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Molecular Signaling Pathway in Manganese-Induced Neurotoxicity

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ABSTRACT

Exposure to high levels of manganese [Mn] has been shown to cause a Parkinson's-like syndrome known as 'Manganism. Present study has attempted to elucidate the cellular and molecular pathways involved in manganese toxicity employing an immortalized dopaminergic cell line. Mn-treated cells exhibited a concentration-dependent apoptosis that was caspase dependent. Mn induced a rapid surge of intracellular reactive oxygen species [ROS] generation, followed by p38 mitogen-activated protein kinase [MAPK] activation and nuclear accumulation of hypoxia-inducible factor-1alpha [HIF-1alpha]. Activation of p38 MAPK and HIF-1alpha accumulation were attenuated by N-acetyl-L-cysteine, GSH [antioxidants], 1400W [specific iNOS inhibitor], or a selective p38 MAPK inhibitor [SB203580]. Finally, RNAi knockdown of HIF-1 alpha protected the cells from Mn. These results indicate that Mn activated the HIF-1alpha-mediated signaling pathway which served as an initiator of Mn induced apoptosis in neuronal cells.

Keywords: Manganese; Neurotoxicity; Apoptosis; MAP Kinase; Oxidative Stress

Abbreviations: BNIP3: Bcl-2/Adenovirus E1b 19kda Interacting Protein; NAC: N-Acetyl Cystinine; DCF-DA-2′,7′-Dichlorofluorescein-Diacetate; FBS: Fetal Bovine Serum; HIF-1α: Hypoxia-Inducible Factor-1α: Mn: Manganese; p38 MAPK: p38 Mitogen-Activated Protein Kinase; NAC: N-Acetyl-L-Cysteine; PBS: Phosphate-Buffered Saline; ROSP: Reactive Oxygen Species; siRNA: Small Interfering RNA; TUNEL: *In Situ* Terminal Deoxynucleotidyl Transferase-Mediated DNA Nick-End Labeling

Introduction

Manganese [Mn] is an essential trace element required for development and multiple physiological functions [1]. However, chronic exposure to excessive Mn levels can lead to a variety of psychiatric and motor disturbances, termed manganism [2,3]. Generally, exposure to ambient Mn air concentrations of more than 5 μg Mn/m3 can lead to Mn-induced symptoms. These exposure levels are encountered in occupational cohorts employed in welding [4,5], Fe and/or Mn smelting [6], mining [7], as well as the manufacturing of batteries [8]. Excess brain Mn represents a risk factor for idiopathic Parkinson's disease [IPD] [9,10]. Mn is known to cause mitochondrial dysfunction [11], including the inhibition of the enzymes of the tricarboxylic acid [TCA] cycle [12,13] and a reduction in the activities of the electron transport chain [14], ultimately resulting in ATP depletion [15]. Some of these mitochondrial events are significantly blocked by antioxidants [16], suggesting the involvement of oxidative stress in the mechanism of Mn-induced mitochondrial dysfunction. Therefore, understanding the underlying molecular mechanism[s] of Mn-induced neurotoxicity is of increasing importance.Apoptosis mediated by hypoxia is linked to upregulation of pro-death Bcl-2 proteins by stimulating hypoxia-inducible factor-1 [HIF-1]. Hypoxia-inducible factor-1 is a transcriptive factor that regulates genes involved in metabolism, angiogenesis, proliferation, and apoptosis. HIF-1 is composed of HIF-1α and HIF-1β subunits. In conditions of normal oxygen concentration, HIF-1β is constitutively expressed,

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whereas HIF-1α undergoes rapid degradation by proteasomes [17]. Under hypoxic conditions, proteasomal degradation of HIF-1α is reduced, leading to HIF-1α accumulation and translocation to the nucleus. In the nucleus, HIF-1α binds to the hypoxia response element [HRE] to activate the transcription of pro-death target genes [18].

