Protective Effects of N-Acetyl-L-cystein on 3,4-Methylene Dioxymethamphetamie-Induced Neurotoxicity in Cerebellum of Male Rats

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ABSTRACT

Introduction: 3-4, methylenedioxymethamphetamine (MDMA) causes apoptosis in nervous system and several studies suggest that oxidative stress contributes to MDMA-induced neurotoxicity. The aim of this study is to examine the effects of N-acetyl-L-Cystein (NAC) as an antioxidant on MDMA-induced apoptosis.

Methods: 21 Sprague dawley male rats (200-250mg) were treated with MDMA (2×0,5mg/kg) or MDMA plus NAC (100mg/kg IP for 7 day). After last administration of MDMA, rats were killed, cerebellum was removed and Bax and Bcl-2 expression was assessed by western blotting method.

Results: The results of this study showed that MDMA causes up-regulation of Bax and down-regulation of Bcl-2 and NAC administration attenuated MDMA-induced apoptosis.

Discussion: The present study suggests that NAC treatment may improve MDMA-induced neurotoxicity.

1. Introduction

3,4-methylenedioxymethamphetamine (MDMA) or ecstasy has excitatory effects on the central nervous system as an amphetamine derivate, particularly on hippocampus, neostriatum and cerebellum(Simantov, 1997, Shankaran,1999 and Farre, 2004). Our previous studies showed that MDMA causes learning memory impairment and apoptosis in brain (Soleimani, 2011). MDMA induces cell death through an apoptotic pathway by releasing cytochrome C and activating the caspases cascade (Jiménez, 2004). Oxidative stress responses involve MDMA-induced neurotoxicity that lead to formation of hydroxyl radicals (Shankaran, 1999), lipid peroxidation (Alves, 2009) and increase in the number of tunnel positive cells in the hippocampus (Riezzo, 2010). Oxidative stress is generated by an imbalance between

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reactive oxygen species (ROS) and antioxidants and may contribute to the neurotoxicity of MDMA in brain (Shankaran, 1999). It has been demonstrated that N-acetyl-L-Cystein (NAC) as a potent antioxidant inhibits activation of c-june terminal kinase and can also prevent apoptosis and promote cell survival (Shimizu, 2002). N-acetyl-L-cysteine (NAC) is a small molecule containing a thiol group, which has antioxidant properties, and is freely filterable with a ready access to blood–brain barrier and intracellular compartments (Farr, 2003). NAC can prevent apoptotic death of cultured neuronal cells (Olivieri, 2001) and promote survival of PC12 cells. Lacking any neurotrophic factor, this protective effect was presumed to be related to its free radical scavenging abilities (Yan et al., 1995). Based on the role of oxidative stress in the neurotoxicity of MDMA in the brain, herein we studied whether administration of NAC could attenuate the MDMA-induced neurotoxicity in the cerebellum.

2. Methods

The present study was carried out in accordance with the protocol approved by Tehran University of Medical Sciences.

2.1. MDMA Preparation

3,4-methylenedioxymethamphetamine was obtained from the Presidency Drug Control Headquarters and solution was made in sterile saline at a concentration that each group received either 1 ml/kg of the drug solution or saline alone.

2.2. Animals

21 adult male Sprague Dawley rats, weighing 200-250 g, were obtained from the Iranian Razi Institute. Rats were allowed to acclimatize to the colony room for 1 week prior to the MDMA administration. Rats were maintained in the colony room at a temperature of 21 ± 1 °C (50 ± 10% humidity) on a 12-h light/12-h dark cycle with access to water and food ad libitum.

