Links Between Paraquat and Parkinson's Disease

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Contents

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Abstract

Parkinson's disease (PD) is the most common degenerative disorder of the aging brain. Several gene mutations have been identified to be involved in familial PD. However, the majority of cases are sporadic and their origin(s) still remain undetermined. The environment is a key contributor to human health and disease. Epidemiological evidence suggests that environmental factors and/or mutations in genes play a role in the etiology of neurodegenerative diseases. Particularly, paraquat (PQ) has been demonstrated to induce neuronal death in cellular and animal models associated with PD. PQ-induced neurotoxicity has provided valuable insight into the mechanisms regulating neuronal cell death by environmental toxicants. However, the molecular mechanisms involved in neuronal cell death by PQ have not been completely identified. Importantly, in vivo studies allow the understanding of how PQ could be associated with PD. This review presents a brief summary of some of the published toxicologic data and critically evaluates whether a relationship exists between PQ exposure and PD.

Keywords

Cell death · Genes · Models · Neurotoxicity · Paraquat · Pesticide

Abbreviations

6-OHDA	6-Hydroxydopamine
ASK1	Apoptosis signal-regulating kinase 1
ER	Endoplasmic reticulum
HDAC	Histone deacetylase
JNK	c-Jun NH_2-terminal kinase
LB	Lewy bodies
LRRK2	Leucine-rich repeat kinase 2
MAPKs	Mitogen-activated protein kinases
MPP^+ 1	Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NF-κB	Nuclear factor-kappa B
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
PD	Parkinson's disease
PINK1	PTEN-induced putative kinase 1
PQ	Paraquat
ROS	Reactive oxygen species
SIRT1	Silent mating-type information regulation 2 homolog 1

SNpc	Substantia nigra pars compacta
WAVE2	Verprolin-homologous protein 2

1 Introduction

Paraquat (PQ, methyl viologen), 1,1′-dimethyl-4,4′-bipyridinium (Fig. 1), is a salt synthesized in 1882 by Weidel and Russo. Its redox properties were described by Michaelis and Hill in 1933 (Gonzalez-Polo et al., 2014). At first, it was used as an indicator of oxidation reduction, since, in the absence of molecular oxygen, donating an electron to PQ (PQ^{2+}) generated a monocationically stable violet or blue form, commonly known as methyl viologen (Gonzalez-Polo et al., 2014). Its properties as a potent herbicide were not discovered until 1955, and, some years later, in 1962, it was actively introduced to the global markets. PQ is registered and used in approximately 100 countries worldwide, being the second most widely used herbicide in the world after glyphosate. However, its use was banned in the European Union (EU) since 2007 (Ko et al., 2017), unlike the import of products from outside the EU that have been treated with PQ. PQ is included in the family of herbicides known as bipyridines. It is a contact herbicide, nonselective and systemic, and does not damage the plant. Among its advantages, PQ increases the yields by acting fast on sprayed weeds, maintains soil fertility (reduce erosion), and is rapidly inactivated in soil. Despite its benefits for environments (save soil structure and biodiversity), there is controversy surrounding the use of PQ in agriculture. It has clear toxicity to workers, especially if proper precautions are not taken. PQ is corrosive to the eyes and skin and is highly toxic for human because no antidote exists (Ko et al., 2017). However, the Environmental

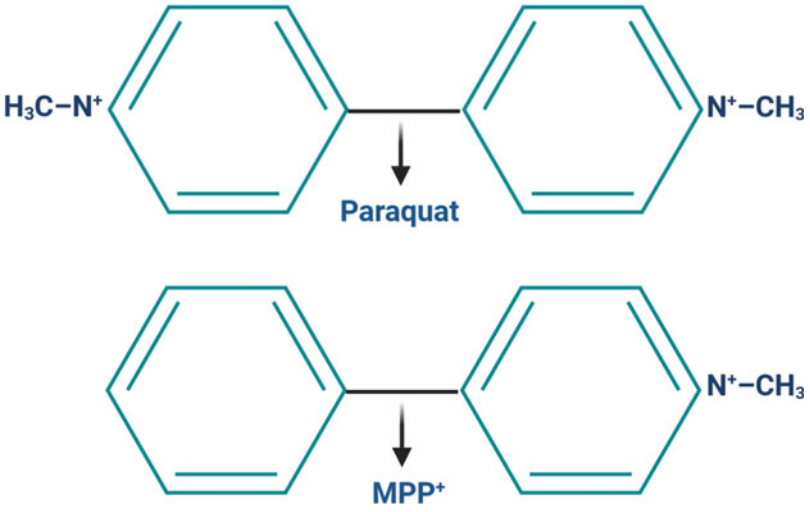

Fig. 1 Chemical structures of PQ and MPP+. PQ (N,N′-dimethyl-4-4`bipiridinium) is a pesticide structurally similar to the active metabolite of the neurotoxin called MPP+, widely accepted as a model of parkinsonism

Protection Agency (EPA) considers the potential risks of PQ insufficient for workers when used according to label instructions. Thus, in the USA, PQ use is still allowed as a restricted use pesticide under certification training. The inappropriate use or accidental ingestion of PQ is associated with kidney and respiration failure leading inevitably to death. In Korea, the prohibition of PQ use reduced significantly the pesticide-associated mortality (Ko et al., 2017).

