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Research Article

Over-activation of the *Drosophila melanogaster hsp83* gene by selenium intoxication

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Abstract

Selenium is an important dietary micronutrient and an essential component of selenoproteins and the active sites of some enzymes, although at high concentrations it is toxic. We investigated diphenyl diselenide ($(C_6H_5)_2Se_2$) for its effects on the developmental stages of *Drosophila melanogaster* and found that in the larval and pupae stages the toxic threshold for this compound when added to the banana-agar medium on which the larva were fed was 350 μmol. In adult flies, fed on the same media, there were no observable toxic effects below 500 μmol but there were toxic effects above 600 μmol, indicating that adult flies were more resistant to selenium intoxication. In larvae, a when diphenyl diselenide was present above the toxic threshold there was increased activation of the *hsp83* heat shock protein gene. Selenium promotes oxidation of sulfhydryl groups and affects the folding of proteins and this could explain the over-expression of *hsp83* because the product of this gene is involved in protein folding and defense responses, including the response to heat shock.

Key words: gene activation, *hsp83* gene, selenium, diphenyl diselenide.

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Introduction

Cellular responses to stress are evolutionarily ancient, ubiquitous, and essential mechanisms for the continued survival and reproduction of organisms (Lindquist, 1986; Feder and Hofmann, 1999). An important part of the cellular response to heat stress is played by a group of genes coding for heat shock proteins (HSP) or stress proteins, because their expression can be induced by high temperatures and a whole range of other stress factors (Sørensen *et al.*, 2003; Sørensen *et al.*, 2005). Stress responsive genes are of interest to the study and understanding of not only environmental stress resistance and stress response in general but also protein folding-mediated diseases in humans, immunological responses, animal breeding, genetic stress, protein quality control, developmental biology and gene regulation (Sørensen *et al.*, 2005).

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Selenium, a nonmetal related to sulfur and tellurium, has long been recognized as a dietary antioxidant and as an important dietary micronutrient in mammals (Walter *et al.*, 1972; Combs and Combs, 1986; Leibovitz *et al.*, 1990; May, 2002). At the molecular level, selenium is an essential component of the active sites of the enzymes glutathione peroxidase (Enzyme Commission number = EC 1.11.1.9), iodothyronine 5'-deiodinase (EC 1.97.1.10) and mammalian thioredoxin reductase (TrxR, EC 1.8.1.9) and is also present in several other mammalian selenoproteins. A deficiency in dietary selenium results in decreased levels of selenoproteins, thus compromising biological processes that are maintained by these proteins (Martin-Romero *et al.*, 2001). Both glutathione peroxidase and TrxR catalyze reactions that are essential to the protection of cellular components against oxidative and free radical damage. As a consequence of the growing recognition of the important biological role of selenium, a number of novel pharmaceutical agents which are selenium-based or which target spe-

cific aspects of selenium metabolism are under development (May, 1999; Meotti *et al.*, 2004).

A variety of seleno-organic compounds are now considered as potential antioxidant and chemopreventive pharmacological agents (Commandeur *et al.*, 2001; Klotz and Sies, 2003). Müller *et al.* (1984) described 2-phenyl-1,2-benzisoselenazol-3(2H)-one, a synthetic lipid-soluble selenium-containing heterocyclic also known as PZ 51, DR3305 or Ebselen, which mimics glutathione peroxidase activity an which has been successfully used experimentally and pre-clinically in a variety of situations where free radicals were involved (Parnham *et al.*, 1987; Harman *et al.*, 1992; Kobayashi *et al.*, 2001; Kono *et al.*, 2001; Porciúncula *et al.*, 2001; Rossato *et al.*, 2002a, 2002b; Farina *et al.*, 2003; Klotz and Sies, 2003). Diselenides are good candidates as antioxidant agents because they have some chemical and biochemical characteristics in common with Ebselen in that they exert glutathione peroxidase-like activity and can react with sulfhydryl groups to form selenosulfide (-Se-S) and disulfides, (Engman *et al*., 1994; Barbosa *et al.*, 1998). Of particular importance is diphenyl diselenide ($(C_6H_5)_2Se_2$), the simplest of the diaryl diselenides, which has been shown to be even more active as a glutathione peroxidase mimic (Wilson *et al.*, 1989) and less toxic to rodents than Ebselen (Meotti *et al.*, 2003; Nogueira *et al.*, 2003). Furthermore, diphenyl diselenide shows anti-inflammatory and antinociceptive activity (Nogueira *et al.*, 2001) and has a neuroprotective effect on *in vitro* models of ischemia (Ghisleni *et al.*, 2003) and glutamate neurotoxicity (Porciúncula *et al.*, 2001; Rossato *et al.*, 2002a).

