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METALLOTHIONEINS AS EARLY AND SENSITIVE BIOMARKERS OF REDOX SIGNALING IN NEURODEGENERATIVE DISORDERS

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ABSTRACT

***Metallothioneins (MT-1-4) are versatile, redox-sensitive, low molecular weight cysteine-rich, metal binding proteins, which were discovered for the first time by Marghoshes and Vallee in horse kidneys and in the rodent brain by our group. It is now well recognized that MTs are capable of preventing oxidative stress and apoptotic cell death in the CNS. Increasing body of evidence suggests that MTs promote neuronal survival and regeneration in vivo. MTs are neuroprotective against, metal ion toxicity, oxidative stress, and cytokines injury due to cerebral ischemia or infection; hence could be considered as early and sensitive biomarkers of redox signaling in neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), Multiple System Atrophy (MSA), stroke, and epilepsy. However the exact molecular mechanism of MTs-mediated neuroprotection in CNS in these and other neurodegenerative disorders remains elusive. By using MTs gene manipulated mice and aging mitochondrial knock out (RhOmgko) cybrids as experimental models of PD and microPET neuroimaging with 18F-DOPA and 18FdG, we have established that MTs may provide dopaminergic neuroprotection by (i) augmenting coenzyme Q10 (CoQ10) synthesis, (ii) attenuating α-Synuclein (α-Syn) nitration (iii) preserving mitochondrial glutathione,(iv) enhancing neuromelanin synthesis, (v) preserving ferritin, (vi) preventing metal ion accumulation, (vii) acting as free radical scavengers, attenuating peroxynitrite ion neurotoxicity, maintaining intracellular redox balance, or through all these mechanisms. Whether, augmentation of CoQ10, glutathione, ferittin, melatonin, and neuromelanin synthesis in metallothionein transgenic (MTtrans) mice CNS occurs independently, is dependent on each other, or occurs synergistically remains unknown. Although we have discovered that 3-morpholinosydnonimine (SIN-1) and 1-methyl 4-phenyl, 1,2,3,6-tetrahydropyridine (MPTP)-induced nitration of α-Syn is attenuated in MTtrans mice striatum, we know very little about the exact functional significance of these findings. In this report, we have focused on the neuroprotective role of MTs in SIN-1 and MPTP-induced oxidative and nitrative stress with a primary objective to explain the basic molecular mechanism of MTs-mediated neuroprotection in PD and other neurodegenerative disorders. We have now proposed that MTs are capable of inhibiting broadly classified neurodegenerative α-synucleinopathies*.

Key words: *Metallothioneins; peroxyynitrite; α-synuclein nitration; biomarkers; α-Synucleinopathies; neuroprotection*

[I] INTRODUCTION

Metallothioneins (MTs), a class of low molecular weight, cysteine-rich, ubiquitous intracellular proteins with high affinity for metal binding including zinc, occur in all eukaryotes, was first identified in the horse kidneys [1] and subsequently in the rodent brain [2]. Rodents possess four isoforms of MTs (MT-1 to MT-4) [3]. Only three isoforms are expressed in the brain namely MT-1+2 (which are also widely expressed and regulated coordinately) and MT-3 (also known as growth inhibitory factor). MTs bind zinc and copper and function in metal ion regulation and detoxification in the CNS as well as peripheral tissues [4].

Recent evidence suggests that MTs could be significant antioxidant proteins as these proteins are dramatically increased in brains of GFAP-IL6 transgenic mice as a physiological adaptation to cope with the CNS injury due to induced cytokine trigger [5]. Cross-breeding GFAP-IL6 mice with MT-1+2 null mice provided a progeny with significantly altered CNS structure as well as function, suggesting that MT-1+2 proteins are valuable

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factors against cytokines-induced CNS injury [6]. Furthermore, high throughput gene screening using serial analysis of gene expression (SAGE) has provided evidence that MT-2 is an important neuroprotective gene as it is three fold induced within 2-16 hrs of focal cerebral ischemia [7].

Although the exact cause of neurodegeneration of nigrostriatal dopaminergic (DA) neurons in PD, particularly among aging male white population remains unknown, increase in mitochondrial iron [8-17], calcium overload [18], lipid peroxidation [19,20], superoxide dismutase (SOD) [21-24] , haem oxygenase-1 [25], reduction in ferritin/transferrin receptors [26-29], ubiquinone-NADH oxido-reductase (complex-1), glutathione peroxidase, glutathione ascorbate [30-32], calcium binding proteins [33,34], neuromelanin [35-38], dietary folate deficiency and elevated homocycteine [39], dopamine autooxidation [40,41], and numerous other possible factors have been implicated in the etiology of PD. Some of these observations have been reproduced in animal models using 6-hydroxy-dopamine (6-OH-DA), MPTP, iron overloading, and β-carbolines, although none of them represent accurate model for PD in humans [42]. Recently we have reported that iron can induce endonuclear translocation of α-Syn and disrupt mitochondrial oxidative phsophorylation, which is prevented by specific iron chelator, deferoxamine in the SK-N-SH neurons [43]. Iron-induced NFκβ induction and neurotoxicity were attenuated by CoQ10 treatment [44]. Current chemotherapy of PD in addition to symptomatic Levo-DOPA treatment, includes neuroprotective strategies with antioxidants such as Selegiline, Rasalgine, and free radical scavengers such as CoQ10 [45, 46] . However their clinical applicability forms a major challenge for future research. It has been reported that CoQ10 could prevent cognitive decline in aging PD and AD patients and its beneficial effects are related to the dose administered. CoQ10 was well-tolerated up to 1200 mg/day without side effects [45, 46]. Although several possible molecular mechanisms of MTs-induced neuroprotection have been proposed, based on our discoveries we have proposed MTs-induced CoQ10-mediated neuroprotection in PD [47]. Furthermore, we have proposed that MTs can serve as early and sensitive biomarkers of neuroprotection as these versatile proteins are directly implicated in inhibiting neurodegenerative α-Synucleinopathies as discussed in this brief report.

1.1. Experimental Models of a-Synucleinopathies

We developed α-Synuclein-MTs triple knockout mice (α-Syn-MTtko) mice, MTs-over-expressing weaver mutant (wv/wv-MTs) mice, and aging mitochondrial genome knock out (RhOmgko) dopaminergic (SK-N-SH) neurons in culture as experimental models of PD in our labs. MTs gene manipulated mice and aging mitochondrial knock out (RhOmgko) cybrids were used with a primary objective to explore the basic molecular mechanism(s) MTs-mediated of neuroprotection in neurodegenerative disorders. MT-1, 2 and ferritin expression in RhOmgko neurons was reduced, while α-Syn expression was elevated. RhOmgko neurons had significantly high α-Synuclein indices (Nitrated α-Syn/native α-Syn), intramitochondrial metal ions (Fe3+, Cu2+, Zn2+, and Ca2+), and reduced MTs and ferritin. Mitochondrial glutathione, SOD, and catalase activities were also down-regulated in RhOmgko neurons. Transfection of RhOmgko neurons with ubiquinone-NADH –oxidoreductase (complex-1) gene partially restored the antioxidant balance and preserved ferritin and MTs function. MPP+-induced capase-3 activation, protein carbonylation, nitration, lipid peroxidation, and 8-OH-2dG synthesis were also attenuated upon transfecting RhOmgko neurons with complex-1 gene. Furthermore, transfection of SK-N-SH neurons with MTsense reduced, with MTantisense increased, and with MT-1scarmbled oligonulceotides did not produce significant change in mitochondrial 8-hydroxy 2-deoxy guanosine (8-OH-2dG) levels, suggesting that MTs-mediated CoQ10 synthesis provides neuroprotection in dopaminergic neurons. Hence MTs gene induction or treatment strategies to enhance brain regional MTs would provide neuroprotection in various neurodegenerative disorders [47].