In this sense, the structural similarity between PQ and the active metabolite of the neurotoxin MPTP) (MPP^+, Fig. 1) widely accepted as a model of parkinsonism, together with some correlations observed in epidemiological studies between the use of PQ and the development of Parkinson's disease (PD), led to the postulation of the existence of a relationship between the pesticide and the origin of the disease (Pezzoli & Cereda, 2013). PQ is currently considered as a valid model for studying neurotoxicity based on oxidative stress, as in the case of MPP^+, and investigations are ongoing concerning the relationship that may exist between application and exposure to this pesticide and the development of PD, which is a widely accepted fact in the case of MPP^+, leading to an accumulation of very strong and important evidence in the case of PQ, oxidative stress, and cell death. The same active ingredient that makes PQ a perfect herbicide also makes it a perfect toxic for any mammalian cell (Gonzalez-Polo et al., 2014). The redox cycling of PQ (Fig. 2) on biological systems has two important implications: one is the generation of reactive oxygen species (ROS), and the other is the depletion of reducing equivalents (NADH, NADPH, etc.), which are necessary for the proper functioning thereof, affecting different cellular processes, such as the synthesis of fatty acids. Like inside plant cells, within the model's neurons, PQ needs a donor of electrons in order to be reduced. The potential standard reduction (E^o) of a compound indicates the affinity of this structure to accept electrons. PQ has an E^o of 0.45 V. The potential E^o of the redox couples NAD^+/NADH and $NADP^+$/NADPH are 0.32 V and 0.324 V, respectively, which means that PQ, under physiological conditions and with the aid of diaphorase cells, could accept electrons from either of the reducing agents (Gonzalez-Polo et al., 2014). The potential E^o of MPP^+ is 1.18 V, which indicates that PQ has a greater ability to accept electrons than MPP^+. Among the cellular enzymes

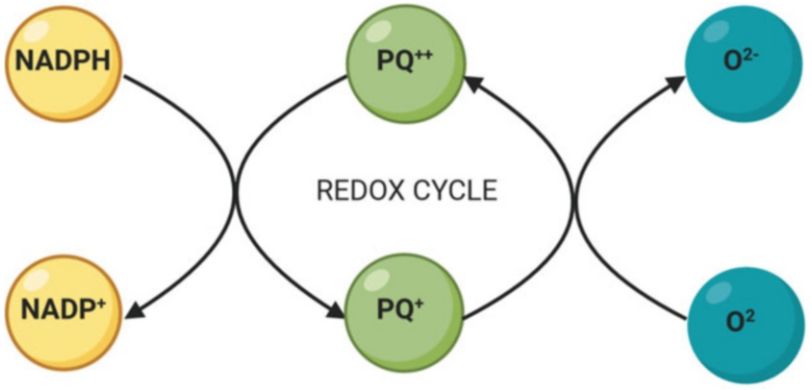

Fig. 2 Redox cycle of PQ. The reduction-oxidation cycling of PQ induced cell death involves ROS

that can give electrons to PQ (PQ enzymes with diaphorase), those that can be highlighted are the following: mitochondrial complex I (NADH: ubiquinone reductase complex), thioredoxin reductase, NADPH-ferredoxin oxidoreductase, NADPH oxidase, etc. The mitochondria, therefore, become a major source of ROS generation within the PQ-induced mechanism, which may induce PQ-diaphorase activity at one or more points during the breathing cycle (Gonzalez-Polo et al., 2014). Once the PQ has been reduced, it can be oxidized by a molecule of oxygen and generate a superoxide molecule ($O^{\bullet -}$), which occurs in cell oxidative stress and will switch to different routes that trigger cell damage in different components and initiate the activation of different cellular mechanisms, such as apoptosis or autophagy. Apoptosis induced by PQ has been demonstrated to involve mainly the intrinsic mitochondrial pathway. It is activated by a wide variety of cytotoxic stimuli or environmental stressors. Although the mechanisms by which these stimuli trigger apoptosis differ between them, they convey the release of pro-apoptotic proteins from the mitochondria, including cytochrome c. In PD, cell death by apoptosis has been proposed to result from mitochondrial dysfunction and silent mating-type information regulation 2 homolog 1 (SIRT1) downregulation (Ding et al., 2016), leading to an increase in oxidative stress and a decline in ATP production. On the other hand, converging evidence suggests that the impairment of homeostasis mechanisms processing damaged organelles and misfolded proteins plays a critical role in the pathogenesis of PD (Huang et al., 2020). Impairment of the autophagy-lysosomal pathway was involved in the development of PQ-induced PD (Hirayama et al., 2018). The activation of autophagy was observed in fibroblasts from PD patients harboring the G2019S (*leucine-rich repeat kinase 2*) *LRRK2* mutation (Bravo-San Pedro et al., 2013) and in PD patients' cells exposed to MPP^+ (Yakhine-Diop et al., 2014). In this sense, PQ is also able to induce autophagy in dopaminergic cellular models and mitophagy in human lung cells (Sun et al., 2018). In idiopathic PD cells, mitophagy impairment is in part due to the decrease of (SIRT1) activity (Yakhine-Diop et al., 2019). Interestingly, elevated concentration or long exposure to PQ decreases the expression of SIRT1 protein (Ding et al., 2016), which is in accordance with the 4 mM PQ-inhibited autophagy in PC12 cells (Zhou et al., 2017). Moreover, PQ induces epigenetic changes by reducing the histone deacetylase (HDAC) activity in dopaminergic cells (Song et al., 2011). It has been recently demonstrated that there was an imbalance between histone acetyltransferase and HDAC activities (Yakhine-Diop et al., 2019) that provoked acetylome changes in idiopathic PD (Yakhine-Diop et al., 2018). Therefore, PQ is considered as one of the most useful models for studying neurotoxicity based on the generation of oxidative stress (such as PD), playing a key and fundamental role in the production of superoxide anions in the redox cycling of the herbicide, which can induce apoptosis and impair the autophagy process.

