Investigating the Role of Paraquat on Parkinson's Disease

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Abstract

The effects of the herbicide paraquat in the pathogenesis of Parkinson's Disease (PD) was investigated. Paraquat is a common pesticide known for its toxicity in humans, but recent studies suggest that exposure can induce PD. Data was collected through a systematic literature review in 12 papers. Results showed a positive correlation between exposure to paraquat and death of dopaminergic neurons, suggesting that pesticide exposure is a risk factor for Parkinson's Disease.

Keywords: Parkinson's Disease, paraquat, alpha-synuclein, dopamine neurons

Introduction

Parkinson's Disease

Parkinson's Disease (PD) is a progressive neurodegenerative disease that causes physical tremors and impairs movement. This arises through the degeneration of dopamine-containing cells in the central nervous system. The disease was first described by James Parkinson in an article published in 1817 on the shaking palsy. When first differentiating PD from other neurodegenerative diseases, Jean-Martin Charcot examined patients and classified them by the differences in their tremors (Charcot, 1872). He first characterized PD as a movement disorder because the main symptoms specific to the disease included muscle rigidity, loss of movement, involuntary movement, and resting tremors. Additionally, other non-motor symptoms including cognitive, speech, mood, olfactory, and urinary dysfunctions are present in PD patients. Some scientists have proposed that PD first affects areas of the brain without dopaminergic neurons,

with alpha-synuclein proteins traveling up the brainstem and finally to the SNpc, known as the Braak staging of PD (Braak et al., 2005). These symptoms reflect the multiple areas of the brain which are affected, resulting in symptoms other than the main movement disabilities thought of as PD. Similar to other neurodegenerative diseases like Alzheimer's disease, the range of symptoms for an affected person is variable, and a person with PD often has non-motor as well as motor symptoms. Currently, around one to two people in every thousand have PD at any point in time, and an estimated 10 million people are currently living with PD today (Tysnes and Storstein, 2017). These statistics make PD the second most common neurodegenerative disease globally to Alzheimer's, both of which have no cure.

Pathogenesis of PD

Although the exact cause of PD is unknown, the misfolding of alpha-synuclein, a protein abundant in the substantia nigra pars compacta (SNpc) part of the basal ganglia of the midbrain, was discovered to be a pathogenesis of PD (Ghosh et al., 2017). This protein is found at the tips of nerve cells in set areas called presynaptic terminals and continuously misfolds into larger masses in people with PD. When the alpha-synuclein protein aggregates beyond a certain size, it is called a Lewy body; these Lewy bodies begin to disrupt the function of nerve cells (Breydo, 2012). Through the disruption of cellular homeostasis, the transmitting neurons are not able to

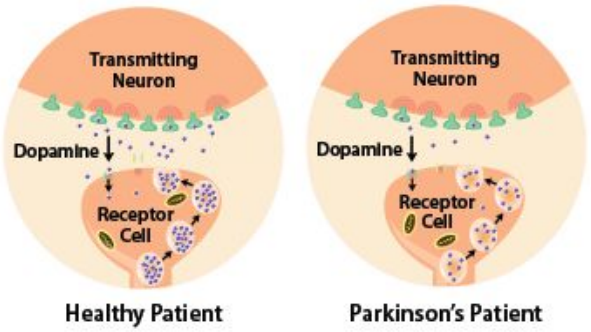

Fig. 1 The picture shows the difference between the dopamine production at the synapse in a healthy person and a PD patient. This results in a reduced dopamine uptake into the receptor cell vesicles decreasing the motor function in affected PD patients. Obtained from: NIH., 2018

produce enough dopamine to properly regulate human bodily movement (Stefanis, 2012). While the formation of Lewy bodies in the neurons of the brain, specifically in the basal ganglia, is consistent throughout most cases of PD, the exact factors which create a predisposition for a person to have PD have yet to be found. In addition, since the formation of Lewy bodies and the subsequent death of dopaminergic neurons in the brain occur as a person develops PD symptoms, the neurodegenerative disease is difficult to diagnosis at an early stage. Likewise, there is no cure for the disease, and the current best options for treatment involve using drugs to supplement missing dopamine to compensate for the loss of dopamine-producing neurons. For this reason, levodopa-carbidopa has been the staple oral drug for patients with PD. The levodopa is converted to dopamine after crossing the blood-brain-barrier while the carbidopa acts as an inhibitor to keep levodopa from converting to dopamine before reaching the brain (Gandhi, 2019).

Economic Impact

While PD and its symptoms cause great distress for those who have the disease, there are other symptoms, that may occur during the pathogenesis of PD, there are many social factors that have an adverse effect on the pathogenesis of this disease. As many people who experience PD have trouble doing daily activities properly, the need for a proper caretaker, if the family is not available, is a factor to consider. The estimated national cost of having PD between all patients in 2010 was approximately $14.4 billion dollars (Kowal et al., 2013). Medical hospitals can provide these caretakers, but the time and cost for a patient with advanced PD heed on their quality of life. The average medical costs for someone who has PD is roughly $22,800 per patient, and along with estimated costs of living with the disease such as lower work hours, a person with PD

can expect costs of around $33,800 (Kowal et al., 2013). Overall, the economic costs of a person who has PD is a factor to consider when discussing the impacts PD has on a person.

