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Original article

Insights into the differential toxicological and antioxidant effects of 4-phenylchalcogenil-7-chloroquinolines in *Caenorhabditis elegans*

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ABSTRACT

Organic selenium and tellurium compounds are known for their broad-spectrum effects in a variety of experimental disease models. However, these compounds commonly display high toxicity and the molecular mechanisms underlying these deleterious effects have yet to be elucidated. Thus, the need for an animal model that is inexpensive, amenable to high-throughput analyses, and feasible for molecular studies is highly desirable to improve organochalcogen pharmacological and toxicological characterization. Herein, we use *Caenorhabdtis elegans (C. elegans)* as a model for the assessment of pharmacological and toxicological parameters following exposure to two 4-phenylchalcogenil-7-chloroquinolines derivatives (PSQ for selenium and PTQ for tellurium-containing compounds). While non-lethal concentrations (NLC) of PTQ and PSQ attenuated paraquat-induced effects on survival, lifespan and oxidative stress parameters, lethal concentrations (LC) of PTQ and PSQ alone are able to impair these parameters in *C. elegans*. We also demonstrate that DAF-16/FOXO and SKN-1/Nrf2 transcription factors underlie the mechanism of action of these compounds, as their targets *sod-3*, *gst-4* and *gcs-1* were modulated following exposures in a *daf-16*- and *skn-1*-dependent manner. Finally, in accordance with a disturbed thiol metabolism in both LC and NLC, we found higher sensitivity of *trxr-1* worm mutants (lacking the selenoprotein thioredoxin reductase 1) when exposed to PSQ. Finally, our study suggests new targets for the investigation of organochalcogen pharmacological effects, reinforcing the use of *C. elegans* as a powerful platform for preclinical approaches.

1. Introduction

Organoselenium and organotellurium compounds have been widely studied, mainly due to their pharmacological and toxicological properties [1–3]. Among their many applications are anti-inflammatory, immunomodulatory, hepatoprotective, anti-cancer, as well as anti-Parkinson's and Alzheimer's diseases activities [4–7]. Given their wide-range spectrum of reported effects, organoselenium and organotellurium putative mechanisms of action have been reported. For example, by mimicking glutathione peroxidase (GPX) activity, most organochalcogens effects might be explained by impinging upon redox homeostasis [8]. In addition, interactions with the thioredoxin system (Trx/TrxR) have also been suggested [9,10]. Finally, thiol oxidation or reduction processes appear to be involved in almost all mechanisms of action already described for these compounds [11].

It is commonly hypothesized that antioxidant molecules can act in ways other than simply scavenging free radicals. By modulating the activity and/or expression of endogenous antioxidants, such as superoxide dismutase (SOD), catalase (CAT) and GPX [12–14], antioxidant compounds might improve endogenous defenses against oxidative stress. However, no studies have been carried out on these endogenous antioxidants and the pathways that regulate them as potential targets for antioxidant organoselenium and organotellurium molecules.

Complementary biological models, such as *C. elegans*, offer a powerful tool at the initial steps of basic pre-clinical research. Importantly, a high number of worm genes have clear orthologs in mammals, including many genes associated with human diseases [15]. The antioxidant and detoxification response in the nematode is well conserved

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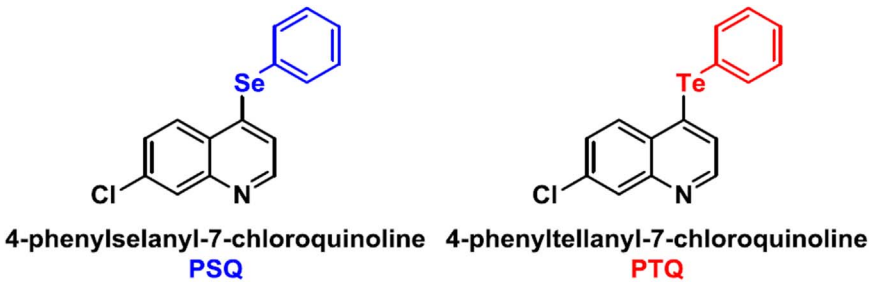

Fig. 1. Structure of 4-phenylselanyl-7-chloroquinoline (PSQ) and 4-phenyltellanyl-7-chloroquinoline (PTQ).

with central regulator proteins such as FOXO/DAF-16, Nrf2/SKN-1, which are also subject to regulation by antioxidants in mammals [14,16–19].

Our group has previously described how two Se- and Te-containing organochalcogen analogues changed the cellular sub-localization of DAF-16 in *C. elegans* [20,21]. Moreover, we described that a range of 4-organylsulfenyl-7-chloro quinolines exerted antioxidant protective effects in *C. elegans* by up-regulating SOD-3 and CTL-1,2,3 GFP reporters levels in a putative hormetic mechanism involving DAF-16 nuclear translocation and transcriptional activation [22]. However, the broad spectrum of action of Se- and Te-containing molecules observed in previous studies may not be explained only by the regulation of the aforementioned proteins. A pilot study performed in our lab demonstrated that Se and Te analogues of the 4-phenylthio-7-chloroquinoline (PSQ and PTQ, respectively, Fig. 1) protected worms from paraquat treatment. These compounds were previously described for their antioxidant action *in vitro* [23], but no *in vivo* study was conducted. Thus, we investigated how these two new compounds, 4-phenylselanyl- and 4-phenyltellanyl-7-chloroquinoline exert protective or toxic effects in *C. elegans* and found that *daf-16* and *skn-1* dependent defensive pathways underlie the toxicity and pharmacology of these compounds.

2. Materials and methods

2.1. General procedure for the synthesis of PSQ and PTQ

4-Phenylselanyl-7-chloroquinoline and 4-phenyltellanyl-7-chloroquinoline were synthesized and characterized as previously described [23]. DMSO (1.0 mL) and KOH (0.3 mmol) were added to a round-bottomed flask containing diphenyl diselenide or diphenyl ditelluride (0.15 mmol) and 4,7-dichloroquinoline (0.3 mmol). The reaction mixture was allowed to stir at 100 °C for 24 h. Next, the solution was cooled to room temperature, diluted with ethyl acetate (15 mL), and washed 3 times with 15 mL water. The organic phase was separated, dried over $MgSO_4$, and concentrated under vacuum. The obtained products were purified by flash chromatography on silica gel using a mixture of ethyl acetate/hexane (20:80) as the eluent. The ^1H NMR and NMR spectra of obtained compounds were in full agreement with their assigned structures.