It was reported that several non-hypoxic stimuli can also stimulate HIF-1 protein levels including vasoactive peptides, cytokine, and hormones [19-21]. All these stimuli can activate the HIF-1 cascade by stimulating ROS production. Therefore, a non-hypoxic stimulus can also activate HIF-1α through redox signaling through activation of certain kinases or inactivation of phosphatases [22]. It was shown that overexpression of p38 MAP kinases can enhance HIF-1α protein levels under normoxic or hypoxic conditions [23]. We have previously reported that in a rat dopaminergic cell line, p38 MAPK is phosphorylated through a ROS-mediated pathway [Toxicologist 2008]. Thus, it is possible that Mn-induced stimulation of p38 MAPK could lead to activation of HIF-1 pathway to initiate a cell death cascade. In the current study, Mes 23.5, an immortalized dopaminergic cell line, was used to investigate Mn neurotoxicity. This cell line displays properties of substantia nigra zona compacta neurons and is considered a dopaminergic cell that expresses tyrosine hydroxylase and synthesizes dopamine [24].

Materials and Methods

Cell Culture

The Mes 23.5 cell line was derived from somatic cell fusion of rat embryonic mesencephalic cells and the murine neuroblastoma-glioma cell line N18TG2. Cells were seeded on poly-l-lysine-precoated plates and maintained in DMEM supplemented with 5% fetal bovine serum [FBS], 2% new born calf serum [NBS], 15 mM HEPES and SATO components [insulin 5 µg/ml, transferrin 5 µg/ml, pyruvic acid 48.6 µg/ml, putrescine 4 µg/ ml, sodium selenite 5 ng/ml, progesterone 6.3 ng/ml] at 37°C in an atmosphere of 5% CO2 and 95% air.

Apoptosis Assay

Apoptosis was quantitated 24 h after exposure to different concentrations of Mn. The insitu terminal deoxynucleotidyl transferase-mediated DNA nick-end labeling [TUNEL] assay was used to confirm apoptosis. The TUNEL assay was performed on paraformaldehyde [4% in PBS] fixed cells using ApoptagTM in situ apoptosis detection kit as described previously [25]. Cells with condensed and fragmented DNA were considered apoptotic.

Measurement of ROS Generation

MES 23.5 cells grown in a polystyrene 96-well plate at a density of 30,000 cells per well were treated with 600 mM of Mn for 3 h. The generation of ROS was assessed by an oxidation-sensitive fluorescent probe DCFH-DA [26]. DCFH-DA is a non-polar compound that readily defuses into cells, where it is cleaved by intracellular esterases to form DCFH and, thereby, is trapped inside the cells. DCFH is oxidized to the highly fluorescent 2,7-dichlorofluorescein [DCF] by ROS. Cells were loaded with 30 mM DCFH-DA [Molecular Probes, Eugene, OR] for 30 min at 37 8C in the dark and then washed with PBS to remove free DCFHDA. After Mn treatment, the culture medium was removed and cells were washed twice with PBS. Fluorescence intensity was monitored with a microtiter plate reader at the excitation wavelength of 485 nm and emission wavelength of 535 nm. Values were expressed as percent of control groups [without Mn treatment].

Western Blotting

Following treatments, whole cell lysates were prepared using a lysis buffer containing 220 mM mannitol, 68 mM sucrose, 20 mM HEPES, pH 7.4, 50 mM KCl, 5 mM EGTA, 1 mM EDTA, 2 mM $MgCl_2$, 1 mM dithiothreitol, 0.1% Triton X-100 and protease inhibitors. Western blotting was carried out using the ECF Western blot kit [Amersham Biosciences, Piscataway, NJ] as described by the manufacture. The primary antibodies were: anti-β-actin antibody [Sigma Chemical Co, St. Louis, Mo], anti-phospho-p38 MAPK antibody, anti-p-38 MAPK antibody. For detection of nuclear HIF-1α, nuclear extracts of the cells were prepared. Briefly, cells were harvested and lysed in buffer containing 10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 1 mM PMSF, supplemented with a protease inhibitor cocktail and centrifuged at 14,000 rpm for 5 min at 4°C. Supernatants were harvested as cytosolic extracts. The pellets were further lysed with buffer containing 20 mM HEPES, 1.5 mM $MgCl_2$, 0.42 mM NaCl, 0.2 mM EDTA, 0.5 mM DTT, 25% glycerol, supplemented with a protease inhibitor cocktail. Thirty minutes later, the supernatants [nuclear extracts] were harvested after centrifugation at 14,000 rpm at 4°C for 10 min. HIF-1α expression in nuclear extracts was detected by Western blotting as described above. The primary antibodies were anti-HIF-1α [Novus, Littleton, CO] and antihistone H3 antibodies [Cell Signaling, Danvers, MA].

