Diethylcarbamazine activates TRP channels including TRP-2 in filaria, *Brugia malayi*

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Diethylcarbamazine is an important classic drug used for prevention and treatment of lymphatic filariasis and loiasis, diseases caused by filarial nematodes. Despite many studies, its site of action has not been established. Until now, the consensus has been that diethylcarbamazine works by activating host immune systems, not by a direct action on the parasites. Here we show that low concentrations of diethylcarbamazine have direct and rapid (<30 s) temporary spastic paralyzing effects on the parasites that lasts around 4 h, which is produced by diethylcarbamazine opening TRP channels in muscle of *Brugia malayi* involving TRP-2 (TRPC-like channel subunits). GON-2 and CED-11, TRPM-like channel subunits, also contributed to diethylcarbamazine responses. Opening of these TRP channels produces contraction and subsequent activation of calcium-dependent SLO-1K channels. Recovery from the temporary paralysis is consistent with inactivation of TRP channels. Our observations elucidate mechanisms for the rapid onset and short-lasting therapeutic actions of diethylcarbamazine.

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The neglected tropical diseases include infections like loiasis and lymphatic filariasis caused by filarial nematode parasites like Loa loa, Brugia spp., and Wuchereria bancrofti. The diseases are transmitted by biting insects that feed on infected hosts' blood, picking up L1 stage microfilaria, which mature in the insect to the L3 stage, before being passed on to a subsequent uninfected host. The transmitted microfilariae mature in the new host to adults where the clinical signs produced depend on the final location of the parasites. Loiasis, or African eye worm, is caused by the filarial worm *L. loa* that threatens more than 29 million people causing itchy swellings of the body known as Calabar swellings. A group of filarial adults like *Brugia malayi* that locate in the host lymphatic system produces lymphatic filariasis. Here, they block drainage of the lymphatics inducing gross swelling of the limbs, itching, and skin infections that produce the clinical condition, elephantiasis. The unpleasant symptoms inhibit productive work of the individual and often cause social rejection. There are an estimated 67.88 million people infected with lymphatic filariasis, which threatens 886 million people in 52 countries. There are no effective vaccines, so Mass Drug Administration (MDA) to control and prevent infection is the only practical option. Diethylcarbamazine (referred to subsequently as DEC), is a valuable and classic drug used for the treatment of loiasis and lymphatic filariasis; it has a very fast therapeutic onset; it removes microfilariae from the blood, but the therapeutic benefits are temporary because the microfilariae return in the blood after only a few hours.

Until now, DEC was not understood to have direct effects on the parasites, but taken to act by stimulating host immune systems. We have discovered that low concentrations of DEC have a direct effect on *B. malayi* parasites, opening Transient Receptor Potential (TRP) channels, which in turn activate calcium-dependent SLOw potassium channels (SLO-1) in their somatic muscle cells, and produce a temporary spastic paralysis. The identification of this site of action of DEC in filaria can explain the rapid onset of action in vivo following administration and its transient therapeutic effect that can be followed by recovery of infection levels after treatment. An entry of calcium and activation of SLO-1 channels by DEC also predicts a synergistic interaction between DEC and emodepside that is under development for treatment of filaria. The new knowledge of the mode of action of DEC will support its continued use and needed combination therapies.

Results

**DEC effects on microfilariae and adult motility.** We tested the effects of addition of different concentrations of DEC on the motility of microfilariae of *Brugia malayi* (Fig. 1a, b). DEC produced rapid (<2 min) coiling, loops, and inhibition of motility that was concentration-dependent. These effects were followed by a gradual recovery that started after 30 min at 1 µM and 24 h at 100 µM. The % motile IC$_{50}$ at 30 min was 4.0 ± 0.6 µM (n = 200/ concentration) (Fig. 1c). We tested the effects DEC on the motility of adult female *B. malayi* (Fig. 2a, b), and found that DEC produced a fast (<30 s) contraction and spastic paralysis that was concentration-dependent and that lasted ~3 h before a gradual recovery over the next 5 h. The IC$_{50}$ for the % motility inhibition at 30 s was 4.4 ± 0.3 µM (n = 12) (Fig. 2c).