1.2. MTs provide CoQ10-mediated neuroprotection

Although beneficial effects of CoQ10 have been reported, the exact molecular mechanism of neuroprotection is yet to be established. We have discovered that MTs provide CoQ10-mediated neuroprotection hence could be used as early and sensitive biomarkers of redox signaling in PD and other neurodegenerative disorders [47]. We have hypothesized that brain regional MTs induction provides neuroprotection through zinc-mediated transcriptional regulation of α-Syn in the dopaminergic neurons. In the absence of MTs, α-Syn can be easily nitrated and aggregated in the perinuclear and endonuclear regions of dopaminergic and other neurons. Enhanced aggregation of α-Syn due to metal ions, oxidative and nitrative stress may also trigger Lewy body synthesis in aging brain. A detailed study is therefore required in this direction, which will provide further insight in pinpointing the exact molecular mechanism(s) of neurodegenerative α-Synucleinopathies such as Parkinson's disease (PD), Alzheimer's disease (AD), multiple system atrophy (MSA) and their effective treatment by brain regional MTs induction as illustrated in **Figure– 1**.

1.3. Molecular mechanism(s) of MTs-induced neuroprotection

The precise neuroprotective mechanism of MTs isoforms in PD and aging CNS remains elusive. Earlier studies suggest that MTs could serve as antioxidant proteins in the CNS [48-50]. We have discovered that MPTP-induced nitration of α-Syn is attenuated in MTtrans mice striatum and Selegiline provides neuroprotection by inducing brain regional MTs [51]. Furthermore, MTs provide neuroprotection through mitochondrial BCl-2 up-regulation, Bax down-regulation, and caspase-3 inhibition [52]. MT isoforms attenuated α-Syn nitration and provided CoQ10-mediated neuroprotection against MPTP neurotoxicity [51, 52]. MTtrans mice synthesized increased neuromelanin (NM) in the substantia niga (SN) and were resistant to MPTP neurotoxicity as compared to MTdko mice in which SN ferritin and neuromelanin (NM)

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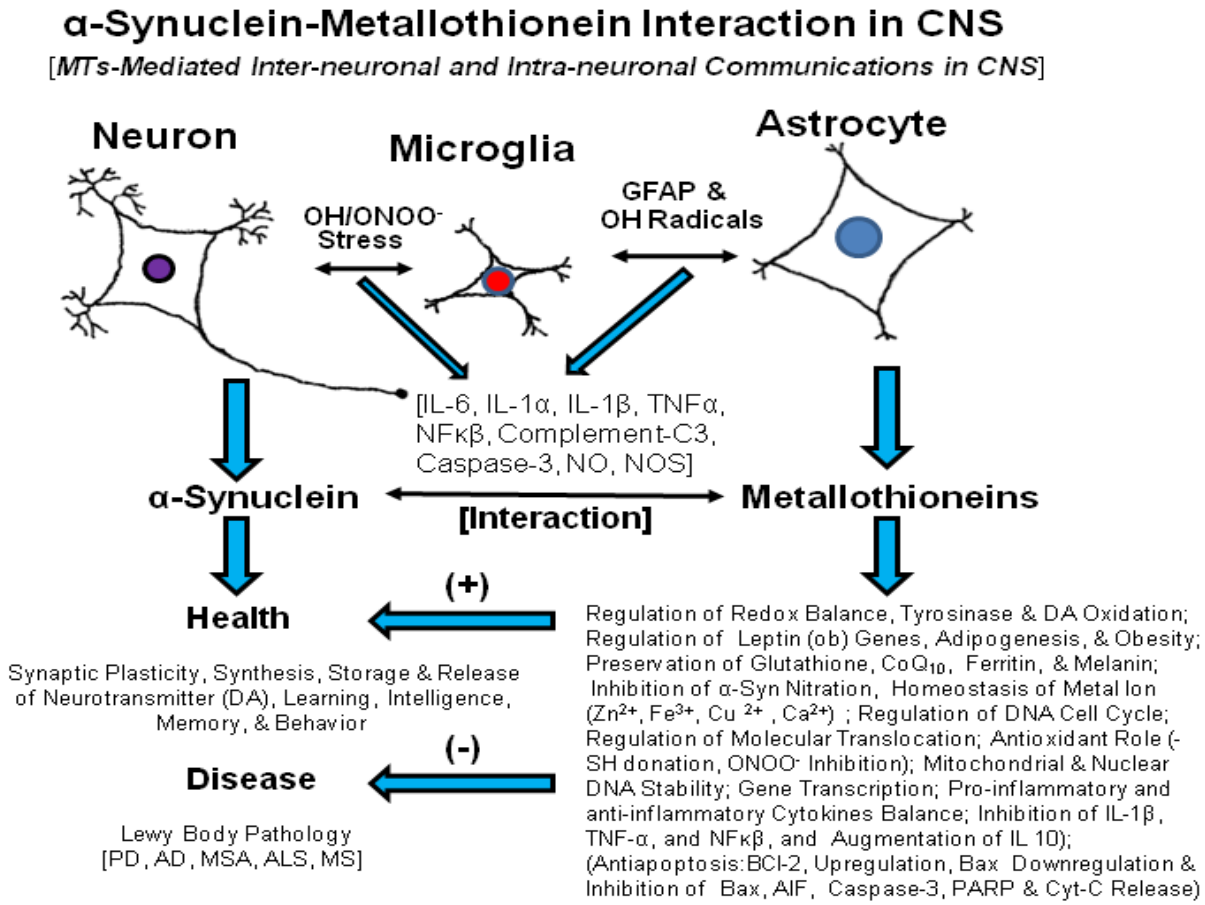
were significantly reduced. These findings have led us to believe that MTs can be used as early and sensitive biomarkers of redox signaling in neurodegenerative disorders including PD, and AD, and stroke. However, the exact functional significance of enhanced NM synthesis in the substantia nigra (SN) of MTtrans mice, reduced NM synthesis in MTdko mice, and its relevance to Parkinsonism is yet to be established.

1.4. Genetic resistance of MTtrans striatal fetal Stem cells to PNs

We have discovered that MTs attenuate 3-morpholinosydnonimine (SIN-1; a potent ONOO- ion generator)-induced oxidative and nitrative stress in the dopaminergic neurons [51, 52]. The striatal fetal stem cells derived from MTtrans mice were resistant to SIN-1-induced lipid peroxidation, caspase-3 activation, and apoptosis. MTtrans striatal fetal stem cells exhibited reduced phosphatidyl serine externalization, plasma membrane perforations, DNA fragmentation, and condensation in response to SIN-1-induced lipid peroxidation as compared to controlwt cells. SIN-1-induced apoptosis was characterized by rounded appearance with reduced neuritogenesis. Controlwt cells exhibited typical membrane perforations, nuclear DNA fragmentation, and condensation in response to SIN-1. SIN-1 induced membrane perforations, DNA condensation and fragmentation in controlwt fetal stem cells. These apoptotic events were attenuated in MTtrans fetal stem cells. MTtrans striatal fetal stem cells also exhibited genetic resistance to dopamine oxidation product, dihydroxy phenyl acetaldehyde (DOPAL)-induced apoptosis. DOPAL-induced apoptosis in controlwt fetal stem cells was represented by peri-nuclear accumulation of mitochondria and translocation of MT-1 in the endonuclear region. DOPAL -induced apoptotic changes were attenuated in MTtrans fetal stem cells.

Fig. 1



Possible Molecular Mechanism of MTs-Mediated Neuroprotection
MTs Inhibit Oxidative & Nitrative Stress to Prevent Neurodegenerative α-Synucleinopathies

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1.5. Genetic susceptibility of aging rhOmgko neurons

Aging mitochondrial genome knock out (RhOmgko) dopamineric (SK-N-SH) neurons were highly susceptible to Parkinosnian neurotoxins (PNs: MPP+, 6-OH-DA, Rotenone, and Salsolinol) and exhibited compromised neuronal recovery in response to antioxidants (Selegiline, CoQ10, and Melatonin). Aging RhOmgko neurons were elliptical in shape, exhibited typical granular appearance, and reduced neuritogenesis. CoQ10 levels were also significantly reduced in RhOmgko neurons. CoQ10 and neuritogenesis were partially restored upon transfecting RhOmgko neurons with mitochondrial genome encoding ubiquinone-NADH-oxidoreductase (Complex-1). Aging RhOmgko neurons exhibited reduced mitochondrial membrane potential ($\Delta\Psi$). Upon chronic exposure to PNs, RhOmgko neurons released cytochrome C and induced further apoptosis, represented by typical zones of growth inhibition. We developed multiple fluorochrome Comet tail assays to further establish the genetic susceptibility of aging RhOmgko neurons. Mitochondrial DNA from aging RhOmgko neurons was susceptible to MPP+-induced neurotoxicity as compared to nuclear DNA. RhOmgko neurons had higher levels of DNA oxidation product, 8-hydroxy, 2-deoxy guanosine (8-OH-2dG), which introduces point mutations by AT to GC transversions. As a matter of fact α-Syn over-expressed RhOmgko neurons exhibited enhanced DNA damage in response to overnight exposure to MPP+ as revealed by significantly increased Comet tails and 8-OH, 2dG synthesis compared to controlwt SK-N-SH neurons.