Other Related Factors

Of all the risk factors for PD, age is among the most prevalent. It is commonly diagnosed to people over the age of 65; PD is present at around one to two percent of the elderly population (De Rijk et al., 1995). When a person develops PD after 50 years of age, this type is known as late-onset PD; early-onset PD is used to describe people who develop symptoms before 50. As a person ages, the function of organelles which perform autophagy, the disassembly and regulation of undesirable components at the cellular level, decreases. Increased aggregation of proteins such as alpha-synuclein occur as a result of dysfunctional organelles. Specifically, impairment of the mitochondrial complex 1 can increase the rate of reactive oxygen species which cause oxidative stress on the brain, having adverse effects on the energy supply to the body. Sex was also determined to play a factor in the development of PD. In a 3-year study, the disease was shown to be twofold more prevalent in adult males compared with females (Baldereschi et al., 2000).

Another supporting factor in people who are more at risk for PD is due to their genetic susceptibility. Research suggests that there are genetic factors linked to disease pathogenesis, most notably mutations in the LRRK2 and GBA gene. Many other mutations are found to be linked to the development of PD, and all of these mutations involve autosomal dominant inheritance, in which a person has one mutant and one normal gene on a pair of chromosomes. These mutations are proposed to increase the risk of PD at an earlier age (Gan-Or et al., 2010). While the occurrence of PD may be heavily based upon a genetic predisposition, recent research looks at the environmental risks associated with PD. Factors such as head trauma, bodily health,

and area of residence have been researched as a risk in this neurodegenerative disease. Ultimately, PD is definitely a multi-factor disease in which genetic dispositions, gender, and age all play a role in a person's likelihood, making for questions if the outside environment has any role in its development.

Environmental Factors

Of all the factors increasing the risk for a person to develop PD, the newest proposed element involves exposure to pesticides. First research when examining the correlation between the pesticides and the pathogenesis of neurodegenerative diseases pointed towards an increased risk of PD for those living in rural areas (Liou et al., 1997). Scientifically, paraquat (N, N′-dimethyl-4,4′-bipyridinium dichloride) is considered a viologen, and the chemical is known to cause toxicity among organisms. The chemical accumulates in the kidney and the lung, creating toxicity in these regions of the body upon consumption (Franco et al., 2010). Following the first study conducted with the emphasis on pesticide exposure to neurodegenerative diseases led to other researchers delving into the topic. Epidemiological studies reveal that residential exposure to paraquat in agricultural areas is associated with over a twofold increase in the incidence of PD (Gorell et al., 1998). While these studies were conducted to find the increasing prevalence of PD with exposure to paraquat and other pesticides, scientists also began conducting research at the cellular level to discover the mechanisms of the herbicide. The agrochemical paraquat, a toxic chemical used for weed and grass control, is primarily looked at when discussing pesticide exposure and PD because of its widespread use.

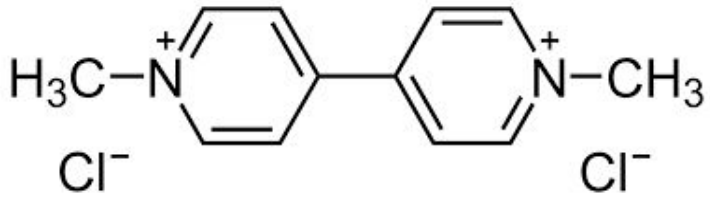

Fig. 2: The diagram shows the chemical structure of paraquat dichloride.

Seen in figure 2, paraquat is a polar molecule due to its polar charged amine (nitrogen-containing) groups and is in the group of heterocyclic amines. The compound has multiple atoms with two amine groups, and its use as a weed killer raised concerns due to research about its relation to PD.

Paraquat Use and PD Prevalence

When analyzing PD prevalence in the United States in relation to the concentration of paraquat in the United States, current statistics did not release information regarding the amounts of paraquat used. The newest data in which incidents of PD and concentrations of paraquat were both released included maps of PD incidents in 2003 and concentrations of paraquat based on pounds per square mile used from 1991 to 1993.

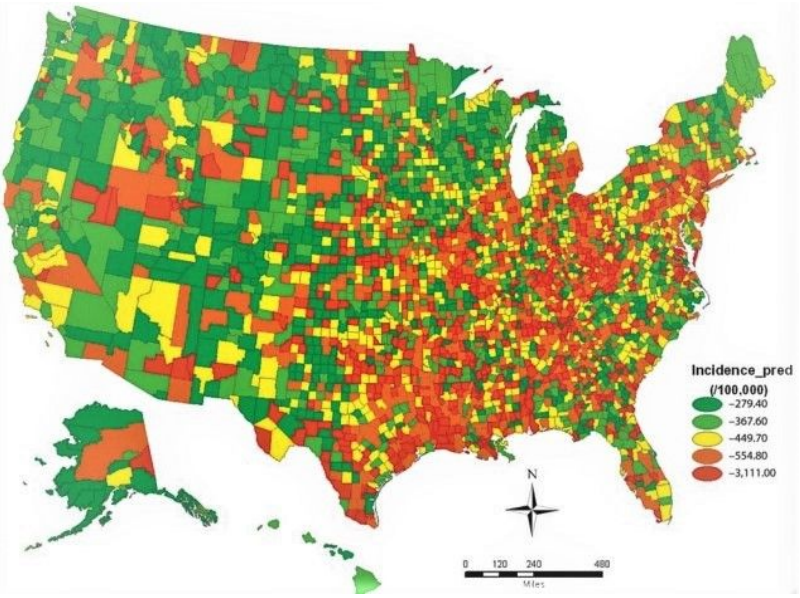

Fig. 3: This map shows the 2003 geographical distribution of Parkinson's Disease in the United States taken from Medicare beneficiaries per 100,000. Retrieved from (Willis et al., 2010).

