ISSN No:-24562165

The Role of Alleviate Oxidative Stress of Oxytocin in The Neurodegenerative Disorders

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Abstract:- **Neurodegenerative diseases are characterized by progressive damage in neural cells and neuronal loss; bearing in mind the relevance of oxidative stress in neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, and the antioxidant properties of oxytocin, which we previously demonstrated, we studied the effects of oxytocin administration 5µM oxytocin for 24h, on enzymatic activities of superoxide dismutase (SOD) and malonic dialdehyde (MDA) level in the Neuro2a cells (N2a cell lines) which treated by 250 µM hydrogen peroxide(H_2O_2) for 24h. In these cells, oxytocin treatment provided a significantly lower SOD enzymatic activity and significantly higher MDA concentration. It is concluded that, due to its antioxidant effect, oxytocin can be considered a candidate for developing a new therapeutic modality in neurodegenerative diseases.**

Keywords:- *Neurodegenerative Diseases, Oxidative Stress, Oxytocin, Superoxide Dismutase, Malonic Dialdehyde, Neuro2a cells.*

I. INTRODUCTION

Oxidative stress (OS) is the imbalance between reactive oxygen species generation and cellular antioxidant potential. This may lead to various pathological conditions and diseases, especially neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) [1]. Neurodegenerative disorders are generally age-related, and while AD primarily leads to hippocampal degeneration, PD, on the other hand, leads to the dysfunction of the nigrostriatal dopaminergic system. They share some similar neuropathological hallmarks, cognitive impairment and motor disorders [1-4], more than 190 neurological disorders are associated with oxidative stress[1].

Oxytocin (OXT) is a hormone, a nine amino acid neuropeptide produced by the hypothalamus and released into circulation with some essential physiological properties, including a direct action on uterus contraction during parturition and milk discharge reflex during lactation [2]. oxytocin release in response to reproductive, stressful, and social stimuli has also been described within distinct brain regions, also plays a role in interactive actions among stress and food eating and contributes to adaptive active coping behaviour[3, 4]. Oxytocin can also have anti-oxidant properties and perform an alleviated role in various kinds of damages in neurodegenerative diseases such as PD and AD [5, 6] and attenuate stress responses in social situations. OXT and its receptor are distributed widely throughout the body, suggesting multiple roles, including paracrine and endocrine functions of OXT [2]. More recently, in vitro and in vivo studies have revealed an important effect of OXT on cell survival and against neuronal cell death [7, 8].It was shown that oxytocin administration antioxidant therapy has been suggested for preventing and treating neurodegenerative diseases.

In the present study, we examined the effect of oxytocin on hydrogen peroxide (H_2O_2) -induced oxidative stress in neurodegenerative diseases and the potential neuroprotective effect of oxytocin.

II. MATERIALS & METHODS

A. Cell Culture and Treatment

Mouse neuroblastoma N2a cells line were maintained in a medium containing 50%DMEM and 50% Opti-MEM supplemented with 5% FBS and kept at 37ºC and 5% CO2 (GibcoBRL, Grand Island, NY, USA). The culture medium was replaced every three days. Cells were grown to approximately 70% - 80% confluence in twelve-well culture plates for cell lysates preparation. N2a cells line were treated with 5µM oxytocin or 250 µM H_2O_2 for 0-24h by adding 1µL of oxytocin or H_2O_2 to the wells. At the end of incubation, after 24h, cells were rinsed twice in 1X PBS (pH 7.5)[9, 10].

B. ELISA Assay

The measurement of SOD and MDA was conducted to assess the oxidative stress as described previously [11, 12]. Cells were cultured in a twelve-well plate after exposure to 5µM oxytocin or 250 µM H_2O_2 for 24h, then cells were washed down from the wells with 1 X PBS (pH 7.4) and lysed with 4 X lysis buffer, followed by sonication for 15 times on the ice. Then, the cells were centrifuged at 10,000 g for 15 min at 4°C.

C. Measurement of Superoxide Dismutase (SOD)

Superoxide dismutase activity in the supernatant was determined with a commercial kit from Jiancheng Bioengineering Institute (Central Road, Nanjing, China)

based on its vital role in antioxidant balance in the body. This enzyme can scavenge superoxide anion radicals and protect cells from damage. This Kit uses the WST-1 method to determine SOD activity. The mauve product (nitrite) produced by the oxidation of the WST-1 method has an absorbance at 450 nm. One unit of SOD activity was determined as the amount that reduced the absorbance at 450 nm by 50%.