1.6. Multiple genes RT-PCR analysis and MTs neuroprotection

We have investigated the transcriptional activation and inactivation of multiple candidate genes involved in neurodegeneration and neuroprotection by employing multiple gene RT-PCR analysis. During exposure to PNs (MPP+, 6-OHDA, Rotenone, and Salsolinol), various apoptotic genes were transcriptionally activated in the DA-ergic (SK-N-SH) neurons. Pre-treatment with antioxidants (Selegiline, CoQ10, and Melatonin) attenuated these neurodegenerative changes and provided neuroprotection by increasing the expression of redox-sensitive genes (MT1-, BCl2, mitochondrila genome (MG), poly-ADP- ribosyl polymerase (PARP). SIN-1, MPP+, and 6-OH-DA significantly enhanced c-fos, c-jun, caspase-3, and α-Syn expressions and inhibited PARP, BCl2, and MG expressions. Furthermore, Selegiline pre-treatment significantly attenuated SIN-1, MPP+, and 6-OH-DA-induced changes in gene expression involved in DA-ergic neurodegeneration [52]. Several other candidate genes might be induced or repressed simultaneously during the progression of PD. To further explore MTs-mediated neuroprotection, it would be interesting to investigate various other redox-sensitive genes by microarrays biotechnology that are implicated in neurodegeneration and/or neuroregeneration using various PNs such as SIN-1, 6-OH-DA, MPTP, and Salsolinol-induced experimental models of oxidative and nitrative stress. Studies in this direction may provide a better functional relationship between MTs and CoQ10 and perhaps furnish novel therapeutic strategies in PD and other neurodegenerative disorders such as AD and stroke. For details please refer [53, 54].

1.7. Induction and translocation of MTS

Cell culture studies have shown that induction and translocation of MTs in the nucleus is to protect from DNA damage, apoptosis, and regulate gene expression during certain stages of the cell cycle [55]. MTs can bind directly with ONOO- to prevent DNA and lipoprotein damage [56]. [3H]NMR-TCOSY spectroscopic and scanning tunneling microscopic studies have demonstrated that MTs bind with ATP across the mitochondrial membranes to become conformationally-active and regulate electron transport chain through zinc release [57]. Similar to MT-1 and MT-2, MT-3 isoform protect against DNA damage induced by Fe3+ and H_2O_2, which is inhibited by alkylating –SH groups by treatment with ethylene diamine tetra- acetic acid (EDTA) and N-ethylmelameide. Furthermore, MT-3 scavenged reactive oxygen species (ROS) and superoxide ions, generated by xanthine/xanthine oxidase system to provide neuroprotection [58].

We have recently discovered that MT-1 is translocated to endonuclear region in response to MPP+ in the mice striatal fetal stem cells [59]. We also have proposed that MTs gene susceptibility might be one of the several possible molecular mechanisms of Parkinsonism and other neurodegenerative disorders among aging white population. Hence MTs may be used as multipurpose, early and sensitive diagnostic indicators of neurodegenerative process. MTs induction in CNS during aging may provide genetic resistance to PD. This hypothesis was supported by our recent discoveries demonstrating that SN neuromelanin of MTtrans mice is significantly elevated [59]. Furthermore, SIN-1-induced ONOO-mediated oxidative and nitrative stress in the DA-ergic neurons is attenuated by MT-1 gene induction in the mice striatum and SK-N-SH neurons [59]. MTs act as potent scavengers of free radicals by engaging their –SH moieties on the cysteine residues. CoQ10 and glutathione also provide neuroprotection by acting as potent free radical scavengers. However, it remains unknown whether glutathione and neuromelanin increase their metabolism or reduce their catabolism. Indeed brain regional CoQ10 and glutathione in the striatum and NM in substantia nigra are higher in MTtrans as compared to controlwt and MTdko mice. Moreover the striatal CoQ10 remained preserved even after chronic treatment of MPTP in MTtrans mice, further confirming our hypothesis that MTs provide neuroprotection by augmenting mitochondrial bioenergetics [59].

1.8. MTs provide neuroprotection by preserving neuronal ferritin

Following neurotoxin exposure, both α-Syn and MTs are induced and translocated in the perinuclear and endonuclear regions. Induction and translocation of α-Syn was attenuated by Selegiline pre-treatment. Similarly, overnight exposure to SK-N-SH neurons to $FeSO_4$ induced lipid peroxidation and structural degradation of plasma membrane. $FeSO_4$ induced molecular

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translocation of α-Syn in the nuclear region, while ferritin remained restricted to the cytosolic region. Since ferritin is a large molecular weight protein (440 kDa), while MT-1 and α-Syn are low molecular weight proteins (6-7 kDA & 17 kDa respectively); during oxidative and nitrative stress, ferritin remains restricted to the cytoplsamic regions, where as MT-1 and α-Syn can translocate freely in the mitochondrial and nuclear compartments and vice versa to provide neuroprotection. A further study is required in this direction to pinpoint the exact functional significance of ferritin in relation to α-Syn and MTs in progressive neurodegenerative disorders [59].

1.9. Neuroprotection by MTs genes

To establish the neuroprotective potential of MTs, we used MTs gene manipulated mice and cultured human dopaminergic (SK-N-SH) neurons and examined the effect of various Parkinsonian neurotoxins (PNs) and antioxidants. MT-transgenic (MTtrans) mice are black and lean, agile and vigilant, where as MTdko mice are brown and obese, with lethargic and reduced vigilant status. They have reduced body hair and SN neuromelanin (NM), developed skin de-pigmentation, and increased susceptibility to PNs, such as MPTP, 6-OH-DA, rotenone, and Salsolinol as a function of aging. Treatment with CoQ10 (10 mg/kg i.p) for 7 days partially alleviated neurodegenerative symptoms in aging MTdko mice. Leptin (ob) gene mRNA expression and abdominal adipose tissue were also increased in MTdko mice as compared to controlwt and MTtrans mice during sexual maturity. MTtrans mice lived long (3.2 ±0.3 years) as compared to controlwt (2.8 ±0.35 years) and MTdko (2.5±0.3 years) mice. The striatal fetal stem cells derived from MTtrans mice embryos were genetically resistant to bacterial and fungal infection and had significantly elevated CoQ10, glutathione, and neuromelanin as compared to controlwt and MTdko mice. Furthermore, MTtrans fetal stem cells were resistant to SIN-1-induced apoptosis and survived longer (75 ±8 days) than control (64 ±5 days) and MTdko (55±6 days) striatal fetal stem cells. In aging RhOmgko neurons, in addition to CoQ10, glutathione was also depleted. MPP+ (100 μM) treatment for 7 days further depleted CoQ10, glutathione, and neuromelanin synthesis. MPP+-induced reduction in CoQ10 and glutathione synthesis were restored to normal upon treating with either Selegiline (10 μM) or MT-1 (100 nM). These observations provided us a lead to further learn the basic molecular mechanism of neuroprotection in PD and other neurodegenerative disorders and propose MTs as early and sensitive molecular markers of neuroprotection/neurodegeneration. For details please refer [60].

