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The Neurotoxicity of Polystyrene Nanoplastics on Mitochondrial Function in Rat Brains.

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1 Introduction

Background

Nanoplastics (NPs) are becoming increasingly present in our lives and bodies (1). NPs can cross the blood brain barrier in animal models that predict that the plastics can enter also human brains (2). The presence of such plastics has been shown to cause mitochondrial dysfunction in many models (3). Mitochondrial dysfunction is strongly linked to neurodegenerative diseases such as Alzheimer's and Parkinson's disease (4).

Aims

This study aimed to confirm that excess **reactive oxygen species** (ROS) are produced in the presence of polystyrene nanoplastics (PS-NPs). The **electron transport chain** (ETC) was also examined to uncover the mechanism of the neurotoxic effects of the NPs.

2 Methods

The ETC complexes' activity was measured in a spectrophotometer.

- Complex I activity was measured by following the oxidation of NADH at λ=340nm.
- Complex II activity was measured by following the reduction of DCPIP at λ=600nm.
- Complex II/III was measured by following the reduction of cytochrome c at λ=550nm.
- Complex IV activity was measured by following the oxidation of cytochrome c at λ= 550nm.

The PS-NPs were incubated with the mitochondria in the assay components for 15 minutes at 37 °C.

H2O2 production was followed using Amplex Red at λ= 561 to 581 nm and the results were calculated using a H2O2 standard curve.

3 Results

3.2 Reactive Oxygen Species

The physiological levels of hydrogen peroxide (H_2O_2) in intact non-synaptic mitochondria (NSM) were determined to be 4.2nM, with the addition of a Glutamate/Malate solution and succinate (SA). There was a 17% and 73% increase in H_2O_2 production for 100 and 250mg/L respectively (figure 1).

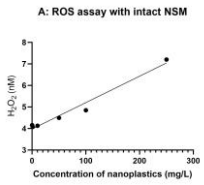

Figure 1: ROS assay with intact mitochondria. *Results presented are displayed as the mean of H_2O_2 produced. N=3, 1 sample of NSM (0.1mg/ml) used.*

The physiological levels of hydrogen peroxide in ruptured NSM were determined to be 2.9nM after the addition of NADH (figure 2). There was a 89% and 298% increase for 100mg/L and 250mg/L respectively in the production of H_2O_2 with the addition of NADH.

The physiological levels of hydrogen peroxide in ruptured synaptosomes (SY) were determined to be 5nM after the addition of NADH. There was a 75% and 104% increase for 100mg/L and 250mg/L respectively in the production of H_2O_2 with the addition of NADH.

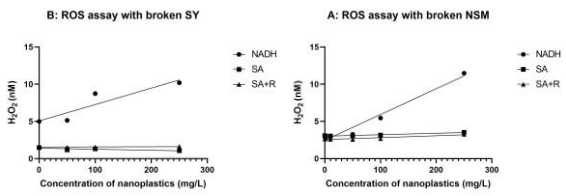

Figure 2: ROS assay with ruptured mitochondria. *A: ROS assay with NSM (0.1mg/ml) and B: ROS assay with SY (0.1mg/ml). The samples were freeze-thawed three times, n=3 with one sample used for each. NADH, succinate (SA) and SA + rotenone (R) were added to the samples separately. Results are displayed as mean H_2O_2 produced.*

Acknowledgments

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3.1 Electron Transport Chain

The specific activity of complex I was found to be 61 ± 0.4 nmol/min/mg in NSM and 40 ± 0.3 nmol/min/mg in synaptosomes (figure 1).

The specific activity of complex II from NSM was determined to be 319 ± 1.1nmol/min/mg and 246 ± 0.2 nmol/min/mg in the SY (figure 1). There was evidence of inhibition in SY at the highest concentration of nanoplastics (250mg/L) after the addition of DQ (22.6% inhibition, $p<0.05$).

The specific activity of complex II/III was found to be 177 ± 7.9 nmol/min/mg for NSM (figure 1). Dose responsive inhibition was seen at 50, 100 and 250 mg/L of nanoplastics with 27%, 40% and 87% inhibition respectively. The specific activity was determined to be 58 ± 0.7 nmol/min/mg for SY. Dose responsive inhibition was also seen in SY at 100 and 250 mg/L with 27% and 54% inhibition respectively.

The specific activity of complex IV was determined to be 298 ± 3.3 nmol/min/mg for NSM and 45 ± 0.5 nmol/min/mg for SY (figure 1).

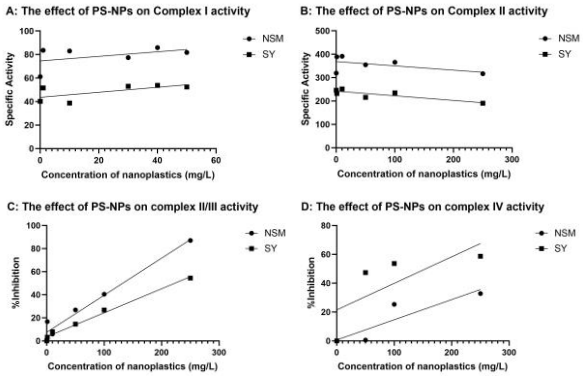

Figure 1: Examining the effect of PS-NPs on the ETC. ***A****: The effect of PS-NPs on complex I activity for NSM (20µg) and SY (20µg). The NPs absorbed at 340nm so the following concentrations were used for this complex: 1, 10, 30, 40 and 50mg/L.* ***B****: The effect of PS-NPs on complex II activity. The % inhibition of SY (20µg, $p<0.05$) and NSM (10µg) are both shown.* ***C****: The effect of PS-NPs on complex II/III activity for both NSM (20µg, $p<0.05$) and SY (20µg, $p<0.05$).* ***D****: The effect of PS-NPs on complex IV activity, for both samples (10µg). The results were expressed as first order decay rate constants (K). The NPs were incubated with the mitochondria for 15 minutes for each complex. The Shapiro-wilk test was used to check for normality and the one-way ANOVA test was used to test the significance of the data. N=3 from one preparation of mitochondria sample.*

4 Conclusion

This study found preliminary evidence that PS-NPs have a damaging effect on non-synaptic mitochondria and synaptosomes. Inhibition of complex II/III for both NSM and SY was found, although the effects seen were stronger in NSM. Further studies need to be done to determine the mechanism of inhibition found in the complex II/III assay.

The preliminary results did not show any inhibition for complex I or IV. However, partial inhibition was found in the complex II assay in the synaptosome sample.

Increased levels of ROS production were found in both NSM and SY. Further studies need to determine if the increased levels of ROS are causing oxidative stress or are elevated as signalling molecules in stress response. No evidence of reverse electron transport through complex I was found.

Refences

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