2 Epidemiologic Evidence

As evidence emerges that several genes are involved in the pathogenesis of PD, the role of environmental chemicals in the etiology of this disease has become intensely debated. Several studies reported increased risk associated with exposure to either insecticides or herbicides ranging between 33% and 88% (Pezzoli & Cereda, 2013).

The relationship between exposure duration and PD risk was investigated in several studies and showed a positive correlation with the duration of exposure to and high doses of herbicides and insecticides. PQ exposure was shown to be significantly associated with PD and was two times more risky than any other chemical products (Pezzoli & Cereda, 2013). Airbone PQ increases the risk of developing PD in people over 80 by 24%. This is because the burden of PQ in the brain was higher in people over 50 years old, increasing the risk of PD with age (Cheng et al., 2018).

PQ has been shown to interact synergistically with other pesticides and insecticides. It has been reviewed that exposure to a combination of maneb (a dithiocarbamate fungicide) and PQ increases PD risk, particularly in younger subjects and/or when exposure occurs at younger ages (Gonzalez-Polo et al., 2014). PQ and maneb altered the expression of genes and metabolites. The toxicity of their combination involves the activation of different mechanisms. For instance, while maneb significantly increases nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-regulated genes, PQ does not (Huang et al., 2020). However, metabolites associated with oxidative stress and mitochondrial energy metabolism are inherent to their addictive toxicity (Roede et al., 2014). Combined exposure of rotenone and PQ has been linked experimentally to pathophysiological mechanisms implicated in human PD. Combined ambient exposure to ziram and PQ as well as combined ambient exposure to maneb and PQ at both workplaces and places of residence showed substantially increased risk of PD. Those exposed to ziram, maneb, and PQ together experienced the greatest increase in PD risk. However, other studies have not found a significant association, although PD risk was still elevated (Gonzalez-Polo et al., 2014).

3 Relation Between Environmental and Genetic Factors in Parkinson's Disease: The Paraquat and PARK Genes Connection

The etiology of PD is unknown, but it likely has a multifactorial origin, involving both genetic and environmental factors, such as exposure to PQ. The interaction of both factors is possibly responsible for the selective death of dopaminergic neurons observed in this disease. Outside of the studies that have identified human mutations as the basis of the disease, the high number of individuals with sporadic PD has an unknown etiology. Although this interaction is not clear, there exist many studies with results that indicate the direct interaction of PQ with PARK genes (Table 1).

One of the most important genes related to PD is *PARK 1/4* (*α-synuclein*). Mutations in *α-synuclein* gene were identified as the first genetic cause of PD and play an important role in the Lewy bodies (LB) formation and could establish interactions with toxicants. It is well recapitulated how the presence of PQ produced the fibril formation of recombinant α-synuclein *in vitro* and increased α-synuclein levels in the ventral mesencephalon and frontal cortex of mice (Gonzalez-Polo et al., 2014). Multiplications of the α-synuclein locus, duplications, and triplications cause direct effect on PD severity with an inverse correlation between gene dose and age at onset. Mutations in SNCA

Table 1 Summary of genetic ("PARK") loci associated with a monogenetic form of PD