RNA Interference

Pre-designed siRNA for HIF-1α [Santa Cruz Biotechnology, Santa Cruz, CA] was used to knock down gene expression. The silencer negative control siRNA, which does not target rat, mouse or human genes, was used as a negative control [Ambion, Austin, TX]. Transient transfection of siRNA was performed with Lipofectamine 2000™ [Invitrogen, Carlasbad, CA].

Statistics

Data were expressed as mean ± SEM. One-way analysis of variance [ANOVA] followed by Tukey-Kramer procedure for multiple comparisons was used to determine statistical differences between treatments. Differences were considered significant at $P<0.05$.

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Results

Manganese-Induced Apoptosis Correlates with Increased HIF-1 Alpha Expression

In Mes 23.5 cells, Mn induced a concentration-dependent apoptotic cell death over the range of 200–600μM, (Figure 1A). The percentage of cells exhibiting positive TUNEL staining was significantly higher in the Mn-treated group than in the control cells. A similar increase in oxidative stress has been observed in cells treated with varying concentrations of manganese [data not shown]. To establish whether Mn could alter HIF-1alpha protein level, cells were exposed to Mn [200–600 mM] for 24 h and nuclear expression was analyzed by Western blotting. Mn exposure induced a concentration-dependent increase of HIF-1 alpha expression paralleling the cell death response. HIF-1 alpha was expressed at a low level under control conditions, whereas exposure to Mn [600 mM] rapidly increased expression (Figure 1B).

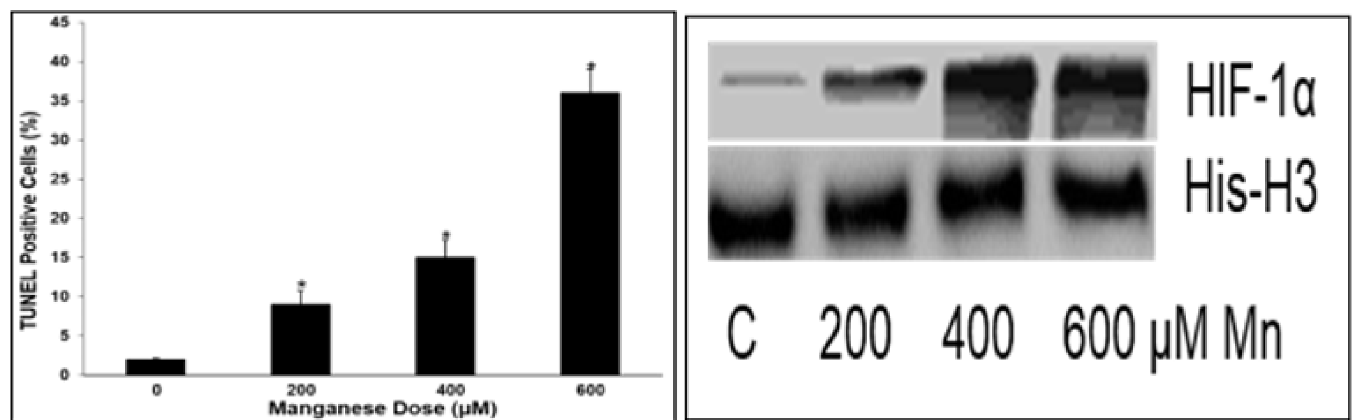

Figure 1:
A. Mn-induced apoptosis. Mes 23.5 cells were treated with Mn (200-600 μM) for 24 hrs. Apoptosis was determined using TUNEL assay.
B. Mn-induced HIF-1α expression. Mes 23.5 cells were treated with varying concentrations of Mn for 12 hrs. and cell lysates were subjected to nuclear fractionation followed by Western blotting to determine HIF-1α levels.

Oxidative Stress-Mediated p38 MAPK Pathway is Involved in HIF-1α Activation by Mn

It has been shown that the p38 MAPK activation is an upstream initiator of Mn-induced apoptosis in brain cells. In the present study, manganese activated p38 MAPK within 2 hrs. and peaked at 4 hrs. (Figure 2A). The activation, as measured by protein phosphorylation, was blocked by SB203580, a p38 MAPK antagonist, whereas the control peptide SB202474 did not alter phospho-p38 expression. Pretreatment with the antioxidant NAC also reduced p38 MAPK activation, showing that ROS is an initiating signal for the kinase. (Figure 2B) Since p38 MAPK can regulate HIF-1α accumulation in the nucleus, it was determined whether HIF-1α was activated by Mn. Increased HIF-1α expression was observed within 1 h of Mn exposure, and maximal levels were detected at 3 h (Figure 3A). Also, total cellular HIF-1α levels were increased by Mn [data not shown]. The increased HIF-1α expression was markedly suppressed by NAC, CAT, 1400W, and SB203580 (Figure 3B). Thus Mn-induced HIF-1α accumulation is dependent on oxidative stress and p38 MAPK activation. On the other hand, pretreatment with antioxidants and p38 MAPK inhibitor resulted in a significant reduction of apoptosis (Figure 3C).