D. Measurement of Malondialdehyde (MDA)

Malondialdehyde, a metabolite of lipid peroxides, was used to measure lipid peroxidation. MDA was estimated with a commercial kit from Jiancheng Bioengineering Institute (Central Road, Nanjing, China) by measuring the malondialdehyde reacted with the thiobarbituric acid (TBA) effect. N-butyl alcohol was used for extraction during this process. 4ml N-butyl alcohol was added into each tube of a mixed solution prepared according to the instruction. Then we centrifuged the newly mixed solution at 3,000 g for 10 min, put it at 4 °C overnight, and the supernatant was measured at a wavelength of 532 nm.

E. Statistical Analysis

All data are expressed as means ± SEM. Data were compared using Student's t-test. Differences among means were analyzed utilizing one-way ANOVA, with time, and treatment, as the independent factors. The $P<0.05$ was considered statistically significant. Data were represented graphically using GraphPad Prism version 8 (GraphPad Software, San Diego, Ca).

III. RESULTS & DISCUSSION

F. Results

It is known that oxidative stress plays an important role in mediating the dopaminergic neuronal loss in PD, AD and other neurodegenerative diseases [13, 14]; we then evaluated different oxidative biomarkers in Mouse neuroblastoma N2a cells line for confirming the anti-oxidative effects of OXT, we treated N2a cells line with 250μM H_2O_2 and evaluated an in vitro oxidative status. We found that treatment of the cells with 5μM OXT effectively rescued the decreased SOD activity and the increased MDA level triggered by H_2O_2 treatment (Fig. 1A, B). Overall, these results showed the anti-oxidative effect of OXT in *vitro*. OXT reduces oxidative stress.

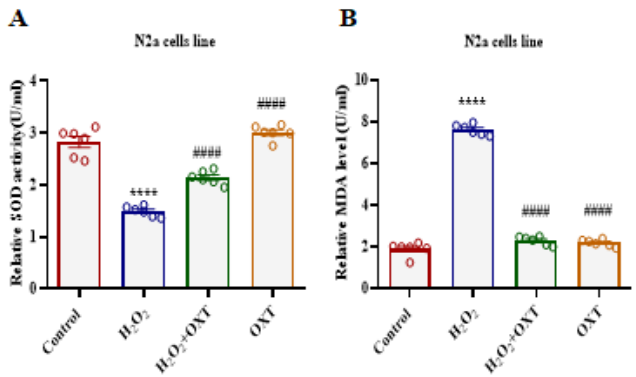

Fig 1 Oxytocin (OXT) attenuated hydrogen peroxide (H2O2)-induced oxidative damage:

*SOD and MDA were determined with commercial kits to evaluate the level of oxidative damage in the present study. The levels of SOD in N2a cell line (A). The contents of MDA in N2a cell line (B). The data were expressed as Mean ± SEM. (**** $p < 0.001$ vs. control; #### $p < 0.001$ vs. Model). SOD, superoxide dismutase; MDA, Malondialdehyde.3.2. Table (Subsection – 2 of section -3)*

G. Discussion

Oxidative stress and the generation of free radicals catalyzed by redox metals have been shown to play a significant role in the regulation of in vivo/in vitro redox reactions, the leading cause of neurodegeneration [15]. There is considerable evidence that oxidative stress also plays a significant role in modulating biochemical changes resulting from aging and neurodegenerative disorders such as PD, AD, Amyotrophic lateral sclerosis (ALS), and multiple sclerosis [16]. Even more, the effects of OXT administration on the oxidative stress demonstrated a possible decrease in the MDA level in N2a cells in vitro were examined [17]. Here, we found that OXT prevent damage to N2a cells line by H_2O_2 through its antioxidative effect. We found that following treatment with H_2O_2 there is a significant increase in the level of MDA and a decrease in SOD activity, whereas OXT treatment decreases the level of MDA and increases the activity of the SOD in vitro. These findings indicated that treatment by OXT can improve the SOD activity, and scavenge lipid peroxidation product MDA, inhibit oxidative stress, alleviate the ROS caused damage to dopaminergic neurons. Additionally, OXT also inhibits NADPH oxidase and decreases NADPH-dependent superoxide activity[18, 19]. Even more, other authors described treatment with OXT, the high content of MDA was rejected, the reduced activities of SOD and GSH-Px were recovered, and the decreased content of GSH were increased[5]. This implies that OXT attenuates the oxidative damage induced by H2O2. These results suggested that OXT may protected mitochondrial function and suppressed ROS production in N2a cells, as our previous study that showed restored the level of SOD and suppressed the level of MDA, indicating an anti-oxidative effect of OXT in MPTP mouse model[6].

IV. CONCLUSION

In conclusion, this study demonstrates that oxytocin treats neurodegenerative diseases in N2a cells, that through its modulation of oxidative stress.

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