Salsolinol, a dopamine-derived tetrahydroisoquinoline, induces cell death by causing oxidative stress in dopaminergic SH-SY5Y cells, and the said effect is attenuated by metallothionein.

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Abstract

The endogenous neurotoxin, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol), has been considered a potential neurotoxin in the etiology of Parkinson’s disease (PD). Salsolinol and \(N\)-methyl(\(R\))-salsolinol were identified in the brains and cerebrospinal fluid (CSF) of PD patients. Oxidative stress is known to be one of the major contributing factors in the cascade that may finally lead to the cell death in PD. The present study was undertaken to understand the role of salsolinol in oxidative-mediated neuronal toxicity in dopaminergic SH-SY5Y cells, and the neuroprotective effects of metallothionein (MT) against salsolinol toxicity in MT overexpressing (MT\textsubscript{trans}) fetal mesencephalic cells. Salsolinol increased the production of reactive oxygen species (ROS) and significantly decreased glutathione (GSH) levels and cell viability in SH-SY5Y cells. Salsolinol also decreased intracellular ATP levels and induced nuclear condensation in these cells. Salsolinol-induced depletion in cell viability was completely prevented by \(N\)-acetylcysteine in SH-SY5Y cells, and also prevented by MT in MT\textsubscript{trans} fetal mesencephalic cells compared to control\textsubscript{wt} cells. The extent of nuclear condensation and caspase activation was also less in MT\textsubscript{trans} cells than control\textsubscript{wt} cells. These results suggest that salsolinol causes oxidative stress by decreasing the levels of GSH and by increasing ROS production, and these events may lead to the death of dopaminergic cell. Furthermore, MT overexpression may protect dopaminergic neurons against salsolinol-induced neurotoxicity, most probably by the inhibition of oxidative stress and apoptotic pathways including caspase-3 activation.

Keywords: Salsolinol; Glutathione; Metallothionein; Oxidative stress; Parkinson’s disease

1. Introduction

The endogenous neurotoxin, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol), has been considered a potential causative factor for Parkinson’s disease (PD) \[14,31,35,37]\. Recent reports indicated the presence of this toxin and its derivatives in certain regions of human brain \[25]\. Salsolinol was found to occur in several regions of the brain, whereas \(N\)-methyl(\(R\))-salsolinol distributed selectively in the nigrostriatum \[25,44]\. \(N\)-methyl(\(R\))-salsolinol was also detected in the human intraventricular fluid \[24]\. In addition, increased levels of salsolinol have been detected in cerebrospinal fluid of Parkinsonian patients \[31]\. It was shown that salsolinol can be synthesized in vivo by three different mechanisms, namely, non-enzymatic Pictet–Spengler condensation of dopamine with aldehydes leading to the formation of racemic salsolinol isomers; non-enzymatic condensation of dopamine and pyruvate to form 1-carboxyl-tetrahydroisoquinoline, followed by decarboxylation and reduction to form (\(R\))-salsolinol; and enantio-selective synthesis of (\(R\))-salsolinol from dopamine and acetaldehyde by (\(R\))-salsolinol synthase.
Among these three pathways, it has been observed that (R)-salsolinol is mainly synthesized from dopamine and acetaldehyde by salsolinol synthase, in vivo [34,46]. Several studies indicated that salsolinol is toxic to dopaminergic neurons in vitro as well as in vivo. Salsolinol is known to inhibit tyrosine hydroxylase and monoamine oxidase [2] as well as mitochondrial complex-I and complex-II enzyme activities [26,30,31] in neuronal cells. Storch et al. [42] reported that salsolinol causes a rapid depletion of ATP in dopaminergic cells. However, the precise biochemical and molecular mechanisms underlying the oxidative stress-mediated neurotoxicity of salsolinol is still poorly understood.

Among several causative factors, oxidative stress is known to be a major contributing factor to the biochemical cascade leading to dopamine cell degeneration in PD [6,9,11,20,21]. Furthermore, several biological markers of oxidative damage were found elevated in substantia nigra pars compacta (SNpc) of PD brains [39]. Catecholamine-related neurotoxins are also involved in the neuronal cell death via increasing oxidative stress [7,8,19]. Reactive oxygen species (ROS) can be neutralized by an enzyme defense mechanism comprising superoxide dismutase (SOD) and catalase (CAT) or non-enzymatically by the defense mechanism comprising superoxide dismutase oxygen species (ROS) can be neutralized by an enzyme. Reduced glutathione provides another important non-enzymatic cellular defense against free radicals. The level of GSH is selectively reduced in the substantia nigra of PD patients and this phenomenon does not occur in other neurodegenerative disorders [41].