Gene	Locus	Protein name	Inheritance	Function	Description
PARK 1/4	4q21.3–q22	α-Synuclein	AD	LB component	α-Synuclein
PARK 2	6q25–27	Parkin	AR	E3 Ubiquitin ligase	Parkin RBR E3 Ubiquitin protein ligase
PARK 3	2p13	¿?	AD	¿?	
PARK 5	4p14	UCHL-1	AD	Hydrolase	Ubiquitin C-terminal hydrolase
PARK 6	1p35–36	PINK1	AR	Mitochondrial kinase	PTEN-induced putative kinase 1
PARK 7	1p36	DJ-1	AR	Antioxidant protein	Parkinsonism-associated deglycase
PARK 8	12q12	LRRK2	AD Risk factor	Kinase, GTPase	Leucine-rich repeat kinase
PARK 9	1p36	ATP13A2	AR	ATPase, cationic transport	Cation-transporting ATPase 13A2
PARK 10	1p32	¿?	AD Risk factor	¿?	
PARK 11	2q36–q37	GIGYF2	AD AR?	Receptor tyrosine kinase signaling	GRB10 interacting GYF protein 2
PARK 12	Xq21–q25	¿?	X-linked Risk factor	¿?	
PARK 13	2p13	HtrA2/Omi	AD	Serine protease	HtrA serine peptidase 2
PARK 14	22q13.1	PLA2G6	AR	Phospholipase A2	Calcium-independent phospholipase A2 enzyme
PARK 15	22q11.2	FBXO7	AR	E3 ubiquitin ligase	F-box protein 7
PARK 16	1q32	RAB7L1	Risk factor	¿?	
PARK 17	16q12	VPS35 (2011)	AD	Endosomes	Vacuolar protein sorting-associated protein 35
PARK 18	3q27.1	EIF4G1 (2011)	AD	Translation	Eukaryotic translation initiation factor 4 gamma I
PARK 19	1p31.3	DNAJC6	AR	Endosomes	HSP90 auxilin
PARK 20	21q22.11	SYNJ1	AR	Endosomes	Synaptojanin 1

(continued)

Table 1 (continued)

Gene	Locus	Protein name	Inheritance	Function	Description
PARK 21	3q22.1	DNAJC13	AD	Endosomes	Receptor-mediated endocytosis 8
PARK 22	7p11.2	CHCHD2	AD	Mitochondria-mediated apoptosis and metabolism	
PARK 23	15q22.2	VSP13C	AR	Mitophagy	Vacuolar protein sorting-associated protein 13C
	lq22	GBA	AD, AR in GD Risk factor	Lysosomes	
	17q21.31	MAPT	Sporadic Risk factor	Microtubules	

¿?, Unknown; AD, Autosomal dominant; AR, Autosomal recessive

lead to a DOPA-responsive early-onset parkinsonism are characterized by Dihydroxyphenylalanine (DOPA)-responsive. This pathology is often severe and accompanied by dementia, characterized by nigral neurodegeneration and widespread brainstem and cortical LB pathology (Del Rey et al., 2018). Chronic oral exposure to PQ (10 mg/kg per day) increases, in transgenic mice overexpressing A53T human mutant α-synuclein, the phosphorylation level of α-synuclein at serine 129. This pathological modification appears first in the enteric nervous system and perhaps later in the brain of mice. However, any modification was observed in wild-type mice exposed to the same doses of PQ over 6 weeks, but they displayed a reactive gliosis (Naudet et al., 2017). In *in vivo* study, the combination of PQ and human α-synuclein overexpression leads to severe oxidative stress and neurotoxicity. PQ-induced oxidative stress increases the level of oxidized/nitrated α-synuclein and promotes its aggregation into oligomeric species (Musgrove et al., 2019). α-Synuclein accumulation is enhanced within dopaminergic neurons, but other neuronal population is targeted such as cholinergic neurons of the enteric nervous system (Naudet et al., 2017). Hence, cholinergic cells of the dorsal motor nucleus of the vagus are susceptible to PQ-induced oxidative stress in mice (Musgrove et al., 2019).

Mutations in *parkin* cause an early-onset autosomal recessive PD, leading to mitochondrial dysfunction and oxidative stress in human samples and animal models. Several studies have demonstrated that the environmental stress also modulates the corresponding protein, for instance, PQ reduces parkin protein level in neuroblastoma cells (Huang et al., 2020). In fibroblasts from PD patients with *parkin* mutations, PQ treatment altered mitochondrial membrane potential and integrity and significantly increased protein oxidation (Gonzalez-Polo et al., 2014). In transgenic knockdown Parkin fly, PQ-induced oxidative stress provokes neurodegeneration

(Ortega-Arellano et al., 2019). Moreover, *parkin* mutant flies enhanced sensitivity to PQ toxicity (Gonzalez-Polo et al., 2014), and parkin knockdown exacerbates apoptosis by decreasing PQ-induced mitophagy in A549 cells (Sun et al., 2018), claiming that parkin could have a protective role.

