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Research Article

Dmp53, *basket* and *drICE* gene knockdown and polyphenol gallic acid increase life span and locomotor activity in a *Drosophila* Parkinson's disease model

Hector Flavio Ortega-Arellano, Marlene Jimenez-Del-Rio and Carlos Velez-Pardo

Neuroscience Research Group, Medical Research Institute, Faculty of Medicine, University of Antioquia, Medellin, Colombia.

Abstract

Understanding the mechanism(s) by which dopaminergic (DAergic) neurons are eroded in Parkinson's disease (PD) is critical for effective therapeutic strategies. By using the binary tyrosine hydroxylase (*TH)-Gal4/UAS-X RNAi Drosophila melanogaster* system, we report that *Dmp53*, *basket* and *drICE* gene knockdown in dopaminergic neurons prolong life span ($p < 0.05$; log-rank test) and locomotor activity ($p < 0.05$; χ^2 test) in *D. melanogaster* lines chronically exposed to (1 mM) paraquat (PQ, oxidative stress (OS) generator) compared to untreated transgenic fly lines. Likewise, knockdown flies displayed higher climbing performance than control flies. Amazingly, gallic acid (GA) significantly protected DAergic neurons, ameliorated life span, and climbing abilities in knockdown fly lines treated with PQ compared to flies treated with PQ only. Therefore, silencing specific gene(s) involved in neuronal death might constitute an excellent tool to study the response of DAergic neurons to OS stimuli. We propose that a therapy with antioxidants and selectively "switching off" death genes in DAergic neurons could provide a means for pre-clinical PD individuals to significantly ameliorate their disease condition.

Keywords: Basket, Dmp53, Drice, Drosophila, paraquat.

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Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder affecting millions of people worldwide (Alves *et al.*, 2008). This neurological condition is clinically characterized by motor disorders (Jankovic, 2008). Pathologically it is prominently characterized by progressive loss of 50-70% of dopaminergic (DAergic) neurons located in the *substantia nigra*, abnormal protein aggregation, oxidative stress (OS) and mitochondrial dysfunction (Zhou *et al.*, 2008; Cuervo *et al.*, 2010; Xie *et al.*, 2010). Despite intense investigation, the molecular mechanism(s) of cell loss is not yet fully established for target therapeutic strategies (Dexter and Jenner, 2013). This unmet need might partially explain the failure to establish definitive anti-Parkinson drugs (Rodnitzky, 2012). Since replacement of deficient dopamine constitutes a first-line symptomatic treatment (Gazewood *et al.*, 2013), there is an urgent need to identify the molecular components involved in the DAergic neuronal demise.

Send correspondence to Carlos Velez-Pardo. Neuroscience Research Group, Medical Research Institute, Faculty of Medicine, University of Antioquia (UdeA), Calle 70 No. 52-21, and Calle 62 No. 52-59, Building 1, Room 412, SIU, Medellin, Colombia. E-mail: carlos.velez@neurociencias.udea.edu.co.

Paraquat (PQ, methyl viologen dichloride; or 1,10-dimethyl-4,40-bipyridinium dichloride) is among the most consistently associated environmental risk factors for PD (Tanner *et al.*, 2011; Wang *et al.*, 2011). PQ might cause neuronal deterioration via OS and mitochondrial damage (Franco *et al.*, 2010). Because ethical and policy issues limitation in human research, most of the PQ toxic effects have been studied in *in vitro* and *in vivo* models of PD. Specifically, our research group has shown that PQ induces apoptosis - a type of programed cell death - by an OS-mediated mechanism (Jimenez-Del-Rio and Velez-Pardo, 2008). The major molecular events involve generation of $O_2^{\cdot-}$/ H_2O_2, activation of the transcription factor p53, JNK (c-Jun N-terminal) kinase, mitochondria depolarization, caspase-3 activation and chromatin condensation/DNA fragmentation. *Drosophila melanogaster* has been used as biological tool to inquire on PD process (for a review see Muñoz-Soriano *et al.*, 2011). Remarkably, PQ selectively destroys DAergic neurons in the fly (Chaudhuri *et al.*, 2007), via OS and mitochondrial damage (Bonilla *et al.*, 2006; Hosamani and Muralidhara, 2013). In agreement with this data, Vrailas-Mortimer *et al.* (2012) have shown that some commercially available antioxidant supplements confer significant protection to *D. melanogaster* against PQ and H_2O_2. Furthermore, we have recently shown that