In the present study, we investigated salsolinol-mediated oxidative stress by studying the changes in glutathione (GSH), metallothionein (MT) and ROS levels in dopaminergic SH-SY5Y cells. Furthermore, we also tested whether MT overexpression protects salsolinol-induced apoptosis in mice fetal mesencephalic cells.

2. Materials and methods

2.1. Reagents

Minimum essential medium (MEM), Dulbecco’s modified Eagle’s medium (DMEM), Hanks balanced salt solution (HBSS), F-12 medium, fetal bovine serum, fetal calf serum, heat-inactivated horse serum, penicillin and streptomycin were all purchased from Gibco BRL (Carlsbad, CA). High protein binding polystyrene multiwell plates, MTT (Thiazolyl blue), bovine serum albumin, monochlorobimane (MCB), glutathione, metallothionein, (±)-salsolinol and N-acetylcysteine (NAC) were each obtained from Sigma (St. Louis, MO). 2′,7′-Dichlorodihydrofluorescein diacetate (H2DCFDA) was purchased from Molecular Probes (Eugene, OR) and ATPlite kit from Packard (USA). Monoclonal antibody to metallothionein was purchased from DAKO (Carpinteria, CA) and antibody to cleaved caspase-3 from Trevigen (Gaithersburg, MD). Nunclon™ culture flasks were obtained from Nalge Nunc International (Rochester, NY). Tissue culture plates (flat-bottom cells) were obtained from Sarstedt (Newton, NC). The human dopaminergic neuroblastoma SH-SY5Y cell line was purchased from American Type Culture Collection (ATCC) (Manassas, VA). All other chemicals used in this study were analytical grade and obtained essentially either from Sigma or Fisher Scientific (USA). Metallothionein transgenic and control wild-type mice were procured from Jackson Laboratories (Bar Harbor, ME).

The experimental protocols used for the mice in this study were approved by the local Animal Care Committee and were strictly maintained according to NIH guidelines. Pregnant mice control wild-type (controlwt) and metallothionein transgenic (MTtrans), of 18th day gestation, were used for the preparation of mesencephalic cells from fetuses.

2.2. Cell culture

2.2.1. SH-SY5Y cell culture

SH-SY5Y cells were grown in a medium containing minimum essential medium, F-12 medium, HBSS (2:1:1) with 10% fetal bovine serum and the cell culture flasks were placed in a humidified incubator with 5% CO2 in air at 37 °C.

2.2.2. Mice fetal mesencephalic cell culture

Mesencephalic regions from mice fetal brains (controlwt and MTtrans) were carefully dissected under aseptic conditions using a stereo-binocular dissecting microscope, and placed in 0.1% trypsin in Ca–Mg free HBSS and incubated for 15 min at 37 °C. After the incubation, the tissues were washed three times with HBSS solution. They were resuspended in approximately 1 ml of HBSS and dissociated by passing through a pasteur pipette with narrow tip. Cells were collected and plated into 35 mm tissue culture dishes at a density of 2 × 10⁴ cells/cm² with 2 ml of supplemented minimum essential medium [MEM + Earls’s salts (1:1) with glutamine 2 mM, glucose 600 mg/100 ml, penicillin 20 units/ml, streptomycin 20 μg/ml] for 30 min followed by conditioned medium [MEM 80%, fetal calf serum 10%, heat-inactivated horse serum 10%] for 4 h. Attached cells were then cultured in synthetic medium containing equal volumes of Ham’s F-12 and DMEM supplemented with an additional 120 mg/100 ml of glucose, 5 μg/ml of bovine insulin, 100 μg/ml of human transferrin, 20 nM progesterone, 100...
µM putrecine, 20 nM selenium dioxide with 20 units/ml penicillin and 20 µg/ml streptomycin. The culture was maintained in a humidified incubator with 5% CO2 at 37 °C. After 4–6 days, the division of non-neuronal cells was halted by addition of 15 µg/ml of fluorodeoxyuridine and 35 µg/ml of uridine. These cultures were maintained for up to 8 weeks and used for all biochemical analysis.

