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Increased Mitochondrial Superoxide Dismutase 2 Level but Not Cytosolic SOD1 Is Associated with Oxidative Stress Of SH-SY5Y Neuroblastoma Cells Induced By Carbamates Pesticide

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Abstract

Neurodegenerative diseases are associated with oxidative stress. By using SH-SY5Y human neuroblastoma cells as a model, we assessed that pesticide containing carbamates as an active group targets mitochondria and responsible for oxidative cell death. Our results showed that when SH-SY5Y cells were exposed in time dependant manner with carbamates compound, cell death and mitochondrial SOD enzyme level were increased. However, there was no change in the level of cytosolic SOD1 enzyme was observed in time dependant manner in SH-SY5Y cells. These findings revealed that the cytotoxicity and oxidative stress in SH-SY5Y is linked with mitochondrial SOD2 induced by carbamates pesticide. These data also indicate that mitochondria are important early targets of carbamate-induced oxidative neurotoxicity.

Introduction:

Pesticides have an active compound that is carbamates and are esters of carbamic acid. These compounds are referred to as N-methylcarbamates. Derivatives of carbamic acid, thiocarbamic acid, and dithiocarbamic acid are used as herbicides [1]. However, over exposure of humans and animals to these pesticides often results in poisonings. N-methylcarbamate insecticides produce their toxicity by inhibiting acetylcholinesterase enzyme. As a result, the toxic signs are of hypercholinergic activity. Carbamate-induced excitotoxicity also involves hyperactivation of N-methyl-d-aspartate receptors. Studies reported that carbamates causes DNA damage, chromosomal aberrations and oxidative stress in mouse and human lungs cell lines [2].

Number of studies has documented that the exposure of environmental pollutant, pesticides and fungicides are associated with neurodegenerative diseases like Parkinson's disease. Parkinson's disease (PD), which is a progressive neurodegenerative disorder affecting mainly the elderly people, appears in two major forms, familial and sporadic, and the latter variety accounts for nearly 90–95% of PD subjects [3]. The pathological hallmark of PD is the degeneration of dopaminergic neurons of substantia nigra that project into striatum. The underlying mechanisms of dopaminergic neuronal death in sporadic PD are still

uncertain, but mitochondrial dysfunctions, oxidative stress, proteolytic impairment with abnormal accumulation of proteins like alpha-synuclein, and inflammatory reactions are key elements in this complex pathogenesis [4].

Oxidative stress is one of the mechanisms highly associated with neurodegenerative diseases. A number of researchers have recently suggested that mitochondria were at the center of the neuronal oxidative stress and also oxidant-induced neuronal death. Superoxide dismutase (SOD) is one of the major antioxidant enzymes in eukaryotes which convert free radicals in the form of superoxide anions to hydrogen peroxide. There are three types of SOD found in cells which have different function and location within cells [5].

The copper/zinc SOD (Cu/ZnSOD or SOD1) is a cytosolic enzyme accounting for 70–80 % of the total cellular SOD activity. The manganase superoxide dismutase (MnSOD or SOD2) is a key mitochondrial antioxidant enzyme coded by the Sod2 gene. Extracellular copper/zinc SOD (SOD3), a minor SOD coded by the Sod3 gene, is only expressed in a limited number of tissues (lung, kidney, and fat tissue) [6].

In the present study, therefore, we sought to demonstrate that mitochondria are crucial initial targets of carbamates-induced oxidative cell death in SH-SY5Y neuroblastoma cells. We used N-succinimidyl N-methylcarbamate (NSNM), a surrogate chemical containing a functional methyl carbamate group to understand the mode of action of this class of compounds in cultured neuroblastoma cell line-SH-SY5Y. SH-SY5Y cells are widely used in PD research.

Materials and Methods

The reagents used in the study were obtained from the following sources: Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotic solution, N succinymidyl N methyl carbamates (NSNM) and 6 hydroxydopamine (6 OHDA) from SigmaAldrich. Superoxide dismutase kit was procured from IBL international company. All common chemicals and reagents used were obtained from Himedia, India.

Cell culture

For the present study SH-SY5Y (a human dopaminergic neuroblastoma cell line) were used and was gifted by Dr. Amit Deshpandey (Deshpandey laboratories, Bhopal, India) and was maintained in DMEM medium containing 10% FBS, 1 % antibiotic solution in an incubator at 37°C and 5% CO2. Cells were subcultured in a ration of 1:3 on every third day. For cell expansion and experiments with isolated cells, the SH-SY5Y cells were detached with 1X trysin-EDTA (0.25%). There was a control sample which remained untreated and received the equal volume of medium and a positive control 6OHDA which exerts neurotoxic effect in SH-SY5Y cells. All different treatments were carried out in triplicate.