1.10. CoQ10 attenuates SIN-1 apoptosis

It is well known that mitochondrial complex-1 is down-regulated in the nigrostriatal dopaminergic neurons of PD patients. Hence treatment with CoQ10 provides neuroprotection in RhOmgko neurons (A cellular model of PD). Furthermore, oxidative and nitrative stress of ONOO- might be involved in the etiopathoigenesis of PD. Therefore we used SIN-1 to induce neurodegeneration and CoQ10 to provide neuroprotection in human dopaminergic (SK-N-SH and SH-S-Y5Y) neurons in culture. To establish the neuroprotective potential of CoQ10 in RhOmgko and MT gene-manipulated neurons against SIN-1-induced ONOO- oxidative and nitrative stress, RhOmgko neurons were transfected with complex-1 gene, MT1sense, MT1antisense, and MT1scrambled oligonucloetides employing Qiagen Effectine transfection reagent, DNA enhancer and pEGFP-N1 vector. For stable transfection, the neurons were selected with G-418 (250 μg/l), and enriched by limiting dilution technique. The neurons were grown in eight chambered microscopic slides and at sub-confluent stage treated overnight with SIN-1 (100 μM) and/or CoQ10 (10 μM), washed thrice with Dulbecco's phosphate buffered saline (pH 7.4), and stained with three fluorochromes. FITC-conjugated ApoAlert (Annxin-V) antibody (Green) to determine the extent of phsophatidyl serine externalization, propidium iodide (red) to image fragmented DNA, and DAPI (Blue) for imaging the structurally-intact DNA. The fluorescence images were captured by SpotLite digital camera and analyzed with ImagePro computer software. The digital fluorescence images captured at three different wavelengths were merged to determine the structural and functional integrity of plasma membrane, mitochondria, and nuclear DNA simultaneously. This unique approach correlated and confirmed our novel multiple fluorochrome Comet tail experiments and suggested that SIN-1-induced oxidative and nitrative stress can be prevented by MT-induced CoQ10 synthesis in the dopaminergic neurons, whereas down regulation of MTs in aging suppresses mitochondrial CoQ10 synthesis and accentuates apoptosis as observed in MT-1antisense-transfected dopaminergic neurons; thus compromising neuronal recovery in response to exogenous CoQ10 administration. MPP+-induced reduction in glutathione was ameliorated upon pre-treatment with Selegiline (10 μM) in controlwt and aging RhOmgko neurons. Glutathione synthesis was augmented upon exposure to control and aging RhOmgko dopaminergic neurons to MT-1 for 48 hrs. Moreover the striatal glutathione levels were significantly high in MTtrans as compared to MTdko mice. MTtrans mice possessed significantly higher SN neuromelanin. However SN-neuromelanin in MTdko mice was significantly reduced as compared to controlwt and MTtrans mice. The exact functional significance of these observations remains unknown [61]. Since weaver mutant (wv/wv) mice exhibited ONOO- stress, progressive dopaminergic degeneration, postural irregularities, and body tremors as function of aging, we proposed to transplant genetically-resistant MTtrans fetal mesencephalic stem cells in the striatal region of these genotypes and monitor the graft outcome by 18F-DOPA, 18FdG, and 18F-rotenone microPET neuroimaging as described in our recent report [62].

1.11. Attenuation of a-syn nitration by MTs

We have discovered that α-Syn nitration can be attenuated by MTs gene induction and enhanced by MT gene down-regulation in the mice striatum as well as in the DA-ergic neurons [62]. Aging RhOmgko neurons exhibited enhanced α-Syn nitration upon overnight exposure to SIN -1 (10 μM). Furthermore, transfection of aging RhOmgko neurons with complex-1 attenuated SIN-1-induced α-Syn nitration. SIN-1-induced α-Syn nitration was suppressed in MT-1sense, enhanced in

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MT1antisense, and did not produce significant change in MT1 scrambled oligonucleotide-transfected neurons. Selegiline pre-treatment attenuated SIN-1-induced α-Syn- nitration in MT1sense oligonucelotide transfected neurons. SIN-1-induced nitration of α-Syn was also enhanced in α-Synwt and A53T α-Syn over-expressed HEK cells. A30P α-Syn mutants did not exhibit significant induction of α-Syn nitration in controlwt, α-Synwt, A53T and A30P α-Syn over-expressed HEK cells, suggesting that induction of wild type or A53T mutant α-Syn can enhance α-Syn nitration and hence aggregation to induce Lewy body pathology during the progression of sporadic or familial type of PD.

1.12. Selegiline provides neuroprotection by MTs induction

Recently we have reported that Selegiline a monoamine oxidase inhibitor provides neuroprotection by enhancing MTs expression and through several other anti-apoptotic molecular mechanisms unrelated to MAO-B inhibition [63, 64]. Overnight exposure to MPP+ (100 μM) induced mitochondrial swelling, loss of intra-mitochondrial cristae, and accumulation of water due to metal ion overload in SK-N-SH neurons. These changes at the ultra-structural level were attenuated by Selegiline pre-treatment. Selegiline provided neuroprotection by enhancing mitochondrial as well as cytosolic MTs. Furthermore, Selegiline elevated mitochondrial CoQ10 levels in control and aging RhOmgko neurons, however neuronal recovery was compromised due to elevated levels of α-Syn in RhOmgko neurons.

1.13. Original discoveries on MTs

We developed α-Synuclein-MTs triple gene knock out (α-Syn-MTtko) mice by crossbreeding α-Synuclein knock out males with MTs-gene double knock out females. The progeny was genotyped with tail DNA analysis employing PCR and immunoblotting. Absence of three genes (MT-1, MT-2, and α-Syn) confirmed that these genetically-engineered animals can remain alive even in the absence of three genes. Newly developed α-Syn-MTtko and MTdko mice were highly susceptible to PNs-induced Parkinsonism, however MTs-over-expressing weaver (wv/wv-MTs) mice developed some genetic resistance to PNs. Since brain regional concentrations of CoQ10 were significantly reduced in α-Syn-MTtko and MTdko , we developed a sensitive procedure for the estimation of CoQ10 and other metabolites from these genetically-susceptible genotypes [65]. Typical features of α-Syn-MTtko mice included brown coat, while controlwt litter-mates had a black coat. α-Syn-MTtko mice exhibited stiff tail, reduced body movements, and lethargic behavior. These genotypes were obese as compared to controlwt and MTtrans mice. Hair, skin, and SN melanin were significantly reduced in α-Syn-MTtko mice as compared to controlwt and MTtrans mice. Mitochondrial CoO10 were also significantly reduced in α-Syn-MTtko mice striatum. Ferritin content was also significantly reduced in α-Syn-MTtko and MTdko mice striatum as compared to controlwt and MTtrans mice, whereas iron content of ferritin was significantly increased in MTdko and α-Syn-MTtko mice striatum as compared to contolwt mice. SN-melanin of α-Syn-MTtko and MTdko mice was heavily impregnated with toxic metal ions [Fe_3+, Cu_2+, Zn $_2$+, and Ca_2+] as compared to controlwt mice. The melanin contents of skin, hair, and substantia nigra of MTdko, α-Synko, and α-Syn-MTtko mice were significantly reduced as compared to controlwt and MTtrans mice. Aging MTtrans mice exhibited genetic resistance to MPTP (30 mg/kg, i.p for 7 days)-induced Parkinsonism as compared to MTdko mice. MTtrans mice could walk with their stiff tail while MTdko mice became completely immobilized following chronic MPTP intoxication. MTdko mice had significantly reduced melanin in their skin, hair, and substantia nigra and were highly susceptible to MPTP-induced Parkinsonism. Aging MTtrans mice were lean, agile, with soft shiny black coat on their body, whereas aging MTdko and α-Syn-MTtko mice were obese, lethargic, and developed skin de-pigmentation. MTdko mice had reduced striatal CoQ10 and these genotypes were highly susceptible to MPTP Parkinsonism. In order to further establish MTs-mediated CoQ10 neuroprotection in DA-ergic neurons, we have conducted several experiments on MT-gene manipulate mice and aging mitochondrial genome knock out (RhOmgko) DA-ergic (SK-N-SH) neurons. MT-1 and 2 genes provided neuroprotection by inhibiting MPTP-induced mitochondrial oxidative and/or nitrative stress, α-Syn nitration, preserving brain regional CoQ10, ferritin, and neuromelanin in the striatum. MPTP-induced α-Syn nitration and carbonylation were also attenuated in MTtrans mice striatum as compared to controlwt and MTdko mice. MTdko and α-Synko mice were highly susceptible to mitochondrial complex-1 inhibitors, MPP+, 6-OHDA, Rotenone, and Salsolinol-induced neurotoxicity. Selegiline provided better neuroprotection against MPTP in MTtrans mice striatum as compared to MTdko and α-Synko mice. Indeed Selegiline induced neuroprotection by MT-1 induction and suppression of α-Syn nitration. MTtrans mice striatum exhibited reduced α-Syn expression and increased ferritin immunoreactivity, whereas, ferritin immunoreactivity was reduced and α-Syn expression was increased in MTdko mice striatum. Chronic treatment of MPTP induced severe Parkinsonism, characterized by facial twitches, postural irregularities, body tremors, muscle rigidity, and immobilization in controlwt and MTtrans mice, suggesting their resistance to Parkinsonism.

