HHS Public Access

Author manuscript

J Biochem Mol Toxicol. Author manuscript; available in PMC 2015 December 14.

Published in final edited form as:

J Biochem Mol Toxicol. 2014 May ; 28(5): 191–197. doi:10.1002/jbt.21552.

The Perplexing Paradox of Paraquat: the Case for Host-Based Susceptibility and Postulated Neurodegenerative Effects

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Abstract

Paraquat is an herbicide used extensively in agriculture, and has also been proposed to be a risk factor for Parkinson's disease. To date, experimental, clinical, and epidemiological data on paraquat neurotoxicity have been equivocal. In this short review, we discuss some technical and biological mechanisms that contribute to inconsistencies regarding paraquat neurotoxicity. We hypothesize that individual genetic variations in susceptibility generate major differences in neurotoxic risk and functional outcome. Identifying these heritable sources of variation in host susceptibility, and their role in complex gene-environment interactions, is crucial to identify risk biomarkers, and to devise better prevention and treatment for those exposed to paraquat and other potential neurotoxicants.

Keywords

host susceptibility; complex traits; toxicogenetics; paraquat; Parkinson's disease; neurodegeneration; iron

Introduction

It is becoming increasingly evident that many neurodegenerative diseases can be classified as familial vs. sporadic. Familial forms are usually manifest at earlier stages of life, and can have high heritability. Consequently, these forms have been more tractable for genetic analyses, thus well-defined gene variants have been linked to risk of Alzheimer's (1) and Parkinson's diseases (2). Conversely, the etiology of sporadic neurodegenerative diseases is

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far more complex, and almost certainly involves gene-environment interactions (3). Moreover, heritable factors likely involve multiple genes making the scientific investigation into the etiology of these diseases challenging since not all “susceptible” genotypes are exposed to environments that contain risk factors. Sporadic Parkinson’s disease (sPD) provides a useful example. Suggested environmental risk factors for sPD include rural living and the drinking of well water (4). Rural living also is physically associated with agriculture and the consequent exposure to chemicals used to control weeds, fungi, and insects. Some of these chemicals have been shown to be associated with increased risk of neurodegenerative diseases. Identifying those individuals carrying toxicant-sensitive genotypes is particularly challenging, but can provide the foundation for making inroads into the investigation into host susceptibility.

One such chemical, paraquat, is an herbicide that is used nearly worldwide in agriculture, and in other scenarios where weed control is desired. When handled improperly or ingested deliberately, it can cause acute pulmonary damage and death. Possibly of even broader impact is the fact that chronic exposure to paraquat has also been associated with a higher risk of sPD, although inconsistencies in epidemiological and animal studies have led to controversy. Our goal is to review the literature that may guide future research directions that can test the hypothesis that there is a relationship between paraquat exposure and risk of sPD.

Is paraquat a red herring?

In an article entitled “Paraquat: the red herring of Parkinson’s disease research,” Miller (5) argued that the risk for developing PD following paraquat exposure was over-stated. His view was that although paraquat is structurally similar to the well-known dopamine toxicant MPP^+, there were few data linking paraquat to disruption of mitochondrial complex I. In addition, he stated that in animal models paraquat causes no more than 25% loss of dopamine neurons in the substantia nigra pars compacta (SNc), whereas early symptoms of sPD require upwards of 70% loss of these neurons. Others have rebutted this assertion noting that not only has paraquat been demonstrated to destroy dopamine neurons in rodents, but paraquat toxicity is likely a complex interaction of both biological and environmental factors (6;7). Lopachin and Gavin (7) further noted that MPP^+ toxicity may not be exclusively related to mitochondrial toxicity, and there is little evidence that paraquat and MPP^+ are toxicologically similar despite their structural similarity. Indeed, Choi et al. (8) have shown that, at least in cell culture, complex I inhibition by paraquat is not required for dopaminergic neuron destruction by paraquat. Nevertheless, Miller’s perspective (5) has been influential, possibly due to inconsistencies in epidemiological data about the influence of paraquat on risk of sPD. We offer an alternate viewpoint that is based on a widely-held hypothesis that sPD is a complex disease influenced by the interaction of genetic and environmental factors. We hypothesize that the genetic make-up of PQ-exposed animals (human or laboratory) plays a major role in host-based susceptibility (i.e., the variability in the risk of sPD in paraquat-exposed individuals).