2.2. C. elegans strains and maintenance

NGM plates seeded with live *Escherichia coli* OP50 bacteria were used for growth and maintenance of the worms, as previously described by Brenner [24]. The *C. elegans* strains Bristol N2 (wild type); CF1553, *muls84 [sod-3p::gfp; rol-6(su1006)];* GA800, *wuls151 [ctl-1(+); ctl-2(+); ctl-3(+); myo-2p::gfp]*; CL2166, *dvIs19* [(*gst-4p*::*gfp*::*nls*] III; LD1171, *ldIs3* [*gcs-1p*::*gfp*; *rol-6*(*su1006*);)]; TJ356, *zIs356* [*daf-16p*::*daf-1a/b*::*gfp*; *rol-6*(*su1006*)]; CF1038, *daf-16(mu86) I*; VC1772, *skn-1*(*ok2315*) *IV/nT1 [qIs51] (IV;V)*; RB1823, *gst-4* & *msp-38*(*ok2358*) IV; VC337, *gcs-1(ok436)/mIn1 [mIs14 dpy-10(e128)] II*; GA480, *sod-2(gk257) I; sod-3(tm760) X*; VC754, *ctl-2(ok1137) II*; PS3551, *hsf-1(sy441) I*; CF1407,*daf-16(mu86) I*; *muls71[daf-1ap::gfp::daf-1a(bKO); rol-6(su1006)]* and VB1414, *trxr-1(sv47) IV* were maintained and handled at 20 ± 1 °C. We also generated three strains: *skn-1*(*ok2315) IV/nT1 [qIs51] (IV;V); muls84 [sod-3p::gfp; rol-6(su1006)]*; *skn-1*(*ok2315) IV/nT1 [qIs51] (IV;V); ldIs3* [*gcs-1p*::*gfp*; *rol-6*(*su1006);*)]; *skn-1*(*ok2315) IV/nT1 [qIs51] (IV;V); dvIs19* [(*gst-4p*::*gfp*::*nls*] *III* using standard methodology [25]. Synchronous L1 population was obtained by isolating embryos from gravid hermaphrodites using bleaching solution (1% NaOCl; 2,4% NaOH), followed by M9 buffer washing until all bleaching solution was removed from the egg preparation, as previously standardized [24].

2.3. Compounds exposure and survival/LD_{50} assessment

Approximately 1500 synchronized L1 worms were exposed for 30 min to the compounds in liquid media containing 0.5% NaCl (5% DMSO from compounds stock solution) in absence of bacteria. L1 worms were chosen for these treatments because exposure at the L1 larval stage offers the possibility to observe effects during the entire lifespan of the worm and because the treatment can be done in absence of *Escherichia coli*, thus eliminating the possibility of bacterial metabolism. LD_{50} curves for PSQ and PTQ were obtained by using concentrations that ranged from 1 μM to 1000 μM, and then nonlethal doses were chosen for stress-resistance assay. At the end of treatment, worms were washed three times in 0.5% NaCl to remove the treatments and transferred onto NGM recovery plates seeded with *E. coli* OP50 (these procedures were performed for all assays). Stress resistance was conducted by exposing worms to 500 μM paraquat (Gramoxone 200 ®) for 30 min prior to compound treatment. Paraquat was removed before compound treatment, which also lasted 30 min. Nonlethal concentrations chosen were 10 μM PSQ and 1 μM PTQ. Lethal concentrations were also chosen according to LD_{50} values (560 μM PSQ, 42 μM PTQ) for the toxicological assessments. All the experiments were carried out in duplicate and repeated independently at least four times.

2.4. Lifespan, development, and reproduction

We assessed the lifespan of 20 worms per plate in duplicate for each group/genotype treated with PSQ and PTQ at both LC and NLC. All experiments were repeated 3 or 4 times, with 120–160 worms assayed per each group/genotype. Worms were transferred daily into a new NGM/OP50 plate until death. Worms were scored as dead when no pharyngeal pumping was observed and there was no response to gentle prodding. We also evaluated worms development by scoring all of them into each of the larval stages of development, 48 h after compound exposure, following standard phenotypes [24]. Reproduction was also evaluated by scoring the total number of laid eggs during the first 3 days of their fertile period.

2.5. Fluorescence measurement

Fluorescence was measured using a plate reader CHAMELEON™V Hidex Model 425-106. All experiments were carried out in duplicate and repeated independently at least five times.

2.5.1. GFP-tagged protein levels

Fluorescence levels of transgenic worms expressing GFP reporter for proteins of our interest were measured at emission 535 nm/excitation 485 nm using 2000 worms at L4 larval stage and normalized to protein levels.

2.5.2. Reactive oxygen species (ROS)

1500 treated worms were incubated for 1 h in M9 buffer containing 50 μM H_2DCF-DA. Non-incorporated dye was removed by consecutive washes. Fluorescence levels were measured by using emission 535 nm/excitation 485 nm for 30 min in order to obtain a kinetic curve for ROS.

2.5.3. Reduced and oxidized glutathione ratio (GSH/GSSG)

2000 worms treated in M9 buffer were frozen and thawed 3×, then homogenized by sonication and an ortho-phtalaldehyde solution (2 mg/mL) was added, according to Cohn and Lyle [26] with minor modifications in order to be adapted to 96-well plates assay. Fluorescence levels were measured by emission 528 nm/excitation 485 nm.

2.6. Thiobarbituric Acid Reactive Species (TBARS) and Non-proteic reduced thiols (NPSH) levels

Malondialdehyde (MDA) levels were assessed following the protocol described by Ohkawa et al. [27], with minor modifications in order to adapt the experiment to 96-well plates. Non-protein levels of –SH groups were determined following the protocol described by Ellman [28], using worm homogenates (2000 worms frozen and thawed 3×, sonicated and re-suspended in M9 buffer), with minor modifications in order to adapt the experiment to 96-well plates.