2.3. MTT assay

SH-SY5Y cells or fetal mesencephalic cells were seeded in 96-well plates at a density of 0.5 × 10^5 cells/well. The cultures were grown for 24 h followed by new medium containing various concentrations of salsolinol alone or co-incubation with N-acetylcyesteine. Cell viability was determined by MTT assay [42]. Briefly, after incubation for 24 or 48 h in case of mesencephalic cultures, 30 µl of MTT reagent (0.5 mg/ml MTT in PBS containing 10 µM HEPES) was added to each well and incubated in a CO2 incubator for 2 h. The medium was aspirated from each well and the culture plate was dried at 37 °C for 1 h. The resulting formazan dye was extracted with 100 µl of 0.04 N HCl in isopropanol and the absorbance was measured in a microplate reader (Molecular Device, Sunnyvale, CA) at 570 and 630 nm.

2.4. Fluorescence microscopic study

For nuclear staining, SH-SY5Y cells were grown in chamber slides (lab tek-II) and treated with salsolinol for 24 h. After incubation, the chambers were briefly rinsed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 30 min. Finally, nuclear stain DAPI (50 pmol/ml) was added and kept under dark for 10 min. The slides were washed again with PBS, removed from the chambers, and examined using a fluorescence microscope (Olympus BX60). Digital images were obtained using an Image-Pro advanced computer software.

Fetal mesencephalic cells from control and MTtrans mice were grown on sterile microscopic coverslips in culture dishes. After sufficient growth 100 µM of salsolinol was added and the cells incubated for 48 h. Medium was then removed, and the cover slips washed with 1 × PBS. The cells were fixed in 4% paraformaldehyde solution for 30 min. The cover slips were incubated with cleaved caspase-3 antibody for 3 h followed by FITC-conjugated secondary antibody for 1 h. Finally the cover slips were treated with the fluorochrome DAPI (50 pmol/ml) for 10 min. The cover slips were washed again with PBS and examined using a fluorescence microscope for digital imaging.

2.5. Reactive oxygen species (ROS) assay

ROS levels were determined by the method of Bondy and Guo [3], using H2DCF-DA, which is de-esterified to 2′,7′-dichlorofluorescein acid, and then oxidized by ROS to fluorescent 2′,7′-dichlorofluorescein (DCF). SH-SY5Y cell cultures were treated with salsolinol for 24 h and then 20 µM H2DCF-DA was added and the cells incubated for an additional 30 min at 37 °C in the dark. DMSO (100 µl) was then added to each well and cells incubated for another 15 min at 37 °C in the dark. Fluorescence was measured using a microtiter spectrofluorometer (Packard Fluorocount™, Packard Bioscience, Downer Grove, IL) at 490 and 530 nm. Sample values were normalized according to their protein values.

2.6. Glutathione assay

Reduced glutathione (GSH) levels were measured using a fluorimetric assay method with monochlorobimane (MCB). Cells were treated with salsolinol for 24 h and the medium was removed, the cells were collected in 0.1 M PBS, sonicated and centrifuged at 14,000 rpm for 10 min. The supernatant was used to determine GSH. Ninety microliters of supernatant (sample) was incubated with 10 µl of MCB at 37 °C for 2 h and fluorescence was measured in a fluorimetric plate reader (Molecular Device) at 380/460 nm. GSH values were calculated using a GSH-standard curve. Sample values were normalized to protein values.

2.7. Enzyme-linked immunosorbent assay (ELISA) for metallothionein

Metallothionein isoforms (MT-I and MT-II) were measured by ELISA using a monoclonal anti-metallothionein antibody (DAKO-MT, E9) which cross-reacts with both MT isoforms. SH-SY5Y cells were treated with different concentrations of salsolinol and incubated for 24 h. Following treatment, the cells were washed twice with PBS, sonicated and centrifuged at 14,000 × g for 10 min. The supernatants were used to determine MT. Briefly, wells were coated with 500 ng of MT using bicarbonate buffer (50 mM, pH 9.8) in high-protein binding 96-well microtiter plates overnight at 4 °C. After blocking with 5% bovine albumin, 50 µl of sample or MT standards were added to the wells and incubated with 50 µl of MT antibody (1:1000) for 24 h at 4 °C. The wells were then washed and incubated with secondary antibody conjugated to alkaline phosphatase (1:5000) for 3 h at 37 °C in assay buffer. After washing, the wells were incubated with p-nitrophenyl phosphate substrate in 1% diethanolamine, pH 9.8 for 30 min at 37 °C. The color was read in a microtiter plate reader (Molecular Device) at 405 nm. Sample MT concentrations were calculated using a MT standard curve and normalized to protein values. MT levels were also estimated in control and MTtrans mesencephalic cells.
2.8. Assay for ATP