Following the above, PTEN-induced putative kinase 1 (*PINK1*, *PARK6*) is a gene that is strongly related to early-onset PD. PINK1 mutations lead to mitochondrial abnormalities and neurodegeneration (Gomez-Sanchez et al., 2016). In *PINK1* silenced cells, PQ reduces cell viability and generates a high level of apoptosis cell death (Gonzalez-Polo et al., 2014). However, another study showed that PQ-induced apoptosis could be mediated by mitophagy through PINK1/parkin pathway (Sun et al., 2018). Although PINK1 is crucial in the clearance of mitochondria, it has been published that its deficiency enhances mitochondrial turnover and autophagy (Gomez-Sanchez et al., 2016). In *C. elegans*, the loss of *pink-1* function produced a higher sensitivity to PQ, whereas the genetic deletion of *lrrk-1* could compensate for both of these deficiencies suggesting a functional linkage between *lrrk-1* and *pink-1* (Gonzalez-Polo et al., 2014). Accordingly, LRRK2 is a modulator of PQ-induced inflammatory process. In wild-type and G2019S *LRRK2* mice, PQ causes an augmentation of corticosterone and cytokine levels (Rudyk et al., 2019). PQ alone has no effect on the anti-inflammatory receptor, CX3RC1, but does increase verprolin-homologous protein 2 (WAVE2) level protein (Rudyk et al., 2019). LRRK2-deficient mice were protected from the loss of dopaminergic neurons and motor impairment induced by subsequent treatments of lipopolysaccharide (LPS) and PQ. Indeed, LRRK2 knockdown provokes an inverse modulation in CX3RC1 and WAVE2 protein levels to inhibit microglia activation (Dwyer et al., 2020).

Mutations in DJ1/PARK7 are related to a rare early-onset PD. It was the case of a 49-year-old male, who poorly responded to levodopa treatment and presented severe degeneration in the *substantia nigra pars compacta* (SNpc) and locus coeruleus with diffuse LBs and glial inclusion. In *in vitro* study, the overexpression of L172Q *DJ1* mutation decreases the levels of DJ1 protein (Taipa et al., 2016). Moreover, 300μM PQ significantly decreases DJ1 protein in SH-SY5Y cells (Huang et al., 2020). Although the function of DJ1 is unclear, several studies suggest their possible involvement in the cellular response against oxidative stress, because DJ1 has the capacity to eliminate ROS by auto-oxidation. Mutations in the DJ1 protein can cause mitochondrial susceptibility against toxins such as MPP^+. There are cellular and animal models available to study the interaction between DJ1 and the herbicide PQ (Gonzalez-Polo et al., 2014). The idea that DJ-1 stimulates the autophagy response against PQ and reduces apoptotic cell death response could open the lines in research to the development of new treatment strategies against PD (Gonzalez-Polo et al., 2014).

4 Cellular Models in Paraquat Neurotoxicity

Different cellular models are used to determine the neurotoxicity of PQ and the distinct mechanisms that are triggered. Nevertheless, there are discrepancies on data depending on the concentration used and the incubation time or manner. In PD

pathogenesis, mitochondrial dysfunction leads to an increase in oxidative stress and a decline in ATP production and oxygen consumption and generally causes cell death by apoptosis (Huang et al., 2020). SH-SY5Y neuroblastoma cells is the most used because of its high sensitivity to PQ (Hirayama et al., 2018). Among other pesticides, PQ has been linked to PD by epidemiological studies. Some studies have shown that PQ induces activation of pro-apoptotic Bcl-2 family leading to release of cytochrome c (Gonzalez-Polo et al., 2014). Therefore, in SK-N-SH neuroblastoma cells, PQ increases ROS generation, lipid peroxidation, DNA damage, and apoptotic cell death (Ravi et al., 2018). However, in other study, 600μM PQ triggers a necrotic cell death in SH-SY5Y cells (Hirayama et al., 2018). On cultured rat neurons, the PQ-induced neuronal death was significantly enhanced with a nontoxic concentration of $CuCl_2$ supplementation. Indeed, copper contributes to PQ neurotoxicity through the formation of free radicals that damage mitochondria. However, the inhibition of N-Methyl-D-Aspartate (NMDA) receptor or the removal of glutamine from the culture medium decreases PQ cytotoxicity and increases cell survival (Stelmashook et al., 2016). Interestingly, there are many works which demonstrate that, individually, PQ and maneb (and other pesticides related to PD) activate Bak, but, together, they trigger Bax-dependent cell death, reduce the dopamine content in (mouse/neuroblastoma cells) (Rasheed et al., 2020), and cause toxicity in the nigrostriatal dopamine system (Colle et al., 2018). PQ neurotoxicity has also been reported to require the activation of stress-activated protein kinases (SAPKs) (Niso-Santano et al., 2010). Although the mitochondrial pathway of apoptosis has been largely linked to PQ-induced cell death, other signaling pathways have also been implicated in PQ toxicity. PQ has been shown to induce DNA damage and endoplasmic reticulum (ER) stress. ER stress is associated with the activation of inositol-requiring enzyme 1 (IRE1), apoptosis signal-regulating kinase 1 (ASK1), and c-Jun NH_2-terminal kinase (JNK) (Niso-Santano et al., 2010). In this sense, PQ-induced oxidation of thioredoxin (Trx) has been reported as a possible mechanism for the activation of the ASK1/JNK signaling pathways (Niso-Santano et al., 2010). Nrf2-dependent regulation of antioxidant proteins has been shown to be protective in PQ toxicity (Cai et al., 2019). Accordingly, Nrf2-dependent regulation of thioredoxin levels determines the sensitivity of PQ toxicity by the activation of ASK1/JNKp38 signaling (Niso-Santano et al., 2010). Also, PQ-induced cell death depends on Nrf2-regulated miR-380-3p in N2A cells (Cai et al., 2019).

