High concentrations of the anthelmintic diethylcarbamazine paralyze *C. elegans* independently of TRP-2

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Abstract

Diethylcarbamazine (DEC) has been used to treat lymphatic filariasis in tropical countries since the 1940s. Its mode of action is still unclear, with several reports suggesting a host immune system-mediated mechanism. We previously demonstrated that DEC causes transient spastic paralysis in the filarial nematode *Brugia malayi* due to the activation of TRP-2. Here we show that DEC causes transient paralysis in *C. elegans* at high concentrations and is 200x less potent compared to its effect on *B. malayi*. *C. elegans* trp-2(sy691) mutants are like the wild-type and only paralyzed by high concentrations of DEC. Our results demonstrate that high concentrations of DEC cause paralysis of *C. elegans* independent of TRP-2.
Figure 1. DEC inhibits C. elegans motility independent of TRP-2
Diethylcarbamazine (DEC) is a classic anthelmintic used to treat diseases caused by filarial nematodes like lymphatic filariasis and loiasis. DEC is very effective in clearing the microfilaria from the blood, but the therapeutic effects are transient as the microfilariae return in the blood after a few hours (Hawking and Laurie 1949). The mode of action of DEC has not been well understood, and many reports suggest that DEC acts by stimulating the host immune system (Kanesa-thasan et al. 1991; Maizels and Denham 1992; Peixoto and Silva 2014). The direct effect of DEC on the whole worms was first reported in filaria B. malayi (Verma et al. 2020). Electrophysiological, genetic, and behavioral assays revealed DEC acts by targeting the TRP channels, TRP-2 in the muscle cells of B. malayi (Verma et al. 2020).

The lack of genetic approaches in a parasitic nematode model led us to investigate the effects of DEC on the free-living nematode C. elegans. Characterizing the effects of DEC in C. elegans would provide a model to investigate the pharmacology and the physiology of DEC mediated paralysis. To the best of our knowledge, a direct effect of DEC on C. elegans has not been previously reported. The presence of homologs for TRP-2 in C. elegans (www.parasite.wormbase.org) suggests that TRP-2 could be the putative target for DEC. The activation of TRP channels in these tissues might not be sufficient to cause potent paralysis. We used a bus-12(e2740) that has a fragile cuticle (Darby et al. 2007) and found DEC had similar effects compared to the N2 (Fig 1E and F). The IC$_{50}$ for bus-12 was 0.5±0.04mM, indistinguishable from N2, indicating DEC passes through the cuticle, and its lack of potency may be due to reduced effect on its target receptor.

In B. malayi, current responses to DEC are abolished when the TRPC gene trp-2 is knocked down (Verma et al. 2020), indicating that TRP-2 could be the putative target for DEC. The C. elegans genome encodes for three TRPC genes, trp-1, trp-2, and spe-41, whereas the parasitic worm genomes encode trp-2 and spe-41. SPE-41 is enriched in the male sperm head (Kim et al., 2016) and may not play a role in DEC-mediated paralysis. Phylogenetic analysis in parasite wormbase (www.parasite.wormbase.org) suggests that C. elegans’ TRP-2 is the closest ortholog to the filarial TRP-2s and is present in a different node to TRP-1. To test if DEC acts through TRP-2 in C. elegans, we used trp-2(sy691), a null allele (Feng et al. 2006), and assayed the effects of DEC on motility. We observed that DEC caused transient paralysis in the trp-2 mutants similar to the N2 strains with an IC$_{50}$ of 0.4±0.1mM Fig 1G and H). Our results suggest that, unlike filaria, TRP-2 might not be the putative target for higher concentrations of DEC in the free-living nematode C. elegans.

Our results demonstrate that DEC causes transient paralysis in C. elegans at higher concentrations independent of TRP-2. TRP-2 is expressed in the body wall muscle of B. malayi (Verma et al. 2020) and not in C. elegans, where it is expressed neuronally (Hunt-Newbury et al. 2007). According to Wormbase, most of the TRP channels in C. elegans are expressed neuronally or in the intestine. The DEC activation of TRP channels in these tissues might not be sufficient to cause potent paralysis. Also, DEC might be targeting a different TRP channel in the muscle cells of C. elegans with a lower potency. Our results suggest DEC has a parasite-specific effect in paralyzing the worm, and it causes transient paralysis at very high concentrations in C. elegans, independent of its putative parasitic target TRP-2.

**Methods**

**Strains:**

A, B: High concentration of DEC causes flaccid paralysis in C. elegans. Still images of N2 worms thrashing (A) and paralyzed (B) in the presence of 1mM DEC. C, E & G: Show time-dependent effects of high concentrations of DEC on the locomotory speed of N2, bus-12(e2977), and trp-2(sy691). Worms treated with 1mM and 3mM DEC undergo paralysis immediately after treatment and recover completely in 40 minutes. N=15, 2-way ANOVA, p<0.001. D, F & H: Show concentration-response plots for the motility of N2, bus-12(e2977), and trp-2(sy691) in the presence of DEC, measured after 0.5 minutes post-treatment. The IC$_{50}$ values for all strains were not significantly different from each other. N=15, 2-way ANOVA, p>0.05.
Strains used in this study, Wild-type (Bristol N2), bus-12(e2977), and trp-2(sy691), were obtained from the Caenorhabditis Genetic Centre (The University of Minnesota, Minneapolis, MN, USA). All C. elegans hermaphrodites were maintained on 60mm Petri dishes containing standard NGM, spread with E. coli OP50 as food stored at 20°C. Well-fed, motile young adult worms, approximately three days after hatching, were used in all experiments.