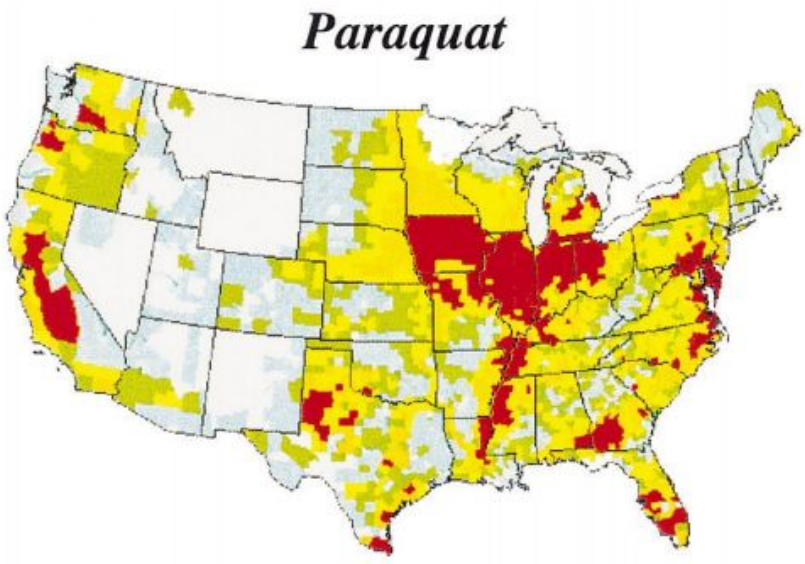

Fig. 4: This map shows the concentration of paraquat use in the United States, Retrieved from (Thiruchelvam et al., 2000).

The maps of figures two and three elaborate on the rising issue of pesticide use in the United States. There is a strong correlation between the areas of concentrated paraquat use, most notably

in the Midwest and the geographic incidence of PD in Medicare beneficiaries. Taken from the National Center for Food and Agricultural Policy statistics on use between 1991-1993. The colored map ranges from white (no paraquat use) to red (high paraquat use) based on pounds per square mile of the pesticide. The pesticide use for paraquat was a total of 885,677 pounds in the nation, covering 1,710,991 acres of land.

Mechanisms of Paraquat

Paraquat, as an herbicide which is not formulated to kill specific types of weeds, is used as a general plant extermination pesticide. It kills plant cells through the production of oxygen free radicals, damaging plant membrane proteins (Ramachandiran et al., 2007). In a reaction known as the Fenton reaction, the conversion of hydrogen peroxide made during oxidative stress put upon the plant to a hydroxyl free radical causes plant cell death.

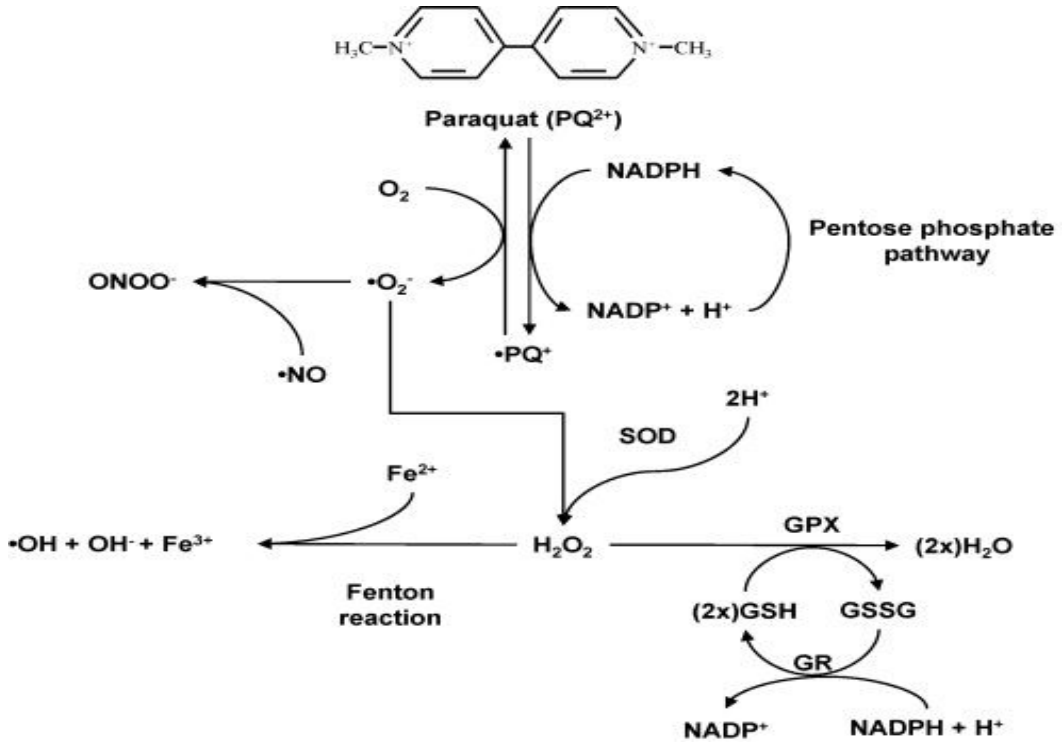

Fig. 5: This flowchart examines the relevance of the Fenton reaction to the process of redox cycling with paraquat.