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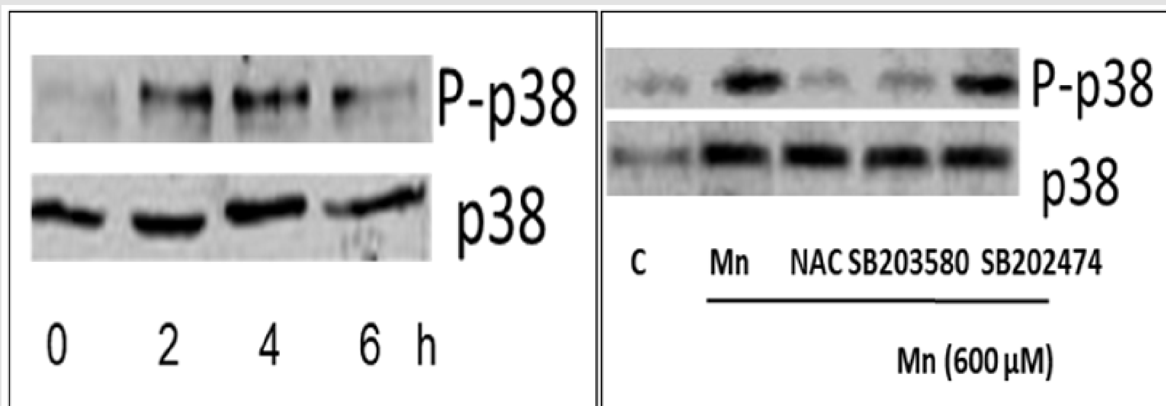

Figure 2:
A. Mn exposure leads to activation of p38 MAP Kinase. Cells were treated with 600 μM of Mn for 2 to 6 hrs. Expression and phosphorylation of p38 MAP Kinase determined by Western blotting.
B. Effects of antioxidant and a p38 inhibitor. Cells were pretreated for 30 min with NAC (0.5 mM), SB203580 (20 μM) prior to treating with 600 μM Mn for 6 h. Phosphorylation of p38 MAP Kinase determined by Western blotting.