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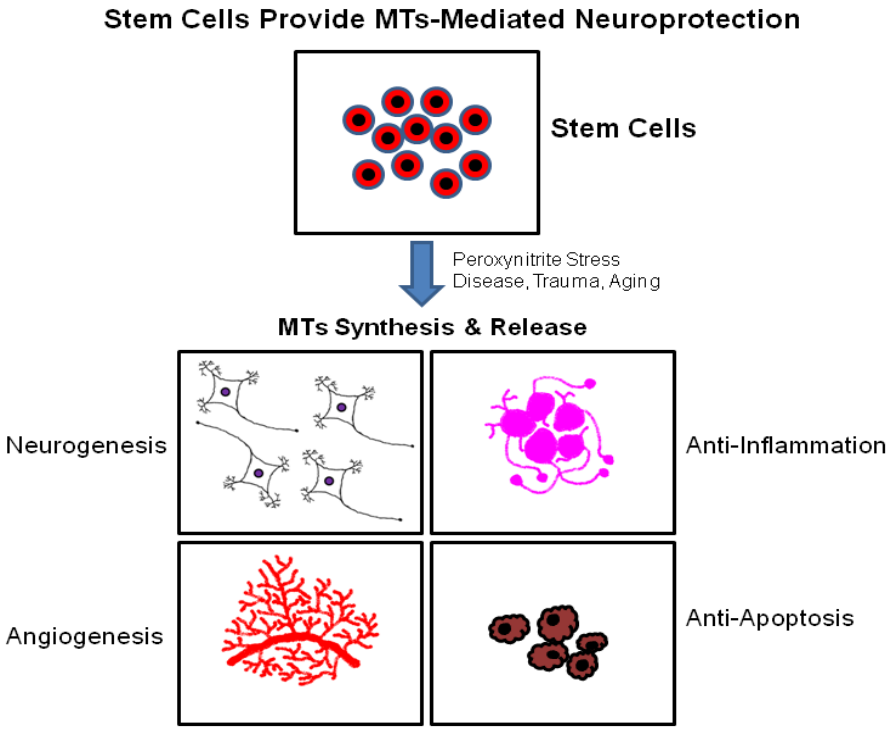
Fig. 2



1.14. Neuroprotection by MTs-induced SN-neuromelanin

Out of total four million PD patents in the world, 1 million exist in USA and every year 50,000 new cases are added. The exact cause of increased incidence of PD among aging white population as compared to aging black population remains enigmatic [66]. It is known that melanin acts as an antioxidant to protect brain from iron-induced oxidative stress which is significantly increased in PD patients. Hence the incidence of PD is low among black population as compared to white population in the world. Recent studies have suggested that a loose association between iron and NM may result in increased production of free radicals. Currently, it is unknown whether neuromelanin (NM) in Parkinsonian brain differs from that found in healthy tissue and thus may perform a different role. Indeed neuromelanin (NM) from substantia nigra (SN) of PD patients possessed lower magnetization as compared to healthy controls [67]. Interestingly, as observed in MTdko mice, SN neuromelanin (NM) contents are also reduced in PD patients [68]. We and other investigators have shown that NM provides neuroprotection against toxic ONOO- ions and can bind iron to prevent Fe3+-mediated toxic hydroxyl (OH) radical generation, proposed to be involved in the etiopathogenesis of PD [69].

Table-1

S.No	Striatum	Group	Substantia Nigra
1	0.4±0.03	Control wt	1.9±0.3
2	0.3±0.02	MTdko	1.4±0.2
3	1.0±0.05	MTtrans	2.5±0.3

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1.15. MTs preserves synaptosomal dopamine transporter (sDAT)

In order to determine whether MTs attenuate sDTA down-regulation in PD, we prepared animal models of Parkinsonism by chronically injecting MPTP (10 mg/kg i.p for 7 day) and/or Selegiline (10 μM) in aging C57BL/6J mice. sDAT and dopamine (DA) metabolism were estimated from the mice striatum and aging RhOmgko neurons with a primary objective to establish the neuroprotective potential of MTs in PD and other neurodegenerative disorders such as AD and stroke. sDAT was estimated by injecting 50 μCi [3H] DA. Manzidole (10 mg/kg, i.p) was used as a DA uptake inhibitor to determine specificity of the assays. After 4 hrs, the radioactivity was stabilized by decapitation, and was measured from the striatal synaptosomal fraction, employing Perkin-Elmer TriCarb β-scintillation counter above background. To establish whether MTs induction improves sDAT in DA-ergic neurons, we transfected SK-N-SH neurons with MT-1antisnese, MT1sense, and MT1scrambled oligonucleotides. Chronic treatment of MPTP inhibited striatal sDAT and DA synthesis, while Seligiline pre-treatment ameliorated sDAT and DA synthesis. sDAT and DA synthesis were reduced in MTdko mice striatum as compared to MTtrans mice. In aging RhOmgko neurons sDAT and DA synthesis were reduced. Transfection of RhOmgko neurons with complex-1 gene ameliorated sDAT and DA synthesis. sDAT and DA synthesis were increased in MT1sense transfected, reduced in MT-1antisense-transfected, and remained unaltered in MT1scrambled neucleotide-transfected neurons. These findings suggested that sDAT and DA synthesis in the DA-ergic neurons are suppressed by PNs such as MPTP whereas Selegiline can improve sDAT function and DA synthesis in PD patients by augmenting MTs synthesis. sDAT and DA synthesis are also suppressed in aging RhOmgko cells, while transfection with complex-1 gene can ameliorate sDAT and DA synthesis. In aging RhOmgko neurons MT-1 gene expression is also suppressed, whereas transfection with complex-1 improves MT-1 expression suggesting that sDAT and DA synthesis can be improved in the DA-ergic neurons by MTs gene induction and vice versa.

1.16. Recent studies on MTs-mediated neuroprotection

Recent studies have shown that MTs mitigate age-dependent secondary brain injury [70] and are known to attenuate apoptosis and pro-inflammatory response during cerebral malaria in mice [71]. Further studies have investigated the molecular mechanisms underlying the differentiation and survival-promoting effects of MT and a peptide modeled after MT, EmtinB [72]. Both MT and EmtinB stimulated neurite outgrowth and promoted survival in vitro in primary cultures of cerebellar granule neurons. The expression and surface localization of megalin (a known MT receptor) and the related lipoprotein receptor-related protein-1 (LRP) were expressed in these neurons. MT and EmtinB induced their neuronal effects through binding to receptors belonging to the low-density lipoprotein receptor family (megalin and LRP), thereby activating signal transduction pathways resulting in neurite outgrowth and survival. Further studies have shown that a peptide modeled after the β-domain of MT, EmtinB, induces neurite outgrowth and increases neuronal survival through binding to receptors of the low-density lipoprotein receptor family (LDLR). Two MT α-domain-derived peptide sequences termed EmtinAn and EmtinAc, each consisting of 14 amino acids, as stimulators of neuronal differentiation and survival of primary neurons have been identified. In addition, a peptide derived from the N-terminus of the MT β-domain, EmtinBn, has been shown to promote neuronal survival. The neuritogenic and survival promoting effects of EmtinAc, similar to MT and EmtinB but not EmtinAn, were dependent on the functional integrity of LDLR. EmtinAn and EmtinAc induced activation of extracellular signal-regulated kinase (ERK) and protein kinase B (PKB/Akt), suggesting that multiple functional sites of MT could serve to cross-link MT receptor(s) to promote signal transduction involved in neurite outgrowth and survival [73].

