Investigation of the therapeutic effects of J-147 in two animal models of Parkinson's disease

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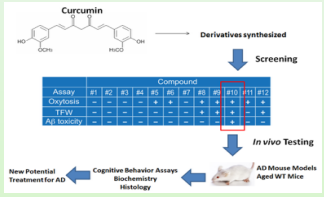
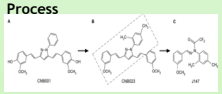
Abstract

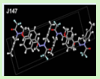
Human idiopathic Parkinson's disease (PD) is a progressive motor system disorder caused by the extensive depletion of dopaminergic neurons in the nigrostriatal pathway. PD affects approximately one million people in the United States. Here, we tested the therapeutic potential of the neuroprotective compound J147 in two well-established rodent models of PD. J147 was developed on the basis of preventing nerve cell death in a wide range of brain toxicities associated with old age, and J147 is poised to begin clinical trials for the treatment of Alzheimer's disease. **The hypothesis is that J147 may have therapeutic efficacy in other neurological disorders marked by nerve cell death (in this study, PD).**

J147 Drug Synthesis



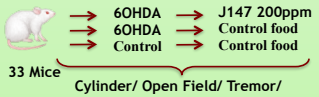
Schematic For Alternative Drug Discovery Process



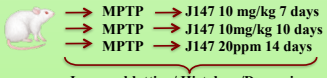


Project Background

1. J147 in 6OHDA Animal Model



2. J147 in MPTP Animal Model



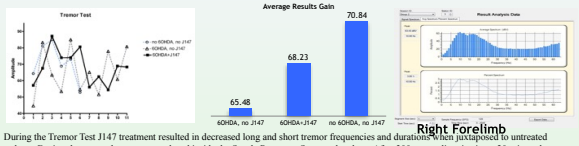
The first Parkinson's model was generated by unilateral injection of 6-hydroxidopamine (6-OHDA) into the striatal pathway, and the second via intraperitoneal injection of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). Levels of the neurotransmitter dopamine were assayed by HPLC. Tyrosine hydroxalase (TH) and inflammation markers were determined by Western blotting. This was followed by behavioral studies comparing changes between the J147-treated and control groups.

The data showed that J147 treatment reduces the loss of TH-positive neurons, decreases inflammation, and ameliorates the dopamine depletion following the 6-OHDA and the MPTP treatments. In addition, J147 improved some of the behavioral motor deficits assessed by the rotometer and tremor tests. These results plus the outstanding pharmacological properties of J147 suggest that J147 has the potential for the clinical treatment of Parkinson's disease.

Results

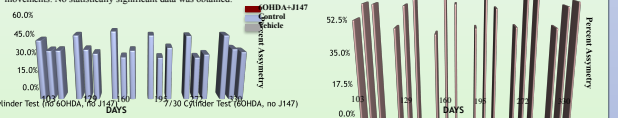
Figure I-VI J147 Reverses Some Aspects of PD Pathology in the 6OHDA Rodent model

I



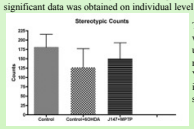
During the Tremor Test J147 treatment resulted in decreased long and short tremor frequencies and durations when juxtaposed to untreated rodents. During the test each mouse was placed inside the Startle Response System chambers. After 300 sec acclimatization a 20 min. voltage recording was performed at the rate 454 recordings per sec. The system utilized an ultra sensitive movement sensor to record continuous movement waveforms and to measure performance, between 5-15 Hz, was then analyzed. The Tremor Monitor System recorded, analyzed and reported rapid repetitive movements in mice. The Tremor Monitor software differentiated tremor events from ambulatory/stereotyped movements. No statistically significant data was obtained.

II



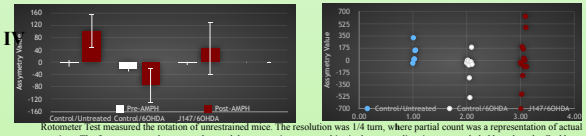
The Cylinder Test was performed on 33 C57BL6/J rodents during 13months at 6 different time frames to measure cerebral asymmetry in forelimb motor function. J147 treatment reduced right-side bias after 160 days of J147 treatment. After 7 days acclimatization, rodents were initially selected into 3 groups, n=11. Group 1—Control, Groups 2—6OHDA alone (control food), Group 3—6OHDA+J147 10mg/kg/day in 200ppm food. The 6OHDA lesion resulted in rodents' right side bias when leaning with hindlimbs on the cylinder. "Best performers"— in the 6OHDA J147-untreated group showed a stable asymmetry response throughout the study (5/11 rodents); in the 6OHDA+J147 group showed the initial asymmetry recovered after 160-195 days of J147 drug treatment (5/11 rodents); non and weak responders to the lesion 2/22. Consequently, statistically significant data was obtained on individual level evaluations over the course of the study; on group level— no statistical significance.