Sporadic Parkinson's disease is a complex trait influenced by multiple genes and environmental factors

After Alzheimer's disease, PD is the second most common neurodegenerative disorder, afflicting more than 1% of individuals over 65 years of age (9). The primary symptoms are resting tremor, progressive rigidity, hyperkinesias, and finally, difficulty in initiating voluntary movement and postural instability. The pathobiological hallmarks of PD include dopaminergic (DA) neuron loss in the substantia nigra pars compacta (SNc), and > 60% decrease in striatal dopamine concentrations at first clinical diagnosis. The suggested causes are many, and many investigators (e.g., 10;11) have hypothesized that sPD is a complex trait involving multiple cellular processes, each of which is modulated by multiple gene variants acting in concert with environmental factors, together causing a net increase or decrease in risk.

sPD is of particular concern, although not limited to, rural communities

In 1993, the National Institutes of Health launched a large study of agricultural health that continues to the present. Participating agencies include the National Cancer Institute, the National Institute of Environmental Health Sciences, the Environmental Protection Agency and the national Institute for Occupational Safety and Health. Although most of the research published from this effort concerns pesticide exposure and various cancers, the NIEHS epidemiology group led by Kamel has addressed the association between exposure to pesticides and neurodegenerative disease, including Parkinson's disease (12–15). This group has advanced our knowledge about the associated risk and most recently showed that identification of subpopulations within epidemiological studies (16) can elucidate the basis for individual differences in susceptibility to paraquat neurotoxicity.

A meta-analysis of more than 200 studies examining risk factors for sPD revealed significantly increased risk for sPD from pesticides, rural living, and consumption of well water (4;17). Exposure to insecticides and herbicides used in agriculture has been implicated in increased sPD risk. One of the most commonly used herbicides (although now banned in much of Northern Europe) is paraquat (PQ). PQ has a long environmental half-life and has been implicated as a risk factor for sPD. In the environment, PQ leaches into ground water and can remain at 80% retention in river water for nearly two years (18;19).

Is exposure to PQ a risk factor for sPD?

The evidence for PQ as risk for sPD is mixed, both in clinical-epidemiological and preclinical studies. Among nine epidemiological studies, six showed that PQ increased risk (20–25), whereas three did not (17;26;27). Berry et al. (28) were highly critical of both epidemiological and preclinical studies that had associated PQ exposure and sPD, arguing that the epidemiological studies failed to show a convincing link between PQ and sPD because of poor definition of exposure. They also argued that preclinical studies forced a comparison with the toxicology of MPTP, and ignored toxicokinetic issues necessary for PQ to enter neurons (28).

Why the disparity?

Epidemiological studies tend to sample populations broadly, and may therefore yield either Type I or Type II errors when subgroups of the sampled population respond differently to pathogens, toxicants, etc. This may be relevant in evaluating the PQ-induced risk for sPD. Indeed, in a recent study, Goldman et al. (16) showed that individuals homozygous for a null mutation in the glutathione S-transferase theta 1 gene and who were exposed to PQ had an odds ratio (OR) of 11.1 for developing sPD as compared to an OR of 1.5 for those not carrying the mutation and exposed to PQ. Although the number of subjects was small, the data implies that there subpopulations of humans (and mice?) that are differentially susceptible to the neurotoxic effects of PQ (and probably other toxicants as well). The task then becomes one of identifying those susceptible and those factors that confer individual differences in risk.

What about the preclinical research?

The principal pathophysiological sign of PD is loss of dopamine neuron perikarya in the SNc, with consequent loss of DA in the striatum. MPTP, a pro-neurotoxicant that targets the SNc DA neurons, is commonly used to model the disease *in vivo* and *in vitro*. MPTP intoxication provides a superb model of the symptoms and signs of PD in humans and non-human primates (29–31), and also responds essentially identically to approved symptomatic antiparkinson drugs (32). Thus, it has become the research standard often used to evaluate both the effects of other toxicants, and also therapeutic interventions. Although heuristically useful, the MPTP model has, at least to date, failed to translate into successful disease-modifying therapies for sPD, the reasons for which are not relevant here. What is important is how this may educate us on the role of environmental chemicals as a contributor to sPD, specifically in that it is highly likely that there are individual differences in toxic responses to MPTP. Thus, we have shown recently that MPTP neurotoxicity varied dramatically among inbred mouse strains (11;33). These data suggest that responses to PQ also are very dependent on factors such as age, strain, species, etc. Can this explain why a recent study by Breckenridge et al. (34) did not find PQ-related toxicity in C57BL/6J mice, whereas other studies have reported PQ-associated loss of DA neurons (35–38), as well as loss of striatal DA function and associated behavioral problems (36)?