**DEC effects on muscle currents.** We examined the effects of 10 µM DEC on the electrophysiology of the body wall muscle cells under whole-cell patch-clamp in 6 separate muscle cells from five different *B. malayi* female worms. We found in each of the preparations that DEC increased a voltage-sensitive outward current (Fig. 3a–d), similar to what we had previously described in *Ascaris suum*, to include a calcium-dependent SLO-1 K current. In addition to the effects on the voltage-sensitive outward current, the main effect of DEC was to produce a concentration-dependent increase in the standing outward current (Fig. 3e), that had an EC$_{50}$ of 13.9 ± 1.3 µM (n = 6) (Fig. 3f). This outward potassium current was inhibited by the addition of iberiotoxin (30 µM), a selective inhibitor of calcium-dependent BK (SLO-1 K) channels (Supplementary Fig. 1).

Upon closer inspection of the standing currents caused by DEC, we observed in many recordings, an inward current that preceded the outward SLO-1 K current (Fig. 4a, b). These inward currents suggest that DEC activates SLO-1 by activating an...
inward current that carries calcium through the plasma membrane. This entry of calcium through the membrane increases SLO-1 K currents and can also explain the contraction and spastic paralysis produced by DEC.

Evidence of TRP channels. To study these inward currents further, we blocked the outward SLO-1 K currents by using 4-aminopyridine (4AP), a potassium channel antagonist. The application of 5 mM 4AP inhibited the potassium currents and produced a significant inward current, which reached a steady level after 1.5 min. Following the addition of the 4AP, a steady current was reached, the application of DEC produced a steady inward current (Fig. 4c, d). In the presence of 4AP, we found that DEC produced a concentration-dependent reversible inward current with an EC50 of 39.1 ± 0.6 µM (n = 5) (Fig. 4e, f). This inward current was reversibly inhibited by 10 µM 2APB (Fig. 4g, h), and 10 µM SKF96365, (Supplementary Fig. 2), both non-selective TRP channel blockers, suggesting that the inward current produced by DEC is due to opening of TRP channels.

TRP channels are a group of non-selective tetrameric cation channels permeable to cations including sodium, potassium, and calcium. We were able to inhibit the SLO-1 K channel currents of B. malayi muscle induced by DEC by perfusing the preparations with calcium-free bath solution (Supplementary Fig. 3). Under these conditions, application of 30 µM DEC produces only an inward current likely carried by sodium and potassium through open non-selective TRP channels but cannot increase intracellular calcium to activate the SLO-1K channels. However, returning the calcium to the perfusing bath solution allowed activation of the SLO-1K channels with the entry of calcium through the TRP channels (Supplementary Fig. 3).

As a further test for the presence of TRP channels in B. malayi muscle cells, we tested capsaicin that activates TRPV1 channels in vertebrates. We found that the effect of 30 µM capsaicin in the presence of 4AP was also able to induce an inward current like 30 µM DEC (Supplementary Fig. 4). The effect of co-application of 30 µM DEC and 30 µM capsaicin was to produce inward currents that were additive rather than mutually exclusive, suggesting that the two compounds do not act on the same receptor but on different types of TRP channels.

Expression of TRP channels in adults and microfilaria. We used primers for PCR, based on the genome of B. malayi, to detect expression of TRP channel transcripts in whole adult female worms (Supplementary Fig. 5a), and found ocr-1, osm-9, cup-5, trp-2, gon-2, ced-11, and trpa-2 transcripts. We observed expression of trp-2, gon-2, and ced-11 in microfilariae but did not detect expression of other TRP channel transcripts (Fig. 5b).

In order to identify which TRP channel subunits expressed in single muscle cells of adults, we performed RT-PCR on cytoplasm collected from single muscle cells with a patch pipette. As shown in Fig. 5c: osm-9, gon-2, ced-11, and trp-2 were expressed in muscle cells. OSM-9 are TRPV-like channel subunits and may explain responses of muscle cells to capsaicin (Supplementary Fig. 4); TRP-2s are TRPC-like channel subunits; and GON-2 and CED-11 are TRPM-like channel subunits.

TRP channel subunits required for DEC responses. In order to identify which TRP channel subunits are required for the motility inhibitory response to DEC, we used dsRNA exposure for 4 days to knock down osm-9, gon-2, ced-11, and trp-2 (Fig. 6a). Knock-down of: gon-2 + ced-11 + trp2, gon-2 + ced-11, or trp-2 but not osm-9 produced a significant loss of the inhibitory effect of 30 µM DEC on motility (Fig. 6b). These observations suggest that TRP channels with TRP-2, CED-11, and/or GON-2 channel subunits are activated by DEC to produce the spastic paralysis that inhibits motility of adult B. malayi.