III



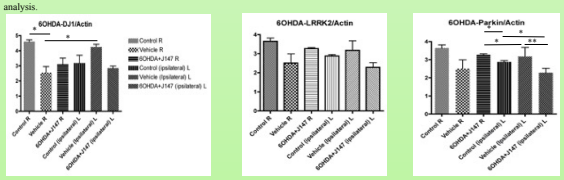
The Open field test (OFT) showed that 6OHDA+J147 treated mice had higher path length and velocity, lower duration to stay in corners and lower acclimatization time when placed into the unfamiliar environment. The open field is an arena with walls to prevent escape. The field is marked with a grid and square crossings. The centroid of this area is exported as calibrated X- and Y-coordinates at a frame rate of 5 frames per second (fps). The area around the body of the mouse is marked red. Rearing and time spent moving are used to assess the activity. Statistical significance was observed on individual level, not on group level.

IV



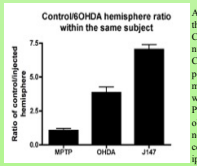
Rotometer Test measured the rotation of unrestrained mice. The resolution was 1/4 turn, where partial count was a representation of actual rotations. The few extreme values were observed due to consistent strong bias in the wrong direction; not excluded based on the Grubbs outlier analysis.

V

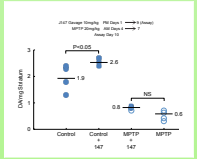


Statistically significant data was derived from the immunoblotting experiments performed. Interestingly in the 6OHDA model J147 treatment increase DJ1 (compered to Vehicle), PARKIN (ipsilateral side compared to Control and Vehicle same side);

VI



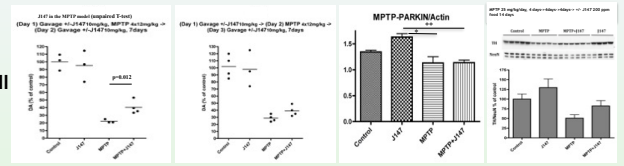
An interesting observation was derived from the High Performance Liquid Chromatography, which suggested increased number of dopaminergic neurons in the Contralateral hemisphere. This observation points to brain plasticity as compensatory mechanism of the contralateral hemisphere with the introduction of J147 treatment. The PD associated motor dysfunctions, as observed from behavior assays were normalized by the contralateral hemisphere compensation for the neuronal loss in the ipsilateral side of the brain.



Results

Figure VII J147 Normalizes the TH positive neuronal Cells in the MPTP Rodent model

VII



Histology was performed subjects, selected from the 6OHDA J147-untreated, and the 6OHDA+J147 treated groups, n=3. Interestingly, a statistically significant increase in cell number was observed in the contralateral hemispheres.

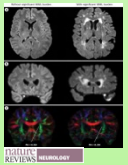
Discussion

- J147 was developed using an alternative drug discovery paradigm based on efficacy in *in vitro* cytotoxic assays that reflect multiple aspects of CNS- associated pathologies.
- J147 is a highly potent and neurotrophic compound.
- Oral administration of J147 showed a neuroprotective effect.
- J147 treatment promoted motor function stabilization by increasing DA levels in the contralateral (non-lesioned) hemisphere.
- J147 resulted in motor function enhancement and inducing TH.
- J147 is a new neuroprotective compound. It has a range of biological activities relevant to human PD and other CNS disorders. The combined properties of J147 indicate potential—as a—promising PD therapeutic, by slowing disease progression through neuroprotection and improving motor function.

Conclusion

Our laboratory designed an alternative drug discovery paradigm to potentially treat sporadic PD that may be linked to mitochondrial and environmental toxins. J147 has highly potent, orally active, broadly neuroprotective properties when investigated *in vivo* in 6OHDA and MPTP animal models of PD.

Future Directions



- J147 has the potential to reverse some of the PD associated phenotypes. Yet we strive to further investigate the neurogenic properties of J147 by performing long-term BrdU labeling to lineage trace newborn cell contribution.
- Investigate J147 in a model that best represents comorbidpresence of age-associated white matter lesions (WMLs).
- Perform the genetic studies, using various different PD models while increasing the rodents' size in our study.

Acknowledgement

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