21 rats were assigned as follow:

1. Sham group (n=7): that received normal saline ,1cc/kg, IP, daily for 1 week

2. MDMA groups (n=7): that received 10 mg/kg MDMA, IP, daily for 1 week.

3. Treatment group (n=7): that received 100 mg/kg NAC 30 minutes before MDMA administration, daily for 1 week.

2.3. Western Blotting Experiment

The animals were killed by cervical dislocation, the brain was rapidly removed and hippocampus dissected out on ice, frozen in liquid nitrogen and kept at -80 °C until use. The frozen hippocampi were homogenized with ice-cold lysis buffer (containing Ripa Buffer with protease inhibitor cocktail 1:10) for 1 h, centrifuged (Eppendrof ,Hamburg, Germany) at 12000 g for 20 min and 4°C and the supernatant was removed and conserved. After determining the protein concentration with a Bio-Rad assay system (Bio-Rad–San Francisco, CA, USA), aliquots of 100 µg of protein from each sample were denatured with sample buffer (6.205 mM Tris-HCl, 10% glycerol, 2% SDS, 0.01% Bromophenol Blue and 50 mM 2-ME) at 95°C for 5 min and separated on 10% sodium dodecyl sulfat polyacrylamid gel electrophoresis (90 min 120 voltage). Then the proteins were transferred to Hybond-PTM membrane (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). Membranes were blocked with 5% nonfat milk dissolved in TTBS buffer (Tris 50mM, NaCl 1.5% and Tween 20; 0.05% pH 7.5) for 1 h. They were stained with anti-bcl-2 and anti-bax monoclonal antibody (1:1000 Sigma Aldrich, Saint Lauis, MO, USA) for 2h followed by secondary antibody alkaline phosphatase-conjugated anti-mouse antibodies (1:10000, Sigma Aldrich, Saint Lauis, MO, USA) for 1 h. Bands were detected by Chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate in the presence of nitroblue tetrazolium. β-actin antibody (1:1000, Sigma Aldrich, Saint Lauis, MO,USA) was used to detect endogenous standard for normalization. The bands from various groups which corresponded to the appropriate molecular weight for each subunit were analyzed and values were compared using densitometric measurements using an image analysis system (UVI-doc, Houston, Texas, USA).

2.4. Statistical Analysis

The data was presented as the mean ± S.E.M and the results were analyzed by one-way ANOVA and post-hoc comparison was performed using Tukey test. The P. value ≤ 0.05 was considered statistically significant.

3. Results

As it is clear in SDS page (Figure 1) and analyze of densitometric average, MDMA treatment caused an increase in BAX protein expression in comparison to the sham group and pretreatment with NAC led to a decrease in Bax expression in comparison to the MDMA group. However, this difference was not statistically sig-

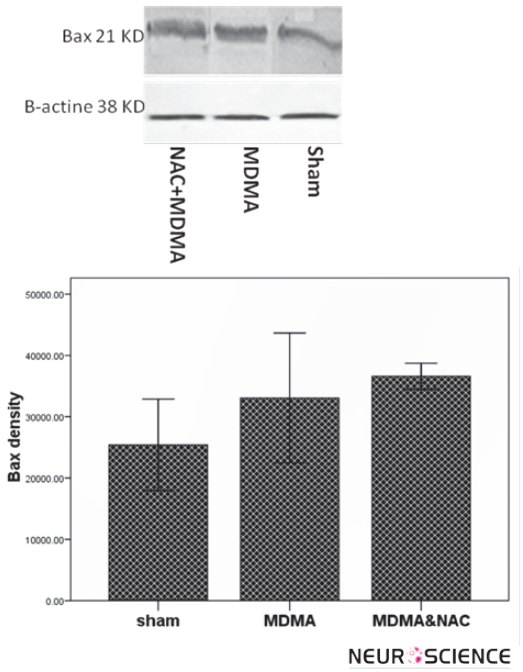

Figure 1. Bax protein expression in nitrocellulose membrane in sham, MDMA 10mg/kg and NAC 100mg/kg plus MDMA groups(above).MDMA administration caused increase Bax protein expression and NAC pretreatment led to decrease that in comparison MDMA group.