The fruit fly, *Drosophila melanogaster*, has been used for over a century as a model for probing the mysteries of genetics and biology (Ashburner, 2005; Tickoo and Russell, 2002). Comparative studies between the fly and human genomes have identified strong evolutionary conservation from insects to mammals at both sequence (structure) and pathway (function) levels (Rubin *et al.*, 2000; Ballatori and Villalobos, 2002). The use of sophisticated genetic approaches combined with emerging genomics technologies suggest that the fly has much to offer as a tool for understanding basic cellular processes and provides an attractive and complex model system for exploring the molecular basis of human diseases such as cancer, Alzheimer's disease and Huntington's disease (Rubin *et al.*, 2000; Tickoo and Russell, 2002).

Heat shock proteins (HSP) are members of a larger group of proteins called molecular chaperones, characterized by their ability to affect the structure or folded state of other proteins and are a family of proteins that are constitutively expressed at high level, with levels increasing when cells are stressed. The HSP include both highly conserved and more variable members, and are induced in all cells in response to heat stress (Lindquist, 1986). In *Drosophila*, six major HSP (Hsp-*83*, Hsp-*70*, Hsp-*27*, Hsp-*26*, Hsp-*23* and Hsp-*22*) are induced in response to heat stress, with Hsp83 being constitutively expressed and upregulated several times during heat stress. The functions of Hsp83 include regulating the activity of other proteins such as specific steroid hormone receptor molecules (Picard *et al.*, 1990; Parsell and Lindquist, 1993). Heat stress causes trimerization and activation of the constitutively expressed heat shock transcription factor (HSF), which is required for *hsp* gene induction (Jedlicka *et al.*, 1997; Westwood *et al.*, 1991). The *hsp83* gene is a member of the *hsp90* gene family, which has been highly conserved through evolution (King and Tower, 1999).

In this paper, we describe the toxicological effect of diphenyl diselenide during different stages of the *D. melanogaster* life cycle and present *hsp83* transcription data on the potential of this seleno-organic compound to promote cellular stress.

Material and Methods

Drosophila stock used and rearing conditions

We obtained *Drosophila melanogaster* (Harwich strain) from the National Species Stock Center, Bowling Green, OH, USA. The flies were reared in 2.5 cm x 6.5 cm bottles containing 5 mL of cornmeal medium (Klein *et al.* 1999) at constant temperature and humidity (20 °C ± 1 °C and 60% relative humidity). Analyses of viability and developmental rate of the flies in different diphenyl diselenide molarities were performed in banana-agar medium (20% v/v homogenized banana; 1% (w/v) brewer's yeast; 2% (w/v) sucrose; 1% (w/v) powdered milk; 1% (w/v) agar; and 0.08% (v/w) methyl p-hydroxybenzoate (Nipagin® Delaware, Brazil). Diphenyl diselenide (molecular formula $(C_6H_5)_2Se_2$ (molar mass 312.13 g mol^{-1}, CAS 1666-13-3). Diphenyl diselenide was synthesized according to literature methods (Paulmier, 1986) dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA) and added to the medium to produce diphenyl diselenide concentration, from 300, 350, 400, 450, 500, 600, 700, 800, 900 and 1000 μmol $(C_6H_5)_2Se_2$/kg of medium. In the control treatment only DMSO was added to the medium.

To test egg to adult survival and developmental rate in different diphenyl diselenide molarities about 200 adult flies were transferred to a 300 mL empty bottle, which was inverted over a Petri dish containing banana medium to allow the flies to deposit fresh embryos. After 30 min the eggs were picked with a histological needle and rinsed in 0.5% (v/w) sodium hypochloride solution, after in distilled water and transferred to medium containing the desired molarity of diphenyl diselenide. Thirty eggs were placed per bottle and observed daily to accompany the developmental stages. Five replicates were prepared per treatment.