Because worm motility is governed by both somatic muscle and neuronal activity and trp-2, ced-11, and gon-2 may have a different role in nerves and muscle, we examined the effect of dsRNA knockdown on DEC activated currents in muscle, again in the presence of 4AP. Fig. 7a shows, in the presence of 4AP, that effects of DEC on control worms are to produce inward currents by opening TRP channels, and the effects of acetylcholine by opening nicotinic receptor channels is also to produce inward currents. Following the removal of 4AP, emodepside (a SLO-1K channel agonist) produces outward currents. Fig. 7b, d shows that the effect of trp-2 knockdown is to block the effect of DEC without reducing the acetylcholine or emodepside induced currents. Fig. 7c, d shows that the effect of ced-11 + gon-2 knockdown is to produce a significant reduction but not a block of the effect of DEC; the effect of B. malayi.
of trp-2 knockdown is ~2× that of ced-11 + gon-2 knockdown response. The responses to acetylcholine and emodepside were not significantly affected by the knockdown. Thus, the DEC current-responses require TRP-2 subunits; GON-2, and CED-11 support the DEC response. These observations can be explained if DEC activates heteromeric TRP channels\textsuperscript{15} in B. malayi muscle with TRP-2 subunits that are essential and that includes GON-2 or CED-11 subunits as optional subunits.

**Arachidonic acid, lipoxygenases, cyclooxygenases, and PUFAs.** The inhibition of lipoxygenase and/or cyclooxygenase that block the synthesis of physiologically active prostanoids in the host and parasites has been discussed as a mechanism of action of DEC\textsuperscript{6–8}. But we now know that particular nematodes do not have cyclooxygenases or lipoxygenases: C. elegans and B. malayi lack identified cyclooxygenases or lipoxygenases in the databases (WormBase).

Nevertheless, nematodes still produce poly-unsaturated fatty acids (PUFAs) with two types of cytochrome oxidase CYP450 enzymes acting on arachidonic acid: (1) \(\omega\)-hydroxylases producing 20-hydroxyeicosatrienoic acids (HETEs); and (2) epoxygenases producing epoxyeicosatrienoic acids (EETs)\textsuperscript{16}. The PUFAs produced by these CYP450 enzymes can explain the ability of B. malayi...
to produce prostacyclin, PGE$_2$ and PGD$_2$ from arachidonic acid in the absence of cyclooxygenases and lipoxygenases.$^{17,18}$ In *B. malayi*, the homolog of the mammalian ω-hydroxylase is BM42071, and the homolog of the mammalian epoxygenases that are inhibited by miconazole is BM38240$^{19}$. Thus, it was possible that DEC acted by inhibiting CYP450 enzymes.

We tested the effect of arachidonic acid and found that its effect was similar to DEC (Fig. 8a, b), but the onset was much slower than DEC, taking several minutes to plateau. This suggests metabolism of arachidonic acid, to an active metabolite was required to produce the effect on the TRP channels. The peak amplitude of the current response to DEC was also reduced in the

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**Fig. 4 DEC induced inward currents at −40 mV revealed by blocking SLO-1 K currents with 5 mM 4AP.**

- **a.** Representative DEC biphasic holding currents, in the absence of 4AP, following the application of 10 μM DEC at −40 mV. Note that an inward (downward) current precedes the outward current.
- **b.** Box-plot representing mean ± SE of the initial inward peak current (−35.2 ± 2 pA) and the subsequent peak outward current (180.8 ± 16.8 pA) following 10 μM DEC (n = 5 from five worms).
- **c.** Application of 5 mM 4AP produces a steady inward current due to the blocking of the steady outward potassium current. The application of 3 μM DEC during the plateau of the 4AP effect produces a steady inward current.
- **d.** Box-plot of the mean 3 μM DEC inward current during the plateau of the 4AP effect, −109.9 ± 12.6 pA (n = 7 muscles from five worms).
- **e.** DEC concentration-dependent inward current-responses in the presence of 5 mM 4AP, −40 mV, EC$_{50}$ 39.1 ± 0.6 μM (n = 5 muscles from five worms; for some concentrations the SE plotted are smaller than represented points on the graph).
- **f.** Application of 2APB (30 μM) as an inhibitor of TRP channels inhibits the inward DEC (30 μM) current seen in the presence of 5 mM 4AP. Box-plot of the 30 μM DEC induced inward currents before (−620.9 ± 47 pA) and in the presence of 30 μM 2APB (−26.9 ± 4.7 pA) as an inhibitor of TRP channels (paired t-test, p = 0.0001; 95% confidence interval was −720 to −470; n = 7 from six worms).
presence of arachidonic acid that may be explained by both the arachidonic acid metabolite and DEC acting as agonists on the same TRP channels.

