The Effect of Chlorpyrifos on the Concentration of α-synuclein

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Science, Biology

Introduction:

People are continuously exposed to different chemicals, such as pesticides and food additives, whether they realize it or not. One class of pesticides commonly found in the environment are organophosphates. Organophosphate pesticides are found primarily in residential areas. They are used in an attempt to increase crop yields and backyard garden harvests. Chlorpyrifos (CPF) is the most widely used organophosphate pesticide in the world. It is known for causing neurotoxic effects in low doses (Polláková *et al.*, 2012, Chen *et al.*, 2017).

Synucleins are small proteins found in all neurons in the brain. The average α-synuclein fibrils are approximately 200nm and weigh 14.5 kDa (Alpha Synuclein, 2018). Extracellular forms of synucleins function as receptors and are involved in the regulation of intracellular processes. However, the accumulation of α-synuclein protein causes neurodegenerative complications, and evidence suggests that it plays a role in causing Parkinson's disease (Surguchev *et al.*, 2019). Bartels *et al.* found that overexpression of the α-synuclein protein aggregated in a neuronal cell culture model, causing cytotoxic effects (2019).

This study examined the effect of CPF on the concentration of α-synuclein in dopamine-producing neurons. The dopamine-producing neurons were treated with different concentrations of CPF for 24 and 48 hours, and the concentration of α-synuclein was determined. The hypothesis was that as the concentration of CPF increased, there would be a significant increase in α-synuclein concentration in the cells.

Materials and Methods:

Neuroblastoma cells (SH-SY5Y) were maintained in HAM F12 media with 10% fetal bovine serum in a humidified incubator at 37°C in 5% CO_2. Once the cells reached 80% confluency, they were differentiated with 10^{-7} M retinoic acid for three days. After three days, the cells were treated with either 25 µM or 35 µM CPF, or 0.2% ethanol (control) diluted in media for 24 or 48 hours. After 24 or 48 hours, cells were trypsinized and lysed with lysis buffer (50µl 100mM NaF, 25µl 200mM Na_3VO_3, and 25µl 1:200 Protease Inhibitor Cocktail III to 5 ml RIPA Lysis Buffer). Lysates were stored in 1.7 ml tubes at -20°C. Three replicates were conducted. A BCA Assay was completed to determine total protein concentration in each lysate.

The cell lysate was diluted 1:1 with sample buffer. Diluted lysates were boiled for 5 minutes and immediately placed on ice to cool before conducting a Western blot. A total of 23 µg of protein was loaded into each lane of a 12% SDS-PAGE gel. After electrophoresis, proteins were transferred to a nitrocellulose membrane which was treated with anti-α-synuclein (1:5000, Abcam) on a gentle rocker at 4ºC overnight. The next day, the membrane was washed with 1X TBS and treated with goat anti-rabbit antibody (1:10,000, Abcam) at room temperature for 1 hour on a rocker. The concentration of α-synuclein was determined using an Fc Odyssey (LiCor). The data were analyzed with a two-way ANOVA (Jamovi) with a Tukey's post-hoc analysis.

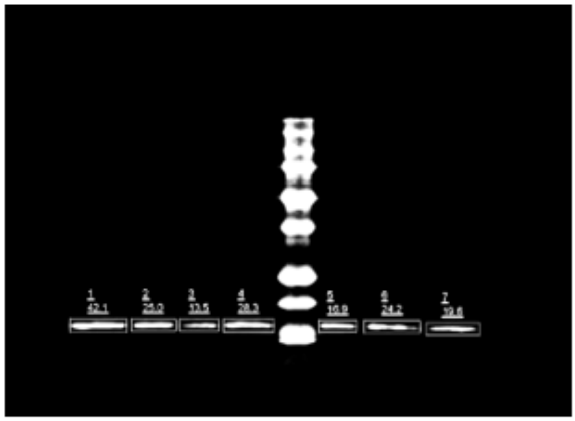

Figure.1 Representative α-synuclein concentration at 0 µM, 25 µM, and 35 µM CPF for 24 hours and 48 hours.

Results:

The average signal intensity of α-synuclein of cells treated for 24 hours with 25 µM CPF was 1.48 (± 0.7) and was 1.35 (± 0.6) for cells treated with 35 µM CPF. The average signal intensity of α-synuclein of cells treated for 48 hours with 25 µM CPF was 1.26 (± 0.3) and was 1.04 (± 0.26) for cells treated with 35 µM CPF.

There was a significant difference in α-synuclein concentration due to treatment (p = 0.05). There was a significant difference in α-synuclein concentration between the control and cells treated with 25 µM CPF (p = 0.03). There was not a significant difference between the control vs. 35 µM CPF treated cells (p = 0.38), nor between 25 µM CPF vs. 35 µM CPF treated cells (p = 0.47). However, α-synuclein concentration was higher to some degree in all cells treated with CPF compared to control. There was not a significant difference between 24 and 48 hours (p = 0.14), and treatment and time do not interact in a significant way (p = 0.56).

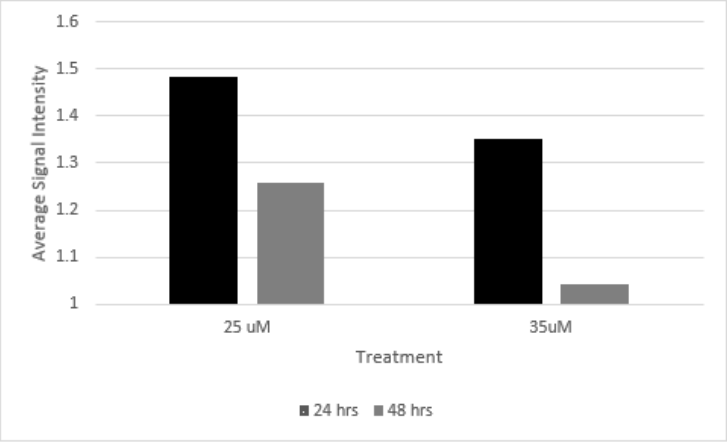

Figure.2 Average α-synuclein signal intensity of each treatment as a percentage of control.

Discussion:

There was a significant increase in α-synuclein protein in cells exposed to 25 µM CPF compared to the control. The effect was observed at 24 hours with no apparent increase or decrease over time. This partially supports the hypothesis that an increased concentration of CPF will cause an increase in α-synuclein in the cell. However, there was no significant difference in α-synuclein concentrations beyond 25 µM CPF. The 35 µM CPF concentration could have caused cell death, such that any effect of CPF on α-synuclein was insignificant. Another possibility is that the increase in α-synuclein proteins at 35 µM CPF could have caused cytotoxic effects that killed the cells (Bartels *et al.*, 2019). The current study is supported by other research that found neurotoxic effects at concentrations as low as 0.1 µM (Polláková *et al.*, 2012).

Exposure to CPF could be a contributing mechanism to the accumulation of the α-synuclein protein. Such an accumulation can play a role in the development of Parkinson's disease (Surguchev *et al.*, 2019). Further studies could include using lower concentrations of CPF. For example, research suggests that the length of neurites is decreased after exposure to 20 µM CPF for 24 hours (Powell & Pomeroy-Black, 2019). Experiments could also incorporate other organophosphate pesticides, such as parathion and malathion. If accumulation of α-synuclein occurs due to exposure to specific pesticides, then scientists may be able to find an inhibition to express α-synuclein formation in people chronically exposed to these pesticides.

Literature Cited:

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