There is an increasing body of evidence demonstrating that MTs express in astrocytes following CNS injury, exhibit both neuroprotective and neuroregenerative properties and are critical for neuronal recovery. As MTs lack signal peptides, and have well characterized free radical scavenging and heavy metal binding properties, their neuroprotective functions have been attributed to these intracellular roles. However, it is being realized that the neuroprotective functions of MTs may also involve an extracellular component. Therefore, it is being realized that the protective functions of MT in the CNS should be widened from a purely astrocytic focus to include extracellular and intra-neuronal roles. These actions of MTs represent a novel paradigm of astrocyte-neuronal interaction after injury and may have implications for the development of MT-based therapeutic agents in future [74]. Furthermore, neuroimmunomodulatory properties of MTs may have therapeutic potential for the treatment of traumatic brain injury [75]. It has been demonstrated that Lead (Pb) exposure causes increased co-localization of MT and Scna proteins only in WT cells. In WT mice after chronic Pb exposure Scna was localized in renal cells forming IBs, whereas MT-null mice did not form Lewy bodies (LBs). Thus, Scna is considered an important component of Pb-induced LBs and, with MT, may play a role in LBs formation [76]. Recent studies have demonstrated that MT-2A is capable of protecting against amyloid-β (Ab) aggregation and toxicity. Given the recent interest in metal-chelation therapies for AD that remove metal from Ab leaving a metal-free Ab that can readily bind metals again, it now believed that MT-2A might represent a unique therapeutic approach as the metal exchange between MT and Ab leaves the Ab in a Zn-bound, relatively inert form [77]. MT induced astrogliosis was permissive to neurite outgrowth and was associated with decreased chondroitin sulphate proteoglycan (CSPG) expression suggesting that MTs have an

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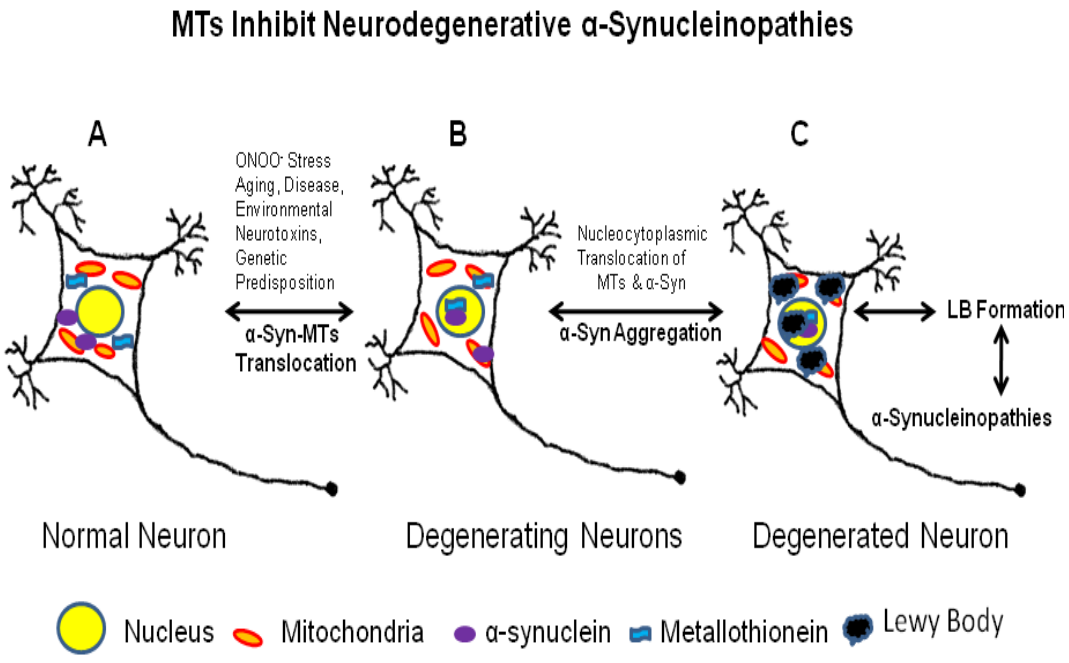
important role in mediating astrocytic responses to traumatic brain injury [78].

1.17. Proposed hypothesis

Recently point mutations in α-Syn (A30P & A53T) have been implicated in the etiopathogenesis of PD [79, 80]. We have shown that over-expression of even wild type α-Syn enhances cell proliferation, which is attenuated in A30P and A53T-α-Syn over-expressed HEK-293 cells. Indeed A53T-α-Syn over-expressed cells were highly susceptible to Rotenone-induced apoptosis, as represented by aggregation and translocation of nitrated α-Syn in the perinuclear and endonuclear regions, reductions in mitochondrial CoQ10, MTs, and ΔΨ, and zone of growth inhibition due to cytochrome C release, suggesting that MTs induction provides mitochondrial as well as nuclear DNA stability.

Usually both α-Syn as well as MTs reside in the cytosolic compartment during normal physiological conditions. However in the absence of MTs, α-Syn can be easily nitrated and aggregated in the perinuclear and endonuclear regions. Enhanced aggregation of α-Syn due to metal ions accumulation and oxidative and nitrative stress may trigger Lewy body synthesis in the aging brain. Hence α-Syn-MTs interaction is very important physiological event in a healthy brain. Impairment in this interaction might lead to various neurodegenerative disorders collectively called as neurodegenerative α-Synucleinopathies. Since we have now experimental evidence that brain regional MTs induction provides neuroprotection through zinc-mediated transcriptional regulation of various redox-sensitive genes in the DA-ergic neurons, further studies in this direction will pinpoint the exact molecular mechanism(s) of neurodegenerative α-Synucleinopathies and eventually their effective treatment by brain regional MTs induction. Hence MTs can be used as early and sensitive biomarkers of redox signaling for better prognosis and effective clinical management of neurodegenerative disorders such as PDS, AD, MSA, MS, and stroke as illustrated in **Figure– 3**.

Fig. 3



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[II] CONCLUSION

We have discovered that MTs provide neuroprotection by attenuating α-Synuclein nitration, oxidation, and carbonylation, and through augmented CoQ10 synthesis via mitochondrial complex-1 rejuvenation. Based on these findings we have proposed that MTs provide neuroprotection by preventing broadly classified α-Synucleinopathies; hence can serve as early and sensitive biomarkers of neurodegeneration/neuroprotection.

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CONFLICT OF INTEREST

Authors declare no conflict of interest in this study.