polyphenols [e.g., gallic acid (GA), propyl gallate (PG), epicatechin (EC)], which are well-known antioxidant and gene modulators, were able to protect, rescue and restore impaired movement activity in *Drosophila* induced by acute (Jimenez-Del-Rio *et al.*, 2010) or chronic (Ortega-Arellano *et al.*, 2011) PQ exposure. It has also been shown that pharmacological inhibition of JNK increased life span and locomotor activity (*i.e.*, climbing) compared to flies treated with PQ (Jimenez-Del-Rio *et al.*, 2008). Taken together, these findings suggest that polyphenols might modulate life span and movement capabilities in *D. melanogaster* exposed to PQ, and that JNK signalling might be involved in those effects. Since there is compelling evidence that the apoptosis pathway is conserved between *Drosophila* and mammalian cells (Oberst *et al.*, 2008; O'Riordan *et al.*, 2008; Mollereau, 2009), and essential signalling molecules involved in the OS response in mammalian cells, such as p53, JNK and caspase-3 are highly similar to *Drosophila Dmp53* (43%), *basket* (87%) and *drICE* (61%), respectively (Reiter *et al.*, 2001; Chien *et al.*, 2002), our hypothesis is that a decreased gene expression of *Dmp53, basket and drICE* in DAergic neurons might have a beneficial impact on flies exposed to PQ in terms of DAergic neurons survival, life span and climbing capabilities. Moreover, transgenic flies treated with antioxidants might show even longer life span and functionality than transgenic flies treated with PQ alone.

To evaluate this premise, a genetically amenable binary tyrosine hydroxylase (*TH)-Gal4/*upstream activator sequences (*UAS-X)* RNA interference (RNAi) *D. melanogaster* system was used, wherein each gene/protein (X) can be silenced by RNAi (Kennerdell and Carthew, 2000) in DAergic neurons. RNAi provides an easy and powerful technique to suppress gene expression by specific removal of mRNA molecules transcribed from endogenous genes (Dietzl *et al.*, 2007). Therefore, RNAi could result in a potent targeted therapeutic strategy in PD (Gavrilov and Saltzman, 2012). Although systematic RNAi analyses have revealed the role of cell death machinery components (*e.g.*, caspases, and caspase-adaptors) in *Drosophila* eye development (Leulier *et al.*, 2006), and JNK and caspase-3 RNAi experiments were done in wing imaginal discs (Umemori *et al.*, 2009), no data is yet available to determine whether *Dmp53, basket* or *drICE RNAi* have any impact on life span or movement activity in the fly.

The aims of the present investigation were to study the life span and locomotor activity of *Dmp53, basket and drICE* gene knockdown in *D. melanogaster* (*e.g.*, *TH-Gal4*$^{+/-}$, *UAS-Dmp53 RNAi*$^{+/-}$; *TH-Gal4*$^{+/-}$*,UAS-basket RNAi*$^{+/-}$; *TH-Gal4*$^{+/-}$, *UAS-drICE RNAi*$^{+/-}$) chronically exposed to 1 mM PQ added to a 1% glucose feeding regimen for 15 days, and to determine whether polyphenols such as GA affect life span and locomotor activity of transgenic flies exposed to PQ for 15 days. GA was selected based on its effectiveness against PQ toxicity (Jimenez-Del-Rio *et al.*, 2010; Ortega-Arellano *et al.*, 2011). Taken together, our findings suggest that PQ induces DAergic neuronal deterioration in *Drosophila* by a p53-, JNK- and caspase-3-dependent mechanism similar to that seen in human cells (Jimenez-Del-Rio and Velez-Pardo, 2012). It is inferred that silencing specific gene(s) involved in neuronal cell death might constitute an excellent tool to study the response of DAergic neurons to OS stimuli. Given that GA proved to be an effective antioxidant against PQ, polyphenols therefore become an important source for long-term prophylactic applications. Understanding the relevant events of cell death in DAergic neurons is a necessary step to better establish genetic and/or antioxidant therapy approaches in PD. Consequently, we proposed that a therapy with antioxidants and selectively "switching off" of death genes in DAergic neurons should provide a means for pre-clinical PD individuals to significantly ameliorate their disease condition.

Materials and Methods

Fly stocks and culture

Wild type Canton-S and fly lines were cultured under standard conditions, as described elsewhere (Ortega-Arellano *et al.*, 2011). The genotypes were established by standard genetics. Fly Stocks obtained from the Bloomington Stock Center (BSC) were: *TH-Gal4* (#8848), and *UAS-GFP* (#1521). *UAS-dsRNAi* (double-stranded RNA interference) lines obtained from the Vienna Drosophila RNAi stock center (VDRC) were: *UAS-drICE RNAi* (#28006), *UAS-basket RNAi* (#34138), and *UAS-Dmp53 RNAi* (#10692). Male *TH-GAL4*$^{+/+}$ flies were crossed with wild type Canton-S females to obtain heterozygous female flies (fF1, *TH-GAL4*$^{+/-}$, Figure 1A). Male *TH-GAL4*$^{+/+}$ flies were crossed with *UAS-X RNAi* females (Figure 1B) to obtain heterozygous female flies (fF1) (*TH-GAL4*$^{+/-}$, *UAS-Dmp53 RNAi*$^{+/-}$). Since female flies were shown to be particularly sensible to PQ (Jimenez-Del-Rio *et al.*, 2008), the fF1 generation was collected under brief CO_2 anesthesia within 2 to 3 days after eclosion for further experiments.