The ATP content in the cells was measured using the ATP Lite kit. SH-SY5Y cells were treated with various concentrations of salsolinol for 24 h. After incubation, the medium was removed, the cells washed once with PBS, and lysed by adding 100 μl of lysis solution. Twenty-five microliters of lysate plus 50 μl of substrate solution was transferred into microtiter plate wells (black color 96-well microtiter plate) and the plates were shaken for 2 min in an orbital shaker at 700 rpm. The plates were dark adapted for 10 min and the luminescence was measured in a Packard TopCount microplate scintillation and luminescence counter (Packard Bioscience).

2.9. Protein determination

Protein determination was carried out by the Bradford method [4], using Bio-Rad protein determination kit and bovine serum albumin as a standard.

2.10. Statistics

Experimental data were analyzed using analysis of variance (ANOVA) and Student’s t-test to compare experimental and control groups. Sigma-Stat statistical software was employed. All values are represented as mean ± S.E.M. The significance was taken when p values were 0.05 or less.

3. Results

3.1. Dose-dependent effects of salsolinol on cell viability

Salsolinol dose-dependently decreased cell viability in SH-SY5Y cells after exposure for 24 h (Fig. 1). At 500 μM concentration, salsolinol decreased cell viability to 32% of controls. Salsolinol-induced depletion in cell viability was completely prevented by N-acetylcysteine (Fig. 2). Fluorescent microscopic studies also revealed that salsolinol decreases the number of DAPI-positive cells after 24 h treatment with two different concentrations of salsolinol (100 and 500 μM) (Fig. 3). Nuclear condensation was observed after salsolinol (C) treatment compared to control cells which have intact large nucleus (A). At 100 μM, 51% cells, and at 500 μM, 97% cells with condensed nucleus was observed by salsolinol (Fig. 3).

3.2. Effects of salsolinol on GSH, MT and ROS level

Salsolinol decreased GSH levels in a dose-dependent manner after 24 h (Fig. 4). A significant decrease in
glutathione levels occurred at the low concentration of salsolinol (50 µM). At 50 µM, salsolinol decreased GSH levels significantly to 42.9 ± 2.75% of control value, and at 100 µM GSH decreased further to 19.6 ± 1.22% (p < 0.05) of control value. At 50 µM, the cell viability also decreased to 80% of control, suggesting that depletion of glutathione may be a primary cause for the cell death. However,

Fig. 3. Real-time digital fluorescence microscopic images of SH-SY5Y cells after exposure to salsolinol for 24 h. Cells were stained with nuclear stain DAPI. A: control; B: cells treated with 100 µM salsolinol; C: cells treated with 500 µM salsolinol (blue = DAPI-positive cells).

Fig. 4. The effect of salsolinol on reduced glutathione (GSH) level in SH-SY5Y cells. Cells were treated with salsolinol for 24 h. GSH levels were determined using a fluorimetric assay based on the high binding of the dye, monochlorobimane (MCB). Data represent mean ± S.E.M. of four separate determinations. The symbol * indicates significance between control and salsolinol-treated groups at p < 0.05.

Fig. 5. The effect of salsolinol on reactive oxygen species (ROS) level in SH-SY5Y cells. ROS levels were measured by fluorimetric method using H$_2$DCF-DA after treating the cells with salsolinol for 24 h. Values represent mean ± S.E.M. of four separate determinations. The results are presented as percent of untreated controls. The symbol * denotes significance between control and salsolinol-treated groups at p < 0.05.
salsolinol at higher concentrations increased ROS and MT levels in dopaminergic SH-SY5Y cells after 24 h (Figs. 5 and 6).

3.3. Effects of salsolinol on intracellular ATP level

The effects of salsolinol on intracellular ATP content in SH-SY5Y cells were determined using the ATP Lite kit after...
incubating the cells for 24 h. Salsolinol caused a dose dependent decrease in intracellular ATP content. At 500 μM, salsolinol decreased ATP levels to ~ 69% of control (Fig. 7).