Cell treatment

Cells were treated with NSNM in time dependent manner for 24, 48 and 72 hrs, keeping dose constant i.e. 0.005 µM. Dose was decided by calculating IC50 and literature search. Stock solution of NSNM was prepared in Dimethyl sulphoxide (DMSO).

Trypan Blue Assay:

10000 cells were seeded per well in a 6 well plate. Cells were treated with 0.005 µM NSNM and incubated at 37 °C, 5% CO2 for different time points. At the end point, the cells were gently scrapped and single cell suspension was prepared by retropipetting. The cell suspension was mixed with trypan blue 1:1 and counted in a neubauer chamber. The viable cell counting was represented as cells per ml.

Cell cytotoxicity assay:

MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. Cells were plated in 96-well culture plates at a density of 5 × 103 cells/well. After overnight incubation, cells were treated with NSNM in a time (24hr, 48hr and 72 hr) in triplicate. After the exposure time cells were then incubated with 20µl of 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide solution (0.5mg/mL) for 3-4hrs. The formazan particles were then solubilized with 200 µl dimethyl sulfoxide and kept on orbital shaker for 5 mins. The absorbance was read at 570 nm and expressed relative at 630 nm with the microplate reader. The results were expressed as survival percentage with respect to an untreated control . % cell viability = OD of sample ×100/OD of blank

Superoxide dismutase assay

Cells were treated with 0.005 µM NSNM for 24, 48 and 72 hrs. Cells were trypsinized and centrifuged at 1200 rpm for 10 minutes. Supernatant was collected in fresh tube for enzymes activity. To separate Cu/Zn SOD and MnSOD, supernatant was centrifuged at 10,000g for 15 min at 4 C. The resulting supernatant contains Cu/Zn SOD and pellet contains MnSOD. Protocol was performed as per kit insert. Optical density was calculated at 450 nm. Optical density is directly proportional to enzyme activity.

Statistical analysis

Student t test was used to calculate the difference between the groups. P value less than 0.05 was considered significant.

Results

MTT results showed that on increasing exposure time of 0.005 uM NSNM, SH-SY5Y cells viability decreases significantly ($p \leq 0.05$). At 72 hr cell viability was found to be 94 % ± 5.6 as compared to control. Figure 1.

On increasing time duration of exposure of NSNM, Mn SOD activity was found be increased statistically as compared to Cu/Zn SOD ($p \leq 0.05$). At 72 hr of treatment Mn SOD activity was found to be 1.0 ± 0.2 OD and Cu/Zn SOD 0.2 ± 0.01 OD. OD of Mn SOD and Cu/Zn SOD on exposure with 6OHDA were found to be 1.4 ± 0.5 and 1.0 ± 0.5 respectively. Figure 2.

Discussion

SOD is an enzyme which is first line of defense on cytotoxic insult. This study revealed that, on exposure of NSNM in SH-SY5Y cells, there is the difference in activity of SOD 1 and SOD 2 was found in time dependant manner. Mitochondrial SOD was actively involved as compared to cytosolic SOD. There is mounting evidence suggesting that elevated mitochondrial superoxide production is a contributing factor in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis [7]. Our results are in agreement with other studies that shows mitochondria is affected by NSNM. A study conducted by Fukui and Zhu 2010 [8] showed that mitochondrial SOD activity was elevated as compared to cytosolic SOD after glutamate exposure in HT22 neuronal cells. Another study done by Biosa et al., 2019 [9] showed that SOD 1 and SOD 2 rescue SH-SY5Y cells from the toxic effect of dopamine derived products.

Conclusion

Mitochondrial SOD2 enzyme plays an important role in SH-SY5Y neuroblastoma cells against NSNM induced oxidative stress. The marked difference in activity of SOD2 versus SOD1 suggests that mitochondrial oxidative stress is a critical early event in NSNM-induced oxidative stress. With this finding it might be concluded that mitochondrial SOD2 in the nervous system may represent an effective therapeutic strategy against oxidative stress.

References

1. Nicolopoulou-Stamati, P, Maipas, S, Kotampasi, C, Stamatis, P, Hens, L. 2016 Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Front Public Health.18;4:148.
2. Azad, GK, Singh, V, Tomar, RS. 2014 Assessment of the biological pathways targeted by isocyanate using N-succinimidyl N-methylcarbamate in budding yeast Saccharomyces cerevisiae. PLoS One. 24;9(3):e92993.
3. Nandipati, S, Litvan, I 2016 Environmental Exposures and Parkinson's Disease. Int J Environ Res Public Health. 3;13(9):881.
4. Chin-Chan, M, Navarro-Yepes, J, Quintanilla-Vega, B. 2015 Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Front Cell Neurosci. 10;9:124.
5. Uttara, B, Singh, AV, Zamboni, P, Mahajan, RT. 2009 Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. ;7(1):65-74.
6. Culotta, VC, Yang, M, O'Halloran, TV. 2006 Activation of superoxide dismutases: putting the metal to the pedal. Biochim Biophys Acta.;1763(7):747-58.
7. Bostantjopoulou, S, Kyriazis, G, Katsarou, Z, Kiosseoglou, G, Kazis, A, Mentenopoulos, G. 1997 Superoxide dismutase activity in early and advanced Parkinson's disease. Funct Neurol. ;12(2):63-8. PMID: 9238339.
8. Fukui, M, Zhu, BT. 2010 Mitochondrial superoxide dismutase SOD2, but not cytosolic SOD1, plays a critical role in protection against glutamate-induced oxidative stress and cell death in HT22 neuronal cells. Free Radic Biol Med. 15;48(6):821-30.