2.7. Gene expression

Relative levels of gene expression following PSQ and PTQ treatments *per se* were measured using TaqMan gene expression assay probes (Life Technologies ®). For this purpose, we used Trizol (Life Technologies ®) in order to extract total RNA following compounds exposure in L1 worms. 1 μg of total RNA was used to synthesize cDNA using High Capacity cDNA Reverse Transcription Kit (Life Technologies ®). Quantitative reverse-transcription PCR (BioRad ® CFX96) was conducted in triplicates using *afd-1* (β-actin homolog) gene as housekeeping. We determined fold difference using comparative $2^{-\Delta\Delta Ct}$ method. The following probes were used: *gst-4* (Ce02458730_g1), *gcs-1* (Ce02436726_g1), *sod-3* (Ce02404515_g1), *afd-1* (Ce02414573_m1). *trxr-1* primer was as previously reported [29].

2.8. Statistical analysis

All the figures and statistical analysis were generated with GraphPad Prism 6 (GraphPad Software Inc.). Dose-response curves were generated in order to obtain the LD_{50} for each compound. To analyze ROS levels, a repeated measures ANOVA and the Tukey *post-hoc* test were applied. Lifespan was analyzed by Kaplan-Meier Log Rank test. A one-way ANOVA was applied for all the other assays, followed by Tukey *post-hoc* test. *p* values lower than 0.05 were considered statistically significant.

3. Results

3.1. PSQ is less toxic than PTQ and both reverse oxidative damage induced by paraquat in short and long-term manners

We determined that PSQ ($LD_{50:}$ 560 μM) was less toxic than its tellurium analogue PTQ ($LD_{50:}$ 42 μM) (Fig. 2a). LD_{50} approximate values (600 μM for PSQ and 50 μM for PTQ, to minimize pipetting/dilutions errors) were used in all toxicological assessments and used as lethal concentrations (LC). LC caused a significant lifespan decrease (Fig. 2b), delayed development (only for PTQ at LC Fig. 2c), decreased egg-laying (Fig. 2d), and increased ROS levels (Fig. 2e). Next, we tested multiple sub-lethal concentrations (LD_{95}) (data not shown) and based on that we selected 10 μM (PSQ) and 1 μM (PTQ) as non-lethal concentrations (NLC) for pharmacological assays. PSQ and PTQ at NLC reversed paraquat-induced mortality (Fig. 2f) and reduced paraquat-induced ROS levels in L4 worms (Fig. 2g). Furthermore, paraquat-induced a decrease in lifespan that was restored by PTQ but not by PSQ post-treatment (Fig. 2h).

3.2. Redox-related parameters suggest a common mechanism between PSQ and PTQ toxicology and pharmacology

PSQ and PTQ at LC increased TBARS levels in worms (Fig. 3a); PTQ decreased non-proteic -SH groups (Fig. 3b) and reduced GSH/GSSG ratio (Fig. 3c), whereas PSQ did not change these parameters. Both PTQ and PSQ, at NLC, restored all these parameters in paraquat-treated worms (Fig. 3a, b, and c).

3.3. daf-16 and skn-1 pathways are involved in stress resistance provided by PSQ and PTQ at non-lethal concentrations

DAF-16 and some of its targets, *sod-3* and *ctl-2,* are involved in acute and long-term stress resistance after food restriction and exposure to high temperatures [30]. Herein, we observed that *sod-3* and *daf-16* were both necessary for PTQ and PSQ to rescue from paraquat-induced mortality (Fig. 3d). Furthermore, *sod-3* was necessary for PTQ-restoring effect against paraquat-induced lifespan reduction (Table 1). *sod-3* mRNA and SOD-3::GFP levels increased soon after PTQ and PSQ exposure at NLC (Table 2), whereas *daf-16* knockout suppressed the increase of *sod-3* mRNA and SOD-3::GFP levels. In contrast, *ctl-2,* the major catalase isoform in *C. elegans*, does not appear to be involved in any of these processes (Fig. 3d and Table 1). Accordingly, we did not evaluate its expression or GFP levels in the present study.

gcs-1 and *gst-4* are both transcriptional targets of SKN-1 and their deletion blunted the protective effect of PSQ and PTQ against paraquat-induced mortality (Fig. 3e). In addition, only *skn-1* and *gcs-1* were necessary for PTQ-induced lifespan recovery following paraquat exposure (Table 1). In addition, PSQ at NLC reduced *gst-4* and increased *gcs-1* and *sod-3* mRNA levels, which was corroborated by protein GFP levels (Table 2). However, *skn-1* knockout did not change any expression pattern observed in wild-type worms after exposure to NLC of PSQ.

3.4. daf-16 and skn-1 pathways are involved in PSQ and PTQ toxicity

Regarding the DAF-16 pathway, both PSQ and PTQ were significantly more toxic in the absence of *sod-3* (Fig. 4a and b). *sod-3* mRNA and SOD-3::GFP levels were increased soon after PTQ and PSQ exposure at LC (Table 3). In addition, in a *skn-1* deficient background, *sod-3* expression was not affected.

With respect to *skn-1* and its targets, both PTQ and PSQ were significantly more toxic in the absence of *gst-4* (Fig. 4a and b). Moreover, PTQ at LC increased both *gst-4* mRNA and GFP levels, whereas *skn-1* knockout blocked the increase in *gst-4* mRNA and GFP levels. PSQ at LC did not cause changes in *gcs-1* and *gst-4* levels (Table 3).