Meclofenamic acid, indomethacin, and miconazole. Next, we tested the effect of 30 μM meclofenamic acid, an inhibitor of mammalian 5-lipoxygenase and cyclooxygenase and found Supplementary Fig. 5a, b, no effect on the DEC induced currents. We also tested the effect of 30 μM indomethacin as a cyclooxygenase inhibitor and found that it also produced no significant effect on the amplitude of the response to 30 μM DEC (Supplementary Fig. 5c, d).

Without the effect of meclofenamic acid and indomethacin on the amplitude of the peak DEC responses, we tested 10 μM miconazole as an inhibitor of epoxygenase CYP450 enzymes. We found that, like arachidonic acid, miconazole produced a slow inward current and reduced the amplitude of the response to DEC (Fig. 9a, b), suggesting that the inhibition of epoxygenases may divert the metabolism of arachidonic acid through hydroxy-lases20 to metabolites that also can activate the TRP-2 channels. The current-responses to both arachidonic acid and miconazole were slow, taking several minutes to plateau, in contrast to the faster action of DEC. The slower time course of arachidonic acid and miconazole can be explained if accumulation of metabolites arachidonic acid are required for activation of Brugia TRP channels and if DEC acts directly on these TRP channels.
Discussion

DEC has direct effects on *Brugia malayi* microfilariae and adults inducing spastic paralysis. Fig. 10 shows a parsimonious explanation for our observations. (1) The rapid effect of DEC inducing an inward current and secondary activation of SLO-1 K; (2) the effect of TRP antagonists; (3) the trp-2, gon-2 + ced-11 knockdown effects on currents and; (4) the effect and slow time course of arachidonic acid and miconazole inducing inward currents. We point out that the slow time course of the effect of arachidonic acid and miconazole inducing inward currents.

Intravenous DEC produces a rapid (within minutes) decline in the level of microfilariae and adult *B. malayi*. Until now, DEC was not understood to have direct effects on the parasites, but taken to act by stimulating host immune systems.

Fig. 8 Arachidonic acid slowly increases the inward current and reduces the peak response of 30 µM DEC in the presence of 5 mM 4AP to block SLO-1 K currents. a Representative inward currents showing that the application of 30 µM arachidonic acid produces a slowly increasing inward current that reduces the peak response to 30 µM DEC (5 mM 4AP present to block SLO-1 K). b Whisker-plot of peak inward currents induced by 30 µM DEC (DEC: −685.5 ± 74 pA), 30 µM arachidonic acid (AA: −350 ± 39 pA), and 30 µM DEC in the presence of 30 µM arachidonic acid (DEC + AA: −192 ± 60 pA). The peak DEC + AA current response, and the AA current response are significantly less than the peak DEC alone current (one-way ANOVA with Bonferroni post-hoc test; p < 0.0001 for DEC vs. DEC + AA, 95% confidence interval was −700 to −300; and p = 0.008 for DEC vs. AA, 95% confidence interval was −600 to −100).
of DEC on microfilariae and adult females in vitro was also to produce a concentration-dependent inhibitory effect (microfilariae EC$_{50}$ 6 µM; adult EC$_{50}$ 3 µM) on motility with the motility returning gradually after 3–5 h (Figs. 1a, b and 2a–c). These concentrations are close to peak plasma concentrations in excess of 5 µM following treatment with DEC for lymphatic filariasis but they are below the EC$_{50}$ for SLO-1 K (14 µM) and the EC$_{50}$ for the TRP muscle current (39 µM). This is explained by the classic phenomena of "Spare Receptors" where a full biological response (muscle paralysis) can be achieved with only a proportion of activated receptors (TRP channels here) when there is a signaling cascade following receptor activation that produces loss of muscle contractility.

A number of authors attribute the therapeutic effects of DEC to be host mediated by their immune systems partly because of a lack of effects previously detected on the parasites in vitro. We think it is possible that the effects on motility have been overlooked because they are transient in nature.

