HEALTH RESEARCH ABSTRACT SUBMISSIONS #46

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Title of Research *	A Dopamine-Derived Neurotoxin, 3,4-Dihydroxyphenylacetaldehyde, and Microglia: Metabolism, Activation, and Toxicity
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Introduction & Purpose *

The cause of the hallmark dopaminergic cell death of Parkinson's Disease (PD) is unknown, but recent research indicates oxidative stress and the endogenous neurotoxin, 3,4-dihydroxyphenylacetaldehyde (DOPAL), to play roles in the disease pathogenesis. DOPAL is generated from dopamine (DA) by monoamine oxidase (MAO) and oxidized to 3,4-dihydroxyphenylacetic acid by aldehyde dehydrogenase. The dopamine metabolite is highly toxic to dopaminergic cells and needs to be rapidly metabolized to prevent toxicity. Non-neuronal cells express high levels of MAO-B, but formation and metabolism of DOPAL within these cells has not previously been measured. Microglial cells have been shown to be activated in the striatal region of PD brains, however the mechanism of this activation has not been demonstrated, and could be due to DOPAL or DOPAL-protein adducts. Activated microglia cause injury to dopaminergic neurons via a host of mechanisms, including reactive oxygen species production, release of cytokines, and phagocytic activity. The ability of DA, DOPAL, and other DA metabolites to activate BV-2 microglial cells was previously unknown, and this research was designed to explore that interaction.

Experimental Design *

DOPAL was biosynthesized from dopamine (DA) by an existing route with mouse liver MAO. BV-2 Microglia were cultured as previously described. Krebs-Ringer-HEPES Buffer (pH 7.4) was used for all metabolism experiments. BV-2 microglia were treated with DA or DOPAL (at varying concentrations) at 37°C, with extracellular aliquots taken at time points and subsequently analyzed by HPLC. Cell viability was determined using both the standard MTT reduction assay (measuring mitochondrial reductase activity) and the lactate dehydrogenase assay (measuring cell permeability). BV-2 microglia were also treated for 4 hours (at 37°C, 5% CO2) with DA and metabolites at varying concentrations in Krebs-Ringer-HEPES Buffer, after which time 1 mL of DMEM media was added, and the cells were incubated for another 20 hours, at which time the media was collected and frozen. Positive control for BV-2 activation was generated by treating cells with 4 µg/mL lipopolysaccharide (LPS) as above. A TNF-α Sandwich ELISA kit (BioLegend) was used by manufacturer's instructions.

Results *	BV-2 microglia metabolize DA and DOPAL to DOPAC and DOPET in a dose-dependent manner. DA and DOPAL are not toxic to BV-2 microglia after four hours. DOPAL is able to elicit release of TNF-alpha, an indicator of microglial activation. Treatment with DA and DOPAC does not produce significant TNF-alpha release, implicating DOPAL as the ultimate toxicant.

Conclusions *

BV-2 microglia have the necessary enzymes to biotransform DA and DOPAL to downstream metabolites (DOPAC and DOPET). Also, microglia produce much higher levels of DOPAC than DOPET after
2 hours. Within BV-2 microglia, DOPAL exhibits low toxicity after 4 hours. In addition, DOPAL is able to activate BV-2 microglia at significant levels with respect to control. Finally, DOPAL is a stronger activator of microglia than DA and DOPAC. DOPAL-mediated activation of microglia demonstrated in this study could represent a mechanism for inflammation and dopaminergic cell death detected in patients with PD.

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