3.5. Other stress resistance genes may also be involved with PSQ toxicity

In addition to *daf-16* and *skn-1*, *hsf-1* is central to *C. elegans* redox state, controlling aging and immunity by means of heat-shock proteins expression [31]. We further investigated whether heat shock transcription factor 1, encoded by *hsf-1* in *C. elegans,* is involved in PSQ and PTQ effects. We found that none of the observed changes were due to *hsf-1* modulation (Fig. 3f and Table 1). Additionally, we investigated the single selenium containing protein in *C. elegans* proteome, thioredoxin reductase 1, which is encoded by *trxr-1*. Interestingly, we found

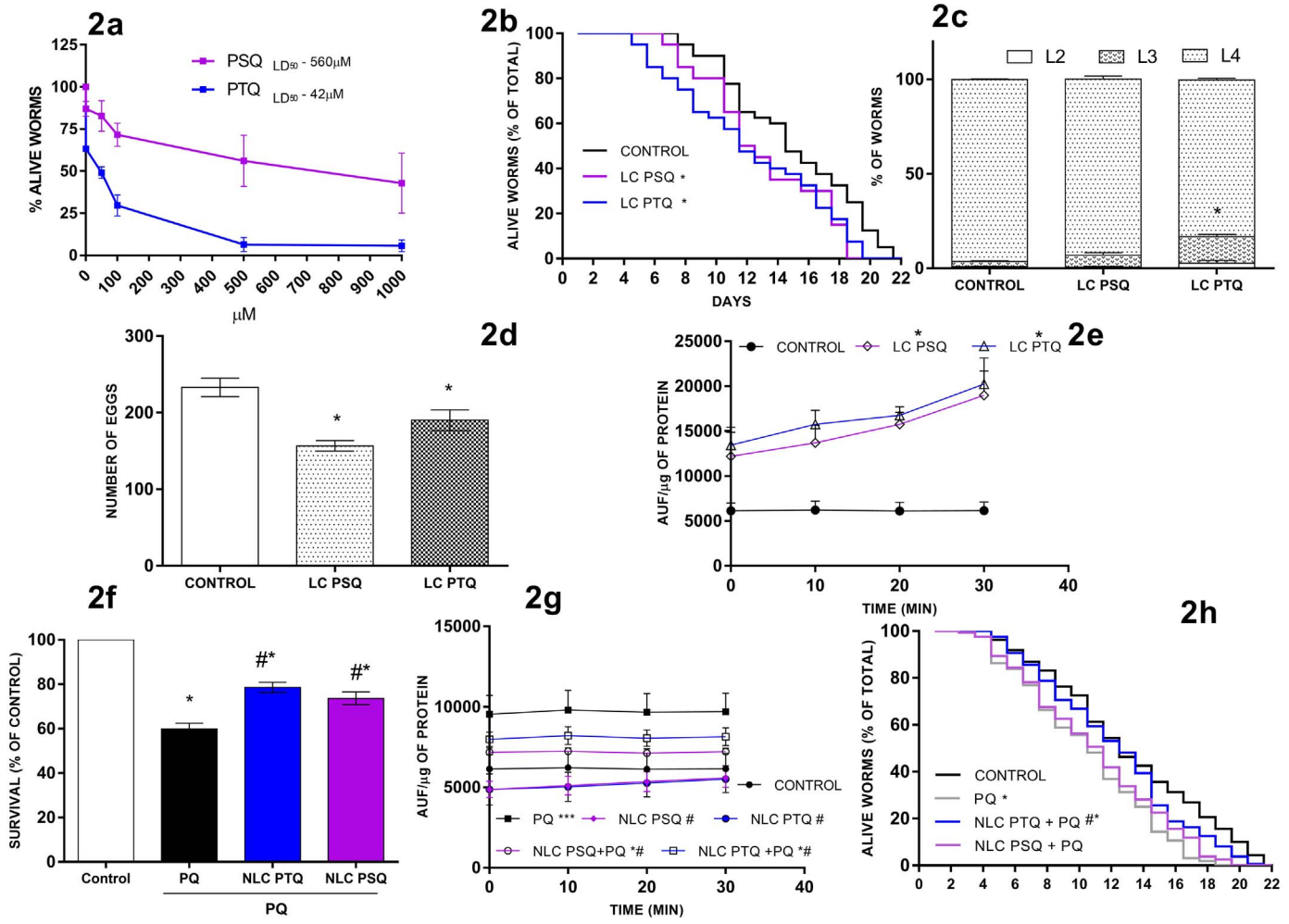

Fig. 2. Physiological alterations after LC and NLC exposure A) LD50 survival curves. Concentrations are represented as log of μM for better plotting. B) Lifespan was significantly decreased in worms exposed to LC PSQ and PTQ. C) Development was impaired after LC PTQ exposure, as shown by the higher number of worms that did not reach the L4 stage at the proper time. D) Number of eggs is reduced after both LC PTQ and PSQ exposure. E) H2DCF-DA oxidation in all curve points. F) Survival is decreased by paraquat, but both NLC PSQ and PTQ attenuate it. G) ROS formation is higher in the PQ only group, but both NLC compounds partially rescue it. H) Lifespan is shortened by PQ, but NLC PTQ is able to partially recover it. * means $p < 0.05$ or higher significance when compared to control. # means difference when compared to PQ-only group.

it to be involved in PSQ toxicity, as in its absence PSQ toxicity was significantly increased (Fig. 4a). Furthermore, in wild-type worms *trxr-1* expression was increased following PSQ toxic exposure (Table 3). Accordingly, this enzyme appears to be important in protecting worms from PSQ toxicity.

4. Discussion

Here, we demonstrated that 4-phenylchalcogeno-7-chloro quinolines containing Se (PSQ) or Te (PTQ) display both anti and pro-oxidant properties *in vivo* in *C. elegans,* depending on the compound's concentration tested. In fact, redox regulation is coupled with pharmacological and toxicological effects of both chalcogen-based compounds, as illustrated by thiol modification patterns and complex modulation of genes involved in cellular redox control. Additionally, TRXR-1, the sole *C. elegans* selenoprotein, appeared to be involved in mediating PSQ toxicity.

LD_{50} values were found to be 560 μM and 42 μM for PSQ and PTQ, respectively (Fig. 2a). In contrast, we have previously reported that LD_{50} values for a Te compound (telluroxylofuranoside), belonging to another class of organochalcogens, was considerably lower than its Se analogue [20]. However, as Se is an essential element for many forms of life [32] and Te may only be found in several bacteria, but not in more complex organisms [33,34], it is reasonable that PSQ would be less toxic than PTQ. We also confirmed that the toxic effects were indeed linked to the presence of Se and Te by using 4,7-chloroquinoline (Q), an analogue molecule lacking these atoms, which showed a higher LD_{50} (Fig. S1ab).