PQ caused a tyrosine nitration and lipid peroxidation. Moreover, the oxidative stress induced by PQ generates a protein aggregation of the plasma membrane Ca^{2+}-ATPase and its degradation by calpain (Gonzalez-Polo et al., 2014). Low concentrations of PQ induce autophagy, which is followed by apoptosis and modulated by DJ1. Because the inhibition of autophagy potentiated apoptosis induced by PQ, it was proposed that autophagy might be acting as a protective mechanism against cell death progression and might be a new strategy for the treatment of neurodegenerative diseases (Gonzalez-Polo et al., 2014). The fact that PQ induces the accumulation of autophagic vacuoles and increases the degradation of proteins in the cytoplasm of SH-SY5Y cells indicates that the increased oxidative stress can activate autophagy in the initial stages of mitochondrial dysfunction, having a protective role of

Table 2 List of studies on the induction of apoptosis, necrosis, and/or autophagy by PQ

Model	Apoptotic major findings	References
Cerebellar granule cells	Production of free radicals	Stelmashook et al. (2016)
Pheochromocytoma PC12 cells	Decrease in cell respiration Reduction of polarized mitochondria	Zhou et al. (2017)
SK-N-SH neuroblastoma cells	Lipid peroxidation	Ravi et al. (2018)
	DNA damage	
SH-SY5Y human neuroblastoma cells	Decrease of the mitophagy inducer BNIP3 (Bcl-2/adenovirus E1B 19-kDa-interacting protein 3) protein	Hirayama et al. (2018)
	Necrosis	
Primary culture of rat embryonic neural stem cells	Decrease in cell proliferation	Colle et al. (2018)
	Autophagic major findings	
SH-SY5Y human neuroblastoma cells	Abnormal aggregation of α-synuclein	Wu et al. (2020)
	Increase of SQSTM1/p62 protein	
	Impaired autophagy and mitophagy	Huang et al. (2020)
	Decrease of ATG12 and ATG7	
	Decrease of DJ1 and Parkin	
	Decrease of lysosome function	Hirayama et al. (2018)
Pheochromocytoma PC12 cells	Decrease in LC3-II level Increase in p62 expression	Zhou et al. (2017)

PQ-induced cell death. However, studies carried out in PC12 cells by Zhou et al. (2017) or in SH-SY5Y cells by Wu et al. (2020) did not observe an activation of autophagy despite the increase of ROS. Most of the studies regarding the molecular mechanisms of PQ-induced apoptosis and autophagy have been directed toward neuronal cell (Gonzalez-Polo et al., 2014) (Table 2). However, it is obvious that the complexity of brain tissue organization is given by the interaction of different neurons with glial cell types. PQ induces microglial and astrocyte activation, which seems to precede PD neurodegeneration. Despite their activation, the role of survival astrocytes is to protect neurons against PQ toxicity (Bhatia et al., 2019). In fact, C57BL6 mice orally exposed to PQ displayed an activation of enteric glial cells without any locomotor deficiencies or α-synuclein accumulation (Naudet et al., 2017). In PQ-treated U118MG astroglia, the upregulation of secretogranin III (SGC3) protein may act as indicator in astrocyte activation (Zhan et al., 2018). Moreover, PQ induces microglial neuroinflammation through toll-like receptor 4 (TLR4)-nuclear factor (NF)-κB pathway, resulting in neuronal cell loss. Mitogen-activated protein kinases (MAPKs) are involved in the production of pro-inflammatory cytokines in microglia, but the inhibition of MAPK phosphorylation partially attenuates PQ-induced microglial inflammation (Wang et al., 2020).

Therefore, low PQ concentrations were toxic to neurons only in the presence of microglial cells. Also, in neuron-microglia cultures exposed to PQ, microglia was a source of PQ-derived oxidative stress (Gonzalez-Polo et al., 2014). Indeed, NADPH oxidase from glial cells mediates the generation of ROS. Therefore, in mutant mice lacking the gp91phox subunit of NADPH oxidase, PQ-induced oxidative stress and the neuron-to-neuron transfer of human α-synuclein were reduced (Musgrove et al., 2019). However, in primary culture of rat embryonic neural stem cells, 1μM PQ-induced ROS decreases cell proliferation (Colle et al., 2018). In other cell lines, Forkhead box O3 (FoxO3) play a protective role by upregulating the expression of antioxidant enzymes and suppressing oxidative stress (Chang et al., 2019).

