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Farnesyltransferase inhibitor attenuates methamphetamine toxicity-induced Ras proteins activation and cell death in neuroblastoma SH-SY5Y cells

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HIGHLIGHTS

- Methamphetamine-induced neuronal cells degeneration.
- Methamphetamine toxicity resulting in Ras activation in neuroblastoma cells.
- Farnesyltransferase inhibitor diminishes methamphetamine-induced Ras activation.

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ABSTRACT

Several lines of evidence support that methamphetamine (METH) toxicity plays a pivotal role in neurodegenerative diseases. However, the molecular mechanisms underlying METH-induced neurotoxicity are still unclear. In addition, Ras modulated death signaling has been continually reported in several cell types. In this study, intracellular Ras-dependent death signaling cascade activation was proposed to contribute to METH-induced neuronal cell degeneration in dopaminergic SH-SY5Y cultured cells. Exposure to a toxic dose of METH significantly decreased cell viability, and tyrosine hydroxylase phosphorylation, but increased c-Jun phosphorylation and active, GTP-bound Ras in cultured SH-SY5Y cells. Farnesyltransferase inhibitor, FTI-277, an inhibitor of the enzyme catalyzed the farnesylation of Ras proteins was able to diminish the toxic effects of METH on induction in cell degeneration, activation in c-Jun-N-terminal kinase cascades, and Ras activation in SH-SY5Y cells. The results of this study show that activation in Ras signaling cascade may be implicated in the METH-induced death signaling pathway in neuroblastoma SH-SY5Y cells.

1. Introduction

Methamphetamine (METH) is a psychostimulant drug which has been sufficiently abused to become an international public health problem. Its effects in the toxicity of mammalian brains have been continually reported to induce neurotoxicity especially long-lasting changes in the central dopaminergic pathway. This substance induces neurodegenerative changes such as decreased dopamine [1], tyrosine hydroxylase (TH) [10], and dopamine transporter (DAT) levels [9] in the monoaminergic neurons. These toxic effects lead to neuronal cell death which finally results in neurodegeneration such as Parkinsonism [4]. The negative neuropsychiatric consequences of METH abuse, leading to neuropathological changes in the brains of these METH addicts have been supported by strong pieces of evidence [18]. METH toxicity causes apoptosis in several regions of the mice brain such as cortex, striatum and hippocampus by reducing mitochondrial respiration, membrane potential and increasing the release of mitochondrial cytochrome c with subsequent activation of the caspase cascade [6]. Intracellular signaling of METH toxicity has also been studied. It has demonstrated that METH can induce reactive oxygen species (ROS) generation and then ROS can stimulate multiple intracellular signaling pathways [3].

Taken together, it can be seen that a major pathway involved in death stimulation in various types of cells requires the sequential activation of Ras, Raf and mitogen-activated protein kinase (MAPK). Ras protein is a GTP-binding protein and known as a major effector molecule which can stimulate many signaling transductions including growth, proliferation, differentiation, motility and cell death [8]. A growing body of evidence indicates an association between Ras protein and a family of c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPK) [22]. Current information of cell death signaling cascades of METH-induced neuronal toxicity has not been clearly elucidated especially the upstream elements of

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JNK activation [7]. Farnesyltransferase inhibitor (FTI) is an inhibitor of the farnesyltransferase (Ftase) enzyme. Ftase catalyzes the farnesylation of Ras proteins. Farnesylation is required for functioning of Ras proteins. FTI-treated mice have been shown to be able to protect the toxin from lipopolysaccharide (LPS)-administrated by decreasing JNK/SAPK activation [19]. However, studies have not been done to elucidate the effective role of FTI on Ras/JNK-dependent death processes induced by METH in dopaminergic cells. Therefore, the aim of this study was to test the hypothesis on METH-induced Ras/JNK-dependent death processes using the potential role of FTI to prevent METH-induced induction in cell death signaling cascades in dopaminergic cell lines.

2. Materials and methods

2.1. Reagents and antibodies

Methamphetamine was obtained from Sigma–Aldrich (St. Louis, MO, USA). For immunoblotting, anti-phosphorylated TH at Ser40, anti-phosphorylated c-Jun at Ser 73 and mouse monoclonal anti-actin were purchased from Chemicon International (Temecula, CA, USA). The Ras Activation assay kit was purchased from Cell Biolabs, Inc. (San Diego, CA, USA). Farnesyltransferase inhibitor (FTI-277) was purchased from Calbiochem (Darmstadt, Germany).

2.2. Cell cultures

SH-SY5Y cells were cultured in cultured flasks with minimum essential medium (MEM)/Ham's F12 (1:1) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin. Cells were maintained in a humidified air incubator with 5% CO_2 atmosphere at 37 °C.