In addition, PSQ and PTQ at LC decreased lifespan, impaired development (PTQ only), reproduction, and increased the amount of ROS. The chemical structure and concentration of organochalcogens are likely determinants of their cellular disposition as pro- or anti-oxidants. Diaryl diselenides and aliphatic or aromatic monoselenides are known to consume thiols prior to becoming nucleophiles and behaving as toxic electrophiles [2,35]. Thus, when using high concentrations of both compounds, we posit a shift in the balance between pro- and anti-oxidant forms.

At non-lethal concentrations (NLC), both PSQ and PTQ were able to attenuate paraquat-induced effects on viability (Fig. 2f), ROS levels (Fig. 2g), lifespan (PTQ only) (Fig. 2h), TBARS levels (Fig. 3a) and GSH/GSSG ratio (Fig. 3c), thus indicating that restoration of worms' redox state improves viability and increases their lifespan [40–42]. It was previously reported that a natural antioxidant, '*açaí*' berry, was able to reduce ROS levels and prevent -SH oxidation and thus able to ameliorate stress resistance in *C. elegans* [45]. Notably, **Q** failed to restore paraquat-induced impairments on viability and lifespan (Fig. S2ab), establishing that Se and Te are necessary for PSQ and PTQ pharmacological action. Organochalcogens have been previously

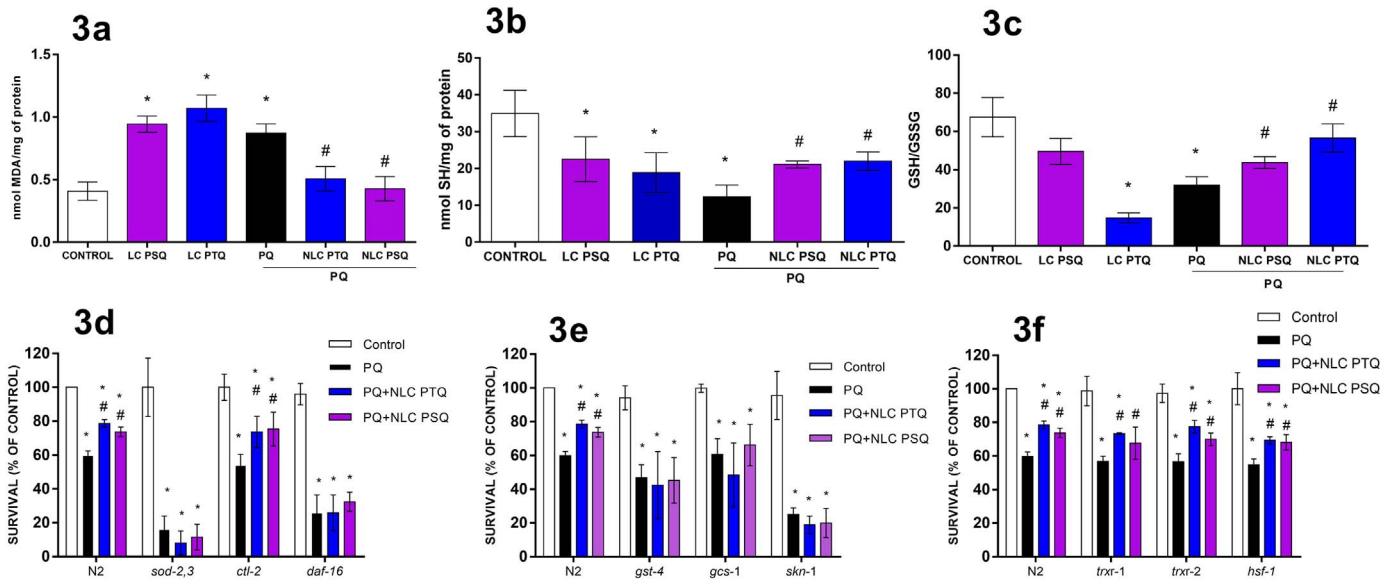

Fig. 3. Effects of PQ, PTQ and PSQ on redox markers and viability A) Thiobarbituric acid reactive species increased greatly after both PTQ and PSQ lethal concentrations exposure. PQT also increased TBARS, but PTQ and PSQ non-lethal concentrations fully restored them. B) Non-proteic SH groups were reduced by PTQ and PSQ lethal concentrations exposure. PTQ also reduced NPSH levels, but PSQ and PTQ post treatment was able to partially recover them. C) GSH/GSSG ratio. D) Survival percentage after paraquat followed by PSQ or PTQ, where * means $p < 0.05$ or higher significance when compared to control. E) Mutants related to the *skn-1* pathway. F) Thioredoxin reductase system and HSF-1. * means $p < 0.05$ or higher significance when compared to control, and # means difference from paraquat-only group.

demonstrated to protect and reverse damage induced by various ROS-based toxicants [20,21,36–39,43,44].

In contrast to the results found at NLC of PSQ and PTQ, at LC the molecules disrupted thiol balance and induced lipid peroxidation (demonstrated with TBARS) in *C. elegans*. Accordingly, organochalcogens have thiol oxidase-like activity, and it is known that thiol homeostasis is essential for *C. elegans* molting and development [29]. Therefore, high amounts of these molecules might impair these functions by modifying thiol redox state. These alterations might have contributed to impaired lifespan, reproduction and development [40–42]. The excessive oxidation of thiols caused by high concentrations of organochalcogenides indicates a possible vicious cycle of thiol oxidation and consequent propagation and amplification of ROS.

To further explore the molecular effects of the organochalcogens on endogenous pathways, we analyzed gene expression patterns. Among the screened *C. elegans* mutants, we observed that the lack of *sod-3* (mitochondrial superoxide dismutase) renders PSQ and PTQ-treated worms more susceptible to paraquat (Fig. 3d and Table 1). We observed increased *sod-3* mRNA expression and SOD-3::GFP levels in wild-type worms treated with PSQ and PTQ at NLC (Table 2). These results

Table 1
Lifespan data. All lifespan curves were analyzed using Kaplan-Meier Log Rank analysis. *sod-3, skn-1* and *gcs-1* were necessary for lifespan recovery by PQT exposure. $p < 0.05$ or less was interpreted as statistically significant.

