Biology

Cytoskeletal toxicity in SH-SY5Y neuroblastoma cells: Effects of chlorpyrifos-oxon on α-tubulin concentration

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Introduction

Organophosphorous (OP) compounds are the most widely used pesticides in the world, and there is escalating concern that low level exposure to these pesticides may interfere with neurodevelopment in children.[1,2] Previous data suggest that exposure to OP compounds during significant stages of brain development can cause permanent neurobehavioral deficits.[2,3,4] Since the year 2000, the OP compound most widely studied with respect to developmental neurotoxicity has been CPF—one of the most widely used OP compounds in the world.[1,5,6]

In vitro studies show that exposure to CPF and its oxon metabolite, CPO, inhibit neurite and axonal outgrowth, which are essential for neuron-neuron connections.[6-12] As well as neuronal and axonal toxicity, many OP compounds, like CPF, have been shown to cause apoptotic death in SH-SY5Y human neuroblastoma cells through disruption in cytoskeletal elements.[13,14] The α-tubulin protein is prevalent in neuroblastoma cells and composes microtubules, which are essential structural elements for neurite and axonal outgrowth. Therefore, to understand CPF's consequence on cytoskeletal elements and development, it is necessary to identify α-tubulin as a key element.

MATERIALS AND METHODS

Cell culture

Human neuroblastoma cells (SH-SY5Y, passages 37-39) were grown in HAM-12 media supplemented with 10% fetal bovine serum, and incubated at 37°C with 5% CO_2. Cells were differentiated by the addition of 10^{-7} M retinoic acid to the growth medium three days prior to treatment with CPO. After CPO was dissolved in ethanol, cells were treated with 10^{-7} M, 10^{-8} M, and 10^{-9} M CPO, or exposed to control media (0.1% ethanol in media) for 4, 12, and 24 hours before being harvested. A total of three replicates were performed. Cells were harvested at 80% confluency, lifted with trypsin-EDTA and lysed with lysis buffer (50 µl 100 mM NaF, 25 µl 200mM Na_3VO_3, and 25µl 1:200 Protease Inhibitor Cocktail III to 5 ml RIPA Lysis Buffer). Lysate was stored at -20°C.

Western Blot Analysis

Upon thawing, cell lysate was diluted 1:1 with β-mercaptoethanol. Lysates were boiled for 5 min and immediately placed on ice to cool before electrophoresis using 7.5% SDS-PAGE gels (Bio-Rad). A total of 31µg of protein was loaded into each lane. Proteins were wet transferred to a nitrocellulose membrane which was treated with anti-α-tubulin (1:200; Santa Cruz) on a gentle rocker at 4°C overnight. The following day, the membrane was washed with 1X TBS and treated with goat anti-mouse IgG-HRP (1:5000; Santa Cruz) at room temperature for 1hr. The α-tubulin protein was detected using an HRP Conjugate Substrate Kit purchased from Bio-Rad. Visual densities of individual bands were then qualitatively assessed (Figure 1).

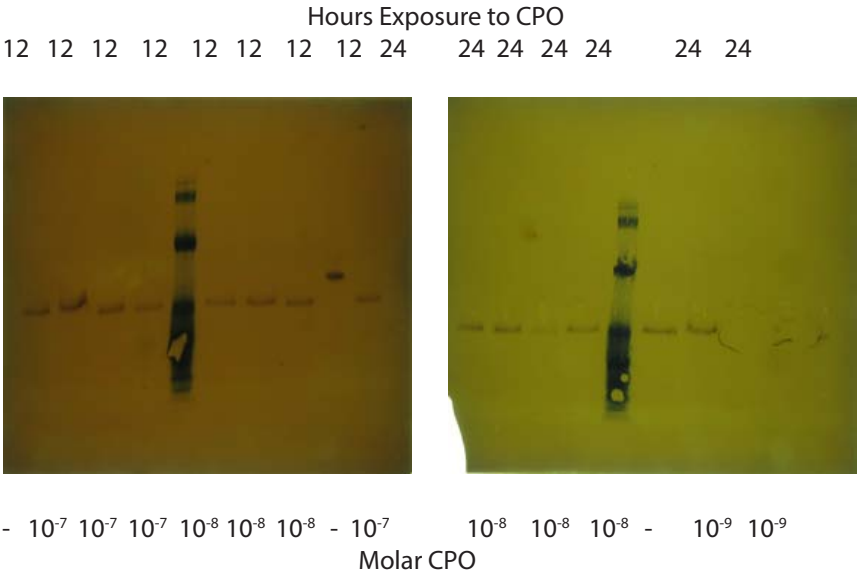

Figure.1 The α-tubulin was detected using an HRP Conjugate Substrate Kit. There were similar band densities at 10^{-7} and 10^{-8} M CPO for 12 hours and at 10^{-7}, 10^{-8}, and 10^{-9} M CPO for 24 hours. A lower band density existed after 24 hours exposure to10^{-7} M and 10^{-8} M CPO than existed after 12 hours exposure to10^{-7} M and 10^{-8} M CPO.

Results and Discussion

Qualitative analysis of the densities of the bands suggests that 24 hours exposure to 10^{-7} M CPO resulted in the smallest concentration of α-tubulin. There was no effect of concentration at 12 hours or 24 hours. This was indicated by similar band densities after 12 hours exposure (10^{-7} M CPO versus 10^{-8} M CPO) or after 24 hours exposure (10^{-7} M CPO, 10^{-8} M CPO, 10^{-7} M CPO). However, there was an effect of time from 12 hours to 24 hours exposure for both 10^{-7} M CPO and 10^{-8} M CPO. A smaller concentration of α-tubulin existed after 24 hours exposure to10^{-7} M and 10^{-8} M CPO than existed after 12 hours exposure to10^{-7} M and 10^{-8} M CPO. Because α-tubulin seemed to remain constant across CPO concentrations at the same time point, but decreased across time points at the same CPO concentration, the data suggests a time dependent response to CPO exposure.

The α-tubulin from all 4 hour exposure samples as well as 10^{-9} M CPO at 12 hours exposure was unable to be detected. Poor transfer from the gel to the membrane likely led to this error. This error confounds the results, but still allows us to see a correlation between time exposed to CPO and α-tubulin concentration. The results of this study support the hypothesis that CPO exposure will cause a decrease in α-tubulin in SH-SY5Y cells. This finding supports the suggestion of other researchers that CPO causes cytoskeletal toxicity by interfering with α-tubulin, which is essential for neurite outgrowth. In fact CPO may be a key OP compound that disrupts early brain development as neurite outgrowth plays a significant role during early brain development. Since studies have implicated several different OP compounds as a contributor to neurobehavioral deficits, more research studying the effect of CPO on early brain development will help reveal the mechanisms by which OP compounds interfere with development of the nervous system.

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