To determine the lifespan of the adult flies when they were unexposed or exposed to diphenyl diselenide freshly ecloded flies were collected and reared on banana-agar medium containing the desired diphenyl diselenide molarities.

For each experiment, 10 vials, each containing 5 males and 5 females, were maintained at 20 °C. Flies were transferred to new vials every 3 days. The number of dead flies was counted every day. Generally, five replicates were prepared per treatment but in some cases up to eight replicates were made.

Molecular methods

To investigate whether exposure to diphenyl diselenide induced cellular stress we estimated the transcriptional expression of the *Hsp83* gene using the Northern blot method. Total RNA was isolated from third-instar *D. melanogaster* larvae which had been exposed to one of following treatments: i) reared completely in banana-agar medium supplemented with 300 µmol of diphenyl diselenide; ii) transferred 8 h before RNA isolation to banana-agar medium supplemented with either 300 or 800 µmol/kg of diphenyl diselenide; iii) transferred 2 h before RNA isolation to a 30 °C chamber to induce heat shock. A group of control larvae were reared on unsupplemented banana-agar medium. Total RNA was isolated with TRIzol® Reagent (Invitrogen Life Technologies) using 30 larvae in each treatment. Total RNA (10 µg) was fractionated on 1.5% (w/v) agarose gel in 4-(2-Hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES)/formamide buffer and transferred to Hybond® N+ nylon membrane (Amersham Bioscience). The membranes were hybridized to the random prime-labeled probes at 65 °C in a mixture containing 0.1% (w/v) sodium dodecyl sulfate, 5% (w/v) dextran sulfate and a 20-fold dilution of liquid block (Gene Image Kit®- Amersham Biosciences) in 5x saline-sodium citrate (SSC) buffer. The filters were washed twice at 65 °C, firstly with 1x SSC and 0.1% (w/v) SDS and then with 0.2% SSC and 1% (w/v) SDS, in both cases under agitation for 15 min. For detection, the Gene Image CPD-Star kit (Amersham Biosciences) was used, following the manufacturer's instructions.

The *phsp83* plasmid (Straten *et al.*, 1997) containing the *D. melanogaster hsp83* gene was used as probe. To control the quantity of RNA applied to the gel, the membrane was re-hybridized with the plasmid containing the 18S rRNA sequence (Straten *et al.*, 1997).

Statistical analyses

The Kaplan-Meier method was used to generate survival curves for all survival experiments using the SPSS 10.0 for Windows Software. Differences in survival between treatment groups were compared using the non-parametric Log-Rank test, where $p < 0.05$ indicates a significant difference between experimental and control groups. The individual p values were adjusted for multiple testing by controlling for false discovery rate using the Benjamini and Hochberg false discovery rate method (Benjamini and Hochberg, 1995). The Northern blots were analyzed with the LabImage® Gel Analysis and Documentation System program version 2.62© 1999-2001 (Kapelan GmbH, German).

Results

In the experiments to test the effects of different diphenyl diselenide molarities on *D. melanogaster* egg-to-pupae developmental rate and viability we found a molarity-dependent relationship between diphenyl diselenide concentration and the rate of development of *D. melanogaster*. As the molarity of diphenyl diselenide increased there was a delay from one to four days for larvae to reach the pupae stage (Figure 1). This data was subjected to chi-squared analysis and the Benjamini and Hochberg false discovery rate method (Benjamini and Hochberg, 1995) and the results presented in Table 1. In most cases a higher diphenyl diselenide molarity was related to a significant reduction in the viability of the flies. The exception was 300 µmol diphenyl diselenide which showed a slightly increased percentage viability (89%) compared with control flies treated with DMSO only, for which only 88% of eggs reached the pupae stage. However, viability decreased when the larvae were exposed to diphenyl diselenide molarities exceeding 300 µmol (Table 1), with the pupal stage being reached by 76% of eggs exposed to 350 µmol diphenyl diselenide, 73% exposed to 400 µmol, 37% exposed to 450 µmol and only 7% exposed to 500 µmol. At diphenyl diselenide molarities exceeding 500 µmol all larvae died. Almost all pairwise comparisons were statistically significant, the exception being the lack of significance between diphenyl diselenide molarities of 350 µmol and 400 µmol (Table 1).

