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Minocycline, levodopa and MnTMPyP induced changes in the mitochondrial proteome profile of MPTP and maneb and paraquat mice models of Parkinson's disease

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ABSTRACT

Mitochondrial dysfunction is the foremost perpetrator of the nigrostriatal dopaminergic neurodegeneration leading to Parkinson's disease (PD). However, the roles played by majority of the mitochondrial proteins in PD pathogenesis have not yet been deciphered. The present study investigated the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and combined maneb and paraquat on the mitochondrial proteome of the nigrostriatal tissues in the presence or absence of minocycline, levodopa and manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP). The differentially expressed proteins were identified and proteome profiles were correlated with the pathological and biochemical anomalies induced by MPTP and maneb and paraquat. MPTP altered the expression of twelve while combined maneb and paraquat altered the expression of fourteen proteins. Minocycline, levodopa and MnTMPyP, respectively, restored the expression of three, seven and eight proteins in MPTP and seven, eight and eight proteins in maneb- and paraquat-treated groups. Although levodopa and MnTMPyP rescued from MPTP- and maneb- and paraquat-mediated increase in the microglial activation and decrease in manganese-superoxide dismutase expression and complex I activity, dopamine content and number of dopaminergic neurons, minocycline defended mainly against maneb- and paraquat-mediated alterations. The results demonstrate that MPTP and combined maneb and paraquat induce mitochondrial dysfunction and microglial activation and alter the expression of a bunch of mitochondrial proteins leading to the nigrostriatal dopaminergic neurodegeneration and minocycline, levodopa or MnTMPyP variably offset scores of such changes.

1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the selective degeneration of dopaminergic neurons of the nigrostriatal pathway. Resting tremor and impaired movement and coordination are reported to be the major symptomatic features of the disease [1,2]. Loss of dopaminergic neurons depletes dopamine content in the dorsal striatum [1]. Anatomically, the disease is characterized by the formation of intra-cytoplasmic protein aggregates called Lewy bodies, in the adjacent neurons [1]. While PD is mainly an aging related disease, inputs of the genetic factors and environmental exposure to pesticides and heavy metals have also been well documented [2]. Several animal models have been developed to study the cellular and molecular pathogenesis and to ascertain the effective therapy to encounter PD [2]. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) not only reproduces some of the basic PD features in primates and rodents but is also regarded as a well established model [1]. Similarly, two commonly used pesticides namely manganese ethylene-bis-dithiocarbamate (maneb) and N,N′-dimethyl-4,4′-bipyridinium dichloride (paraquat)

Abbreviations: PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Maneb, manganese ethylene-bis-dithiocarbamate; Paraquat, N,N′-dimethyl-4,4′-bipyridinium dichloride; MnTMPyP, manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin; Mn-SOD, manganese superoxide dismutase; AOP1, antioxidant-like protein 1; Prx3, peroxiredoxin 3; IDH3α, isocitrate dehydrogenase 3 (NAD^+) α; VDAC, voltage dependent anion channel; BCIP/NBT, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium salt; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; BSA, bovine serum albumin; DHBA, 3,4-dihydroxybenzylamine hydrobromide; $MgCl_2$, magnesium chloride; PMSF, phenylmethylsulfonyl fluoride; TFA, trifluoroacetic acid; IPG, Immobiline pH gradient; NaCN, sodium cyanide; KH_2PO_4, potassium dihydrogen orthophosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NADH, reduced nicotinamide adenine dinucleotide disodium salt; TH, tyrosine hydroxylase; PVDF, Polyvinylidene difluoride; CBB, coomassie brilliant blue; SDH, succinate dehydrogenase; LDH, lactate dehydrogenase; 2-D PAGE, two-dimensional polyacrylamide gel electrophoresis; MALDI-TOF/TOF, matrix assisted laser desorption/ionization-time of flight/time of flight; SEM, standard error of means; DLST, dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex; COX 5a, cytochrome c oxidase subunit 5a; DRP-2, dihydropyrimidinase-related protein-2; Prx2, peroxiredoxin 2; PDH E1α, pyruvate dehydrogenase E1 component subunit α; PEBP1, phosphatidylethanolamine-binding protein 1; UCH-L1, ubiquitin carboxyl-terminal hydrolase isozyme-L1

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in combination also mimic several cardinal features of PD in mice [2]. The combined maneb and paraquat model is environmentally relevant and exhibits the slow and progressive nigrostriatal dopaminergic neurodegeneration similar to sporadic PD [2].