The paraquat ion (PQ^{+2}) is shown to break down in the presence of oxygen which, when added with hydrogen ions ($2H^{+}$) in conjunction with the superoxide dismutase yields hydrogen peroxide. In the Fenton reaction, the hydrogen peroxide accumulates until it decomposes to two superoxides OH and OH^{-}. The process is then repeated as an electron from the paraquat ion is transferred to molecular oxygen, forming a superoxide. In turn, the continued generation of superoxides leads to the formation of hydrogen peroxide which undergoes the Fenton reaction.

Studies have shown that Paraquat has damaging effects on animals and humans in terms of the pathogenesis of PD. One postulated mechanism of paraquat in regards to PD is its ability to increase the aggregation of alpha-synuclein proteins, thus inducing neuronal cell death in the SNpc from the formation of Lewy Bodies (Maturano et al., 2015). This hypothesis is a result of studies suggesting that pesticide exposure leads to mitochondrial stress because the dysfunction of mitochondria results in the formation of superoxides (Gatto et al., 2010). As discussed previously, the oxidative stress put upon the brain from superoxides leads to neuronal cell death.

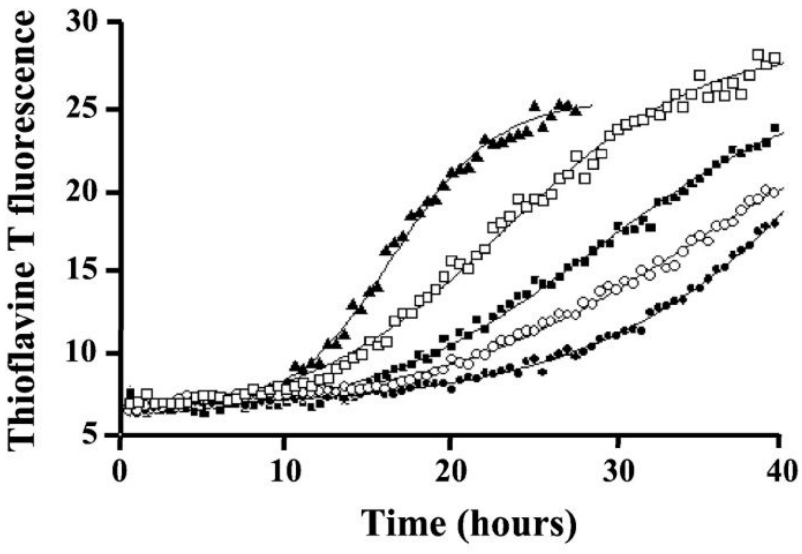

Figure 6: The graph displays the increase of alpha-synuclein expression, monitored by Thioflavine T fluorescence. Retrieved from (Manning-Bog et al., 2003).

As well as forming superoxides, the degradation of mitochondria inhibits the transmission of ATP which maintains neurotransmission (Stykel et al., 2018). Since normal ATP levels protect from cell death, ATP regulators like the mitochondrial complex 1 are important towards

healthy neuron cells. While these proposed mechanisms all contain relevancy when discovering the reason for PD, dopaminergic cell death occurs in all cases of PD. Thus, the data analyzed will focus on variability between dopamine cell loss with a consistent dosage of paraquat for consistency.

Figure 6 shows the mechanisms of paraquat on alpha-synuclein proteins in effect, noting an increase in fibrillation upon administration of paraquat. The five different curves represent varying concentrations of paraquat in vitro as follows: filled triangles, 1000 uM; unfilled squares, 500 uM; filled squares, 100 uM; unfilled circles, 10 uM; filled circles, control group without paraquat administered. When the regulation of alpha-synuclein was considered through an in vitro approach, an apparent increase was noted within all concentrations of paraquat in figure 6 between 10-1000 uM. The graph shows an initial buffer region where fibril formation is not as apparent, followed by a significant jump in fibril formation, and finally a plateau. As shown through the varying concentrations of paraquat used, increase in concentration resulted in higher rates of fibrillation. These results suggest possible validation that paraquat and other pesticides increase the mechanisms that generate

