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#### **4. INTRODUCTION**

We are continuing to examine a number of neuroprotective agents in an MPTP model of PD. We are also continuing to utilize metabolomic profiling to identify novel biomarkers for PD and to investigate whether these occur in animal models of PD. We are continuing to develop and characterize a new animal model of PD by making a knock out of PINK1, a nuclear encoded kinase localized to mitochondria, and which causes autosomal recessive PD. We have completed a study of the effects of human dopaminergic stem cells in a 6-hydroxy dopamine model of PD.

Outline of Research Goals:

Task 1: To determine the ability of pharmacologic agents to prevent MPTP neurotoxicity.

1. Examine neuroprotective effects of phosphodiesterase IV inhibitor rolipram on MPTP.
2. Examine neuroprotective effects of mitochondrial targeted antioxidants (SS02 and SS31), which inhibit the MPT.
3. Examine neuroprotective effects of celastrol and promethazine on MPTP.
4. Examine a novel form of Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) in the MPTP model of PD.
5. Examine the role of caspase 3 activation in activation of microglia and MPTP toxicity.

Task 2: To develop a new transgenic mouse model of PD by knocking out PINK1, a protein in which mutations cause autosomal recessive PD (months 1-48).

Task 3: To utilize metabolomic profiling to develop biomarkers for PD. We will utilize metabolomic profiling to study patients with PD and animal models of PD (months 1-48).

Task 4. To determine the efficacy of human dopaminergic stem cells in the 6-hydroxydopamine (6-OHDA) model of PD (months 6 -24).

## 5. BODY

**Task 1:** To determine the ability of pharmacologic agents to prevent MPTP neurotoxicity.

1. Examine neuroprotective effects of phosphodiesterase inhibitor rolipram on MPTP.

We have completed studies with the phosphodiesterase inhibitor rolipram. We saw protective effects with either 1.2 or 2.5 mg/kg. We also showed significant protective effects against dopamine depletion and loss of tyrosine hydroxylase neurons. These results are now published in *Experimental Neurology*.

2. Examine neuroprotective effects of mitochondrial targeted antioxidants (SS02 and SS31), which inhibit the MPT.

We have made substantial progress in these studies. We found that both of these compounds, which concentrate in mitochondria and have ROS scavenging properties are able to dose-dependently inhibit lipid peroxidation as measured by chemoluminescence. We also found that these compounds protect against MPP<sup>+</sup> toxicity *in vitro*. We carried out studies against MPTP toxicity in mice. We found that both compounds were able to significantly protect against MPTP induced loss of dopamine, as well as loss of tyrosine hydroxylase neurons. The dopamine metabolites DOPAC and HVA showed similar effects. We are working on a manuscript describing these results, as well as other continuing studies. These include studying the neuroprotective effects of these peptides in a chronic MPTP model.

3. Examine neuroprotective effects of celastrol and promethazine on MPTP.

We have completed studies of both of these compounds during our last report. We found marked neuroprotective effects of both compounds. The results were published in the *Journal of Neurochemistry* and in *Neurobiology of Disease* respectively.

4. Examine a novel form of coenzyme Q10 (CoQ10) in the MPTP model of PD.

We carried out a large number of studies, which showed that there were indeed significant protective effects. In particular, we were able to show that coenzyme Q administered in a chronic model of MPTP toxicity not only protected against loss of tyrosine hydroxylase neurons but it also protected against the development of alphasynuclein aggregates. This was a model in which MPTP was administered over one month by Alzet pump. These results we believe are particularly relevant to PD itself. These results are now published in the *Journal of Neurochemistry*.

5. Examine the role of caspase 3 activation in activation microglia and MPTP toxicity.

We continued our studies of matrix metalloprotease 3 (MMP3) and its role as a novel signaling proteinase from apoptotic neuronal cell death that activates microglia. We found that it is of importance in activating NADPH oxidase to generate superoxide and this play a direct role in dopamine cell death. We have recently completed initial studies, which have been published in *FASEB Journal*.

**Task 2:** To develop a new transgenic mouse model of PD by knocking out PINK1, a protein in which mutations can cause autosomal recessive PD (months 1-48).

We have generated the PINK1 knockout mice. Correctly targeted ES cells were used to inject and generate PINK1 knockout mouse founders. We are now continuing to expand the colony. We have found mild defects in motor behavior. We have also found impaired dopamine release using microdialysis. We have observed a number of different phosphorylated proteins on two-dimensional gels. Of particular interest, the protein OMI is phosphorylated by PINK1. This is of interest, since OMI is also implicated in PD. We intend to carry out studies examining these mice both histologically, as well as biochemically. These studies are ongoing. We also carried out studies in *Drosophila* of PINK1 deficiency in collaboration with Dr. Bingwei Lu of Stanford University.