Due to multi-factorial and sporadic nature of PD, all animal models developed so far have some or the other limitations [2]. While transgenic models elucidate the roles played by the selected genes, pathological aberrations in sporadic PD are contributed by multiple genes rather than a few selected genes [3,4]. MPTP and combined maneb and paraquat models were selected over the transgenic models, as environmental toxins are implicated in PD pathogenesis [1,2]. While rotenone model is environmentally relevant and is better than MPTP or combined maneb and paraquat in a few aspects, such as distinct Lewy body formation, it non-specifically affects the brain. MPTP and combined maneb and paraquat models were preferred over the rotenone model since MPTP and paraquat are structurally alike, their preferential target is the nigrostriatal dopaminergic neurons and they lead to the mitochondrial dysfunction and subsequent free radical generation almost in the similar fashion [1,2].

The mitochondrion acts as an epicenter of PD pathogenesis since its impaired function is associated with sporadic PD and a few toxicant-induced PD models [1]. MPTP and combined maneb and paraquat inhibit the mitochondrial complex I and/or III [2,5,6] and induce oxidative stress, neuroinflammation and microglial activation, which subsequently lead to the nigrostriatal dopaminergic neurodegeneration [2,7]. Albeit MPTP and combined maneb and paraquat inhibit the mitochondrial complex I, underlying mechanisms have been found to be unrelated. MPTP inhibits ATP biosynthesis owing to the inhibition of electron transfer from iron–sulfur cluster of complex I to ubiquinone [8] while paraquat generates free radicals mainly through redox cycling by withdrawing the electron from the mitochondrial complex I enzyme [6]. Such differences raise the possibility that diverse steps could be involved after complex I inhibition in both the models.

Proteomic approaches have been used to identify MPTP-induced changes in the expression of multiple proteins associated with defective energy metabolism, ubiquitin proteasome system, apoptosis and mitochondrial function [2]. A few proteins, which decide the fate of the genetic forms of PD, are localized in or interact with the mitochondria and mitochondrial dysfunction could affect neuronal survival [9]. MPTP and maneb and paraquat have been shown to affect the mitochondrial protein complexes, their effects on the proteins of the mitochondrion and the roles of such proteins in the mechanisms of neurodegeneration have not been fully characterized. Mitochondrial proteomics could be used as a precise tool to explicate the inputs of the novel mitochondrial proteins in MPTP- or maneb- and paraquat-induced PD.

Minocycline is a clinically available antibiotic and is an anti-inflammatory molecule. Its neuroprotective efficacy is reported against many neurological disorders employing animal models [10]. Minocycline is also used in animal models of Parkinsonism and found to inhibit microglial activation and neuroinflammation, two key events involved in PD pathogenesis [11,12]. Therefore, the present study investigated the effect of minocycline against toxin-induced rodent models of PD. Similarly, levodopa, a dopamine precursor (in combination with 3,4-dihydroxyphenylalanine decarboxylase inhibitor-carbidopa), is extensively used to ameliorate motor dysfunction and other deleterious effects of dopamine depletion against chemically-induced Parkinsonism [13–15]. While levodopa is used to improve symptomatic features, conflicting reports are available in literature and a few studies failed to detect protective or toxic effect [13]. Toxic potential of levodopa is described in culture cells but in vivo experimentations failed to unequivocally demonstrate whether levodopa accelerates degeneration of dopaminergic neurons of the substantia nigra and causes permanent impairment of their function or not [13]. Effect of levodopa largely depends on the route of its administration, dose and time of exposure [16].

