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Expression of genes related to Parkinson's disease after paraquat treatment in *Drosophila melanogaster*

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ABSTRACT

This study was designed to determine the minimum effective concentration of paraquat that modulated the expression of PKD-related genes in *Drosophila*. We first studied the viability of *Drosophila* and then tested the expression of the PKD-related genes—*Parkin*, *UCH*, and *tau*—in various concentrations of paraquat in the water sucked by *Drosophila*. The lowest effective concentration of paraquat was approximately 20 μM and the gene expression was induced at paraquat doses between 20 mM and 20 μM. *Parkin* and *tau* expression was inhibited, while that of *UCH* was significantly increased.

Next, we examined the expression of the *Parkin* and *UCH* genes in the neurons of *SOD*-reduced mutants under oxidative stress conditions and found that *Parkin* was up regulated, while *UCH* was down regulated. We also found that the expression of *Parkin* was regulated by *JNK*. This study revealed that paraquat affects the expression of PKD-related genes via oxidative stress.

In conclusion, our results showed that paraquat in the water sucked by *Drosophila* altered the gene expression at a minimum concentration of 20 μM, and that it not only promoted but also inhibited PKD-related gene expression via signal transduction mediated by oxidative stress. In order to confirm whether paraquat is a causal factor of PKD, more balanced and in-depth tests seem to be done looking into multiple aspects.

1. Introduction

One of the typical pathologic features in Parkinson's disease (PKD) is nigral degeneration and the development of Lewy bodies containing poorly degraded α-synuclein [1,2]. The quantitative change in α-synuclein is a function of both synthesis and degradation. Consequently, many factors involved in the ubiquitin-proteasome system have been focused on, particularly at the gene level, as a potential cause of familial PKD, namely, ubiquitin carboxy-terminal hydrolase (UCHL) mutation, α-synuclein mutation, and parkin (ubiquitin ligase) deletion and/or point mutation [3].

Contrary to familial PKD, the genetic contribution to sporadic PKD is not clearly understood. Candidate genes associated with the sporadic form of PKD comprise genes related to dopaminergic transmission [4,5], xenobiotic metabolism [6,7], protein aggregation [8–10], and microtubule-associated protein tau (MAPTau) [11,12].

Since the introduction of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [13], the striking similarity between MPTP PKD and idiopathic PKD highlights the notion that exposure to environmental factors might trigger PKD [14–17]. Paraquat (1,1′-dimethyl-4,4′-bipyridium dichloride) has been proposed to act as a mitochondrial poison in the same manner as MPTP. It is structurally similar to MPTP [18] and has been shown to induce the destruction of nigral dopaminergic neurons with a consequent neurobehavioral syndrome in mice [19]. Other evidences favoring paraquat as a cause of PKD are neuronal damage at the genome and proteome levels [20] and epidemiologic evidence linking paraquat with PKD development [21].

Paraquat has been one of the most commonly used herbicides worldwide. Therefore, populations inhabiting rural areas may be exposed to paraquat through environmental contamination via well-water drinking and/or skin contact or inhalation during spraying. Taking into consideration the fact that farmers are invariably exposed to at least trace amounts of paraquat while spraying, the results of the aforementioned study suggest that paraquat may be a possible risk factor for PKD in farmers. However, reports on the effect of paraquat do not explicitly state whether the herbicide is sufficiently detrimental to warrant guidelines to limit its use or exposure to it.

In order to provide a solution to this type of practical problem, further studies should adopt various perspectives to review the effects of paraquat on gene expression. First, paraquat concentrations in experiments should be evaluated to confirm whether these are comparable to the expected levels during spraying in

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Table 1
Drosophila homologue genes in the familial Parkinson's disease

Class	Hunan gene	*Drosophila* gene	Gene No.
Parkinson's disease related	*PARK1* (α-synuciein)	No significant homolog in *Drosophila*	
	PARK2 (*Parkin*)	*Parkin*	CG10523
	PARKS (*UCH-L1*)	*Uch*	CG4265
	NR4A2	*Hr38*	CG1864
	MAPT	*tau*	CG31057

See Ref. [23], http://superfl.ucsd.edu/homophila/.

Table 2
Primer sequences for target genes

Drosophila gene	Product size	Primer sequences
Parkin	483 bp	Forward: AAGCTGTGTAATGGCAAACT Reverse: CAACAGCTTGAAGTGATGAA
Uch	362 bp	Forward: GAGGATCTCTTCTACATGCG Reverse: GCATCCTTCACAAAAGTCTC
tau	438 bp	Forward: GATGAGTCCACTCAGGAGAA Reverse: CCACTGCAACTTTGTTGTAA
rp49	410 bp	Forward: AGATCGTGAAGAAGCGCACCAAG Reverse: CACCAGGAACTTCTTGAATCCGG

farms. Of course, it is very difficult to estimate the level of paraquat exposure in farmers because it varies widely depending on the method of spraying and type of protective equipment used during spraying. However, it is very important to determine the lowest concentration of paraquat that acts as a "possible PKD inducer" in vitro or in experimental animals.