**Drugs:**
Diethylcarbamazine citrate (DEC, CAS-1642-54-2) used in this study was obtained from Sigma Aldrich (St. Louis, MO, USA). 100mM stock DEC was prepared by dissolving in distilled water and stored at -20°C.

**Motility assays:**
Motility assays were performed on young adult hermaphrodites. Fifteen worms from each strain were transferred to a 96 well plate (1 worm/well) containing RPMI media at a final volume of 200µL. After allowing 20 min for acclimatization, DEC was added and video recordings were performed using WormLab software with a purpose-built illumination stand (MBF Bioscience) equipped with a Nikon 60 mm Microlens (Nikon Inc., Melville, NY, USA) and an AVT Stingray F-504B digital camera (Allied Vision Technologies GmbH, Stadtroda, Germany). Videos were captured at 7.5 frames per second and saved as AVI files. To determine the potency of DEC on young adult hermaphrodite, C. elegans were treated with various concentrations of DEC (100µM, 300µM, 1mM, and 3mM). Worm motility was recorded for 30s before the addition of DEC, 30s following the addition of DEC and at 2, 40, and 120-min post-treatment to generate a concentration- and time-course response analysis. Motility was recorded for 30s for all time points.

**Data Analysis and Statistics:**
We used WormLab software (MFB Biosciences, USA) to track and analyze the recorded videos of the Caenorhabditis elegans to determine the average speed of the 45 individual worms (n=15 per strain). Wormlab analyzed, frame by frame, the detected changes in movement to track the total length of forward and reverse movement in micrometers and the average speed of the worm in micrometers per second (µm/s). The data were analyzed using GraphPad Prism 5.0 software (Graphpad Software, Inc., La Jolla, CA, USA).

**Reagents**

| Strain  | Allele      | Available from CGC |
|---------|-------------|---------------------|
| N2      | NA          | Yes                 |
| CB6667  | bus-12(e2977) | Yes              |
| TQ194   | trp-2(sy691) | Yes                |

**Acknowledgments:** We thank the Caenorhabditis elegans Genetics Center for providing us with the necessary strains for this study.

**References**
Cox GN, Kusch M, Edgar RS. 1981. Cuticle of Caenorhabditis elegans: its isolation and partial characterization. J Cell Biol 90: 7-17. PubMed ID: 7251677
Darby C, Chakraborti A, Politz SM, Daniels CC, Tan L, Drace K. 2007. Caenorhabditis elegans mutants resistant to attachment of Yersinia biofilms. Genetics 176: 221-30. PubMed ID: 17339204
Feng Z, Li W, Ward A, Piggott BJ, Larkspur ER, Sternberg PW, Xu XZ. 2006. A C. elegans model of nicotine-dependent behavior: regulation by TRP-family channels. Cell 127: 621-33. PubMed ID: 17081982
Hawking F, Laurie W. 1949. Action of hetrazan on filariasis and onchocerciasis. Lancet 2: 146. PubMed ID: 18146950
Hunt-Newbury R, Viveiros R, Johansen R, Mah A, Anastas D, Fang L, Halfnight E, Lee D, Lin J, Lorch A, et al. 2007. High-throughput in vivo analysis of gene expression in Caenorhabditis elegans. PLoS Biol 5: e237. PubMed ID: 17850180
Kanesa-thasan N, Douglas JG, Kazura JW. 1991. Diethylcarbamazine inhibits endothelial and microfilarial prostanoid metabolism in vitro. Mol Biochem Parasitol 49: 11-9. PubMed ID: 1775151
Maizels RM, Denham DA. 1992. Diethylcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. Parasitology 105 Suppl: S49-60. PubMed ID: 1308929

Peixoto CA, Silva BS. 2014. Anti-inflammatory effects of diethylcarbamazine: a review. Eur J Pharmacol 734: 35-41. PubMed ID: 24726556

Verma S, Kashyap SS, Robertson AP, Martin RJ. Diethylcarbamazine activates TRP channels including TRP-2 in filaria, Brugia malayi. Communications Biology. 2020;3(1):398. doi: 10.1038/s42003-020-01128-4 DOI: 10.1038/s42003-020-01128-4

Kim B, Suo B, Emmons SW. 2016. Gene Function Prediction Based on Developmental Transcriptomes of the Two Sexes in C. elegans. Cell Rep 17: 917-928. PubMed ID: 27732864

**Funding:** NIH NIAID grants R01AI047194 and R01AI155413 to RJM and The EA Benbrook Endowed Chair of Pathology and Parasitology. The funding agencies had no role in the design, execution or publication of this study. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases.

**Author Contributions:** Real Datta: data curation, formal analysis, investigation, methodology, writing - original draft, writing - review editing. Alan Robertson: conceptualization, formal analysis, supervision, writing - original draft, writing - review editing. Richard Martin: conceptualization, funding acquisition, supervision, formal analysis, writing - review editing. Sudhanva Kashyap: conceptualization, data curation, formal analysis, investigation, methodology, supervision, writing - original draft, writing - review editing.

**Reviewed By:** John Chan

**History:** Received March 17, 2022 Revision Received April 4, 2022 Accepted April 20, 2022 Published April 20, 2022

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**Citation:** Datta, R; Robertson, A; Martin, R; Kashyap, S (2022), High concentrations of the anthelmintic diethylcarbamazine paralyze *C. elegans* independently of TRP-2. microPublication Biology. 10.17912/micropub.biology.000548