The effect of different diphenyl diselenide molarities on the pupal stage of the *D. melanogaster* life cycle is

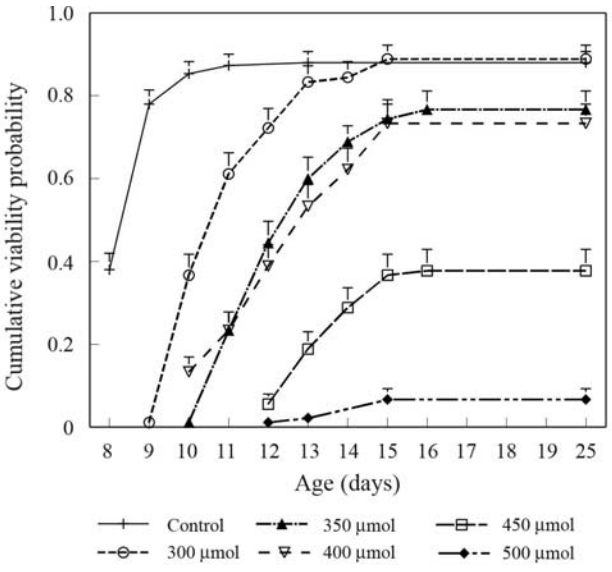

Figure 1 - Diphenyl diselenide molarity and *Drosophila melanogaster* viability and developmental delay curves for the egg to pupa stage. For each of five replicates, 30 eggs were placed on banana-agar containing different molarities of diphenyl diselenide and assessed for viability every day up to the pupal stage, the Y-axis representing the percentage of viable larvae surviving to this stage.

Table 1 - Pairwise chi-squared (χ^2) comparisons of the *D. melanogaster* egg to pupae developmental rate for flies fed on banana-agar medium supplemented with different molarities (µmol/kg of medium) of diphenyl diselenide dissolved in dimethyl sulfoxide (DMSO). The comparisons use the Benjamini and Hochberg false discovery rate method and are ranked (i) in order of decreasing χ^2 value and probability level (p) is in increasing magnitude. The corrected p-value (A) is equal to the p-value multiplied by the rank (i) and divided by the total number of comparisons (A = p i/15) for an error rate = 0.05. The treatments were significantly different in respect to developmental rate when A > p, with the higher diphenyl diselenide molarities showing lower development rates. The control was DMSO only and 30 *D. melanogaster* eggs were used for the control and each diphenyl diselenide concentration.

Diphenyl diselenide molarity	χ^2	p	i	A = 0.05 i/15	Significant (A > p)
0.00 *vs.* 500	157.11	0.000000	1	0.00333	Yes
300 *vs.* 500	145.13	0.000000	2	0.00667	Yes
0.00 *vs.* 450	110.90	0.000000	3	0.01000	Yes
350 *vs.* 500	100.53	0.000000	4	0.01333	Yes
400 *vs.* 500	90.76	0.000000	5	0.01667	Yes
300 *vs.* 450	88.62	0.000000	6	0.02000	Yes
0.00 *vs.* 400	59.45	0.000000	7	0.02333	Yes
0.00 *vs.* 350	58.83	0.000000	8	0.02667	Yes
350 *vs.* 450	38.87	0.000000	9	0.03000	Yes
300 *vs.* 0.00	36.91	0.000000	10	0.03333	Yes
400 *vs.* 450	30.86	0.000000	11	0.03667	Yes
450 *vs.* 500	25.82	0.000000	12	0.04000	Yes
300 *vs.* 400	20.20	0.000000	13	0.04333	Yes
300 *vs.* 350	19.66	0.000010	14	0.04667	Yes
350 *vs.* 400	0.24	0.623300	15	0.05000	No

shown in Figure 2. In the control without diphenyl diselenide the percentage viability at this stage was 80% but the viability increased to 86% at 300 µmol diphenyl diselenide, although there was an average delay of two days for the flies to complete their developmental cycle. However, at higher diphenyl diselenide molarities there was a significant decline in viability from 72% viability at 350 µmol diphenyl diselenide to 3.33% viability at 500 µmol (Table 2) and the development cycle increased from two to seven days as diphenyl diselenide concentration increased. At 600 µmol diphenyl diselenide and above none of the pupae survived. Almost all pairwise comparisons were statistically significant, the exception being the lack of significance between diphenyl diselenide molarities of 350 µmol and 400 µmol (Table 2).