9. Bostantjopoulou, S, Kyriazis, G, Katsarou, Z, Kiosseoglou, G, Kazis, A, Mentenopoulos, G. 1997 Superoxide dismutase activity in early and advanced Parkinson's disease. Funct Neurol.;12(2):63-8. PMID: 9238339.

Figures:

Figure 1. Cell viability after exposure of 0.005 uM NSNM in SH-SY5Y cells.

Figure 2: Mitochondrial SOD1 and Cytosolic SOD2 activity in SH-SY5Y cells in time dependant manner.

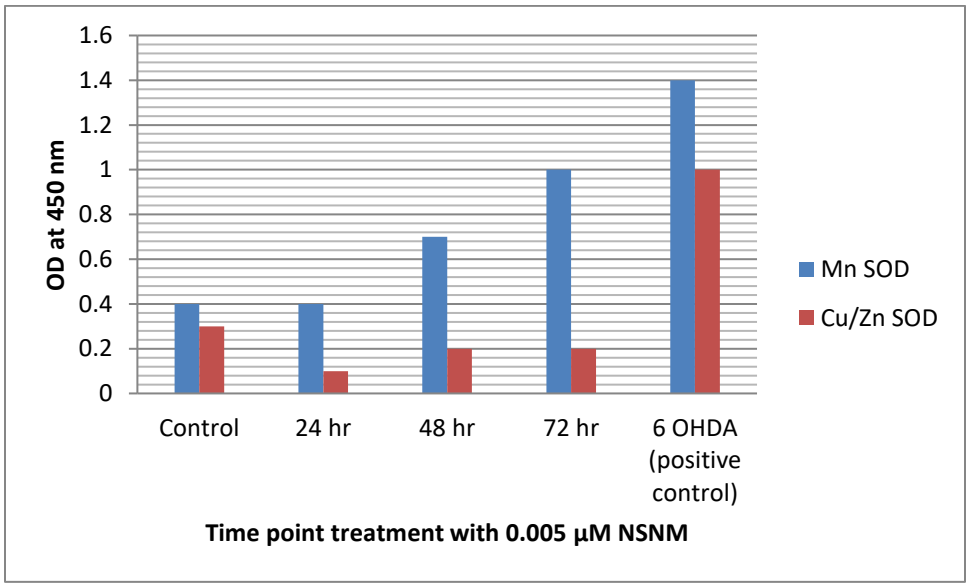

Figure 1

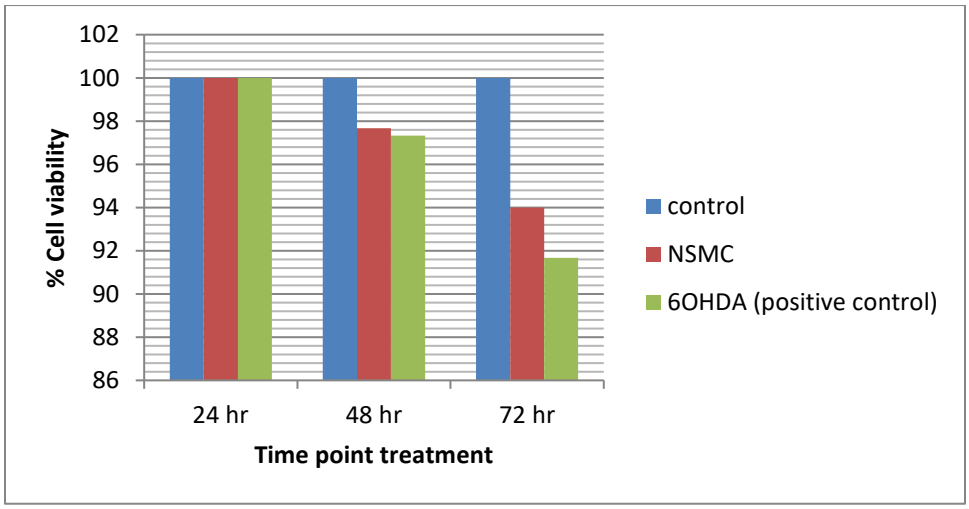

Figure 2