5 Animal Models in Paraquat Neurotoxicity

The identification of animal models that mimic the different human pathologies is an essential tool in order to determine the evolution of certain diseases, to identify treatments, and to develop new therapies (Cristovao et al., 2020). Identifying new treatments to modulate its effects and study the limitations and benefits of these drugs in animal models are vital in order to provide clear justification for clinical trials in human. In vivo studies are needed to investigate the complex etiology of the disease with differences in onset, progression, symptoms, and neuropathology (Colle et al., 2020). There are numerous animal models that currently reproduce several neuropathological features of PD and may show comparable etiologies and pathologies, which will potentially be useful for future therapeutic trials (Cristovao et al., 2020). However, animals do not develop PD spontaneously, and some of them may have important differences from humans and cannot replicate perfectly the clinical syndrome (Kalyn et al., 2019). Transgenic animal models of PD can be employed, but the experimental induction with local or systemic administration of neurotoxins used for modeling PD are pesticides and herbicides (Konnova & Swanberg, 2018). MPTP, rotenone, maneb, and PQ are the main neurotoxins used to create animal models based on degeneration of dopaminergic neurons (Cristovao et al., 2020). The animal models commonly used in research of PD are monkey, rat, or mice; however, rabbits, fruit flies, dogs, nematodes, zebrafish, pigs, and guinea pigs are also used, but to a lesser extent.

The following is a review to collect information from different pesticide animal models used for the studies of progressive PD risk and increase the understanding of how environmental factors are associated with exposure to PQ. Pesticides and herbicides have been mostly used in rodents to try to model PD. The majority of 23,000 animal studies of PD published from 1990 to 2018 involve rodents (Konnova & Swanberg, 2018). These pesticides in PD models are extensively studied by its accessibility for genetic manipulations to allow creation of several transgenic mouse models that can reproduce many features of parkinsonism. They facilitate us a better understanding of the genetic alterations involved in familial PD. Its easy handling allows having robust experimental protocols and route and dose of drug

administration. One of the main advantages of rodent PD models is that nigrostriatal dopaminergic degeneration correlates to motor deficits in mice (Colle et al., 2020) and rats (Cristovao et al., 2020), directly or indirectly related to reactive oxygen species.

5.1 Rat Animal Model

Rats are the most commonly used animals for preclinical trials to study parkinsonian symptoms. Consequently, rats chronically exposed to PQ presented a loss of 41% of dopaminergic neurons and a decrease in motor performance (Cristovao et al., 2020). PQ-treated rats develop α-synuclein inclusions in the SNpc and the dorsal motor nucleus of the vagus, which in turn provokes a reduced gastric tone and motility. Gastrointestinal dysfunctions are considered non-motor symptoms of PD (Anselmi et al., 2017). PQ-induced ROS triggers an increase of intracellular Zn^{2+} in SNpc, via AMPA receptor activation, leading to the degeneration of dopaminergic neurons (Tamano et al., 2019). Moreover, exposure to PQ in rats induces lung, renal, and liver toxicity. At higher doses, observations include decreased body weight, clinical signs like dyspnea, increased respiratory sounds, swellings and sores in the genital area, hematological changes, and effects on organ weight, as well as increased mortality. However, Cristovao (et al. 2020) did not highlight a decrease of weight in rats with chronic low dose of PQ administration. Despite, there was in the SNpc an α-synuclein (phosphorylation at serine 129) aggregation preceded by a microglial activation and ROS generation.

5.2 Mouse Animal Model

Chronic PQ administration in mice induces dopaminergic cell loss in SNpc, a decrease in the level of dopamine, and a decrease in motor activity (motor deficits). However, PQ does not always induce PD symptoms in mice, but its administration triggers apoptotic cell death program through oxidative stress-mediated activation of the JNK signaling pathway, suggesting a possible mechanism for selective dopaminergic neuron loss (Gonzalez-Polo et al., 2014). High doses of PQ are not suitable for modeling PD, since they cause pulmonary fibrosis which could have secondary effects on behavior and cardiac remodeling and dysfunction (Chang et al., 2019). Repeated administration of 10 mg/kg PQ and 30 mg/kg maneb to mice twice a week over the span of 6 weeks produces PD symptoms in mice compared to each compound alone, and the exposure during postnatal period can cause neurotoxicity in the nigrostriatal dopaminergic systems and motor deficit (Colle et al., 2020). With a better understanding of the genetic alterations involved in familial PD, several transgenic mouse models have been generated that can reproduce many features of parkinsonism. Animal models generated by mutations or deletions of the relevant Parkinson's-related genes have also been used to examine the effects of PQ.