The adult flies were more resistant to diphenyl diselenide intoxication than larvae or pupae. Below 500 µmol diphenyl diselenide there was no significant alteration to the lifespan of adult flies (data not shown). However, contrasting with the lower diphenyl diselenide molarities, there was a significant decline in the lifespan of the adult flies at molarities exceeding 500 µmol when compared to the con-

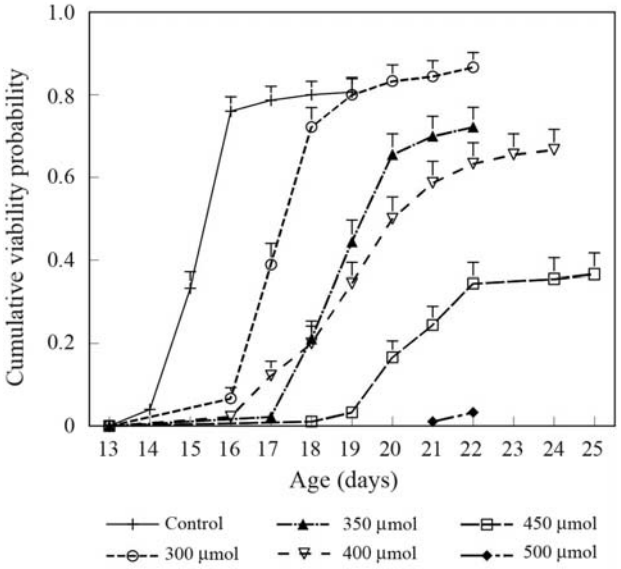

Figure 2 - Diphenyl diselenide molarity and *Drosophila melanogaster* viability and developmental delay curves for the pupa to adult stage. For each of five replicates, 30 eggs were placed on banana-agar containing different molarities of diphenyl diselenide and assessed for viability every day up to adulthood, the Y-axis representing the percentage of viable larvae surviving to this stage.

Table 2 - Pairwise chi-squared (χ^2) comparisons of the *D. melanogaster* pupae to adult developmental rate for flies fed on banana-agar medium supplemented with different molarities (µmol/kg of medium) of diphenyl diselenide dissolved in dimethyl sulfoxide (DMSO). The comparisons use the Benjamini and Hochberg false discovery rate method and are ranked (i) in order of decreasing χ^2 value and probability level (p) is in increasing magnitude. The corrected p-value (A) is equal to the p-value multiplied by the rank (i) and divided by the total number of comparisons (A = p i/15) for an error rate = 0.05. The treatments were significantly different in respect to developmental rate when A > p, with the higher diphenyl diselenide molarities showing lower development rates. The control was DMSO only and 30 *D. melanogaster* eggs were used for the control and each diphenyl diselenide concentration.

Diphenyl diselenide molarity	χ^2	p	i	A = 0.05 i/15	Significant (A > p)
300 *vs.* 500	142.00	0.0000	1	0.00333	Yes
0.00 *vs.* 500	129.24	0.0000	2	0.00667	Yes
350 *vs.* 500	97.44	0.0000	3	0.01000	Yes
300 *vs.* 450	87.93	0.0000	4	0.01333	Yes
400 *vs.* 500	84.23	0.0000	5	0.01667	Yes
0.00 *vs.* 450	74.03	0.0000	6	0.02000	Yes
350 *vs.* 450	36.33	0.0000	7	0.02333	Yes
0.00 *vs.* 400	34.64	0.0000	8	0.02667	Yes
0.00 *vs.* 350	33.16	0.0000	9	0.03000	Yes
450 *vs.* 500	31.78	0.0000	10	0.03333	Yes
300 *vs.* 400	28.59	0.0000	11	0.03667	Yes
300 *vs.* 350	27.82	0.0000	12	0.04000	Yes
400 *vs.* 450	23.13	0.0000	13	0.04333	Yes
0.00 *vs.* 300	16.24	0.0001	14	0.04667	Yes
350 *vs.* 400	1.04	0.3077	15	0.05000	No