Paraquat toxicity assay

The paraquat toxicity assay was performed on virgin 2- to 3-day-old fF1 flies collected overnight and kept on regular food medium. Subsequently, 50 separated adult fF1 flies were starved in empty vials for 3 h at 25 °C. Then, groups of five flies were placed in ten vials containing a filter paper (Bio Rad Mini Trans-Blot 1703932) saturated with 1% glucose (55.5 mM glucose, Gluc) in distilled water (dW) for 24 h. After this time, flies were starved in empty vials for 3 h at 25 °C and transferred to vials with a filter paper saturated with 200 µL (1 mM) PQ in Gluc for 15 days. Filters were changed daily. Red food dye (8 µL/mL) (Red food colour McCormick) was added to ensure homogeneity and food intake. Living flies were counted daily.

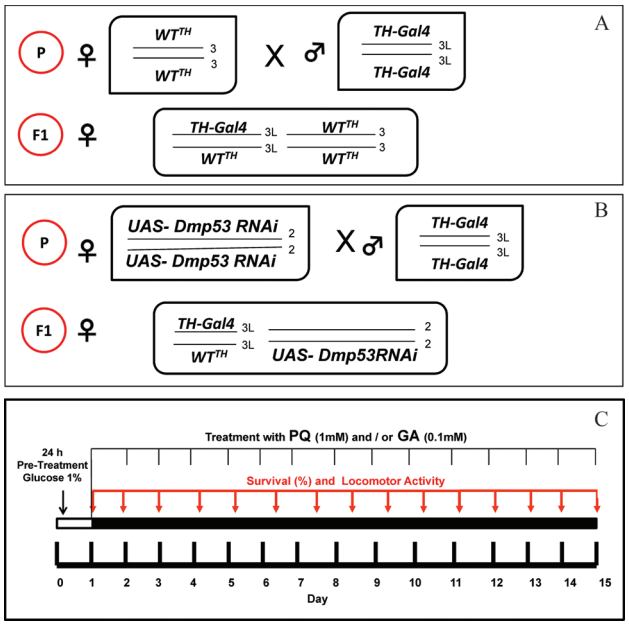

Figure 1 - Scheme for basic fly cross and selection. (A) *TH-Gal4*$^{+/-}$ flies were obtained by crossing *TH-Gal4*$^{+/+}$ (males, n = 10) and wild type Canton S *Drosophila melanogaster* (females, n = 10). After five days of husbandry, parental flies were discarded from the mating tubes. F1 flies were then reared according to standard procedures. (B) *TH-Gal4*$^{+/-}$, *UAS-X RNAi*$^{+/-}$ flies, where *X* represent either *Dmp53*, *basket* or *drICE* gene, were obtained by crossing *TH-Gal4*$^{+/+}$(males, n = 10) and *UAS-X RNAi*$^{+/+}$ (females, n = 10). For the sake of clarity, *TH-Gal4*$^{+/-}$ *UAS-Dmp53 RNAi*$^{+/-}$ transgenic is shown as representative sample. (C) Schematic representation of feeding schedule, paraquat (PQ), and/or polyphenol (gallic acid, GA) treatments in *Drosophila melanogaster*.

Antioxidant assay

The antioxidant assay was also performed on virgin 2- to 3- day-old fF1 flies collected overnight and kept on regular food medium. Subsequently, 50 adult fF1 flies were starved as described above and groups of five flies were placed in ten vials containing a filter paper saturated with 1G in dW for 24 h. Then, flies were fed for 15 days with 200 µL Gluc solution containing 0.1 mM fresh polyphenol solution (*e.g.*, GA) and 1 mM PQ. Filters were changed daily. Red food dye was added as described and survival proportion and locomotion assay (%) were rated at each interval of time.

Locomotion assay

The movement deficit assay was performed on treated and untreated flies according to Ortega-Arellano *et al.* (2011). Briefly, untreated or treated fF1 flies were placed in empty plastic vials. After a 10 min resting period, the flies were tapped to the bottom of the vials and the number of flies able to climb 5 cm in 6 s was recorded at each time interval. The assays were repeated three times at 1 min intervals. For each experiment, a climbing percent (%) was calculated, defined as $1/2[(n_{tot} + n_{top} - n_{bot})/n_{tot}] \times 100$. Data were shown as mean ± standard deviation of the mean (SD).

A Chi Square (χ^2) test was performed to compare the proportion of percentage between independent groups. Differences were considered statistically significant at $p < 0.05$.

