MPP⁺-MEDITATED IMMUNE ACTIVATION IN MACROPHAGES IN A PARKINSON’S DISEASE MODEL

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Introduction

• Parkinson’s disease (PD) is a neurodegenerative disorder characterized by a loss of dopaminergic neurons of the substantia nigra and accumulation of α-synuclein in Lewy bodies
• PD patients present motor, gastric, neuropsychiatric and sensory symptoms [1]
• Pathogenesis of PD is still unclear with genetic predispositions and environmental toxins through nose and intestine
• PD modeled with MPP⁺ (1-methyl-4-phenyl-1,2,3,6-tetrahydro-9-pyridinyl), a neurotoxin with a similar structure to herbicides. It interferes with mitochondrial complex I and could induce an immune response
• Oxidative stress (ROS) is involved in mitochondrial dysfunction, gelatin cell activation and protein misfolding, which can all lead to apoptosis [2]

Objective

To determine early mechanisms of immune activation in PD

Hypothesis

MPP⁺ induces an increase in ROS, which damages mitochondria, thus causing neuronal loss through immune activation

Materials and methods

Cell lines

Raw (mice macrophages), BV2 (mice microglia), N2A (mice neurons) and SHSY5Y (human dopaminergic neurons)

Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Function</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP⁺</td>
<td>MPP⁺ analogue</td>
<td>A</td>
</tr>
<tr>
<td>Cellrox</td>
<td>Oxidative stress</td>
<td>B</td>
</tr>
<tr>
<td>MitoTracker</td>
<td>Mitochondria</td>
<td>C</td>
</tr>
<tr>
<td>LPS</td>
<td>Inflammation marker</td>
<td>D</td>
</tr>
<tr>
<td>MPP⁺</td>
<td>PD model</td>
<td>E</td>
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</tbody>
</table>

Cell culture

All cell lines cultured in DMEM, detached with trypsin/EDTA. 3000 cells/well were put into a chamber coverglass. Dyes were incubated 30 min prior to adding APP⁺ or LPS (Epposucaride)

Image acquisition

Confocal laser scanning microscope (Olympus) was used for time-lapse imaging of cells for 2-3 hours.

Table: Experimental setups

<table>
<thead>
<tr>
<th>Materials</th>
<th>MPP⁺</th>
<th>APP⁺</th>
<th>Cellrox</th>
<th>MitoTracker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>100µM</td>
<td>100ng/ml</td>
<td>LPS</td>
<td>100nM</td>
</tr>
<tr>
<td>During imaging</td>
<td>24 hours</td>
<td>3 hours</td>
<td>24 hours</td>
<td>24 hours</td>
</tr>
</tbody>
</table>

Results

Colocalization of MPP⁺ probe within mitochondria in neurons (N2A)

→ Oxidative stress can occur in nucleus and elsewhere in the cytosol, seemingly in vesicles
→ More oxidative stress in LPS and MPP⁺ conditions

Colocalization of MPP⁺ probe within mitochondria in macrophages (Raw)

→ Oxidative stress can occur in nucleus and elsewhere in the cytosol
→ Slight increase in oxidative stress in MPP⁺ condition

Stimulation of oxidative stress in pro-inflammatory conditions in macrophages (Raw)

→ Oxidative stress can occur in nucleus and elsewhere in the cytosol

Stimulation of oxidative stress in pro-inflammatory conditions in microglia (BV2)

→ Oxidative stress can occur in nucleus and elsewhere in the cytosol
More oxidative stress in LPS than MPP⁺ condition

Conclusions and perspectives

• MPP⁺ could be uptaken in the mitochondria as it is analogue to APP⁺
• Oxidative stress, shown with Cellrox, takes place in mitochondria, elsewhere in the cytosol and seemingly in vesicles
• This is the case for all cell lines, indicating a similar toxicity mechanism of MPP⁺
• More experiments have to be done to showcase a clear toxicity difference between MPP⁺ and non-MPP⁺ conditions

Future experiments

• As more men are affected by PD than women, estrogen were tested as therapeutic agents
• E2 (17β-estradiol), causes negative effects in reproductive organs through ERα and ERβ (estrogen receptors)
• G1 acts only on GPER1 (G protein-coupled receptor-1), a novel receptor, without these side effects [4]
• Incubate raw cells with estrogens (E2) and agonists and antagonists to see effect on immune cells in a MPP⁺ model

Acknowledgments

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Further information