Genotype	Treatment	Mean lifespan (days)	KM analysis (versus vehicle)	KM analysis (versus PQ)	Number of worms (3 different experiments)
	Vehicle	14.8	–	–	160
wt	PQ	11.9	P = 0.0094	–	160
	PQ + NLC PTQ	13.2	P = 0.0341	P = 0.0362	162
	Vehicle	13	–	–	120
daf-16	PQ	8.3	P = 0.0013	–	116
	PQ + NLC PTQ	14.6	P = 0.0484	P < 0.0001	110
	Vehicle	13.4	–	–	120
sod-3	PQ	9.8	P = 0.0096	–	120
	PQ + NLC PTQ	10.4	P = 0.0375	P = 0.7422	101
	Vehicle	13.6	–	–	120
ctl-2	PQ	10.9	P = 0.0109	–	131
	PQ + NLC PTQ	14	P = 0.7891	P = 0.0093	120
	Vehicle	9.8	–	–	84
skn-1	PQ	7.3	P = 0.0483	–	82
	PQ + NLC PTQ	7.1	P = 0.0492	P = 0.9551	80
	Vehicle	14.1	–	–	160
gcs-1	PQ	11.6	P = 0.0142	–	155
	PQ + NLC PTQ	12.1	P = 0.0401	P = 0.0850	160
	Vehicle	11.6	–	–	164
gst-4	PQ	8.7	P = 0.0099	–	160
	PQ + NLC PTQ	13.2	P = 0.0213	P < 0.0001	160
	Vehicle	13.5	–	–	120
hsf-1	PQ	12.1	P = 0.0523	–	122
	PQ + NLC PTQ	15.4	P = 0.0103	P = 0.0076	120
	Vehicle	13.2	–	–	116
trxr-1	PQ	9.9	P = 0.0013	–	114
	PQ + NLC PTQ	12.6	P = 0.0626	P = 0.0046	120

Table 2
mRNA and GFP levels after NLC PSQ and PTQ exposure in different mutant strains. mRNA is expressed as fold change relative to the *afd-1* housekeeping gene, compared to a non-treated group for statistical purposes. GFP is expressed as % of control relative to AUF/mg (Arbitrary Units of Fluorescence) of protein in each sample. mRNA levels were measured immediately in exposed L1 worms, while GFP levels were measured 48 after exposure in L4 worms. That was done trying to match expression and protein levels in more a relevant scenario for *C. elegans*, which is during the L4 stage. * means $p < 0.05$ or higher significance. (There is no available TRXR-1::GFP strain).

Strain	Gene	Treatment (NLC)	Fold change	*T*-test (vs. vehicle)	GFP AUF/mg protein (%)	*T*-test (vs. vehicle)
wt	*sod-3*	PSQ	1.424 ± 0.2507	*	157.11 ± 21.001	*
daf-16	*sod-3*	–	0.9106 ± 0.09184	–	89.122 ± 9.334	–
skn-1	*sod-3*	–	1.479 ± 0.1617	*	166.784 ± 15.47	*
wt	*gcs-1*	–	1.431 ± 0.2543	*	198.78 ± 20.51	*
skn-1	*gcs-1*	–	1.3782 ± 0.1635	*	144.3 ± 12.114	*
wt	*gst-4*	–	0.6259 ± 0.04605	*	67.144 ± 10.23	*
skn-1	*gst-4*	–	0.5364 ± 0.0372	*	71.02 ± 7.114	*
wt	*trxr-1*	–	1.034 ± 0.147	–	–	–
wt	*sod-3*	PTQ	2.087 ± 0.2244	*	170.59 ± 22.15	*
daf-16	*sod-3*	–	0.8241 ± 0.04147	–	87.44 ± 13.21	–
skn-1	*sod-3*	–	1.716 ± 0.3280	*	136.732 ± 7.45	*
wt	*gcs-1*	–	0.9505 ± 0.1779	–	178.8544 ± 31.452	*
skn-1	*gcs-1*	–	1.0126 ± 0.1235	–	152.54 ± 18.844	*
wt	*gst-4*	–	0.8525 ± 0.1194	–	104.48 ± 15.478	–
skn-1	*gst-4*	–	0.8364 ± 0.0372	–	95.475 ± 8.678	–
wt	*trxr-1*	–	0.9482 ± 0.08177	–	–	–

corroborate that the protective effects of these compounds are related to increased *sod-3* expression. Notably, *sod-3* overexpression and/or overactivation are necessary for the induction of pro-longevity response after mild oxidative damage [46]. The quinolinic ring present in these molecules, at high concentrations, may become a superoxide generator [47], explaining why LC upregulated *sod-3* levels and why *sod-3* mutants were more vulnerable to PSQ and PTQ at LCs (Fig. 4a). Thus, higher *sod-3* expression levels might represent a compensatory response to a xenobiotic such as PTQ and PSQ at LC, which was associated with increased ROS, MDA and oxidized thiols.

*gst-4 (*glutathione-s-transferase) is commonly used as a stress marker in *C. elegans* [48], and indeed we have found it to be overexpressed following PTQ at LC exposure (Table 3), thus suggesting its involvement in a detoxification mechanism. Conversely, increased PTQ toxicity in *gst-4* mutants was found (Fig. 4b), reinforcing its detoxifying role. A systematic screen with different xenobiotics found that GST-4, as many other GSTs, possesses a wide range of different substrates [49], thus we cannot state whether our observations are due to direct interaction between PTQ and GST-4 or between ROS sub products and GST-4. As GST-4 consumes GSH, it was not surprising that the levels of GCS-1::GFP, the limiting step on glutathione synthesis, were increased following PTQ exposure at LC (Table 3). In agreement, at LC we observed a decrease in -SH groups and an increase in the oxidized form of GSH (Fig. 3b and c).

Reinforcing the hypothesis that PSQ and PTQ shift between toxic and beneficial effects, *gcs-1* and *gst-4* were necessary for worm lifespan recovery upon paraquat exposure (*gcs-1* only for PTQ). Notably, *gcs-1* mRNA levels were increased following PSQ and PTQ exposure at NLC (Table 3). Higher levels of this enzyme might have assisted in replenishing GSH levels (Fig. 3b and c). Other investigations suggest the role of γ-glutamyl cysteine synthetase (*gcs-1*) and its regulator, *skn-1*, in the cytoprotective effect induced by antioxidant compounds in different model organisms [50–55].