2.3. Cell viability assay

The MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used to determine the ability of viable cells to convert yellow MTT into purple formazan crystal by mitochondrial dehydrogenase. After METH treatment, MTT solution in 0.1 mM phosphate buffer saline (PBS) was added into the wells and incubated for 4 h. After that the extraction buffer (0.04 N isopropanol in HCL) was added to dissolve blue formazan product. The optical densities were measured at 570/630 nm spectral wavelengths using a micro-titer plate reader (Bio-tek Instruments, Winooski, VT).

2.4. Western blot analysis

SH-SY5Y cells lysates were prepared by extracting proteins with lysis buffer (RIPA buffer) supplemented with protease and phosphatase inhibitors. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk in 0.1% Tween in Tris-buffered saline (TBS) and then incubated with primary antibodies overnight at 4 °C (dilution 1:1000 in TBST). The membrane was incubated with a HRP-conjugated secondary antibody and developed signal with Chemiluminescence ECL Plus-Western Blotting detection reagents.

2.5. Immunofluorescence staining

The cytosolic part of cells was visualized using a red-fluorescent mitochondria-staining dye, MitoTracker®Red CMXRos and then cells were washed and incubated overnight at 4 °C with rabbit polyclonal anti-c-Jun phosphorylation (dilution 1:100) or rabbit monoclonal anti-TH phosphorylation (dilution 1:100). Cells were then incubated sequentially with a Texas Red-conjugated anti-rabbit IgG (dilution 1:200) and Dylight 488-conjugated anti-rabbit IgG (dilution 1:200) for 2 h. Cells were mounted with VectaShield and visualized by FluoView 300 confocal laser scanning microscopy.

2.6. Ras activation assay

The activated Ras proteins in the cells were measured using the pan-Ras activation assay kit, which is based on active (GTP-bound) Ras binds specifically to the Ras-binding domain (RBD) of Raf1 (Raf1-RBD). SH-SY5Y cells were lysed by 1× assay/lysis buffer and the lysate was centrifuged and then the supernatant or whole cells lysate (WCL) was collected and proceeded to GTP-bound Ras pull-down procedure (Cell Biolabs, Inc.). The supernatant was added with suspended Raf1-RBD agarose bead and incubated with gentle agitation. After incubation, the sample was centrifuged and then the supernatant was removed and the active (GTP-bound) Ras–Raf1-RBD agarose bead complex was washed 3 times with 1× assay buffer. The precipitated active Ras and total Ras in WCL was detected by Western blot analysis using mouse monoclonal anti-pan-Ras.

2.7. Statistical analysis

All experimental data were reported as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey–Kramer test was selected to analyze all experimental data. Probability (P) values of less than 0.05 were considered statistically significant.

3. Results

3.1. Farnesyltransferase inhibitor (FTI-277) attenuates METH-induced toxicity in SH-SY5Y cultured cells

METH produced a time-dependent decrease in cell viability. The results showed that 1 mM METH for 2, 4, 12, and 24 h gradually decreased cell viability to 93 ± 2.9%, 87 ± 3.2% ($p < 0.01$), 86 ± 2.2% ($p < 0.01$), and 77 ± 1.9% ($p < 0.001$) of the control value, respectively. In order to determine whether FTI-277 causes the inhibitory effect on METH-induced toxicity, SH-SY5Y cells were treated with METH at 1 mM for 24 h with or without pre-treatment with various concentrations of FTI-277 at 0.1, 0.5, 1 and 2 μM for 12 h. Control untreated cells were incubated with culture-medium for 36 h. The results showed that 1 mM METH for 24 h significantly decreased cell viability to 81.53 ± 4.0% ($p < 0.05$) of the control value. Pre-treated cells with FTI-277 at 0.1, 0.5, 1 and 2 μM for 12 h prior to exposure to 1 mM METH were able to restore cell viability to 106 ± 6.8% ($p < 0.001$), 108 ± 6.4% ($p < 0.001$), 100 ± 6.9% ($p < 0.01$) and 95 ± 6.3% of the control values, respectively (Fig. 1). Pretreated cells with FTI-277 at 0.1, 0.5, and 1 μM significantly increased cell viability in METH-treated cells when compared to METH-treated cells without pre-treatment with FTI-277.

The ability of FTI-277 to reverse METH-induced toxicity in SH-SY5Y cells was determined by monitoring phosphorylation of tyrosine hydroxylase (p-TH), the rate limiting enzyme for synthesizing the neurotransmitter dopamine [16]. The green color indicated p-TH positive immunostaining in SH-SY5Y cells. Exposure to 1 mM METH for 24 h resulted in reduction in p-TH immunofluorescence staining when compared with control untreated cells. However, pre-treated cells with 0.1 μM FTI-277 were able to restore p-TH immunostaining in METH-treated cells when compared with METH-treated cells without pre-treatment with FTI-277 (Fig. 2A). The amount of p-TH was also determined in SH-SY5Y cells using Western blot analysis. Treated cells with 1 mM METH for 24 h significantly decreased p-TH levels to 53 ± 8.6%