Why the disparity?

All of these studies used the same C57BL/6 strain. Breckenridge et al. (34), however, used mice that were only two months old (equivalent to late adolescence in humans), and found no effects of PQ. Conversely, studies in which older animals were used have consistently reported PQ-induced effects on dopamine neurons, including animals that were 3–4 mo (37), 4–6 mo (38), and 10 mo of age (36). McCormack et al. (35) tested mice at 6 weeks, 5, or 18 months of age for PQ toxicity (three weekly injections of 10 mg/kg ip), and found that the number of dopamine neurons in the SNc decreased significantly with age, with the greatest loss observed in the 18 mo animals. Since age is the most critical variable in sPD, it is not surprising that it might also be a factor in response to toxicants that might be related to sPD etiology (11;33).

Host characteristics – the key to understanding the complexity of sPD

The epidemiological work of Goldman et al. (16) is illustrative of how identification of host characteristics can elucidate the relative risk for sPD in those exposed to PQ. Similarly, in animal models the age of the animal appears to be a critical factor in susceptibility to PQ neurotoxicity. What other host characteristics may influence PQ toxicity? Recently, we demonstrated that strain differences in reduction in tyrosine hydroxylase-positive (TH^+) neurons in the SNc by PQ is related to PQ-induced iron increases in the ventral midbrain, including the SNc (37). Iron has been shown to act synergistically with PQ in nigrostriatal neuron loss (39). Among the many proposed mechanisms of PQ neurotoxicity, one important factor may be genetic differences in how PQ disrupts iron regulation in the SNc. We hypothesize that those individuals whose exposure to PQ increases iron in the SNc will be most susceptible to PQ-induced parkinsonian toxicity.

Iron regulation as a major factor in the etiology of PQ-induced sPD

Peng et al. (40) demonstrated that PQ applied to a primary culture of mesencephalic neurons did not affect survival of TH+ neurons, yet when the culture consisted of neurons and glia, PQ caused a 40%% reduction in TH+ neurons. Adding iron to the culture medium increased this effect by 10%. This suggests that glia may be critical for PQ neurotoxicity and that iron exacerbates this toxicity. Moreover, Peng et al. (40) also showed that microglial activation *per se* results from iron-related oxidative stress. Inhibiting this oxidative stress by inactivating superoxide reduced the loss of PQ- and PQ+Fe treated neurons from the neuron-glia primary culture (40). More recently, Rappold et al. (41) showed that PQ exists in two oxidative states, PQ^{2+} and PQ^+, and that the reduced form enters cells via the dopamine transporter (DAT) more readily than does PQ^{2+}. Moreover, Rappold et al. (41) also found that microglia reduce PQ^{2+} using NADPH oxidase in humans and microglial nitric oxide synthase in mice.

It appears, then, that a limiting factor for PQ neurotoxicity may be the availability of iron. Iron activates microglia that in turn reduce PQ^{2+} to PQ^+ which can enter neurons via DAT and by organic cation transporter-3 (41). Rhodes and Ritz (42) have proposed that the genetics of iron regulation may play a major role in the degree of sPD risk resulting from PQ exposure. Relevant to this, Wu et al. (43) reported that there was increased iron concentrations in the SN (pars reticulata), globus pallidus, and red nucleus in humans who had suffered acute PQ poisoning at 6, 12, and 24 mo following intoxication, although the increase was significant in the globus pallidus and SNpr only at the last time point. These findings suggest the importance of determining whether acute PQ poisoning may disrupt iron homeostasis over the long term.

Iron accumulation in the SNc *per se* is associated with sPD

Iron regulation in the brain (as well as other tissues) is highly complex and requires the participation of many proteins that sequester, change oxidative state, transport and otherwise control availability of free or loosely bound iron. The genetics and genomics of these proteins provide ample opportunity for individual differences in iron regulation, especially in the SNc. Moreover, iron deposition in the SNc increases progressively with age in

humans (44), rats (45) and mice (46). There is agreement about such changes even using different methodologies such as MRI imaging (47), sonography (48), and postmortem examination. Notably, Sofic et al. (49) showed in postmortem samples that a progressive iron increase in the SNc is a feature of advanced PD. What is not known is whether increases in iron concentration in regions like the SNc are a cause or a consequence (or both) of sPD.