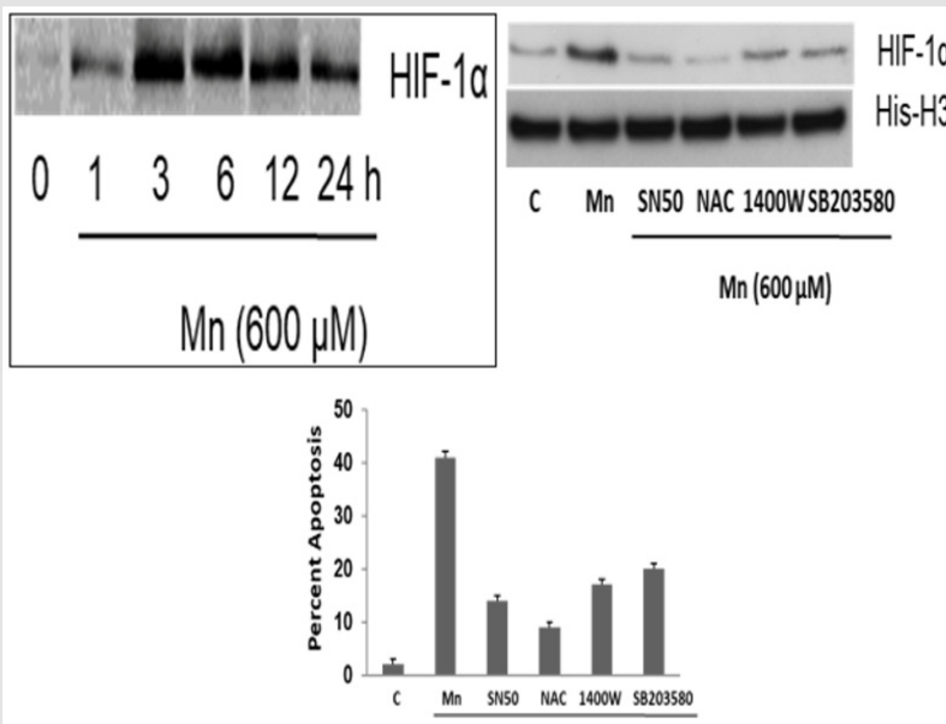

Figure 3:
A. Mn-induced HIF-1α expression. Mes 23.5 cells were treated with 600 μM of Mn. Samples were collected at different time points and cell lysates were subjected to nuclear fractionation followed by Western blotting to determine HIF-1α levels.
B. Effects of blocking oxidative stress and a p38 MAPK phosphorylation on HIF-1α protein expression. Cells were pretreated for 30 min with NAC (0.5 mM), 1400W (0.1 mM), SB203580 (20 μM) or SN-50 (20 μM) prior to 600 μM Mn treatment for 6 h. Cell lysates were subjected to nuclear fractionation followed by Western blotting.
C. Effects of blocking oxidative stress and a p38 MAPK phosphorylation Mn-induced apoptosis. Cells were pretreated for 30 min with NAC (0.5 mM), 1400W (0.1 mM), SB203580 (20 μM) prior to 600 μM Mn treatment for 24 hr. Apoptosis determined by TUNEL staining.

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Downregulation of HIF-1 alpha Prevents Mn-Induced Apoptosis

To determine that HIF-1 alpha is an upstream regulator of cell death, Mn-induced expression of HIF-1 alpha was knocked down by RNAi. The RNAi treatment markedly reduced HIF-1 alpha expression and protected the cells from manganese-induced apoptosis Transfection with a negative control RNAi did not significantly alter induction of HIF-1 alpha expression or cell death by Mn (Figures 4A & 4B).