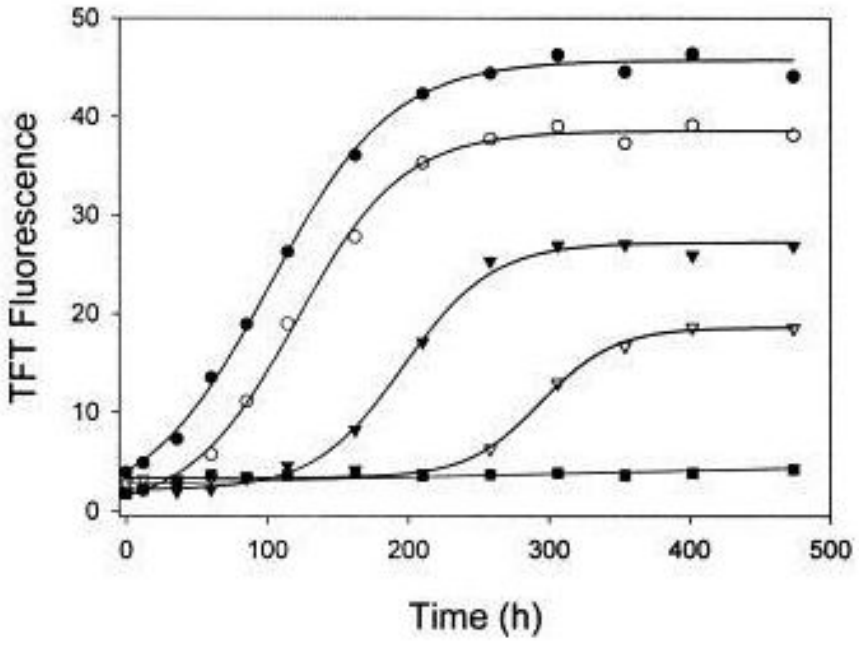

Figure 7: The graph above shows the formation of fibrils marked through Thioflavine T fluorescence as paraquat was administered in vitro. The study analyzed the course of fibrillation over a period of 500 hours. Retrieved from (Uversky, Lee, and Fink, 2001).

PD. When analyzing results from figure 7, a similar conclusion was made by Uversky, Lee, and Fink (2001), demonstrating increased fibrillation after applying 100 uM of the paraquat. In comparison to the experiment conducted by Manning-Bog and his group of researchers, they allotted over two weeks to observe their findings. Nevertheless, both research groups came to the same conclusion that the pesticide stimulated increased fibrillation of the alpha-synuclein protein, an important aspect of the development of PD. Results indicating the increase of fibrillation between the alpha-synuclein protein represents a more narrow perspective with a focus on the rate of fibrillation. As evidenced, the results supplement the main evidence of dopaminergic neuronal cell death by verifying the mechanisms of paraquat at the intracellular level. Ultimately, the mechanisms of paraquat increasing the fibrillation of alpha-synuclein suggest connections towards a bigger scale in which the death of dopamine neurons can occur.

Current Restrictions

The use of paraquat is widespread around the world because of its value as an agrochemical. Due to existing knowledge of the harms of paraquat as a toxic agent, safety precautions have been taken due to the toxicity of paraquat. The primary concern for easy access of paraquat is the use of the chemical for suicidal purposes. In fact, an estimate of around 2,000 people attempt suicide with paraquat in South Korea, the leading nation for suicide attempts using the herbicide (Seok et al., 2009). Because of increasing awareness that more people are using paraquat for suicidal purposes, 32 countries banned the chemical, including South Korea. The bans resulted in significantly fewer suicide rates in South Korea alone, with attempts using paraquat dropping by 46.1% (Myung et al., 2015). The United States identifies paraquat as a restricted chemical, meaning the use of the chemical is only limited to licensed applicators.

However, paraquat has not been banned in the United States and continues to be widely used to maintain control of weeds in crops. Alongside the restricted use of paraquat, the liquid is colored blue to deter users from thinking of it as consumable, a potent smell is added to further deter ingestion, and a vomiting agent is added to make certain the chemical will not be absorbed by the digestive system. Nevertheless, exposure to applicators such as farmers is inevitable, as is the exposure in sources of water and in locations near crops treated with paraquat.

Purpose

Investigating the effects of paraquat and its role in the pathogenesis of PD can serve multiple purposes. Understanding the connection can allow scientists to make necessary warnings regarding the use of paraquat. Also, the pathogenesis of neurodegenerative diseases can be more fully understood, which may promote the discovery of new treatments or cures for the disease. Systematic literature review in the topic of pesticides and PD was only found to analyze single variables to gather the certainty of paraquat's role in developing PD. In analyzing current literature studying the relationship between paraquat and PD, multiple factors play a role in a person's susceptibility. By addressing the correlation of such studies, more or less research can be focused on pesticide exposure, ultimately saving time for where the problems of the pathogenesis of PD needs to be addressed. This paper analyzes multiple studies of paraquat effects on mice brains to determine the likelihood of a positive correlation between paraquat and PD.

Research Question

Does exposure to paraquat result in the loss of dopaminergic neuronal cells? Should paraquat be considered as an environmental risk factor of PD?

Alternative Hypothesis

Considering articles covering the various mechanisms of paraquat and its interactions with the human body, paraquat is hypothesized to result in the loss of dopaminergic neuronal cells. Therefore, the role of paraquat in the pathogenesis of PD must be an environmental risk factor to consider when exposing oneself to pesticides.

Null Hypothesis

Exposure to paraquat does not result in the loss of dopaminergic neuronal cells. Thus, the need to label paraquat as an environmental risk factor is not necessary.

Methods

Many of the systematic literature reviews conducted on the relationship between pesticides and the pathogenesis of PD analyze all studies conducted between paraquat and a biomarker to indicate if PD-like symptoms are present. However, these systematic literature reviews do not separate certain aspects of the research which could cause possible bias. Data was collected across multiple sources using specific selection criteria to ensure neutrality between the studies. In this review, studies were separated into blinded and unblinded tests to ensure that both sides of the experiments were done in a reasonable fashion without concern for partiality.