REFERENCES

[1] Marghoshes M and Vellee BL. [1957] A cadmium protein from equine kidney cortex. *J Am Chem Soc* 79:4813–4814.
[2] Itoh M, Ebadi M, Swanson S. [1983] The presence of zinc binding proteins in brain. *J Neurochem* 41: 823–829.
[3] Palmiter RD, Finley SD, Whitmore TE, Durman DM. [1992] MT-III, a brain-specific member of the metallothionein gene family. Proc *Natl Acad Sci* USA 89:6333–6337.
[4] Quaife CJ, Findley SD, Erickson JC, Forlick GJ, Kelly EJ, et al. [1994] Induction of new metallothionein isoform (MT-IV) occurs during differentiation of stratified squamous epithelia. *Biochemistry* 33: 7250–7259.
[5] Penkowa M [2006] Metallothioneins are multipurpose neuroprotectants during brain pathology. *FEBS Journal* 273: 1857–1870.
[6] Giralt M, Penkowa M, Hernandez J, Molinero A, Carrasco J, et al. [2002] MT-1,2 deficiency increases brain pathology in transgenic mice with astrocyte-targeted expression of interleukin-6. *Neurobiol of Dis* 9:319–338.
[7] Trendelenburg G, Prass K, Priller J, Kapinya K, Polley A, Muselmann C, et al. [2002] serial analysis of gene expression identifies MT-11 as major neuroprotective gene in mouse focal cerebral ischemia. *J Neurosci* 22: 5879–5888.
[8] Ben-Shachar D and Youdim MB. [1993] Iron, melanin, and dopamine interaction: Relevance to Parkinson's disease. Prog Neuropharmacol *Biol Psychiatry* 17: 139–150.
[9] Youdim MB, Ben-Shachar D, Reiderer P. [1993] The possible role of iron in the etiopathology of Parkinson's disease. *Mov Disord* 8:1-12.
[10] DiMonte DA, Schipper HM, Hetts S, Langston JW. [1995] Iron-mediated bioactivation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in glial cultures. *Glia* 15: 203–206.
[11] Lan J and Jiang DH. [1997] Excessive iron accumulation in the brain: a possible potential risk of neurodegeneration in Parkinson's disease. *J Neural Transm* 104:649–660.
[12] Lan J and Jiang DH. [1997] Desferroxamine and vitamin E protect against iron and MPTP-induced neurodegeneration in mice. *J Neural Transm* 104: 469–481.
[13] Yantiri F, Anderson JK. [1999] The role of iron in Parkinson's disease and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine toxicity. *IUBMB Life* 48: 139–141.
[14] Youdim MB, Ben-Schachar D, and Riederer P. [1993] The possible role of iron in the etiopathology of PD. *Mov Disord* 8: 1–12.
[15] Youdim MB, Grunblatt E, and Mandel S. [1999] The pivotal role of iron in NFkappa B activation and negirostriatal dopaminergic neurodegeneration. Prospectus for Neuroprotection in PD with iron chelators. *Ann N Y Acad Sci* 890: 7–25.
[16] Youdim MB, Gassen M, Gross A, Mandel S and Grunblatt E. [2002] Iron chelating, antioxidant and cytoprotective properties of dopamine receptor agonist; apomorphine. *J Neural Transm Suppl* 58: 83–96.
[17] Fredrickson A, Schroder N, Eriksson P, Izquierdo I and Archer T. [2002] Neonatal iron potentiates adult MPTP-induced neurodegenerative and functional deficits 7: 97–105.
[18] Chiueh CC, Miyake H, and Peng MT. [1993] Role of dopamine autooxidation, hydroxyl radical generation, and calcium overload in underlying mechanisms involved in MPTP-induced Parkinsonism. *Adv Neurol* 60: 251–258.
[19] Fahn S and Cohn G [1992] The oxidant stress hypothesis in Parkinson's disease: Evidence supporting it. *Ann Neurol* 32: 804–812.
[20] Schapira AH, Hartley A, Cleeter MW, Cooper JM. [1993] Free radicals and mitochondrial dysfunction in Parkinson's disease. *Biochem Soc Trans* 21: 367–370.
[21] Jenner P. [1989] Clues in the mechanism underlying dopaminergic cell death in Parkinsons' disease. *J Neurol Neurosurg Psychiatry Suppl* (22) 8: 22–28.
[22] Jenner P. [1991] Oxidative stress as a cause of Parkinsons's disease. *Acta Neurol Scand Suppl* 136: 6–15.
[23] Jenner P. [1992] What process causes nigral cell death in Parkinson's disease? *Neurol Clin* 10: 387–403.
[24] Jenner P. [1998] Oxidative mechanisms in nigral cell death in Parkinson's disease. *Mov Disord 13 Suppl* 1: 24–34.
[25] Fernandez-Gonzalez A, Perez-Otano I and Morgan JI. [2002] MPTP selectively induces haem oxygenase-1 expression in striatal astrocytes. *Eur J Neurosci* 12: 1573-1583.
[26] Mash DC, Pablo J, Buck BE, Sanchez-Ramos J and Weiner WJ. [1991] Distribution and number of transferrin receptors in Parkinson's disease and in MPTP-treated mice. *Exp Neurol* 114: 73–81.
[27] Mash DC, Sanchez-Ramos J and Weiner WJ. [1993] Transferrin receptor regulation in Parkinson's disease and MPTP-treated mice. *Adv Neurol* 60: 133–139.
[28] Faucheux BA, Herrero MT, Vilares J, Levy R, Javoy-Agid F, et al. [1995] Autoradioradiographic localization and density of [125]ferrotransferrin binding sites in the basal ganglia of control subjects, patients with Parkinson's disease and MPTP-lesioned monkeys. *Brain Res* 691: 115–124.
[29] Goto M, Mochizuki H, Imai H, Akiyama H and Mizuno Y. [1996] An immune-histochemical study of ferritin in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced hemiparkinsonian monkeys. *Brain Res* 724: 125–128.
[30] Reiderer P and Lang KW [1992] Pathogenesis of Parkinsons' disease. Curr Opin Neurol. *Neurosurg* 5: 295–300.

THE IIOAB JOURNAL
ISSN: 0976-3104

[31] Hom DG, Jiang D, Hong EJ, Mo JQ, and Anderson JK. [1997] Elevated expression of glutathione peroxidase in PC-12 cells results in protection against methamphetamine but not MPTP toxicity. *Brain Res Mol Brain Res* 46: 154–160.

[32] Hirlinger J, Schulz JB, Dringen R. [2002] Effects of dopamine on the glutathione metabolism of cultured astroglial cells: Implications for Parkinson's disease. *J Neurochem* 82: 485–467.

[33] Chiueh CC, Huang SJ, and Murphy DL. [1994a] Suppression of hydroxyl radical formation by MAO inhibitors: a novel possible neuroprotective mechanism in dopaminergic neurotoxicity. *J Neural Transm Suppl* 41: 189–196.

[34] Chiueh CC, Wu RM, Mohankumar KP, Sternberger LM, Krisna G, et al. [1994] In vivo generation of hydroxyl radicals and MPTP-induced dopaminergic toxicity in the basal ganglia. *Ann NY Acad Sci* 738: 25–36.

[35] Drukarch B and Muiswinskel FL. [2001] Neuroprotection for Parkinson's disease: A new approach for a new millennium. *Expert Opin Investig Drugs* 10: 1855–1868.

[36] Zecca L, Fariella R, Riederer P, Sulzer D, Gatti A, Tampellini D. [2002] The absolute concentrations of nigral neuromelanin: assayed by a new sensitive method, increases throughout life and it dramatically decreases in Parkinson's disease. *FEBS Lett* 510: 216–220.

[37] Double KL, Gerlach M, Youdim MB, Riederer P. [2000] Impaired iron homeostasis in Parkinson's disease. *J Neural Transm* 6: 37–58.

[38] Double KL, Ben-Shachar, Youdim MB, Zecca L, Riederer P, Gerlach M. [2002] Influence of neuromelanin on oxidative pathways within the human substantia nigra. *Neurotoxicol Tetratol* 24: 612–628.

[39] Duan W, Ladenheim B, Cutler RG, Kruman iL, Cadet JL, Mattson MP. [2002] Deitary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. *J Neurochem* 80: 101–110.

[40] Kristal BS, Conway AD, Brown AM, Jain JC, et al. [2001] Selective dopamine vulnerability: 3,4-dihydroxyphenylacetaldehyde targets mitochondria. *Free Radic Biol Med* 30: 924–931.

[41] Li SW, Lin T, Minsteer S, and Burke WJ. [2001] 3, 4 dihydroxyacetaldehyde and hydrogen peroxide generate a hydroxyl radical: possible role in Parkinson's disease pathogenesis. *Brain Res Mol Brain Res* 93: 1–7.

[42] Gerlach M and Reiderer P. [1996] Animal models of Parkinson's disease. : An empirical comparison with the phenomenology of the disease in man. *J Neural Transm* 103: 987–1041.

[43] Sangchot P, Sharma SK, Chatsawang B, Govitrapong P, Ebadi M. [2002] Deferozamine attenuates iron induced oxidative stress and prevents mitochondrial aggregation and α-Synuclein translocation in SK-N-SH in culture. *Dev Neurosci* 24: 143–153.

[44] Kooncumchoo P, Govitrapong P, Sharma S, Ebadi M. [2006] Coenzyme Q10 provides neuroprotection in iron-induced apoptosis in dopaminergic neurons. *Mol Neurosci* 28: 125–142.

[45] Beal MF. [2002] Coenzyme Q10 as a possible treatment for neurodegenerative diseases. *Free Radic Res* 36: 455-460.

[46] Shults CW, Oakes D, Kieburtz K, Beal MF, Haas R, et al. [2002] Effects of coenzyme Q10 in early Parkinson's disease: Evidence of slowing of the functional decline. *Arch Neurol* 59: 1541–1550.