5.3 Fruit Fly Animal Model

The fruit fly *Drosophila melanogaster* has a similar number of genes to humans, and it is often used in genetic changes in order to observe the effects on the progression of neurodegenerative diseases (Gonzalez-Polo et al., 2014). A large number of human genes implicated in PD, such as parkin, ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), PINK1, DJ1, and LRRK2, have highly conserved homologs in *D. melanogaster*. Moreover, PQ did not affect the tyrosine hydroxylase proteins in Parkin knockdown flies but reduced their lifespan and movement activity (Ortega-Arellano et al., 2019). Rho kinase (ROCK) inhibitors demonstrate neuroprotective effects in flies subjected to paraquat through parkin pathway dependent on the regulation of mitophagy (Moskal et al., 2020). The α-synuclein or parkin models in flies show that PQ induces DA neuron loss, mitochondrial pathology, locomotor deficits, LB-like protein aggregates, and sensitivity to oxidative stress. In D. *melanogaster*, PQ induces oxidative stress, increases free iron (Fe^{2+}) content and caspase-3 activation, and decreases flies' survival (Dos Santos Nunes et al., 2019). DJ1 plays a critical role in the survival of dopaminergic neurons and response to oxidative stress in PQ-treated flies. There exist many models of fruit flies to study genetic changes, which have shown dysfunction of DA neurons, locomotor deficits, and retinal degeneration after treatment with PQ (Gonzalez-Polo et al., 2014) and dysregulation of the innate immune through the activation of the NF-κB transcription factor and the stress signaling factor JNK by exposure to PQ-induced PD pathogenesis (Maitra et al., 2019).

5.4 Nematode Animal Model

Caenorhabditis elegans is a nematode animal model commonly used in scientific studies. *C. elegans* models have a well-characterized genome, and the adult wild-type worm comprises 1,000 cells, about a third of which are neurons and exactly eight of which are dopaminergic neurons. The dopamine neurons of *C. elegans* are susceptible to neurotoxins and pesticides (Gonzalez-Polo et al., 2014). This has led to a search for genetic patterns among nematodes of resistance to the toxicity of PQ in genes that seem to be involved in the adaptive behavioral and cytoprotective responses (Gecse et al., 2019) and in the oxidative stress, restricted energy metabolism, and reduced stress resistance and longevity (Dilberger et al., 2019).

5.5 Zebrafish Animal Model

Zebrafish is used to study development and gene function, as a potential model of PD. In zebrafish, dopaminergic neurons are anatomically comparable with the nigrostriatal tract in mammals. For this reason, its use is increasing in the study of neurodegenerative diseases associated with mitochondrial dysfunction and oxidative stress (Gonzalez-Polo et al., 2014). It is also used for drug screening due to

several advantages, including its larval transparency, reduced size, and high genetic homology to the molecular, pathological and physiological pathways conseved with mammals (Gonzalez-Polo et al., 2014). These neurons are sensitive to neurotoxins showing a severe neurodegenerative impact of paraquat on dopamine and serotonin protein levels (Wang et al., 2018). However, PQ was the least efficient neurotoxic compound to induce dopaminergic neuronal loss and locomotor defects in zebrafish (Kalyn et al., 2019). MitoPQ induces in zebrafish a mitochondrial superoxide generation (Pinho et al., 2019), with decreased spontaneous movement and brain tyrosine hydroxylase levels.

5.6 Dog Animal Model

The toxicity of accidental PQ ingestion of dogs has been reported in South Africa. Animals presented tachypnea, dyspnea, and cyanosis. Necropsy displayed gastrointestinal irritation, renal failure, and necrosis of the tongue and alveolar cells (Williams et al., 2016).

5.7 Rabbit Animal Model

In vitro and ex vivo, PQ induces cell toxicity in primary rabbit corneal endothelial cells and rabbit corneal tissue specimens, respectively. In vivo, PQ causes a corneal endothelial damage and the transparency of corneal stroma is affected (Hsueh et al., 2020). Nevertheless, there exist no studies demonstrating damage at the neurological level.

5.8 Pig Animal Model

In porcine jejunal epithelial cell line (IPEC-J2), PQ (70μM) increases lactate dehydrogenase (LDH) release, GSSG concentration, and the ratio of GSSG/glutathione (GSH) leading to cytotoxicity. PQ induces oxidative stress in the intestine of piglet through the inhibition of Nrf2 signaling pathway (Xiao et al., 2019) and reproduces acute lung injury (Gonzalez-Polo et al., 2014).

6 Conclusion

Toxicologic studies have suggested that multiple genetic and environmental factors could be involved in the etiology of PD. Studies with transgenic mice suggest that the genetic background may also lead to increased vulnerability to the neurotoxic effects of pesticides such as PQ. The weight of evidence is sufficient to conclude a generic association between PQ exposure and PD exists, but it is not sufficient to conclude that this is a causal relationship. In addition, the multifactorial etiology of

PD hampers unequivocally establishment of the role of any individual contributory causal factor. Further research is needed in order to identify long-term biomarkers of exposure, improve methods for estimating pesticide exposure, and undertake prospective cohort studies of PQ-exposed people. It is also necessary to increase the volume of studies on the molecular basis of PQ toxicity in order to establish a clear relationship between its neurotoxic effects and in vivo neuronal loss that leads to PD.

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