Theoretical Systematic Literature Review was used as the primary research strategy to evaluate the effectiveness of paraquat on PD across multiple authors. Google Scholar,

ScienceDirect, EBSCOhost, NCBI, PubMed, etc. were used to obtain studies gathering information about paraquat. These sources provide detailed information regarding the content of my paper and are reliable sources to use as references. Keywords such as "paraquat and Parkinson's Disease," "paraquat and dopaminergic neurons," "alpha-synuclein and Parkinson's Disease," "prevalence of Parkinson's Disease geographically," etc. were searched to collect necessary articles. Further articles were found using the references section of another author's study. Upon searching for relevant articles using these keywords when collecting data with dopaminergic neurons as the variable, a search count of n=12,500 papers were found with the research database Google Scholar. Of these papers, those which looked at the number of TH+ neurons in the striatum and SNpc of C57BL/6J male mice were n=12. When separating these studies into blinded and unblinded tests, a count of n=7 was determined relevant for unblinded studies while a count of n=5 was determined for blinded studies. The data was graphed using Excel in the form of a bar graph, comparing the amount of TH^+ neurons before and after the administration of paraquat.

Selection Criteria

The following variables were taken into consideration to provide the most stable results possible: type of study (blinded or unblinded) and type of mice. Variability of dosage frequency was not taken into consideration because the p-values which are going to be determined are based on the ratio of affected mice to unaffected mice. Age of mice was considered irrelevant due to meta analysis of the studies comparing the null and positive results which determined age was not predictive of the outcome between positive or null results (Smeyne et al., 2016). Using

these limitations, data was collected from C57BL/6J male mice, and data was separated from unblinded and blinded studies.

Tests were analyzed using the stereological method of an optical fractionator to estimate the total number of tyrosine hydroxylase (TH^+) neurons present in the mice brain (Olsen et al., 2017). The optical fractionator estimates neurons through virtual counting spaces regardless of the shape of the cross-section used, allowing precision when counting estimates of the neurons. The count for the number of dopaminergic cells was accomplished through using tyrosine hydroxylase as a biomarker for the cells as the TH^+ expression can be used for counting the number of neuron cells in the SNpc and striatum (Weihe et al., 2006).

Results

Table 1: The table inputs the mean number of TH+ neurons present in the brain of mice in a blinded setting.

Author	Mean Control Group (# TH+ neurons)	Mean Paraquat Group (# TH+ neurons)
Smeyne (2016)	6302	6294
Breckenridge (2013)	11700	8020
Fernagut (2007)	10416	7708
Khwaja (2007)	6185	4615
Mangano (2011)	4444	3185
P Value	0.02130957256	

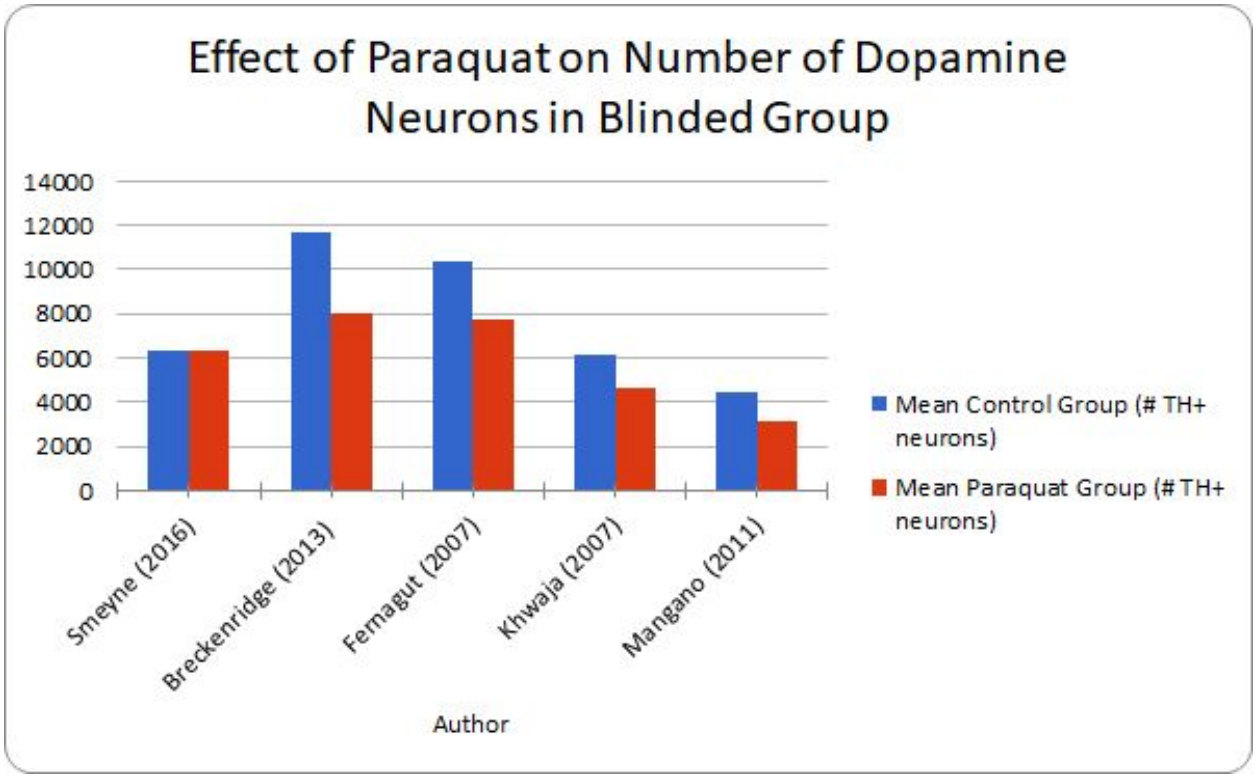

Fig. 8: This figure shows the relationships between paraquat and the number of dopamine neurons in a blinded setting.