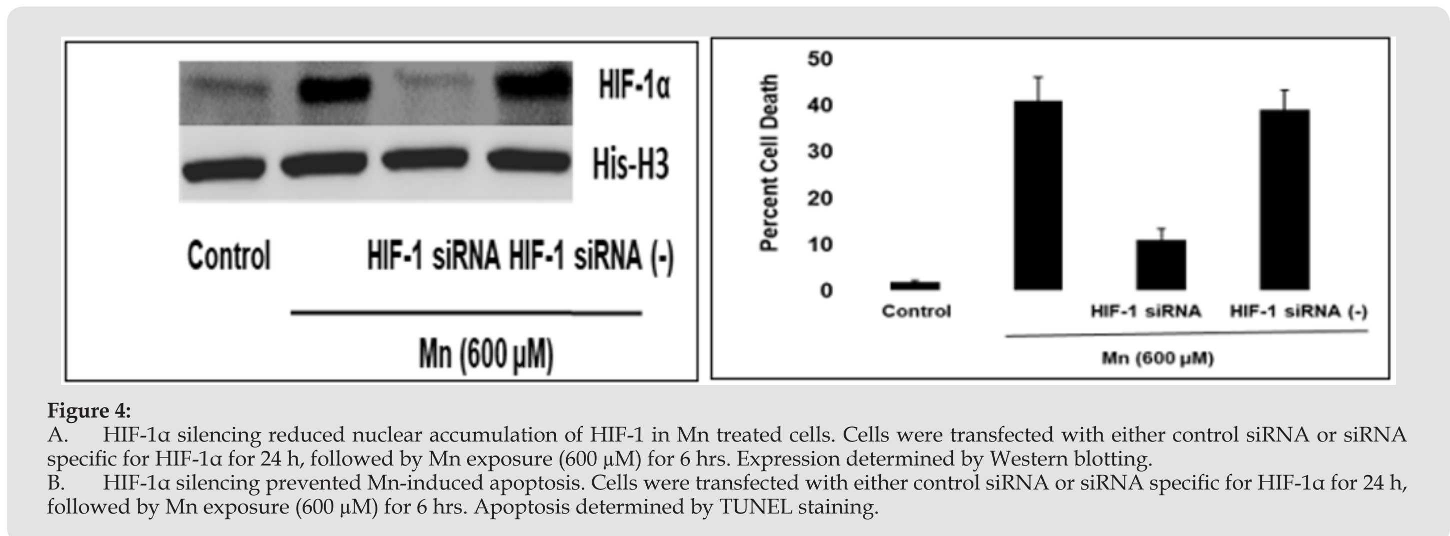

Figure 4:
A. HIF-1α silencing reduced nuclear accumulation of HIF-1 in Mn treated cells. Cells were transfected with either control siRNA or siRNA specific for HIF-1α for 24 h, followed by Mn exposure (600 μM) for 6 hrs. Expression determined by Western blotting.
B. HIF-1α silencing prevented Mn-induced apoptosis. Cells were transfected with either control siRNA or siRNA specific for HIF-1α for 24 h, followed by Mn exposure (600 μM) for 6 hrs. Apoptosis determined by TUNEL staining.

Discussion

In the present study, Mn induced a rapid surge of ROS generation, followed by activation of p38 MAPK and HIF-1 alpha accumulation HIF1 alpha is a transcription factor, hence, it is likely that manganese-induced toxicity is dependent upon gene activation. Both normoxic and hypoxic conditions can upregulate HIF-1. A non-hypoxic stimulus such as oxidative stress induces HIF-1 activation. Substances such as growth factor, thrombin, or insulin can promote HIF-1 expression through increasing cellular ROS generation [19,27,28]. These responses were inhibited by antioxidants or over-expression of redox-modifying enzymes, indicating that an antioxidant state reduces levels of free radicals to limit activation of the HIF pathway, on the other hand, elevated cellular levels of oxidative radicals promote the pathway. In the present study, the initiation signal for HIF-1 accumulation by Mn was reactive oxygen species, whereas treatment with a free radical scavenger such as NAC reduced ROS and prevented apoptosis by inhibiting HIF-1 pathway activation. Present results indicate that p38MAPK signaling is necessary for the induction of HIF-1α by Mn. The p38 MAPK, a member of the MAP kinase family involved in apoptotic cell death can be activated by phosphorylation [29,30].

Numerous studies have shown that stimulation of cellular ROS generation can lead to MAP kinase activation whereas antioxidants can inhibit activation. In the case of Mes 23.5 cells, Mn-induced activation of p38 MAP kinase preceded nuclear HIF-1α accumulation since pharmacologic inhibition of p38 MAP kinase blocked the expression of HIF-1α. Although the mechanism underlying HIF-1α regulation by p38 MAPK is unclear, it appears that p38 MAPK acts as a redox-sensitive factor to induce HIF-1α stabilization, followed by nuclear translocation to promote transcription of target genes. The functional result is a transfer of the redox signal to the nucleus to initiate transcription of pro-death gene products. As a transcription factor, HIF-1α regulates a series of target genes encoding proteins that promote cell survival [31]. On the other hand, HIF-1α can also participate in cell death by activating pro-death genes, including BNIP3, and Nix [32]. BNIP3 expression can be upregulated under both hypoxic and non-hypoxic conditions in cell lines derived from carcinomas, fibroblasts, and macrophages [32,33]. In the nervous system, BNIP3 functions as a pro-death factor in select brain regions following subarachnoid hemorrhage [34] or focal cerebral ischemia [35]. Recently, BNIP3 was linked to brain developmental apoptosis in which BNIP3 mRNA increased in parallel with developmental cell death in neonatal rat brains [36]. BNIP3 induction was shown to be regulated by HIF-1α and induces apoptotic cell death in many non-neuronal cell lines [Bruick [37,38]]. A similar induction of BNIP3 by Mn in a dopaminergic cell line was reported by us. Knockdown of BNIP3 by siRNA transfection protected cells from cytotoxicity, providing strong support for the role of BNIP3 in cell death [26]. Overall, these results, show that in Mn-induced cell death by upregulating HIF-1. Oxidative stress and p38 MAP kinase activation initiate upregulation of HIF-1α mediated signaling and its role in inducing the expression of cell death-promoting genes.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Bowman AB, Kwakye GF, Herrero Hernández E, Aschner M (2011) Role of Manganese in Neurodegenerative Diseases. J Trace Elem Med Biol 25(4): 191-203.

2. Aschner M, Erikson KM, Herrero Hernández E, Tjalkens R (2009) Manganese and Its Role in Parkinson's Disease: from Transport to Neuropathology. Neuromolecular Med 11(4): 252-266.