3.4. Effects of salsolinol on cell viability and nuclear condensation in controlwt and MTtrans mice fetal mesencephalic cells

Increased MT levels were detected in MTtrans cells (5.4 μg/mg protein) compared to controlwt cells (2.3 μg/mg protein) (Fig. 8). A dose-dependent decrease in cell viability in controlwt and MTtrans cells was observed after salsolinol treatment (Fig. 9). However, in MTtrans cells, the viability was decreased to only 14% at 50 μM, 46% at 100 μM, 68% at 200 μM salsolinol concentrations compared to 30% at 50 μM, 68% at 100 μM and 88% at 200 μM for the respective wild-type controls (Fig. 9). Fig. 10 shows the DAPI staining in controlwt and MTtrans cells. In controlwt cells, salsolinol induced notable DNA condensation (64%) when compared to untreated controls (11%). In MTtrans cells treated with salsolinol (100μM) minimal DNA condensation was observed (6%).

3.5. Effects of salsolinol on caspase-3 activation in controlwt and MTtrans fetal mesencephalic cells

Fig. 11 shows the caspase-3 activation in controlwt and MTtrans cells. In controlwt cells salsolinol increased caspase-3 activation (52%) compared to untreated controls (2%). In untreated controls. Fig. 11 shows the caspase-3 activation in controlwt and MTtrans cells. In controlwt cells salsolinol increased caspase-3 activation (52%) compared to untreated controls (2%). In
MT_{trans} cells, however, the caspase-3 activation was very low (15%), even after salsolinol treatment (100 μM), indicating that in MT_{trans} cells the MT gene protects against salsolinol toxicity.

4. Discussion

Salsolinol and N-methyl(R)-salsolinol are known to be involved in the etiology of Parkinson’s disease (PD) [25,31,33]. In control human brains, their distribution have been examined in four regions, frontal cortex, caudate, putamen, and substantia nigra. N-methyl(R)-salsolinol was found to occur selectively in the nigrostriatum, whereas salsolinol distributes uniformly among the other brain regions [25,34]. N-methylation of salsolinol by an N-methyltransferase seems to bring about the selective toxicity to dopaminergic neurons [34]. Elevated levels of salsolinol and N-methyl(R)-salsolinol were found in CSF of patients with PD [24,31], and both these compounds are known to induce cell death in dopaminergic neurons [34,42]. However, the molecular mechanisms involved in the neurotoxicity induced by these compounds are poorly understood. Neurons undergoing oxidative stress and apoptosis have been observed in SNpc of postmortem brains with PD [29,41]. The imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense status is considered to be one of the critical factors in neurodegeneration, including PD. In the present study salsolinol dose-dependently decreased cell viability in SH-SY5Y cells. Biochemical analysis revealed that salsolinol significantly decreased GSH levels in the concentration range between 50 and 500 μM. However, only at higher concentrations, salsolinol significantly increased ROS levels, decreased ATP levels and caused nuclear condensation. As salsolinol at lower concentrations significantly decreased glutathione levels, hence glutathione deficiency may be one of the primary events in the cascade of cell death [8]. GSH functions as an essential antioxidant neutralizing the oxidative stress caused by free radicals. GSH depletion is known to be directly related to superoxide radical generation and is likely to play an important role in glutamate cytotoxicity [8,32]. Hence, the role of GSH, as a reductant, is extremely important particularly in the highly oxidizing environment of the cell. The sulfhydryl moiety of GSH can be used to reduce peroxides formed during oxygen transport.

Although GSH depletion was observed by salsolinol at as low as 50 μM, however, at this low concentration there was no parallel increase in ROS level. It was suggested that total GSH depletion (cytosolic and mitochondrial) is necessary before detection of ROS, because the GSH which is preserved in mitochondria can clear ROS even when cytosolic GSH is completely depleted [47]. In order to test further whether glutathione depletion is critical for loss of cell viability by salsolinol, we co-incubated the SH-SY5Y cells with N-acetylcysteine (NAC), a precursor for GSH synthesis along with salsolinol. NAC significantly prevented the depletion in cell viability caused by salsolinol. The results clearly suggest that salsolinol can cause oxidative stress in dopaminergic cells mainly by decreasing GSH levels and increasing the formation of ROS especially at higher concentrations. In addition, perhaps as a compensatory response, salsolinol increased MT levels in these cells. Evidence that GSH depletion causes neuron cell death has been obtained from cell culture studies using immature cortical neurons and a neuronal cell line. Heales et al. [13] and Li et al. [23] showed that a decrease in GSH triggers the activation of neuronal 12-lipoxygenase, which leads to the formation of peroxides, influx of Ca^{2+}, and ultimately cell death. Jain et al. [16] reported that GSH deficiency leads to mitochondrial damage in the brain. A deficiency in GSH raises the level of mitochondrial hydrogen peroxide which can damage mitochondria and reduce intra cellular ATP content. It is interesting to note that the proper functioning of the mitochondria is essential in maintaining the viable and healthy cells [12]. Abnormal mitochondria generate free radical production and ATP depletion. The mitochondrial pool of GSH is likely to be involved in maintaining intra-mitochondrial protein thiol levels [8] and excessive production of H_{2}O_{2} within mitochondria leads to the depletion of mitochondrial GSH. This in turn causes oxidation of proteins and impairment of mitochondrial function which fosters mitochondrial damage. As GSH synthesis also requires ATP, a deficiency of energy supplied by mitochondria is likely to affect the synthesis and cellular turnover of GSH. Our study strongly suggests that in addition to GSH depletion, salsolinol at higher concentrations induce cell death by generating ROS, depleting ATP and causing nuclear condensation.