Table 2: The table inputs the mean number of TH^+ neurons present in the brain of mice. The correlation between mice not taking paraquat and mice taking 10mg/kg paraquat is shown through the calculated p-value.

Author	Mean Control Group (# TH+ neurons)	Mean Paraquat Group (#TH+ neurons)
McCormack (2005)	11770	9277
Peng (2007)	11770	8089
Yin (2011)	6554	4492
Thiruchelvam (2003)	12889	9148
Jiao (2012)	9818	5236
Choi (2006)	13481	4296
Manning-Bog (2003)	12084	9462
P-value	0.002234864637	

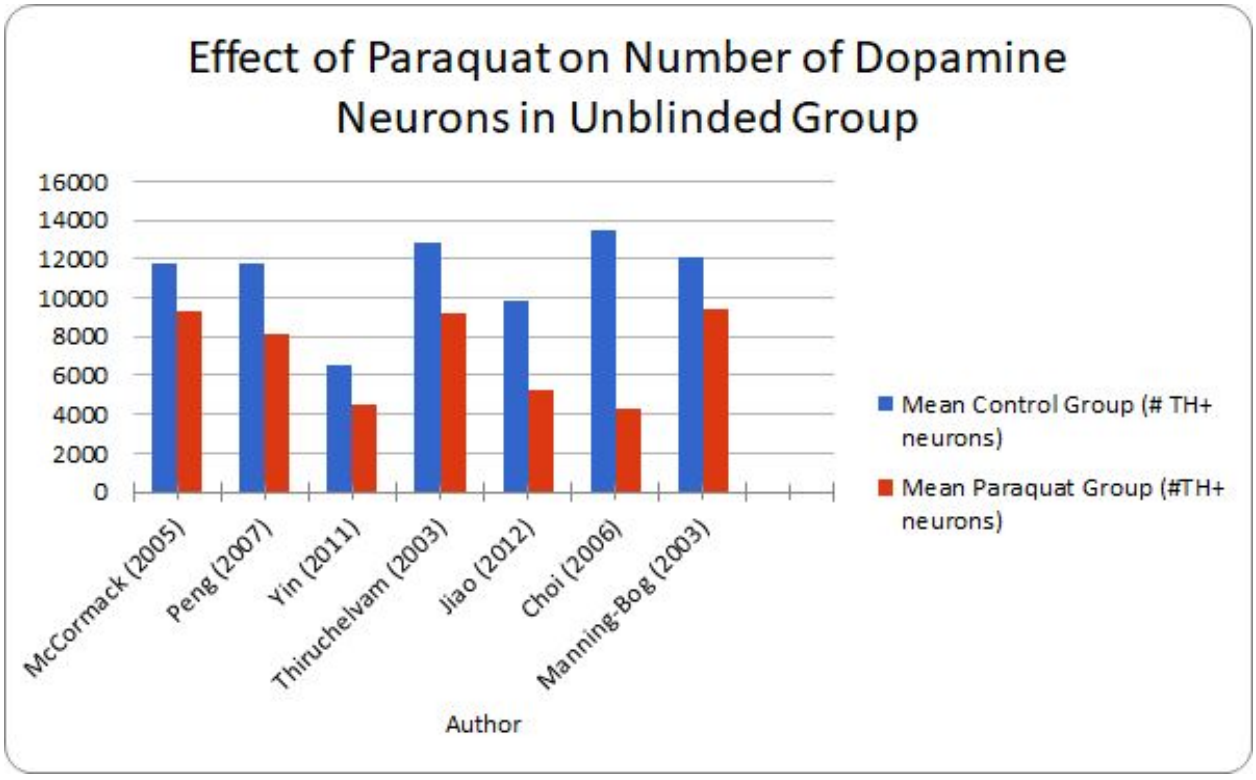

Fig. 9: This graph shows the relationship between paraquat and the number of dopamine neurons in an unblinded setting.

Discussion

The results from published studies analyzing the mean number of TH^+ neurons of paraquat-treated mice compared to control mice groups in an unblinded test environment strongly suggest a correlation between paraquat exposure and PD. In Table 1, a total of 5 papers were found acceptable with the same variables as well as following all the selection criteria discussed in the methodology section. The correlation between mice not taking any pesticide and mice taking paraquat is shown through the calculated p-value. The numbers plotted are taken from blind studies in which data collection was done without knowing if the mice were injected with the dosage of paraquat or not. When conducting a T-test in the blinded tests, a p-value of