It is well-known that free radicals formed by paraquat induce direct cellular injury. However, recent studies have further established that free radicals control gene expression by directly intervening with signal transduction [22]. In this regard, the gene expression as an aftermath of paraquat intervention merits more comprehensive investigations such as gene studies to elucidate the expression of various suspected genes simultaneously at various paraquat concentrations.

Drosophila melanogaster is a complex multicellular organism, and many aspects of its development and behavior parallel those in humans. Further, many of its genes exhibit significant homology to human genes. *Parkin*, *UCH*, and *tau* have been reported to be the PKD-related genes in *Drosophila* [23] (see Table 1). This study was designed to determine the lowest concentration of paraquat in the water sucked by *Drosophila* that modulates PKD-related genes.

2. Materials and methods

2.1. Insect

2.1.1. Drosophila culture and drug treatments

Drosophila melanogaster were kept at 25 °C and cultured using the standard method.

Wild-type *Oregon-R strain*, *elav-gal4*, *and UAS-JNK-dominant negative* flies were obtained from the Bloomington stock center.

The UAS-JNK transgenic fly was a gift from Dr. C. Jung, and the UAS-SOD-1R (RNA interference) and UAS-SOD-2R transgenic flies, from Dr. Phillips. To express these UAS lines, the UAS/gal4 binary genetic system was used [24].

2.2. Median lethal dose of paraquat

First, 5-day-old flies (100 wild-type *Oregon-R strain*) were starved for 6 h in vials containing 1.3% agar without paraquat and were subsequently transferred to vials containing 1.3% agar, 5% sucrose solution, and various concentrations of paraquat [25]. To determine the median lethal dose (LD_{50}) at 20 mM paraquat, the number of dead flies was counted every 6 h for 3 days. At least five independent experiments were performed. The data were presented as the mean and error bar (±SEM). Analysis of variance (ANOVA) (one-way *F*-test) program was used for statistical analyses, and $p < 0.05$ was accepted as statistically significant.

2.3. Reverse transcription polymerase chain reaction analysis

Total RNA was isolated with Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 μg mRNA by using the SuperScript TM III First-Strand Synthesis Kit (Invitrogen, USA). For the semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis, 1 μl First-strand cDNA was subjected to PCR amplification with a primer set of target genes (Table 2). AccuPower® PCR premix (Taq DNA polymerase-based system; Bioneer, Korea) was used in the PCR reactions. PCR was performed at 98 °C for 5 min, followed by 35 cycles at 98 °C for 10 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min with the Thermal Cycler (Applied Bioscience, USA). PCR products were resolved in 1.5% agarose gel and visualized using ethidium bromide staining. Expression of the rp49 gene was used as the control. All experiments were repeated at least 3 times and the data was presented as the mean and error bar (±SEM). The statistical significance was tested by Microsoft Excel-based application for the Student *t*-test statistical analysis.

3. Results

3.1. The LD_{50} of paraquat

The LD_{50} of paraquat at 48 h was 20 mM. The lethality of paraquat increased in a dose- and time-dependent manner (Fig. 1). Therefore, we varied the paraquat concentration in the water sucked by *Drosophila* between 20 mM and 20 μM (1000 times diluted).

3.2. Expression of PKD-related genes

Expression of PKD-related genes in *Drosophila* is presented in Fig. 2. At 20 mM, paraquat treatment for 24 h inhibited the expression of *Parkin* and *tau*, while it significantly increased the expres-

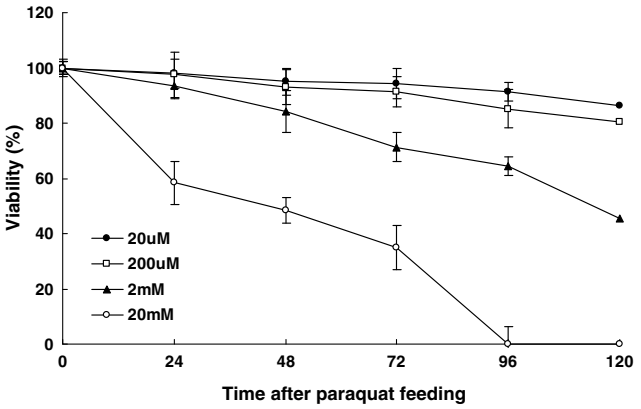

Fig. 1. The viability of flies was measured at paraquat concentrations 20 μM, 200 μM, 2 mM, and 20 mM. The viability decreased on treatment with paraquat in a dose- and time-dependent manner. Note that the viability is significantly low when the flies are exposed to 20 mM than other concentrations through the observation period of 5 days ($p < 0.001$). When the flies were exposed to 20 mM of paraquat, half of the flies died by 48 h.