Survival test

fF1 flies were treated chronically with PQ and polyphenol (GA) as described above for 15 days. Live flies were counted daily in groups of five flies per vial. 50 flies per treatment were used. Survival curves were plotted using the Kaplan-Meier estimator. The statistical significance was calculated using the log rank test implemented in the portable IBM SPSS statistics 19 package program. The null hypothesis in all survival assays was that the exposure of genetically modified *Drosophila* to PQ and/or GA made no difference to the survival of the flies in the absence of these reagents. Differences were considered statistically significant at $p < 0.05$.

Results

The knockdown of *Dmp53*, *basket* and *drICE* genes in dopaminergic neurons increases life span and locomotor activity in *Drosophila melanogaster* exposed to paraquat

We initially wanted to evaluate life span and locomotor activity in *D. melanogaster TH-Gal4*$^{+/-}$, (Figure 1A, control) resulting from the cross between wild type *Canton S* and homozygous *TH-Gal4*$^{+/+}$, exposed to 1 mM PQ in the experimental design (Figure 1C). As shown in Figure 2A, the proportion of surviving *TH-Gal4*$^{+/-}$ flies treated with PQ was significantly diminished compared to *TH-Gal4*$^{+/-}$ flies. Indeed, while 50% of the *TH-Gal4*$^{+/-}$ flies perished by day 14, the*TH-Gal4*$^{+/-}$ plus PQ group did so at day 5. The percentage of locomotor activity (*i.e.*, > 75% climbing performance) remained normal in *TH-Gal4*$^{+/-}$ flies until day 15, whereas climbing performance was drastically diminished already by day 5 when they were exposed to PQ (Figure 2B). Interestingly, knockdown flies *TH-Gal4*$^{+/-}$, *UAS-Dmp53 RNAi* $^{+/+}$ (Figure 2 A,B); *TH-Gal4*$^{+/-}$, *UAS-bsk RNAi*$^{+/-}$ (Figure 3 A,B), and *TH-Gal4*$^{+/-}$, *UAS- drICE RNAi*$^{+/-}$ (Figure 4 A,B) displayed survival percentages and climbing capabilities comparable to control flies (*TH-Gal4*$^{+/-}$). However, in 50% of the transgenic flies treated with PQ the percentage of survival slightly increased (by 2 days) and climbing performance moderately augmented (by 3-5 days) compared to the control group (Figures 2, 3 and 4A,B).

Gallic acid (GA) increases life span and locomotor activity in RNAi fly lines

Polyphenols have shown a high protective effect against PQ in *Drosophila* (Jimenez-Del-Rio *et al.*, 2010). We, thus, investigated herein whether GA also had an effect in genetically modified flies when exposed to 1 mM PQ for

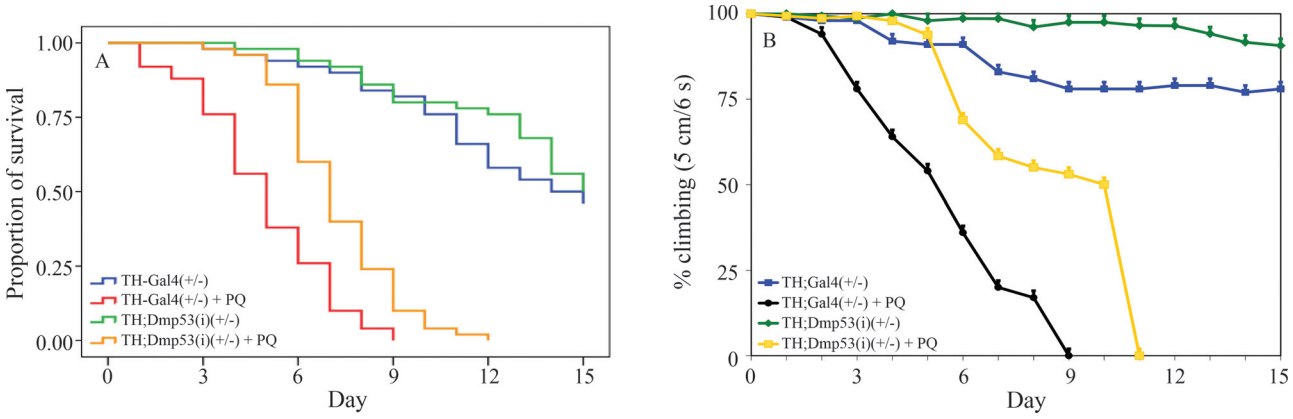

Figure 2 - *Dmp53* gene knockdown increases life span and locomotor activity in *Drosophila melanogaster* exposed to paraquat. Female flies (n = 50 per treatment) were treated as described in *Materials and Methods*. The graphs show that the proportion of survival (A) and climbing performance (B) were significantly increased in *TH-Gal4*$^{+/-}$, *UAS-Dmp53 RNAi*$^{+/-}$ gene knockdown flies compared to the *TH-Gal4*$^{+/-}$ fly line (control). Statistical comparisons between untreated and treated flies showed: (A) a $p < 0.05$ by log-rank test and (B) a $p < 0.05$ by χ^2 test. Comparisons between treated gene knockdown flies and the *TH-Gal4*$^{+/-}$ fly line showed statistical significance.