The biology of PQ neurotoxicity

SPD is a highly complex disease and many factors likely contribute to its etiology. Although we recognize that there are several likely pathways, one major pathway is by way of cooperation between iron and PQ. In our model, in susceptible individuals, PQ disrupts iron regulation in the SNc, causing an increase in iron. This iron influx in turn activates microglia, which reduce PQ^{2+} to PQ^{+}, thus facilitating uptake into DA neurons to produce ROS and cell damage/death (41). We have demonstrated genetic differences in the capacity of PQ to disrupt iron regulation in mice (37), and we propose that the same genetic differences in PQ disruption of SNc iron regulation occurs in humans and thus is another key to understanding individual differences in susceptibility to PQ neurotoxicity. Of course, iron regulation is only one of many suspected host characteristics. Others include polymorphisms in the dopamine transporter gene (50) and in the divalent metal transporter 1 gene (51).

How can we use this information in future PD related research?

The first lesson to be learned here is how to identify those individuals who are at relatively greater risk for PQ-related neurological damage – and not just paraquat but other toxicants as well. Genotyping has become relatively inexpensive and markers identified from genome-wide association studies and from complementary animal studies will prove to be instrumental in prevention (52). The use of genetically defined mice can prove useful in identifying candidate genes as the mouse and human genomes are more than 90% syntenic.

In parallel to studies in animals, it is timely to determine how genetic makeup influences individual susceptibility to environmental toxicants that play a role in the development of sPD, and its progression in human. This shall pave a critical step for personalized prevention and possible treatment. Of course, such studies may also offer insight into general mechanisms of sPD. In addition to there are multifactorial etiological factors (both environmental and genetic) that are involved, there are, however, several other challenges to studying the interaction of genetic and environmental factors in neurodegenerative diseases like PD in humans. These include: 1) a long preclinical period prior to the onset of clinical symptoms and 2) a lack of reliable human biochemical, molecular, or imaging biomarkers.

Imaging using both radioligands that label dopamine terminals and magnetic resonance imaging (MRI) has been applied extensively to study PD-related pathological changes in human subjects (53–55). In particular, diffusion tensor imaging (DTI), by measuring microstructural disorganization, has shown promise as a tool of PD-related changes (53;56;57). In the MPTP mouse model, DTI changes have been associated with dopamine neuron loss in the SN (58). Furthermore, several human studies have demonstrated reduced

FA values in the SN of early PD patients, consistent with the notion that DTI changes may be able to detect nigral changes *in vivo* (47;55). Recently, we have reported that subtoxic, chronic pesticide exposure of asymptomatic agriculture workers leads to DTI changes in the SN similar to those seen in PD patients (59), suggesting that such changes may represent the consequence of pesticide-induced toxicity to nigral circuitry. These findings are consistent with the previously proposed role of pesticide exposure in initiating or accelerating pathological processes that are similar to those occurring in PD. More importantly, the study demonstrated that DTI could detect microstructural changes in the SN possibly caused by pesticides. This approach may be useful in determining if other environmental chemicals alter brain microstructure, and/or can assist in focusing on specific chemicals that affect PD susceptibility.

Overall significance

sPD is more prevalent in rural areas than in urban areas and is most likely the result of gene-environment interactions. Not only is potential exposure to pesticides and some other environmental chemicals more common in rural areas, many individuals living on farms or in the country also drink well water that ranges widely in iron concentration. Iron homeostasis involves many proteins whose expression varies widely in different individuals (60–65). We hypothesize that iron may be an important factor in the toxicity of PQ, and that a systems genetics approach can help to elucidate some of the critical mechanisms. Although the interaction of genetic and environment factors is widely thought to be important for sPD, it has created a complex experimental template. Identification of the genetic mechanisms underlying individual differences in susceptibility to PQ neurotoxicity has great promise in developing new strategies for the prevention and treatment of environmentally-related sPD, and experimental tools may now exist to, at least partially, untangle these factors.

Acknowledgements

This work was supported, in part, by grants from the National Institutes of Health (R01 ES019672, R01 NS060722, U01 NS082151, U01 AA016662, U01 AA013499) and the UTHSC Center for Integrative and Translational Genomics.

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