Role of oxidative stress in pesticides-induced Parkinsonism and altered level of superoxide dismutase (SOD) in PD patients are widely reported [6,17,18]. Use of a levodopa or SOD/catalase mimetic, such as MnTMPyP, could rescue a compromised antioxidant defense system. Moreover, superoxide ion is produced as a result of electron transfer reactions occurring within the mitochondria, assessment of the effect of MnTMPyP on the mitochondrial proteome could highlight the link between oxidative stress and mitochondrial dysfunction. Deficiency of manganese-superoxide dismutase (Mn-SOD) or its altered expression is also associated with oxidative stress caused by paraquat or MPTP [6,17]. Metalloporphyrins, synthetic SOD/catalase mimetics, protect against MPTP- and paraquat-induced toxic effects in rodents [19,20]. Manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP), a metalloporphyrin, inhibits lipopolysaccharide-induced free radical generation and dopaminergic neurodegeneration [21]. Chemical entities, which restore dopamine content, attenuate oxidative stress and inhibit microglial activation, could resist the mitochondrial dysfunction and subsequent events leading to neurodegeneration [17,22,23]. Albeit several studies are performed to measure the neuroprotective efficacies of minocycline, levodopa and MnTMPyP against PD [12,14,21], studies relating with their effects on the mitochondrial proteome have been limited. A few studies based on the mitochondrial proteomics are conducted using MPTP model [24–26] but not even a single study describing the mitochondrial proteome profile of combined maneb and paraquat model is reported to date with the best of our knowledge. Comparative mitochondrial proteome patterns of MPTP and maneb and paraquat at the doses, which induce PD phenotype, could offer clues to understand the similarities and discrepancies between the two. Moreover, changes in the mitochondrial proteome profiles of MPTP and combined maneb and paraquat in the presence or absence of levodopa, minocycline and MnTMPyP could help in identifying their variable effects and mode of actions and elucidating the disparities, if any.

2. Experimental procedures

2.1. Chemicals

Acetonitrile, acrylamide, ammonium bicarbonate, ammonium persulphate, anti-antioxidant-like protein 1 (AOP1)/peroxiredoxin (Prx) 3, anti-isocitrate dehydrogenase 3 (NAD+) α (IDH3α) and anti-voltage dependent anion channel (VDAC) primary antibodies, anti-mouse/rabbit biotin conjugated secondary antibody, antimycin, MPTP, alkaline phosphatase chromogen containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) liquid substrate, bromophenol blue, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 3,3′-diaminobenzidine liquid enhanced system, 3,4-dihydroxybenzylamine hydrobromide (DHBA), dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), fatty acid free bovine serum albumin (BSA), Folin Ciocalteu's reagent, 3-hydroxytyramine hydrochloride, magnesium chloride ($MgCl_2$), mannitol, maneb, N,N′-methylene bisacrylamide, NBT salt, nonidet P-40, paraformaldehyde, paraquat, protease inhibitor cocktail, phenylmethylsulfonyl fluoride (PMSF), rotenone, sodium cyanide (NaCN), sodium deoxycholate, sodium dodecyl sulfate (SDS), sodium fluoride, sodium orthovanadate, sodium pyruvate, sodium succinate, tris-base, N,N,N′,N′-tetramethylethylenediamine, trifluoroacetic acid (TFA), tween-20, triton X-100, ubiquinone and urea were procured from Sigma-Aldrich, St. Louis, MO, USA. Immobiline pH gradient (IPG) strips, IPG buffers and dry strip cover fluid were obtained from GE Healthcare, Chalfont, St. Giles, UK. Copper (II) sulfate 5-hydrate, formaldehyde, glycerol, methanol, MnTMPyP, potassium chloride, potassium dihydrogen orthophosphate (KH_2PO_4), potassium sodium tartrate, silver nitrate, sodium carbonate and sodium thiosulphate were procured from Merck Biosciences, Darmstadt, Germany. Acetic acid, agarose, cytochrome c, dibutyl phthalate xylene, disodium hydrogen