15 days. As shown in Figure 5, the presence of GA in the diet of *TH-Gal4*$^{+/-}$, *UAS-Dmp53 RNAi*$^{+/-}$ fly lines treated with PQ increased the proportion of survival (Figure 5A) and climbing performance (Figure 5B) compared to flies treated with PQ alone. Noticeably, while 50% of the *Dmp53 RNAi*$^{+/-}$ flies treated with PQ and GA survived when scored on days 10 and 13, respectively, 50% survival in the *Dmp53 RNAi*$^{+/-}$ fly line treated with PQ alone could only be scored until day 7 and 10, respectively. Similar results were obtained with the other RNAi transgenic flies (Table 1).

Discussion

By using a *GAL4/UAS-X RNAi* system, we could show that *Dmp53*, *basket* and *drICE* gene knockdown prolonged life span ($p < 0.05$, log rank test) and locomotor activity (*i.e.* climbing capability, $p < 0.05$, χ^2 test) in *D.*

Table 1 - *Dmp53*, *bsk* and *drICE* gene knockdown increase the life span and locomotor activity of *Drosophila melanogaster* chronically exposed to paraquat.

Line	Noxious/ antioxidant	Treatment	Concentration (mM)	Effect on			
				Survival (50%)[a]	K-M, p	Climbing (50%)[b]	χ^2, p
TH -GAL4$^{+/-}$	**PQ**	**1**	**0**	14 ± 0.6	1 *vs.* 2, $p < 0.005$	**> 15**	1 *vs.* 2, $p < 0.05$
	PQ	**2**	**1**	5 ± 0.3	2 *vs.* 4, $p < 0.005$	**5**	2 *vs.* 4, $p < 0.05$
	GA	**3**	**0.1**	15	3 *vs.* 4, $p < 0.005$	**> 15**	3 *vs.* 4, $p < 0.05$
	GA + PQ	**4**	**0.1 + 1**	11 ± 0.4	4 *vs.* 8 12 16, n.s.	**13**	4 *vs.* 8 12 16, n.s.
UAS~Dmp53(RNAi)$^{+/-}$ *TH-Gal4*$^{+/-}$	**PQ**	**5**	**0**	15 ± 0.4	1 *vs.* 5, n.s.	**> 15**	1 *vs.* 5, n.s
	PQ	**6**	**1**	7 ± 0.4	2 *vs.* 6, $p < 0.005$	**10**	2 *vs.* 6, $p < 0.05$
	GA	**7**	**0.1**	15	6 *vs.* 8, $p < 0.005$	**> 15**	6 *vs.* 8, $p < 0.05$
	GA + PQ	**8**	**0.1 + 1**	10 ± 0.5	7 *vs.* 8, $p < 0.005$	**13**	7 *vs.* 8, $p < 0.05$
UAS~ bsk(RNAi)$^{+/-}$ *TH-Gal4*$^{+/-}$	**PQ**	**9**	**0**	15 ± 0.4	1 *vs.* 9, n.s.	**> 15**	1 *vs.* 9, n.s.
	PQ	**10**	**1**	7 ± 0.5	2 *vs.* 10, $p < 0.005$	**9**	2 *vs.* 10, $p < 0.05$
	GA	**11**	**0.1**	15	10 *vs.* 12, $p < 0.005$	**> 15**	10 *vs.* 12, $p < 0.05$
	GA + PQ	**12**	**0.1 + 1**	10 ± 0.5	11 *vs.* 12, $p < 0.005$	**13**	11 *vs.* 12, $p < 0.05$
UAS~drICE (RNAi)$^{+/-}$ *TH-Gal4*$^{+/-}$	**PQ**	**13**	**0**	15	1 *vs.* 13, n.s.	**> 15**	1 *vs.* 13, n.s.
	PQ	**14**	**1**	7 ± 0.5	2 *vs.* 14, $p < 0.005$	**8**	2 *vs.* 14, $p < 0.05$
	GA	**15**	**0.1**	15	14 *vs.* 16, $p < 0.005$	**> 15**	14 *vs.* 16, $p < 0.05$
	GA + PQ	**16**	**0.1 + 1**	11 ± 0.4	15 *vs.* 16, $p < 0.005$	**13**	15 *vs.* 16, $p < 0.05$

[a]represents number of days at which 50% of total flies have been killed.
[b]represents number of days at which 50% of climbing ability is impaired.
Abbreviations: Paraquat, PQ; Galic Acid, GA; K-M, Kaplan-Meier test; n.s., no significance; χ^2, Chi-square test.