3. Ellingsen DG, Konstantinov R, BastPettersen R, Merkurjeva L, Chashchin M, et al. (2008) A Neurobehavioral Study of Current and Former Welders Exposed to Manganese. Neurotoxicology 29(1): 48-59.

4. Bowler RM, Gysens S, Diamond E, Nakagawa S, Drezgic M, et al. (2006) Manganese Exposure: Neuropsychological and Neurological Symptoms and Effects In Welders. Neurotoxicology 27(3): 315-326.

5. Park JD, Kim KY, Kim DW, Choi SJ, Choi BS, et al. (2007) Tissue Distribution of Manganese in Iron-Sufficient or Iron-Deficient Rats After Stainless Steel Welding-Fume Exposure. Inhal Toxicol 19(6-7): 563-572.

6. Myers JE, teWater Naude J, Fourie M, Zogoe HB, Naik I, et al. (2003a) Nervous system effects of occupational manganese exposure on South African Manganese Mineworkers. Neurotoxicology 24(4-5): 649-656.

7. Myers JE, Thompson ML, Naik I, Theodorou P, Esswein E (2003b) The Utility of Biological Monitoring for Manganese in Ferroalloy Smelter Workers In South Africa. Neurotoxicology 24(6): 875-883.

8. Bader M, Dietz MC, Ihrig A, Triebig G (1999) Biomonitoring of Manganese in Blood, Urine And Axillary Hair Following Low-Dose Exposure During The Manufacture Of Dry Cell Batteries. Int Arch Occup Environ Health 72(8): 521-527.

9. Kim Y, Kim JW, Ito K, Lim HS, Cheong HK, et al. (1999) Idiopathic Parkinsonism with Superimposed Manganese Exposure: Utility Of Positron Emission Tomography. Neurotoxicology 20(2-3): 249-252.

10. Racette BA, McGeeMinnich L, Moerlein SM, Mink JW, Videen TO (2001) Welding-Related Parkinsonism: Clinical Features, Treatment, And Pathophysiology. Neurology 56(1): 8-13.

11. Gavin CE, Gunter KK, Gunter TE (1990) Manganese and Calcium Efflux Kinetics in Brain Mitochondria. Relevance To Manganese toxicity. Biochem J 266(2): 329-334.

12. Malthankar GV, White BK, Bhushan A, Daniels CK, Rodnick KJ (2004) Differential Lowering by Manganese Treatment of Activities of Glycolytic And Tricarboxylic Acid (Tca) Cycle Enzymes Investigated In Neuroblastoma And Astrocytoma Cells is Associated with Manganese-Induced Cell Death. Neurochem Res 29(4): 709-717.

13. Zheng W, Ren S, Graziano JH (1998) Manganese Inhibits Mitochondrial Aconitase: A Mechanism of Manganese Neurotoxicity. Brain Res 799(2): 334-342.

14. Rao KV, Norenberg MD (2004) Manganese Induces the Mitochondrial Permeability Transition in Cultured Astrocytes. J Biol Chem 279: 32333-32348.

15. Verity MA (1999) Manganese Neurotoxicity: A Mechanistic Hypothesis. Neurotoxicology 20(2-3): 489-497.

16. Chen CJ, Liao SL (2002) Oxidative Stress Involves Astrocytic Alterations Induced by Manganese. Exp Neurol 175(1): 216-225.

17. Hirota K, Semenza GL (2005) Regulation of Hypoxia-Inducible Factor 1 By Prolyl and Asparaginyl Hydroxylases. Biochem Biophys Res Commun 338(1): 610-616.

18. Greijer AE, vander Groep P, Kemming D, Shvarts A, Semenza GL, et al. (2005) Up-Regulation of Gene Expression by Hypoxia Is Mediated Predominantly By Hypoxia-Inducible Factor 1 (HIF-1). J Pathol 206(3): 291-304.

19. BelAiba RS, Djordjevic T, Bonello S, Flugel D, Hess J (2004) Redox-Sensitive Regulation of the Hif Pathway Under Non-Hypoxic Conditions In Pulmonary Artery Smooth Muscle Cells. Biol Chem 385: 249-257.

20. Haddad JJ, Land SC (2001) A Non-Hypoxic, Ros-Sensitive Pathway Mediates Tnf-Alpha-Dependent Regulation of Hif-1alpha. FEBS Lett 505(2): 269-274.