[47] Sharma S, Kheradpezhou M, Shavali S, Refaey El H, Eken J, et al. [2004] Neuroprotective actions of coenzyme Q10 in Parkinson's diseases. *Method in Enzymol* 382: 488–509.

[48] Aschner M, Cherian MC, Klassen CD, Palmiter RD, Erickson JC, Bush AI. [1997] Metallothioneins in brain: The role in physiology & pathology. *Toxicol Appl Appl Pharmacol* 142: 229–242.

[49] Hidalgo J, Castellano B, Campbell IL. [1997] Regulation of brain metallothioneins. *Curr Top Neurochem* 1: 1–26.

[50] Hidalgo J, Aschner M, Zatta P, Vasak M. [2001] Roles of the metallothionein family of proteins in the central nervous system. *Brain Res Bulit* 55: 133–145.

[51] Ebadi M and Sharma S. [2003] Peroxynitrite and mitochondrial dysfunction in the pathogenesis of Parkinson's disease. *Antiox and Redox Signal*.5: 319–335.

[52] Sharma S Ebadi M. [2003] Metallothioneins attenuates 3-morpholijosydninomine (SIN-1)-induced oxidative stress in dopaminergic neurons. *Antiox Redox Signal* 5: 251–264.

[53] Ebadi M, Govitrapong P, Sharma S, Muralikrishnan D, Shavali S, et al. [2001] Ubiquinone (Coenzyme Q10) and Mitochondria in Oxidative Stress of Parkinson's disease. *Biological Signals and Receptors* 10:224–253.

[54] Ebadi M, Sharma S, Muralikrishnan D, Shavali S, Josh E, Sangchot p et al. [2002] Metallothionein provides Ubiquinone-Mediated Neuroprotection in Parkinson's disease. *Proceedings of Western Pharmacol Soc*. 45: 1–3.

[55] Cherian MG, Apostolova MD. [2000] Nuclear localization of metallothionein during cell proliferation and differentiation. *Cell Mol Biol* 46: 347–356.

[56] Cai L, Klein JB, Kang YJ. [2001] Metallothionein inhibits peroxynitrite-induced DNA and lipoprotein damage. *J Biol Chem* 275: 38957–38960.

[57] Maret W, Heffron G, Hill HA, Djuricic D, Jiang LJ, Vallee BL. [2002] The ATP/Metallothionein interaction: NMR and STM. *Biochemistry* 41: 1689–1694.

[58] You JY, Oh D, Choi CY, Lee DG, Hahm KS, Moon AR, Jeong HG. [2002] Protective effects of metallothionein-II on DNA damage in response to reactive oxygen species. *Biochim et Biophys Acta* 1573: 33–38.

[59] Sharma S and Ebadi M. [2008] Therapeutic potential of metallothioneins in Parkinson's disease. In New Research on Parkinson's Disease. Eds T.M. Hahn & Julian Weener, Chapter -1 pp 1-41. *Nova Science Publishers*.

[60] Ebadi M and Sharma S. [2006] Metallothioneins 1 and 2 attenuate peroxynitrite-induced oxidative stress in Parkinson's disease. J Exp Biol & Med 231: 1576–1583.

[61] Ebadi M, Brown-Borg H, Garrett S, Singh B., Shavali S, Sharma S. [2005] Metallothionein-Mediated Neuroprotection in genetically-Engineered Mice Models of Parkinson's Disease and Aging. *Molecular Brain Research* 134: 67–75.

[62] Ebadi M, Sharma S, Ghafourifar P, Brwon-Borg H, Refaey HEI. [2005] Peroxynitrite in the Pathogenesis of Parkinson's disease. *Method in Enzymology* 396: 276–298.

[63] Sharma S, Carlson E, Ebadi M. [2003] Neuroprotective actions of Selegiline in inhibiting 1-methyl, 4-phenyl pyridinium ion (MPP+)-induced apoptosis in SK-N-SH neurons. *J Neurocytol* 76: 563–571.

[64] Ebadi M, Brown-Borg H, Sharma S, Shavali S, El ReFaey H, Carlson EC. [2006] Therapeutic Efficacy of selegiline in Neurodegenerative Disorders and Neurological Diseases. *Current Drug Targets* 7: 1–17.

THE IIOAB JOURNAL
ISSN: 0976-3104

[65] Sharma S and Ebadi M. [2004] An improved method for analyzing coenzyme Q10 homologues and multiple detection of rare biological samples. *J Neurosci Methods* 137: 1–8.

[66] Lang AE, Lozano AM. [1998] Medical Progress: Parkinson's disease. Part 1 and 2 N. *Eng J Med* 339:1130–1143; 1044–1053.

[67] Bolzoni F, Giraudo S, Lopiano L, Bergamasco B, Fasano M, Crippa PR. [2002] Magnetic investigations of human mesencephalic neuromelanin. *Biochim Biophys Acta* 1586: 210–218.

[68] Drukarch B and Muiswinskel FL. [2001] Neuroprotection for Parkinson's disease: A new approach for a new millennium. *Expert Opin Investig Drugs* 10: 1855–1858.

[69] Stepien K, Wilczok A, Zaidel A, Dzierzega-leczok T. [2000] Peroxynitrite mediated linoleic acid oxidation and tyrosine nitration in the presence of synthetic neuromelanin. *Acta Biochim Pol* 47: 931–940.

[70] Natale JE, Knight JB, Cheng Y, Rome JE, Gallo V. [2004] Metallothionein I and II mitigate age-dependent secondary brain injury. *J Neurosci Res* 78: 303–314.

[71] Wiese L, Kurtzhals JAL, Penkova M. [2006] Neuronal apoptosis, metallothionein expression and proinflammatory responses during cerebral malaria in mice. *Expt Neurol* 200: 216–226.

[72] Ambjørn M, Asmussen JW, Lindstam M, Gotfryd K, Jacobsen C, et al. [2008] Metallothionein and a peptide modeled after metallothionein, EmtinB, induce neuronal differentiation and survival through binding to receptors of the low-density lipoprotein receptor family. *J Neurochem* 104:21–37.

[73] Asmussen JW, Ambjørn M, Bock E, Berezin V. [2009] Peptides modeled after the alpha-domain of metallothionein induce neurite outgrowth and promote survival of cerebellar granule neurons. *Eur J Cell Biol* 88:433–443.

[74] Chung RS, Penkowa M, Dittmann J, King CE, Bartlett C, et al. [2008] Redefining the Role of Metallothionein within the Injured Brain. EXTRACELLULAR METALLOTHIONEINS PLAY AN IMPORTANT ROLE IN THE ASTROCYTE-NEURON RESPONSE TO INJURY. *J Biol Chem* 283:15349–15358.

[75] Chung RS, Leung YK, Butler CW, Chen Y, Eaton ED, et al. [2009] Metallothionein Treatment Attenuates Microglial Activation and Expression of Neurotoxic Quinolinic Acid Following Traumatic Brain Injury. *Neurotoxicity Res* 15:381–389.

[76] Zuo P, Qu W, Ryan N. Cooper RN, Goyer RA,. Diwan DA and Waalkes MP [2009] Potential Role of a-Synuclein and Metallothionein in Lead-Induced Inclusion Body Formation. *Toxicological Sciences* 111:100–108.

[77] Chung RS, Howells C, Eaton ED, Shabala L, Zovo K, et al. [2010] The native copper- and zinc-binding protein metallothionein blocks copper-mediated Abeta aggregation and toxicity in rat cortical neurons.

[78] Leung YKG, Pankhurst M, Dunlop AS, Ray S, Dittmann J, et al. [2010] Metallothionein induces a regenerative reactive astrocyte phenotype via JAK/STAT and RhoA signalling pathways. *Expt Neurol* 221: 98–106.

[79] Giasson BL, Duda JE, Murray IV, Chen Q, Souza JM, et al. [2003] Oxidative damage linked to neurodegeneration by selective α-synucelin nitration in synopathy lesions. *Science* 290: 985–989.

[80] Duda JE, Giasson Bl, Chen Q, Gur TL, Hurtig HI, et al. [2003] Widespread nitration of pathological inclusions in neurodegenerative synucleinopathies. *Am J Pathol* 157: 1439–1345.

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