trol group without diphenyl diselenide (Figure 3, Table 3). Between 500 µmol and 1000 µmol diphenyl diselenide there was, with one exception, no significant difference in the lifespan of the flies, suggesting a similar level of toxicity. The exception was that when 600 µmol diphenyl diselenide was compared to the control group without diphenyl diselenide there was no statistically significant difference (Table 3) but there was a statistically significant difference between the 600 µmol and 800 µmol diphenyl diselenide molarities. These experiments were replicated five times and produced similar results (data not shown), with 500 µmol diphenyl diselenide failing to produce significantly different results from the control without diphenyl diselenide in some replicates while in other replicates 600 µM diphenyl diselenide failed to produce significantly different results from the control. These results suggest that the interval between 500 µmol and 600 µmol is the threshold at which diphenyl diselenide affects *Drosophila* adult lifespan. We also found that on the first five days the mortality of the adult flies exposed to various diphenyl diselenide molarities was very similar to that for unexposed control flies but, subsequently, the lifespan of the flies decreased, mainly for the flies exposed to the higher diphenyl diselenide molarities.

Northern blot analyses showed differential expression of *hsp83* in the different treatments (Figure 4). The treatments were able to increase the transcription of this gene, though not to the same intensity as heat shock treatment. Quantification of *hsp83* expression with the LabImage program gave a Northern blot value of 1.197 ± 0.053 for larvae maintained on medium containing 300 µmol diphenyl diselenide and 1.421 ± 0.051 for larvae growing on the same medium but which were also heat shocked. In comparison, larvae maintained on medium containing 800 µmol diphenyl diselenide gave a Northern blot value of 1.19 ± 0.036 compared with 1.604 ± 0.021 for larvae growing on the same medium but which were also heat-shocked. All these Northern blot values compare to an arbitrarily defined value of 1 given to the control without diphenyl diselenide. The Northern blot values for the diphenyl diselenide treatments were significantly different from control without diphenyl diselenide (both with and without heat shock treatment), suggesting that although this selenoorganic compound is able to induce a cellular stress responses the response is not as strong as that caused by heat shock.

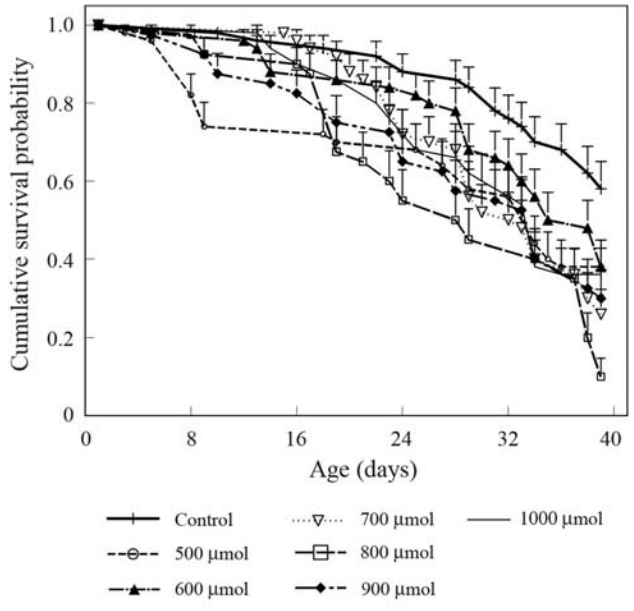

Figure 3 - Diphenyl diselenide molarities and *Drosophila melanogaster* adult survival curves. For each of five replicates, 30 eggs were placed on banana-agar containing different molarities of diphenyl diselenide and assessed for viability every day up to adulthood. Data are expressed as the cumulative survival probability.

