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Lessons from the rotenone model of Parkinson's disease

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In their recent review on environmental toxins and Parkinson's disease (PD)¹, under the heading 'What have we learned from the Rotenone model of PD?', Cicchetti and colleagues do not list a single positive aspect of the model. It is possible, however, to view the rotenone model from a different perspective.

Rotenone was first used to model PD in 1985 when Heikkila injected this mitochondrial complex I inhibitor directly into the brain and showed that at a concentration of 5 mM – approximately 500,000-fold higher than its IC₅₀ of 10 nM – it killed dopaminergic neurons²; however, identical results could have been obtained with a high concentration of virtually any toxin, mitochondrial or otherwise. Later, because there was a growing suspicion that PD might be associated with *systemic* mitochondrial defects, several groups began to experiment with systemic administration of mitochondrial toxins. Ferrante (1997) reported that administration of rotenone (10–18 mg/kg/day) produced 'nonspecific' brain lesions and peripheral toxicity³. In contrast, when Betarbet (2000) titrated the experimental complex I inhibition to a level similar to that reported in platelets from PD patients (using 2–3 mg/kg/day), it produced highly selective nigrostriatal degeneration⁴. Even more remarkably, rotenone-treated rats developed alpha-synuclein-positive cytoplasmic inclusions, similar to Lewy bodies, in nigral dopaminergic neurons – overcoming for the first time a key limitation of other available *in vivo* models. Moreover, rotenone provided the first proof-of-concept that a systemic defect in mitochondrial function could lead to selective nigrostriatal neurodegeneration. And although the rotenone model was developed initially to test the 'mitochondrial hypothesis' of PD, given the epidemiological links to pesticide exposure, it was also of interest that rotenone is a pesticide.

Subsequent studies found that the rotenone model accurately recapitulates many other features of PD⁵, including: accumulation and aggregation of *endogenous, wildtype* alpha-synuclein; alpha-synuclein- and polyubiquitin-positive Lewy bodies and Lewy neurites; apomorphine-responsive behavioral deficits; early and sustained activation of microglia; oxidative modification and translocation of DJ-1 into mitochondria *in vivo*; impairment of the nigral ubiquitin-proteasome system; accumulation of iron in the substantia nigra through a mechanism involving transferrin and transferrin receptor 2; alpha-synuclein pathology in enteric neurons and functional deficits in GI function, including gastroparesis. Additionally, systemic rotenone treatment can recapitulate the retinal pathology, the loss of testosterone and some of

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the sleep disturbances that are typical of PD. It is also worth noting that the transferrin-dependent mechanism of iron accumulation discovered in the rotenone model was subsequently found to be operative in human PD⁶. In other words, the rotenone model *predicted* what would be found in PD. Further, after the initial work in rats, others reported successful application of the rotenone model in species that are more tractable genetically, including mice, *Drosophila* and *C. elegans*.

It is also worth noting that, based on initial reports of rotenone neurotoxicity and the fact that it is a pesticide, epidemiological studies began to look at the potential role of exposure to rotenone *per se* as a risk factor for development of human PD. While the number of individuals exposed to rotenone and other ‘botanicals’ is small compared to other classes of synthetic pesticides, a recent study found an odds ratio of 5.9 (CI 0.6-56.1)⁷ and another found an odds ratio for rotenone of 10.9 (CI 2.5-48.0)⁸. Thus, the rotenone model informed subsequent epidemiological studies, which have suggested a potential role for rotenone in some cases of PD.

For all its strengths, however, the rotenone model has limitations. As expected, this mitochondrial poison, like any other, can produce dose-dependent systemic toxicity and mortality. Moreover, especially when delivered by osmotic minipump, there has been substantial variability in the proportion of animals that become parkinsonian and in the extent of their nigrostriatal lesions. Despite this variability, many groups have reported that rotenone (2-3 mg/kg/day) produces selective nigrostriatal degeneration, generally without nonspecific lesions (e.g., see Fleming⁹). Nevertheless, for unclear reasons, a few labs have reported striatal or other lesions with systemic rotenone. For example, Cicchetti and colleagues (2004) found that rotenone caused “severe digestion problems” with a stomach that was enlarged and full of undigested food¹⁰. Although the authors may not have recognized it as such, this may have been the first indication that rotenone can reproduce gastrointestinal features of PD, such as gastroparesis. In fact, Drolet (2009) recently reported that rotenone accurately recapitulates pathological and functional features of parkinsonian gastrointestinal impairment¹¹. Although the reasons for the discrepancies between labs are uncertain, recent refinements of the rotenone model have apparently made it more reproducible and have reduced nonspecific toxicities¹².

In the end, it is unrealistic to expect to be able to model perfectly in rats all aspects of an age-related disease like PD. Even genetically accurate models PD have met with limited success in replicating key behavioral and pathological features of the disease. Nevertheless, a great deal has been learned – and remains to be discovered – about pathogenic mechanisms using the rotenone model of PD.

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