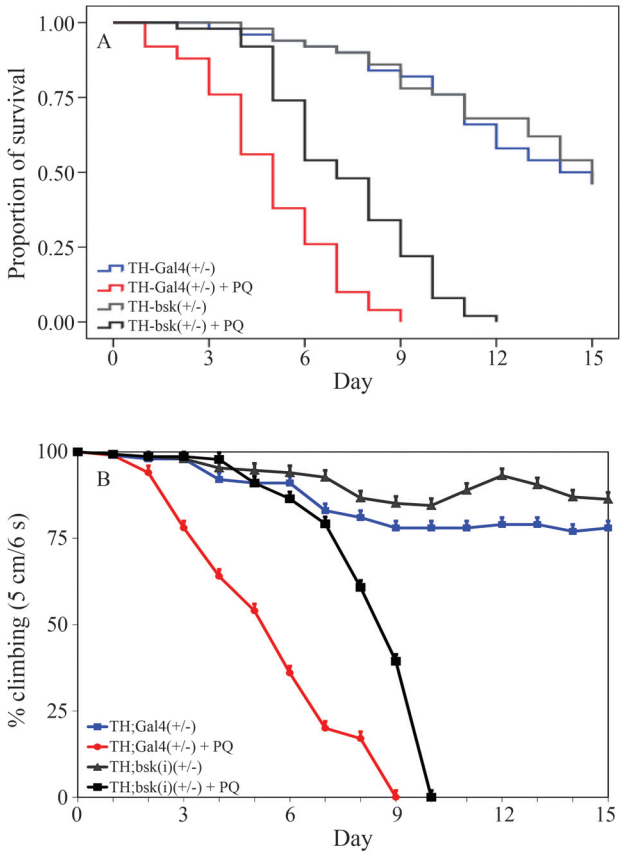

Figure 3 - *Basket* gene knockdown increases life span and locomotor activity in *Drosophila melanogaster* exposed to paraquat. Female flies (n = 50 per treatment) were treated as described in *Materials and Methods*. The graphs show that the proportion of survival and climbing performance were significantly increased in *TH-Gal4*$^{+/-}$, *UAS-bsk RNAi*$^{+/-}$ gene knockdown flies compared to the *TH-Gal4*$^{+/-}$ fly line (control). Statistical comparisons between untreated and treated flies showed: (A) a $p < 0.05$ by log-rank test and (B) a $p < 0.05$ by χ^2 test. Comparisons between treated gene knockdown flies and the *TH-Gal4*$^{+/-}$ fly line showed statistical significance.

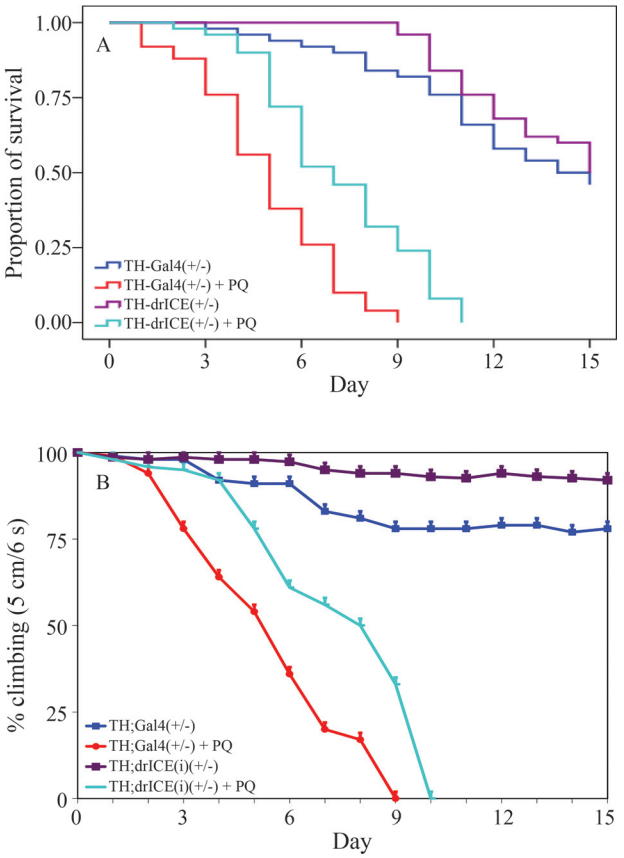

Figure 4 - *DrICE* gene knockdown increases life span and locomotor activity in *Drosophila melanogaster* exposed to paraquat. Female flies (n = 50 per treatment) were treated as described in *Materials and Methods*. The graphs show that the proportion of survival and climbing performance were significantly increased in *TH-Gal4*$^{+/-}$, *UAS-drICE RNAi*$^{+/-}$ gene knockdown flies compared to the *TH-Gal4*$^{+/-}$ fly line (control). Statistical comparisons between untreated and treated flies showed: (A) a $p < 0.05$ by log-rank test and (B) a $p < 0.05$ by χ^2 test. Comparisons between treated gene knockdown flies and the *TH-Gal4*$^{+/-}$ fly line showed statistical significance.

melanogaster lines chronically exposed to PQ compared to controls. These findings comply with the notion that altered gene function, either by mutation or knockdown can modulate the susceptibility to a known environmental PD risk factor such as PQ (Goldman *et al.*, 2012). However, the PQ toxic effect was dependent on the genetic background of the exposed flies. One possible explanation for this finding is that while some DAergic neurons can cope with a rise in OS, others are more vulnerable (Wang and Michaelis, 2010). Because of such selective vulnerability, these neurons are usually the first to exhibit cell death and functional decline (*i.e.* climbing performance).

