A Study on the Mechanism of the Parkinsonian-Causing Drug 1-methyl-4-phenylpyridinium (MPP^+)

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1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease in which the brain's dopaminergic neurons in the midbrain are heavily depleted, lowering the neurotransmitter dopamine (DA) supply available for signal transmission [1]. Loss of dopaminergic neurons through PD results in the characteristic loss of motor control, shaking, and spasticity [1]. Although PD affects over 1.5 million people in the US, researchers do not understand the disease's direct cause and there is currently no known cure [2]. The most common treatment for Parkinsonian symptoms is administration of L-DOPA, the dopamine precursor, to increase the brain's DA level. Short-term treatment with L-DOPA seems to remove the disease's characteristic tremors, but after 3-5 years the side-effects of this drug are often considered worse than the disease itself. It is for this reason that it would be extremely valuable to develop a focused treatment of cure for the disease without the side effects of L-DOPA.

An instance of very early-onset PD was discovered in the 1980s in several young men. The origin of the disease was later traced to a contaminant in the illicit street drugs they had used. This contaminant, called 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was thoroughly investigated by researchers to determine its specific toxicity to dopaminergic neurons. It was found that MPTP is converted to 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase in the brain, and that MPP^+ is the chemical which is selectively toxic to dopaminergic neurons [3]. PD researchers have commonly used MPP^+-induced Parkinsonism as a model to study the biochemical mechanisms that underlie the pathophysiology of PD, but much like the disease itself, the definitive molecular mechanism leading to specific neurotoxicity of MPP^+ has yet to be defined [4].

It is well-established that MPP^+ is taken up by the plasma membrane dopamine reuptake transporter (DAT) into the cytosol of neurons, but its exact intracellular target for the toxicity is highly controversial. While some argue that MPP^+ inhibits mitochondrial complex I leading to ATP-deficient cellular death [5], others believe that MPP^+ interferes with intracellular catecholamine metabolism and increases the reactive oxygen mediated oxidative stress [6]. Although both theories are plausible, no current data clearly explain the severe toxicity of MPP^+.

In our laboratory, we have designed and synthesized a wide array of MPP^+ analogs for use in systematic comparative structure-activity studies on mitochondrial complex I inhibition, oxidative stress due to altered catecholamine metabolism and cellular cytotoxicity. We believe our studies will lead to a better understanding of the molecular mechanism(s) of MPP^+ toxicity and may eventually help to better understand the causes of PD at the molecular level.

2. Experimental

Mitochondrial Complex I Inhibition: Mitochondrial complex I inhibition by MPP^+ is well-known, but detailed structure-activity studies of the inhibition have never been reported. To test the effects of MPP^+ and its analogs on complex I, we have isolated whole mitochondria from bovine liver according to the methods of Hovius and Lambrechts [7] and membrane fragments were obtained by subjecting intact mitochondria to several freeze-thaw cycles. The coenzyme Q dependent complex I activity was determined by measuring the rate of NADH oxidations according to the procedure of Yen, et al. [8] .

Dopamine Uptake Inhibition: Bovine chromaffin granule ghosts have been extensively used as a model in studying the DA uptake and norepinephrine biosynthesis in catecholamine storage vesicles as they contain the same vesicular monoamine transport protein, VMAT-2 [9]. This protein transports catecholamines from the cytosol into the storage vesicle at the expense of a vesicular H^+-ATPase generated pH gradient. The uptake and/or inhibition (with respect to DA) kinetics of MPP^+ and its derivatives for VMAT were measured using resealed granule ghosts as previously described [10]. The K_i

for inhibition of DA uptake was determined for each analog using standard data analysis methods.

Cell Culture/Toxicity Studies: SH-SY5Y human neuroblastoma cells were grown in DMEM high-glucose

media in 5% CO_2. Cells were seeded into 96-well plates and grown to 80-90% confluence prior to experimentation. MPP^+

analog stock solutions were freshly made between 1µM and 1mM. 50 µL of toxin solution/media was added to each cell well and incubated for 24 hrs, followed by addition of 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) solution was added to each well. After 90 min incubation period, 200 µL of acidic detergent solution was added to each well and was incubated overnight. The resulting formazan produced by viable cells was quantified by the difference in absorbance at 570 nm and 650 nm and results are expressed as % of control.

3. Results and Discussion

Although many more MPP^+ analogs have been examined, only the 3'OH, 4'OH and 4'OCH_3 derivatives will be discussed in this article.

Table 1. DA uptake inhibition studies for selected MPP^+ analogs.

MPP^+ Analogs	**DA Uptake Inhibition**	
	K_i for DA Uptake Inhibition (uM)	$K_{m,(DA)}$ / $K_{i\ (Deriv.)}$
MPP^+	92.4 ± 14.1	0.3
4'OH MPP^+	82.3 ± 10.7	0.7
4'OCH_3 MPP^+	105.7 ± 14.2	--
3'OH MPP^+	2.35 ± 0.1	9.7
3'OCH_3 MPP^+	--	--
2'OH MPP^+	--	--

Table 2. Complex I inhibition studies for selected MPP^+ analogs.

MPP^+ Analogs	**Mitochondrial Complex I**	
	IC_{50} for Complex I Inhibition (uM)	Relative to MPP^+
MPP^+	3515	--
4'OH MPP^+	83.5	42.1
4'OCH_3 MPP^+	69.2	50.8
3'OH MPP^+	424.5	8.3
3'OCH_3 MPP^+	449.8	7.8
2'OH MPP^+	306.1	11.5

Tables 1 and 2 show the relative potencies of MPP^+ and its analogs on DA uptake inhibition as well as on complex I inhibition. For analysis, please see conclusion section.

While comparing the overall toxicity of these four compounds over a 24-hour time course, each showed clear cellular death in a dose-dependent manner. While this was the case, no overwhelming difference in toxicity was observed between the four derivatives. All four compounds exhibited 50% cell death at ~75 µM.

4. Conclusions

Although each compound exhibited widely different effects on both complex I inhibition and dopamine uptake inhibition, this does not, as of yet, appear to directly correlate into a structure-activity relationship for overall toxicity. In all MPP^+ analogs tested, an electron-donating group placed in the *p*- (4') position greatly increased potency compared to the corresponding *m*- analog, with –OCH_3 showing the greatest potency for complex I inhibition. If complex I inhibition were the sole mechanism of toxicity, one would expect to see at least a moderate increase in toxicity which parallels complex I inhibition, but this was not the case. Although the structural requirements for complex I inhibition apparently greatly differed from those for dopamine uptake inhibition, neither showed a strong correlation with toxicity as all compounds tested exhibited very similar toxicities over 24 hours.

No definitive conclusions can be drawn yet as this research is still in its early stages. The fact that no direct structure-dependent toxicity was observed with these four analogs does not necessarily preclude these two processes from playing roles in the molecular mechanism. The molecular mechanism of the toxicity of these compounds could be complex and multi-functional and further studies are certainly necessary for full description. Further studies are underway in our laboratory examining these and other cellular processes to more fully characterize the mechanism in question.

5. Acknowledgements

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6. References

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