Table 3 - Pairwise chi-squared (χ^2) comparisons of *D. melanogaster* adult lifespan for flies fed on banana-agar medium supplemented with different molarities (µmol/kg of medium) of diphenyl diselenide dissolved in dimethyl sulfoxide (DMSO). The comparisons use the Benjamini and Hochberg false discovery rate method and are ranked (i) in order of decreasing χ^2 value and probability level (p) is in increasing magnitude. The corrected p-value (A) is equal to the p-value multiplied by the rank (i) and divided by the total number of comparisons (A = p i/21) for an error rate = 0.05. The treatments were significantly different in respect to developmental rate when A > p, with the higher diphenyl diselenide molarities showing lower development rates. The control was DMSO only and 30 *D. melanogaster* eggs were used for the control and each diphenyl diselenide concentration.

Diphenyl diselenide molarity	χ^2	p	i	A = 0.05 i/21	Significant (A > p)
0.00 *vs.* 800	23.82	0.0000	1	0.0024	Yes
0.00 *vs.* 700	11.71	0.0006	2	0.0048	Yes
600 *vs.* 800	8.91	0.0028	3	0.0071	Yes
0.00 *vs.* 900	8.88	0.0029	4	0.0095	Yes
0.00 *vs.* 1000	6.23	0.0125	5	0.0119	Yes
0.00 *vs.* 500	6.07	0.0138	6	0.0143	Yes
800 *vs.* 1000	4.83	0.0280	7	0.0167	No
0.00 *vs.* 600	3.97	0.0464	8	0.0190	No
500 *vs.* 800	3.44	0.0637	9	0.0214	No
700 *vs.* 800	2.71	0.0998	10	0.0238	No
800 *vs.* 900	2.26	0.1328	11	0.0262	No
600 *vs.* 700	2.07	0.1506	12	0.0286	No
600 *vs.* 900	1.43	0.2313	13	0.0310	No
700 *vs.* 1000	0.52	0.4721	14	0.0333	No
500 *vs.* 600	0.40	0.5248	15	0.0357	No
900 *vs.* 1000	0.37	0.5413	16	0.0381	No
600 *vs.* 1000	0.35	0.5524	17	0.0405	No
500 *vs.* 700	0.34	0.5583	18	0.0429	No
800 *vs.* 900	0.16	0.6895	19	0.0452	No
500 *vs.* 1000	0.02	0.8988	20	0.0476	No
700 *vs.* 900	0.01	0.9413	21	0.0500	No

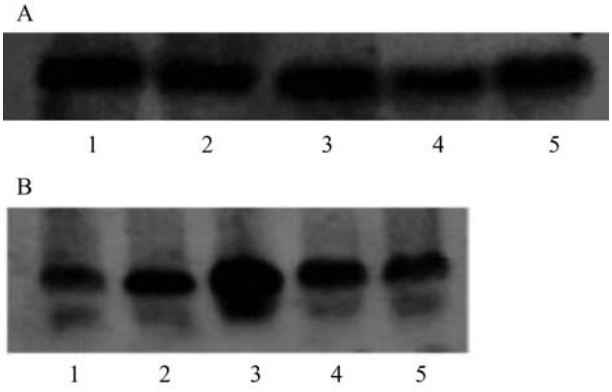

Figure 4 - Northern blot results. A) 18s ribosomal gene B) *D. melanogaster hsp83* gene. Total RNA was isolated from third-instar larvae raised on banana-agar media with or without diphenyl diselenide and with or without heat shock. 1) banana-agar media containing no diphenyl diselenide (control); 2) banana-agar media containing 300 µmol diphenyl diselenide; 3) banana-agar media containing no diphenyl diselenide and heat shocked at 30 °C; 4) banana-agar media containing 300 µmol diphenyl diselenide and heat shocked at 30 °C; and 5) banana-agar media containing 800 µmol diphenyl diselenide.

Discussion

Selenium, while essential to the metabolism of living organisms, presents toxic effects when present at molarities higher than required in the diet. These effects could be observed in our experiments with *D. melanogaster*. The importance of selenium in *D. melanogaster* metabolism is indicated by the fact that when a low concentration of elemental selenium (Rosenfeld and Belth, 1964) or sodium selenite (Lewgoy and Cordeiro, 1971) was added to *D. melanogaster* culture medium there was a significant increase in the viability of the flies, although these authors also showed that high molarities of selenium decreased the viability of the flies.

