deficient mice have been shown to display mitochondrial dysfunction and defect in anti-oxidative defense mechanism \[7, 9\]. Ubisol-Q10 contains, potentially, two potent antioxidants CoQ10 and a pro-drug form of \( \alpha \)-tocopherol (PTS). However, tested alone PTS did not produce any neuroprotection against MPTP (Fig. 1 and [3]) suggesting that the observed effects could mainly be ascribed to the properties of CoQ10 delivered as Ubisol-Q10. In already published in vitro studies Ubisol-Q10 prevents oxidative stress-induced neuronal cell death and inhibits Bax-induced mitochondrial destabilization \[19, 20\]. Ubisol-Q10 could very well maintain the integrity of mitochondria and quench ROS produced in the cells. Ubisol Q10 might be able to compensate to some extent for the deficiency of DJ-1 function as it relates to the mitochondrial integrity and anti-oxidative defense mechanisms.
displayed a higher number of leg slips on each of the three post-injection beam tests than the mice in saline injected control group or the protected MPTP/Ubisol-Q10 group, neither of which showed any significant changes from their respective baseline. These observations were confirmed by a significant interaction between groups and sessions, F(6, 57) = 5.32, p = 0.03. This interaction effect was not significant for sessions involving only the MPTP/water group, F(3, 21) = 6.893, p = 0.002, suggesting that these animals significantly increased their post-injection leg slips above their last pre-injection session level. The performance of DJ-1 deficient mice on the beam test correlated with the MPTP-induced loss of DA neurons. In summary, we have developed a genetic susceptibility model of PD by combining the DJ-1/PARK 7 defects with the systemic exposure to MPTP. We confirmed the hypersensitivity of DJ-1 transgenic animals to MPTP and demonstrated a clear neuroprotection by a prophylactic use of Ubisol-Q10. Since Ubisol-Q10 is already GRAS approved by the FDA, it represents a promising and realistic prospect of preventative therapy for people genetically predisposed to PD.

AUTHOR’S CONTRIBUTIONS

KM, JS, MS, JKS, JC and SP contributed to the planning and execution of the experiments and writing the manuscript. KM, JS and HJ were involved in performing injection and feeding of different regiments, dissections, immunohistochemical analysis and biochemical analysis. JC, DL, KM and JS were involved in the design and execution of the beam test and animal care.

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