21. Biswas S, Gupta MK, Chattopadhyay D, Mukhopadhyay CK (2005) Up-regulation of Gene Expression by Hypoxia is Mediated Predominantly By Hypoxia-Inducible Factor 1 (HIF-1). J Pathol 206(3): 291-304.

22. Ueda S, Masutani H, Nakamura H, Tanaka T, Ueno M, et al. (2002) Redox control of cell death. Antioxid Redox Signal 4(3): 405-414.

23. Kietzmann T, Jungermann K, Görlach A (2003) Regulation of the Hypoxia-Dependent Plasminogen Activator Inhibitor 1 Expression by Map Kinases. Thromb Haemost 89(4): 666-673.

24. Le WD, Colom LV, Xie WJ, Smith RG, Alexianu M, et al. (1995) Cell death induced by beta-amyloid 1-40 in MES 23.5 hybrid clone: the role of nitric oxide and NMDA-gated channel activation leading to apoptosis. Brain Res 686(1): 49-60.

25. Prabhakaran K, Li L, Mills EM, Borowitz JL, Isom GE (2005) Upregulation of Uncoupling Protein 2 By Cyanide Is Linked with Cytotoxicity in Mesencephalic Cells. J Pharmacol Expl Therap 314: 1338-1345.

26. Prabhakaran K, Chapman GD, Gunasekar PG (2009) Bnip3upregulation and Mitochondrial Dysfunction in Manganese-Induced Neurotoxicity. Neurotoxicology 30: 414-422.

27. Calvani M, Rapisarda A, Uranchimeg B, Shoemaker RH, Melillo G (2006) Hypoxic induction of an HIF-1alpha-dependent bFGF Autocrine Loop Drives Angiogenesis in Human Endothelial Cells. Blood 107: 2705-2712.

28. Leek RD, Stratford I, Harris AL (2005) The Role of Hypoxia-Inducible factor-1 in three-dimensional tumor growth, apoptosis, and Regulation by The Insulin-Signaling Pathway. Cancer Res 65: 4147-4152.

29. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, et al. (1995) Pro-Inflammatory Cytokines and Environmental Stress Cause P38 Mitogen-Activated Protein Kinase Activation by Dual Phosphorylation on Tyrosine and Threonine. J Biol Chem 270: 7420-7426.

30. Ho TC, Yang YC, Cheng HC, Wu AC, Chen SL, et al. (2006) Activation of Mitogen-Activated Protein Kinases Is Essential for Hydrogen Peroxide -Induced Apoptosis In Retinal Pigment Epithelial Cells. Apoptosis 11: 1899-1908.

31. Yeo EJ, Chun YS, Park JW (2004) New anticancer strategies targeting HIF-1. Biochem Pharmacol 68: 1061-1069.

32. Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL, et al. (2001) HIF-1-Dependent Regulation of Hypoxic Induction of The Cell Death Factors BNIP3 and NIX in human tumors. Cancer Res 61: 6669-6673.

33. Vengellur A, LaPres JJ (2004) The Role of Hypoxia Inducible Factor 1alpha In Cobalt Chloride Induced Cell Death in Mouse Embryonic Fibroblasts. Toxicol Sci 82: 638-646.

34. Ostrowski RP, Colohan AR, Zhang JH (2005) Mechanisms of Hyperbaric Oxygen-Induced Neuroprotection in a Rat Model of Subarachnoid Hemorrhage. J Cereb Blood Flow Metab 25(5): 554-571.

35. Althaus J, Bernaudin M, Petit E, Toutain J, Touzani O, et al. (2006) Expression of the Gene Encoding the Pro-Apoptotic BNIP3 Protein and Stimulation of Hypoxia-Inducible Factor-1alpha (HIF-1alpha) protein following focal cerebral ischemia in rats. Neurochem Int 48(8): 687-695.

36. Sandau US, Handa RJ (2006) Localization and Developmental Ontogeny of The Pro-Apoptotic Bnip3 mRNA in the postnatal rat cortex and hippocampus. Brain Res 1100: 55-63.

37. Bruick RK (2000) Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc Natl Acad Sci U S A 97(16): 9082-9087.

38. Vande Velde C, Cizeau J, Dubik D, Alimonti J, Brown T, et al. (2000) BNIP3 and Genetic Control of Necrosis-Like Cell Death Through the Mitochondrial Permeability Transition Pore. Mol Cell Biol 20(15): 5454-5468.

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