The results obtained in our present work using the organic compound diphenyl diselenide, as a selenium source were consistant with those previously described by other authors (Rosenfeld and Belth, 1964; Lewgoy and Cordeiro, 1971).

In our study, 300 µmol diphenyl diselenide caused a 6% increase in the viability of *D. melanogaster* larvae/pupae/adult flies, while at molarities higher than 350 µmol it produced toxic effects. These results support other studies described in the literature that show that selenium is toxic to various organisms when present at molarities in excess of dietary requirements (Painter, 1941; Wilber, 1980; Yang *et al.*, 1983; Helzisouer *et al.*, 1985; Navarro-Alarcon and Lopez-Martinez, 2000).

Interestingly, we found that pre-adult stages were more susceptible to selenium intoxication than adult flies, with the baseline diphenyl diselenide concentration producing toxic effect in adult flies (500 µmol) being lethal to larvae. This is possibly explainable by the metabolic differences between these stages, with larvae and pupae possessing cells undergoing active mitotic divisions while adults are post-mitotic. Affleck *et al.* (2006) have shown that methotrexate (an antimetabolite and antifolate used the treatment of cancer and autoimmune diseases and which acts by inhibiting folic acid metabolism) presents toxic and teratogenic effects in both larvae and pupae of drosophila but produces no such effects in adult flies, possibly because adult drosophila are post-mitotic. One explanation for such effects could be that larvae are more susceptible to selenium intoxication because they are in permanent close contact with selenium in the medium on which they are feeding. However, we observed the same toxicity pattern for pupae, which are generally on the surface of the medium and which are not actively feeding. It thus seems probable that the toxic effects of selenium in regard to drosophila pupae are probably related to ingestion and absorption of selenium during the larval stage.

In the experiments designed to investigate the effects of various diphenyl diselenide molarities on the lifespan of adult *D. melanogaster* we found that on the first five days mortality was very similar to that for control flies not exposed to diphenyl diselenide but the lifespan of the flies subsequently decreased, principally flies exposed to the higher molarities. These results were similar to those described by Leal and Neckameyer (2002) who found that there was a delay of about three days in the initiation of feeding by adult *D. melanogaster* when the gamma-aminobutyric acid (GABA) transport inhibitor DL-2,4-diaminobutyric acid (DABA) was added to their medium, possibly because the flies took around three days to start to ingest the medium. This may also have been the case for our experiments with adult flies, although it is also possible that diphenyl diselenide has a delayed effect and only acts about five five days after ingestion.

At toxic molarities we found that diphenyl diselenide induced the *hsp83* cellular stress response gene. The product of this gene is an 83 kD protein reported to be involved in variouse processes, including protein complex assembly, protein folding, the defense response and the heat response (Lange *et al.*, 2000). Several authors have shown that diphenyl diselenide promotes oxidation of sulfhydryl groups in proteins and affects the folding of these macromolecules, such oxidation also being promoted by cadmium and lead as well as selenium (Barbosa *et al.*, 1998; Maciel *et al.*, 2000). We suggest that activation of *hsp83* is an attempt by cells to recover the correct shape of proteins disturbed by high selenium molarities. It is also possible that other stress related genes involved in the refolding of proteins (*e.g. hsp*70 and *hsp23*) could also be activated by diphenyl diselenide.

The increased viability seen by us when diphenyl diselenide was added to *D. melanogaster* culture medium below the toxic threshold concentration suggests that organic selenium compounds can be a source of necessary dietary selenium but, conversely, at levels higher than the

threshold concentration diphenyl diselenide activates the stress responsive gene *hsp83* in *D. melanogaster* larvae. Diselenides such as Ebselen and diphenyl diselenide have been used in pre-clinical trials for their pharmacological properties but our drosophila study has shown that at high molarities these compounds can promote activation of cellular stress protection systems, indicating that further research in this area is warranted. Since cellular stress mechanisms are highly conserved, we suggest that *Drosophila* can be a good model for studying the effects of selenium compounds.

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