A second possibility is that some mutated (Goldman *et al.*, 2012) or experimentally knocked down gene(s) may confer resistance to DAergic neurons against PQ-driven OS, thereby increasing life span and locomotor activity. Our data support the latter hypothesis. In fact, *Dmp53*, *basket* and *drICE* RNAi described herein in an *in vivo* system appears to mirror the pharmacological inhibition of p53, JNK, caspase-3 and cell survival previously seen *in vitro* (Jimenez-Del-Rio and Velez-Pardo, 2008). Therefore, these data suggest that PQ induces a molecular mechanism of cell death in DAergic neurons that is similar in *Drosophila melanogaster*, mice (Peng *et al.*, 2004) and primary human cells (Jimenez-Del-Rio and Velez-Pardo, 2008, 2012).

This conclusion is strongly supported by several observations. First, the *Drosophila* genome contains all the genes encoding canonical mitochondrial (Miwa *et al.*, 2003; Oberst *et al.*, 2008) and cell death proteins (Shi, 2004; O'Riordan *et al.*, 2008; Steller, 2008), suggesting that they function in a similar manner as in mammalian cells (see O'Riordan *et al.*, 2008). Indeed, the *Drosophila* Apaf-1 related killer (Dark) assembles in an apoptosome that functions within the intrinsic cell death pathway similar to that seen in mammals (Yuan *et al.*, 2011). During apoptosis, the initiator caspase Dronc (ortholog of the mammalian pro-caspase-9) is activated by the Dark

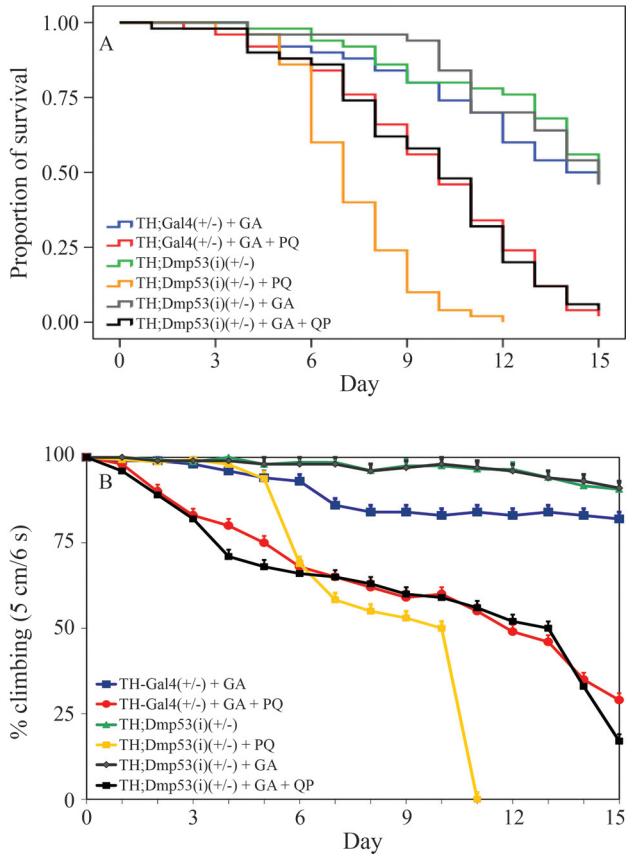

Figure 5 - Gallic acid (GA) increases survival (A) and locomotor activity (B) in gene knockdown *Drosophila melanogaster* lines exposed to paraquat. Female flies (n = 50 per treatment) were treated as described in *Materials and Methods*. The graphs show that the proportion of survival (A) and climbing performance (B) were significantly increased in *TH-Gal4*$^{+/-}$*,UAS-Dmp53 RNAi*$^{+/-}$ flies treated with (1 mM) PQ and (0.1 mM) GA. Similar results were obtained with *TH-Gal4*$^{+/-}$, *UAS-bsk RNAi*$^{+/-}$ and *TH-Gal4*$^{+/-}$, *UAS-drICE RNAi*$^{+/-}$ flies (Table 1). Statistical comparisons between untreated and treated flies showed: (A) a $p < 0.05$ by log-rank test and (B) a $p < 0.05$ by χ^2 test. Comparisons between gene knockdown flies treated with GA plus PQ, and those treated with PQ alone showed statistical significance.

