Methamphetamine Mediated Mitochondrial Dysfunction and Microglial Apoptosis

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Abstract

We hypothesized that increasing concentrations of Meth will cause microglial apoptosis which induces mitochondrial dysfunction leading to neurodegeneration. Our goal was to evaluate if Meth treatment induced the activation of the mitochondrial-dependent intrinsic pathway. Gene expression of pro- and anti-apoptotic genes were quantified with qPCR using RNA extracted from cultured microglial cells line (HTHU). Our results indicated that Meth treatment resulted in a significant increase in the gene expression levels of apoptotic proteins like APAF-1, BAX and BCL-2. Analysis of Western Blot data showed a significant modulation of the expression level of the antiapoptotic protein BCL-2 with varying Meth concentrations. Using immunofluorescence analysis we examined the expression of mitochondrial proteins, Cytochrome C, COX 4 and MCL-1, all of which were significantly activated on Meth treatment indicating the activation of the mitochondrial-dependent (intrinsic) apoptotic pathway. We conclude that Meth causes microglial apoptosis via the mitochondrial-dependent (intrinsic) apoptotic pathway contributing to neurotoxicity and subsequent neurodegeneration.

Methods

Cell Culture: The HTHU microglial cell line originally isolated from tissue biopsy, were a generous donation from Dr. Jonathan Karn (CWRU, Cleveland, OH). Cells were cultured in DMEM:F12 supplemented with 10% fetal bovine serum (FBS), 100 μg/ml penicillin, and 100 μg/ml streptomycin. Cultures were maintained at 37°C in a 5% CO2 humidified incubator.

RNA Extraction & Real time qPCR: Total RNA was extracted from microglial cells treated with Meth for 24hr using Trizol reagent (Invitrogen). 500 ng of total RNA was used for the RT reaction (25 μl total volume) by using the First-Strand cDNA synthesis kit (GE Healthcare, Piscataway, NJ) according to the manufacturer’s instruction. One μl of the resultant cDNA from the RT reaction was used as the template in PCR reactions.

Immunofluorescence: HTHU cells are grown to 70% confluence in a petri dish with a glass bottom and treated with Meth (10-100 nM) for 24hr. Cells are fixed for 10 min at 37°C in 4% formaldehyde, followed by permeabilization with ice-cold 0.5% methanol. Cells are then washed in 1X phosphate buffered saline (PBS) and treated with a specific primary antibodies and detected using fluorescent labelled secondary antibodies. Imaging was performed using the EVOS® FL Cell Imaging System.

Western Blot Analysis: Microglial cells treated with Meth for 48hr followed by protein extraction using the MPEER reagent. The amount of protein used was 50 μg/lane. Standard Western blotting procedures were followed. 1:1000 dilution of BCL-2 antibody was used, followed by AP labeled secondary antibody and development of blot using NBT/BCIP.

Results

Figure 1: CCK-8 Cell viability assay. Meth treatment (0.5-50 μM) did not affect cell viability. >90 % cell viability was observed.

Figure 2: TUNEL staining shows microglial apoptosis. A 46% increase in mean pixel units was observed.

Discussion

Our results show that the balance between pro- and antiapoptotic proteins in Meth treated microglia depends on the concentration of Meth. The ratio of pro- and antiapoptotic proteins is altered by Meth dose and may regulate mitochondrial physiology and cell apoptosis. Our next step is to evaluate Meth induced changes in mitochondrial morphology and function and their subsequent impact on neuronal cell apoptosis.

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