0.02 was found. Since a p-value of P<.05 indicates statistical importance, this number indicates that the dosage of paraquat on C57BL/6J male mice results in significantly less TH^+ neurons present, compared to the control mice who did not take any dosage of paraquat. In Smeyne's study, a null result between paraquat and the death of dopaminergic neurons was found. As the outlier to the other data produced in the systematic literature review, it is important to review possible reasons as to why his study found paraquat to have no effect on the death of the neurons. One explanation could be the handling of the tissues and the fashion in which they were stained. Since TH was used to stain the neurons, improper application of the stain may have resulted in the inconclusive results. As mentioned by Smeyne (2016) in his results process, a similar study conducted by Jiao (2012) stained the SNpc years in advance before completing their analysis through stereology. The overall results in the unblinded study nevertheless indicated a direct effect of the paraquat in reaction towards cell death. The reason for the death of dopaminergic neurons may then be due to the mechanisms of paraquat as a catalyst for increased fibrillation of alpha-synuclein proteins. As mentioned through research done by Maturano (2015), the continued misfolding of the proteins would form Lewy bodies to disrupt cell function. Finally, the death of dopaminergic neuronal cells will follow, apparent in figures 8 and 9.

Similarly, when analyzing the effects in a blinded study environment, results indicate a significant P-value of 0.002 as shown in figure 9. In figure 9, all seven papers analyzed showed a decrease in dopaminergic neuronal cells compared to figure 8 in which one study found null results. Both T-tests indicate positive results regarding the possible connection between the effects of paraquat and the death of dopaminergic neuron cells, and whether the studies were blinded or unblinded was found negligible to the overall results between all experiments.

Due to the findings of both the blinded and unblinded studies, the connection between paraquat introduction and the development of PD is furthered. These results express that the proposed mechanisms for cell death in the brain may be valid. The main argument for the pathogenesis of PD is the accumulation of Lewy bodies, formed from the continuous aggregation of alpha-synuclein proteins. When PD patients are examined post mortem, the common occurrence of these proteins is observed, primarily in the Snpc. From the consistent degradation of dopaminergic neurons throughout the in vivo studies analyzed in this review, paraquat and other pesticides evidently have a positive effect on the increase in alpha-synuclein fibrillation. In turn, the Lewy bodies disallow proper function of the dopaminergic neurons, resulting in loss of dopamine to the brain. As this continues, a patient will eventually have PD from its hallmark symptoms of loss of movement and muscle control.

Because of the decrease in dopaminergic neurons in mice, it is reasonable to infer that paraquat should be considered as an environmental risk towards PD. Seen through the consistent death of dopaminergic neurons in vivo, paraquat is likely to impose environmental risks to the human population which should be taken into consideration when regulating future pesticides.

Conclusion

Ultimately, this systematic literature review showed the connections between paraquat and its role in the pathogenesis of PD. When this review was conducted, a positive correlation was found due to an overall decrease in TH^+ neurons in the SNpc of mice. Almost all papers in this review supported the alternative hypothesis, with one outlier, Smeyne (2016), likely due to miscalculations during the procedure. In sum, the evidence collected from the data suggests that

paraquat is linked to PD by prompting hallmark signs of a person who has PD, with the death of dopaminergic neurons being the focus of the review. Thus, paraquat should be considered as an environmental risk which aids in the development of PD.

Limitations

In graphing the data on alpha-synuclein fibrillation, estimates of the values were needed as the only data available on the papers consisted of graphs. Since there were no set points in a data table, the values were approximated. However, the overall message of the graphs plotted that paraquat causes increased aggregation of alpha-synuclein proteins is still kept. When conducting the systematic literature review, the number of degraded neurons was approximated between all mice in the twelve studies evaluated. However, the number of mice used per study varied between each source. For example, Smeyne (2016) used 14 mice in his study while Breckenridge (2013) used 10 mice. The results of the Smeyne study were null while the Breckenridge study accepted their alternative, making for concern if fewer amounts of mice made for a greater deviation in the average findings for neuronal cell loss. Methods of assessing the number of dopaminergic cells were all done through stereology, but two different parts of the brain were assessed and synthesized. The SNpc and the striatum were used as valid sources to detect the death of dopaminergic neurons in the mice. However, in a human brain, the main area which experiences dopamine cell loss is in the Snpc and not in the striatum. Thus, the results may indicate paraquat effectiveness on mice, but their relation to humans may not be as definitive. Because the studies were done in vivo and in vitro, the human brain might have different effects at the molecular level than the results found through these methods. Even

though global and national data also supports the correlation between paraquat and its environmental risk, its effects on humans could change significantly.

Further Work

Since the link between pesticide use and the development of PD is limited, further work must be done to contribute to human studies in PD. Researchers should further discuss the negative effects of the heterocycle paraquat and the possible actions people can take to assure their safety from the agrochemical. Because the background and research of paraquat's link to neurotoxicity and the pathogenesis of PD mainly consist of in vivo studies, opening up research to more clinical studies can allow for deeper discussion into the link between paraquat and PD. Recently, the first study conducted with human stem cells analyzed the effect of the pesticides paraquat and maneb on mitochondrial transport, finding that exposure to the agrochemical paraquat results in a decrease in function for the mitochondrial complex 1, a proposed mechanism for the pathogenesis of PD (Stykel et al., 2018). With the advancement of technology, these methods can be added upon to further research of pesticides not only in vivo but with actual human stem cells. By coming closer towards studies involving human cells, the link between exposure to pesticides and other environmental risks can be more accurately measured and proposed, addressing the concerns of people living in areas with higher concentrations of pesticides which are typically those who live in more rural locations.

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