apoptosome. This allows Dronc to cleave the execution caspase DrICE (ortholog of the mammalian caspase-3), which, via proteolytic cleavage of the *Drosophila* inhibitor of caspase-activated DNase (dICAD, Mukae *et al.*, 2000) or caspase-activated DNase (CAD, Yokoyama *et al.*, 2000)), culminates in cell death. Second, stress-activated intracellular response signaling molecules (*e.g.* MAP kinase kinase kinase family proteins) are evolutionary conserved and appears to mirror their mammalian counterparts. Indeed, TAK1 (transforming growth factor β-activated kinase-1) activates JNK (Silverman *et al.*, 2003) probably via D-MEKK1, the *Drosophila* ortholog of mammalian MEKK4/MTK1 (Ryabinina *et al.*, 2006). Third, pharmacological treatment with the JNK inhibitor SP600125 has been demonstrated to protect and rescue flies against acute PQ intoxication (Jimenez-Del-Rio *et al.*, 2008).

Interestingly, it has been shown that a Dmp53/JNK (basket)-dependent feedback amplification loop is essential for the apoptotic response to stress in *Drosophila* (Shlevkov and Morata, 2012). It is therefore reasonable to think that PQ might be able to trigger activation of Dmp53, basket and drICE via $O_2^{\cdot-}$/ H_2O_2 signaling and cell death (Steller, 2008) in a similar fashion as proposed in mammalian cells (Jimenez-Del-Rio and Velez-Pardo, 2012). In accordance with this view, several reports have shown that PQ induces activation of JNK associated with OS and cell death *in vitro* and *in vivo* in mammalian DAergic neurons (Chun *et al.*, 2001; Peng *et al.*, 2004; Klintworth *et al.*, 2007; Ramachandiran *et al.*, 2007; Choi *et al.*, 2010; Niso-Santano *et al.*, 2010). But these observations stand in contradiction with others that have shown that overexpression of JNK (Wang *et al.*, 2003; Inamdar *et al.*, 2012) or overexpression of JNK target genes, such as human peroxiredoxin II (hPrxII) and Jafrac1 (a *Drosophila* homolog of hPrxII) (Lee *et al.*, 2009) confer protection against PQ-induced toxicity in DAergic neurons of the fly. The reason for these contradictory observations is not yet known. Therefore, further research will be required to clarify this issue.

Finally, PQ induces PD-like clinical features in *Drosophila,* including resting tremor, bradykinesis, and postural instability with or without neuronal damage. A similar PD-like phenotype can be generated by pharmacological inhibition of the tyrosine hydroxylase enzyme with α-MT (alpha-methyl-tyrosine). In these experiments no neuronal damage was observed, but functional impairment (climbing performance) was evident (Bonilla-Ramirez *et al.*, 2011).

In conclusion, these data suggest that the knockdown of specific gene(s) in the *Drosophila* brain may provide basic information about the mechanism(s) of cell death/survival and clinical behavior in human PD.

Polyphenols have been postulated as potential neuroprotectant molecules in neurodegenerative disorders including PD (Albarracin *et al.*, 2012). In agreement with others (Long *et al.*, 2009; Peng *et al.*, 2011) and our previous investigations (Jimenez-Del-Rio *et al.*, 2010; Ortega-Arellano *et a*l., 2011), we found that GA prolongs life span and climbing activity in wild type or knocked down *D. melanogaster.* These data suggest that GA (and probably other polyphenols) may protect DAergic neurons independently of the genetic background of the fly, either via direct interaction with ROS, enzymes, receptors and/or transcription factors (Fraga *et al.*, 2010), or through antioxidant gene regulatory mechanisms (Kim *et al.*, 1997; Peng *et al.*, 2011). Our findings suggest that GA can protect DAergic neurons against PQ. Consequently, this polyphenol is capable of modifying the life span and locomotor capabilities of flies exposed to OS stimuli.

In summary, induced RNAi of specific pro-apoptotic genes in DAergic neurons in *D. melanogaster* increased survival and climbing performance under PQ treatment.

Since polyphenols such as GA displayed antioxidant capacity, they may certainly contribute to develop nutritional strategies oriented towards preventing PD. Our data suggest that pharmacologically targeted proteins or knockdown of critical death signaling genes, such p53, JNK and caspase-3 in DAergic neurons, together with antioxidant exposure, may retard neural deterioration or neuronal loss, thereby, restoring or prolonging locomotor activity in PD patients. In addition, our *in vivo* data on flies link directly to *in vitro* work done on OS-induced cell death in mammalian cells (Shi, 2004; O'Riordan *et al.*, 2008; Steller, 2008; Jimenez-Del-Rio and Velez-Pardo, 2012). Understanding the mechanism(s) by which dopaminergic neurons are eroded in PD is critical for the development of effective therapeutic strategies.

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