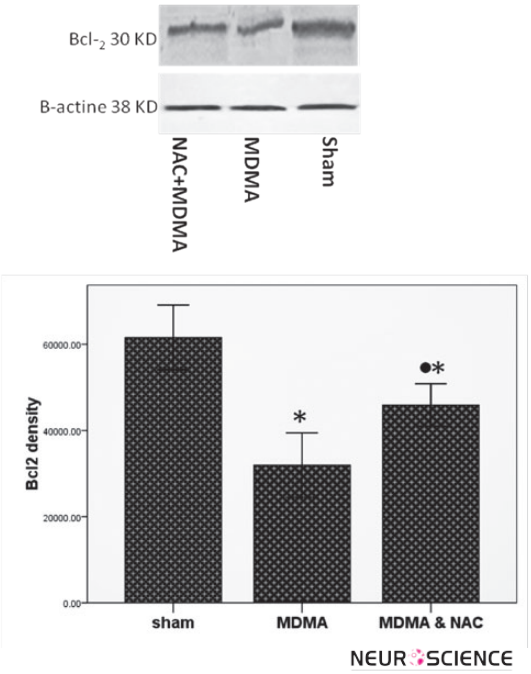

Figure 2. Bcl-2 protein expression in nitrocellulose membrane in sham, MDMA 10mg/kg and NAC 100mg/kg plus MDMA groups(above).MDMA administration resulted in decreas Bcl-2 protein expression (*p<0.001 vs. sham) and NAC pretreatment caused increase Bcl-2 protein(•p<0.05 vs.MDMA)(Below)

nificant (mean=254.1±3.74, 330.67±5.85, 85.03±1.72 and 366.12±10.23 for sham, MDMA and MDMA plus NAC groups respectively) (Figure1).

Furthermore, MDMA administration caused a significant decrease in Bcl-2 protein expression in comparison to the sham group, ($p<0.001$, Figure 2) and administration of NAC before MDMA resulted in significant increase of Bcl-2 expression in comparison to the MDMA group ($p<0.05$, Figure 2).

4. Discussion

In this study we found that repeated administration of MDMA caused an increase in proapoptic Bax protein and decrease in anti apoptotic Bcl-2 protein expression in rat cerebellum that is in consistent with Jayanti et al study that showed injection of methamphetamine, as another amphetamine derivate, causes up regulation of Bax and down regulation of Bcl-2 proteins (Jayanthi,2001). MDMA causes rapid intracellular ca2+ influx, mitochondrial membrane depolarization, ROS production and DNA fragmentation (Montgomery, 2010). Furthermore, MDMA treatment depletes intracellular gluthation (GSH) and finally induces oxidative stress and nuclear death (Upreti, 2011).

In this study we showed both up-regulation and down-regulation of Bax and Bcl-2 proteins, respectively in NAC plus MDMA treated group in comparison to the MDMA group. Our results are consistent with another study which showed that NAC can lead to prominent decrease in PARP apoptosis protein and increase in the expression of Bcl-2 antiapoptotic protein in spinal cord injury (Barbosa, 2010).

Our results demonstrated that NAC can protect brain damage in MDMA treated rats that is in consistent with a study by Fu et al that showed NAC improves β-amiloeid-induced neurotoxicity (Fu, 2006).

It is well known that NAC can act as a precursor for gluthation synthesis as well as a stimulator of the cytozolic enzymes involved in glutathione regeneration (De Flora, 2001). Furthermore, NAC can act by direct reaction between its reducing thiol group and reactive oxygen species (Cuzzocrea, 2000). It has been shown that NAC can prevent programmed cell death in cultured neuronal cells and can increase mitochondrial complex I and IV specific activity both in vitro and in vivo in synaptic mitochondrial preparations of aged mice (Barbosa, 2010). Therefore, it should be noted that as a potent antioxi-

dant, NAC can attenuate the MDMA-induced imbalance in Bcl-2 family.

5. Conclusions

In summary, the present study suggests that NAC as an antioxidant would be a potential therapeutic drug for the neurotoxicity in the brain of MDMA abusers. Since, use of MDMA is a worldwide problem, additional studies are needed to investigate the effects of other antioxidants on MDMA-induced neurotoxicity.

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