**Task 3:** To utilize metabolomic profiling to develop biomarkers for PD.

We made significant progress in our studies of metabolomic profiling. We identified markers, which clearly separate unmedicated PD patients from controls. We examined 25 controls and 66 PD patients. We utilized metabolomic profiling with high performance liquid chromatography, coupled with electrochemical coulometric array detection. However, initial studies were of unmedicated PD subjects as compared to controls to rule out confounding effects of symptomatic medications. We were able to completely separate these two groups. We utilized a partial least squares discriminate analysis. To identity which variables were responsible for this separation, the variable influence on the projection parameter was used to select variables that had the most significant contribution in discriminating the metabolomic profiles. We applied the discrimination analysis to PD patients who were treated with Sinemet or the combination of Sinemet with dopamine receptor agonist. These results showed that once again, we could completely discriminate these groups of patients. These results demonstrate that the metabolomic profiles which differentiate control in PD subjects are not attributable to possible drug effects. We examined a number of specific markers. These included uric acid which we found was significantly decreased in PD patients. We also found increased levels of reduced glutathione, which is of interest, since it is activated by the Nrf2 transcriptional pathway, which is known to be activated in PD. We, therefore, believe that this is a compensatory phenomena. Lastly, we measured 8-hydroxy-2-deoxyguanosine levels. These were significantly increased in the PD subjects. These results were published in *Brain* this year.

In the last year, we have studied patients with LRRK2 mutations. We have studied patients with both the gene, who have not yet manifested the disease and family members who are gene-negative. We can clearly separate PD patients with LRRK2 mutations

from idiopathic PD and controls. Similarly, we could separate the gene-positive patients from both controls and LRRK2 mutation-negative patients. Currently, we are working on the structural elucidation of the remaining analytes separating PD patients from controls using mass spectrometry. We have developed an integrated parallel LCECA/LCMS with post-column flow splitting between the electrochemical and mass spectrometric detectors. These results confirm that metabolomic profiling holds great promise for development of both diagnostic and disease progression markers for PD. Our results to date show that this approach is feasible. We intend to carry out further validation in larger numbers of patients, as well as in patients with specific gene mutations such as LRRK2.

**Task 4:** To determine the efficacy of human dopaminergic stem cells in the 6-hydroxy dopamine (6-OHDA) model of PD.

With regard to this task, we completed it. We carried out the studies in collaboration with Dr. Steve Goldman and Dr. Neeta Roy. We carried out detailed histologic studies. The acquisition of highly-enriched dopaminergic populations is an important prerequisite to using HES-derived dopaminergic neurons for cell-based therapy. We utilized a new strategy improving the efficiency of dopaminergic neurogenesis from human ES cells. This involved co-culture with telomerase immortalized human mesencephalic astrocytes during induction of a dopaminergic phenotype using sonic hedgehog and FGF8. Using this means, we achieved a high efficiency enrichment of dopaminergic neurons. We then studied the ability of these to functionally replace and correct 6-OHDA lesioned adult rat brain. We found that there was a significant substantial and long lasting restitution of motor function. We also measured for motor asymmetry and found that this was also corrected. There was efficient generation of TH positive neurons in all six animals studied. We also looked to see if the engrafted brains still showed evidence of mitosis amongst the engrafted population. We examined BDRU incorporation *in vivo*. This showed that approximately 6½ % of the neurons showed BDRU incorporation. Nevertheless, these are extremely promising results which set the stage for further studies before human transplantation. These studies were published in *Nature Medicine*.

## **6. KEY RESEARCH ACCOMPLISHMENTS**

- A. The finding that mitochondrial targeted antioxidants SS02 and SS31, which inhibit the MPT are neuroprotective against MPTP toxicity in mice, as well as MPP+ toxicity in cell culture.
- B. The finding that a reduced novel form of CoQ10 is effective in the MPTP model of PD. We also showed that CoQ was effective in a chronic model of MPTP toxicity. We furthermore found that MPTP toxicity was significantly attenuated in MMP3 deficient mice, which lack the MMP3 activation in microglia. This was accompanied by a decrease in superoxide generation, which was mediated by NADPH oxidase.
- C. We have developed a knockout model of PINK1. We are continuing to study these mice to enable a full characterization.
- D. We have continued metabolomic profiling and now have shown that we can separate unmedicated PD patients from controls, as well as medicated PD patients from controls. This work has now been published. We also showed that a number of specific metabolites were altered including 8-hydroxy-2-deoxyguanosine and reduced uric acid.
- E. We developed a novel technique for increasing the induction of dopaminergic phenotype in human ES-derived dopaminergic neurons. We demonstrated and published that this was effective in reversing a motor deficit in rats lesioned with 6-hydroxy-dopamine.

## 7. REPORTABLE OUTCOMES

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## **8. CONCLUSIONS**

We accomplished our original research goals. We have characterized a number of agents, which show protection against MPTP toxicity. We have developed a new transgenic mouse model of PD by knocking out PINK1. We are continuing metabolomic profiling studies of PD patients and we have found that we can identify unique biomarkers in patients with LRRK2 mutations. We completed studies of transplantation of human ES cell derived dopaminergic neurons into a 6-hydroxy-dopamine model of PD and have shown successful restitution of behavioral abnormalities.

## 9. REFERENCES

None.

## 10. APPENDICES

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