In order to determine whether these organochalcogens were regulating antioxidant pathways to promote indirect cytoprotection via activation of antioxidant pathways, the expression of DAF-16 and SKN-1 targets were accessed. DAF-16 and SKN-1 transcription factors work in parallel to regulate numerous redox coupled processes by inducing, among many others, *sod-3*, *gcs-1* and *gst-4* genes expression [56,57]. Both transcription factors are responsive to oxidative stress and play a role in lifespan extension and stress resistance [58]. Our results demonstrate *daf-16*-dependent effects for both PSQ and PTQ in restoring paraquat-induced mortality (Fig. 3d) as well as induction of *sod-3* expression (Table 3). Organic Se and Te containing compounds might act as thiol modifiers, for instance, by oxidizing cysteines at Keap1, which allows Nrf2 activation, the human homolog of SKN-1 [59,60]. *skn-1* is necessary for viability and lifespan restoration (only PTQ for lifespan) following paraquat exposure (Fig. 3e and Table 1). As *skn-1* is also able to upregulate *sod-3* expression [61], it is reasonable that *skn-1* and *sod-3* are necessary for lifespan restoration by PTQ at NLC (Table 2). The fact that SKN-1 can also up-regulate *sod-3* mRNA levels may explain why *daf-16* is not necessary for lifespan extension by PTQ at NLC (Table 1). Notably, it has been reported that a quinolinic derivative was capable of improving antioxidant response by means of DAF-16 and SKN-1 regulation [62].

Overexpression of *gcs-1* induced by NLC of PSQ and PTQ was not abolished in *skn-1* mutants (Table 2). It was previously reported that SKN-1 is only able to control *gcs-1* expression in worms' neurons, but not in the pharynx [63]. This indicates that *gcs-1* may be regulated by another transcriptional factor when worms are exposed to PSQ or PTQ. When lethal concentrations were tested, we observed that PSQ did not increase *gcs-1* expression, but increased GST-4 levels in a *skn-1*-dependent manner. In contrast, PTQ at LC increased both GCS-1 and GST-4 levels, even in the absence of *skn-1*.

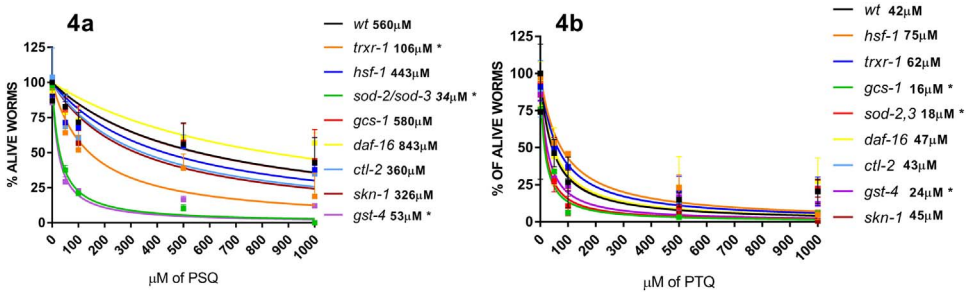

Fig. 4. LD50 curves for different mutants. A) PSQ curve demonstrates that *trxr-1, sod-3* and *gst-4* are involved in the PSQ toxic effects or detoxification. B) The PTQ curve suggests that *sod-3, gcs-1* and *gst-4* are involved in its toxic effects. * means $p < 0.05$ or higher significance when comparing to N2 LD50 curve by repeated measures ANOVA.

Table 3
mRNA and GFP levels after LC PSQ and PTQ exposure in different strains. mRNA is expressed as fold change relative to the *afd-1* housekeeping, compared for statistical purposes to a non-treated group. GFP is expressed as % of control relative to AUF/mg of protein in each sample. mRNA levels were measured in recently exposed L1 worms, while GFP levels were measured 48 after exposure in L4 worms. That was done trying to match expression and protein levels in more a relevant scenario for *C. elegans*, which is during the L4 stage. * means $p < 0.05$ or higher significance. (There is no available TRXR-1::GFP strain).

Strain	Gene	Treatment (LC)	Fold change	*T*-test (vs. vehicle)	GFP AUF/mg protein (%)	*T*-test (vs. vehicle)
wt	*sod-3*	PSQ	1.563 ± 0.2842	*	188.3 ± 22.766	*
daf-16	*sod-3*	–	0.8397 ± 0.1499	–	76.34 ± 15.412	–
skn-1	*sod-3*	–	0.95 ± 0.1367	–	90.145 ± 13.12	–
wt	*gcs-1*	–	0.9163 ± 0.06297	–	113.22 ± 14.341	
skn-1	*gcs-1*	–	0.8926 ± 0.0936	–	108.455 ± 19.78	–
wt	*gst-4*	–	0.869 ± 0.1177	–	122.1 ± 23.41	–
skn-1	*gst-4*	–	1.0234 ± 0.1264	–	87.47 ± 12.146	–
wt	*trxr-1*	–	1.473 ± 0.1483	*	–	–
wt	*sod-3*	PTQ	1.816 ± 0.1976	*	158.2 ± 14.88	*
daf-16	*sod-3*	–	0.9483 ± 0.04519	–	112.334 ± 25.14	–
skn-1	*sod-3*	–	0.8974 ± 0.1362	–	108.648 ± 21.48	–
wt	*gcs-1*	–	0.8945 ± 0.2535	–	177.11 ± 29.475	*
skn-1	*gcs-1*	–	0.9826 ± 0.0838	–	168.478 ± 31.43	*
wt	*gst-4*	–	1.371 ± 0.1936	*	210.989 ± 31.77	*
skn-1	*gst-4*	–	1.234 ± 0.2064	*	165.749 ± 23.568	*
wt	*trxr-1*	–	1.082 ± 0.1058	–	–	–