We have also tested the effects of salsolinol on another antioxidant, metallothionein (MT). MT is known to participate in intracellular defense against reactive oxygen and nitrogen species [5,8,20,21]. This is illustrated by the observation that agents causing oxidative stress such as ethanol, iron, and inflammatory agents, induce the synthesis of MT and animals deficient in MT exhibit increased susceptibility to oxidative stress [22].

In our present study, we have observed an increase in the levels of cellular MT after treatment with salsolinol in SH-SY5Y cells. The increase in MT levels by salsolinol indicate that there is an ongoing oxidative stress in the cell. There is also evidence to suggest that GSH depletion can cause increase in MT [8,36]. Up-regulation of MT also occurs in some neurodegenerative disorders such as Alzheimer’s disease [49], amyotrophic lateral sclerosis [17], brain ischemia [48], and during aging [28]. These findings suggest that MT in the brain may buffer the oxidative stress and prevent tissue damage caused by
different factors. Rojas et al. [40] and Thakran et al. [45] have shown that agents which generate free radicals such as 6-hydroxydopamine, iron, hydrogen peroxide, and various alcohols promote synthesis of MT in cultured hippocampal neurons and in Chang liver cells. All together, these results suggest that the induction of MT that occurs during oxidative stress is a compensatory response in the brain to prevent oxidative stress [1].

Furthermore, in the present study, we report that MT overexpression inhibits salsolinol-induced toxicity in MT<sub>trans</sub> fetal mesencephalic cells. MT<sub>trans</sub> cells showed increased levels of MT compared to control<sub>wt</sub> cells. The cell viability data indicated that MT<sub>trans</sub> cells showed increased resistance to salsolinol-induced toxicity compared to control<sub>wt</sub> cells. Low caspase-3 activation and low nuclear condensation was observed in MT<sub>trans</sub> cells compared to control<sub>wt</sub> cells in response to salsolinol treatment. These results suggest that MT overexpression inhibits caspase-3 induction as well as its activation. It has been reported that salsolinol induces DNA strand breaks in PC12 cells and neurons in the presence of Cu<sup>2+</sup> or iron [18,19,43]. The DNA strand breaks were observed in calf thymus DNA or DNA isolated from PC12 cells after incubation with salsolinol (200 μM) along with Cu<sup>2+</sup>. It was suggested that salsolinol may induce reactive oxygen species which induce DNA fragmentation. In our study, we have observed that the intensity of DNA condensation by salsolinol was attenuated in MT<sub>trans</sub> cells compared to control<sub>wt</sub> cells, suggesting that MT overexpression reduces the DNA damage in MT<sub>trans</sub> cells. It was also suggested that MT can prevent DNA damage induced by peroxynitrite [5].

In conclusion, our study clearly demonstrates that salsolinol causes oxidative stress by depleting reduced GSH levels and by increasing ROS production in dopaminergic SH-SY5Y cells. The reduction in intracellular GSH levels may be critical for dopaminergic cell death in salsolinol-treated SH-SY5Y cells, as addition of N-acetylcysteine completely prevented salsolinol-induced depletion in cell viability. Furthermore, our study also suggest that salsolinol induces apoptosis in mice fetal mesencephalic cells by causing caspase-3 activation and nuclear condensation while MT isoforms provide neuroprotection against salsolinol-induced toxicity.

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