We also investigated another protein related to thiol homeostasis, thioredoxin reductase 1 (TRXR-1). TRXR-1 is the sole selenoprotein in *C. elegans* [64]. Its absence drastically decreased PSQ LD_{50}, thus suggesting for the first time a role in organochalcogen detoxification or even its metabolism (Fig. 4a). Furthermore, *trxr-1* expression is increased upon PSQ lethal concentration exposure (Table 3). TRXR-1 functions together with thioredoxin (TRX), reducing its active center in a NADPH-dependent process. The TRX system functions by reducing disulfides, thus playing an important role in maintaining cellular redox [65]. TRXR-1 has also been tested for its putative participation at inorganic selenium detoxification and/or metabolism, but no link was found [66]. In contrast, we demonstrate for the first time that an organic selenium compound requires *trxr-1* for detoxification and/or toxic effect. Plausibly, PSQ might have oxidized several –SH groups, then requiring *trxr-1* to act by reducing them. TRXR-1 could also act directly on the PSQ molecule, thus reducing/metabolizing it due to its wide range of substrates [67]. Indeed, whereas PSQ toxicity seems to be related to *trxr-1*, PTQ did not change its expression, thus suggesting a Se-specific effect (Table 3). Finally, a recent work suggested that many organoselenium compounds may modulate TRXR expression, whereas the same effect was absent with inorganic selenium [68]. Related to this, novel β-selenoamines were previously tested as TRXR substrates, and one of them was shown to have affinity for mouse hepatic TRXR [69].

Notably, the results presented here indicate hormetic responses to PSQ and PTQ, which can be attributed to the weak electrophile properties of their organic chalcogen moiety. Hormetic responses to other thiol modifiers or pro-oxidant agents have been already indicated in the literature. The concept that low levels of harmful substances can induce protective responses is not recent [70]. Indeed, this would seem to be the case in the present study, since LC and NLC have opposite effects over the same parameters. Accordingly, *daf-16* and *sod* genes have already been described for acting in hormetic responses of different compounds [71]. *skn-1* and its target *gst-4* were described as playing a role in the hormetic effect of nitrogen-rich diet [72] and plumbagin [73], a naphtoquinone similar to the quinolines studied here. However, hormesis is also commonly linked to protection provided by one harmful compound against a different compound [74,75]. Thus, we strongly suggest that this may not be the case for PTQ and PSQ protection against paraquat, as we have performed a protocol of exposure that simulates an antidote treatment, discarding a hormetic protection against paraquat toxicity.

5. Conclusion

We sought to investigate how two new synthetic Se and Te quinolines act in *C. elegans* at toxic and non-toxic concentrations. We posit that PSQ and PTQ may interact with thiol-related proteins, such as SKN-1, which upon oxidation of -SH groups or in the presence of strong electrophiles [30] is able to translocate into the nucleus and orchestrate the increased expression of *gcs-1*, *sod-3* and *gst-4*, thus improving stress response [60]. *daf-16* was deemed indispensable for recovery from paraquat-induced oxidative damage after exposure to both PSQ and PTQ. Reinforcing the hypothesis of thiol modification by PSQ and PTQ, *trxr-1*, the sole selenoprotein of *C. elegans* was shown to be needed to attenuate PSQ toxicity. Our study sheds novel insight into the modulation of endogenous antioxidant defenses by organoselenium and organotellurium compounds, reinforcing their interaction with the Nrf2/SKN-1 and FOXO/DAF-16 transcription factors. Figs. 5 and 6 represent an overall summary of our data. Further studies are required to verify at which specific level these compounds modulate SKN-1 and DAF-16 pathways, as well as to evaluate additional proteins related to TrxR, such as Trx and Prdx.

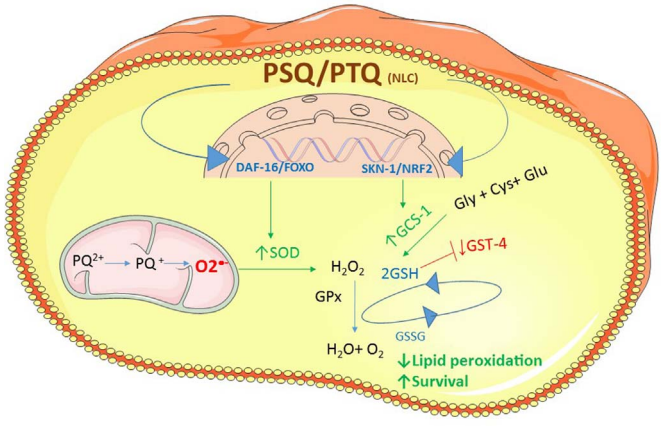

Fig. 5. PSQ and PTQ protective mechanisms against PQ toxicity in *C. elegans*. PSQ and PTQ modulate the translocation of antioxidant transcription factors such SKN-1 and DAF-16, which consequently increase *sod-3* levels, reducing superoxide levels produced by PQ exposure. In parallel through a SKN-1-independent mechanism, both compounds induce the expression of *gcs-1*, thus increasing GSH levels. As a consequence, worms show reduced lipid peroxidation and increased survival when treated with non-lethal concentrations (NLC) of PSQ and TSQ. As these concentrations are not toxic to the worms, *gst-4* levels were not increased.

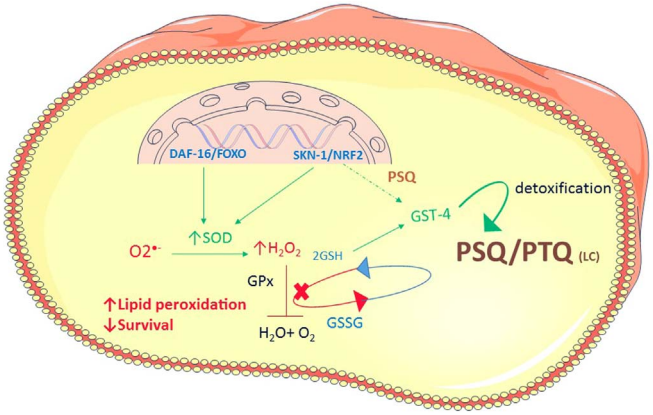

Fig. 6. PSQ and TSQ and toxic molecular mechanisms of action in *C. elegans*. High concentrations of PSQ and TSQ increase the expression of *gst-4*, an important factor in xenobiotic detoxification. This increase is modulated by SKN-1 (for PSQ only). As GSH levels decrease, the detoxification of H_2O_2 is impaired and superoxide may accumulate, thus increasing lipid peroxidation and reducing worms survival. SOD-3 is produced due to nuclear translocation of DAF-16 and SKN-1.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.freeradbiomed.2017.05.020.

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