Piessens & Beledkas reported effects of DEC on host eosinophils, leukocytes, and granulocytes and Cesbron et al., reported effects on blood platelets. These studies and others have been reviewed. Our studies have not tested effects on host immune systems, so we do not exclude such effects of DEC.

However, here we have shown that DEC, at low therapeutic concentrations, produces a fast and temporary spastic paralysis of adult female B. malayi that includes activation of TRP channels that include activation of TRP channels of different capillary beds. TRP channels are tetratimers composed of four subunits that are mostly homo- or heteromeric but can also be heteromic. Most TRP channels are permeable to calcium and subjected to sophisticated auto-regulation mechanisms that self-regulate calcium entry and downstream calcium signaling. We can explain the temporary spastic paralysis of B. malayi by an agonist action of DEC on TRP channels that is followed by auto-regulatory mechanisms leading to a subsequent closure of the TRP channel and recovery of motility. A temporary paralysis of the worms in the blood will lead to trapping of any paralyzed parasites in different capillary beds where they will undergo attack by the mononuclear phagocyte system (MPS) and immune systems that is required for the elimination of the worms. The entry of calcium also activates of calcium-activated SLO-1 K channels. Emodepside activates, and 4AP blocks the SLO-1 K channels. Arachidonic acid is metabolized to inactive PUFAs by Brugia epoxygenases that are inhibited by miconazole; or arachidonic acid is metabolized by ω-hydroxylases to active PUFAs that are increased in the presence of miconazole and that increase opening of TRP channels.
and importance of role of TRP channels in the action of anthelmintic drugs. Finally, TRP channels are also present on many host cells including those involved in immune responses. Entry of calcium through DEC activated host TRP channels may contribute to enhancement of an immune response to the filarial parasite, producing a DEC-induced dual parasite- and host-mediated action.

Methods

Parasite maintenance. *B. malayi* microfilariae and adult worms were provided by NIH/NIAID Filariasis Research Reagent Resource Center (FR3; College of Veterinary Medicine, University of Georgia, Athens, GA, USA). Worm handling protocols were approved by Institutional Bio-safety Committee (IBC) at Iowa State University. Microfilariae and adult worms were maintained in non-phenol red Roswell Park Memorial Institute (RPMI) 1640 media (Life Technologies, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Fisher Scientific, USA) and 1% penicillin–streptomycin (Life Technologies, USA). The worms were stored individually in 24-well culture plates containing 2 ml of supplemented RPMI –1640 media and placed in an incubator at 37 °C supplemented with 5% CO2.

Drugs. Emodepside used in this study was obtained from Bayer Animal Health. Dyethylcarbamazine (DEC), 4-aminopyridine, miconazole, meclofenamate, arachidonic acid, ibuprofen, theophylline, and capsaicin were obtained from Sigma Aldrich (St. Louis, MO, USA). The drugs were dissolved in distilled water or DMSO and diluted in recording solution to obtain final concentration.

*B. malayi adult and microfilariae motility screening*. Microfilariae were concentrated by centrifugation (400 x g for 10 min) and the highly motile microfilariae were collected and quantified microscopically. Forty microfilariae were then separated into each well of the 96 well plates in 200 µl of RPMI culture media. The microfilariae were kept at 37 °C under an atmosphere of 5% CO2 before and after addition of DEC during experiments as per protocol described by Samje et al. The volume was restricted so that the movement of the microfilariae was restricted to two dimensions. To observe the DEC concentration effect, we applied different concentrations of DEC (100 nM, 300 nM, 1, 3, 10, and 100 µM) and used distilled water as the vehicle control. The percent motility at 1 h was used to determine the concentration–response relationships.

**Worm disinsection**. Longitudinal using a tungsten needle. The resulting section was glued along one side using Glushield cant. The base of the chamber was a 24 × 50 mm cover slip coated with a thin layer of Sylgard® (Dow Corning, USA). The worm section was then glued along one side using Glushield "cant" differences between groups. The ectopic expression of two TRP homologs, TRP-2 and TRP-3, in *B. malayi* was identified using the TRP-2FL reporter construct containing a yellow fluorescent protein (YFP) linked to the Ca2+ sensor, Gaussia luciferase, and a lacZ reporter. The resulting construct was delivered to the worm using a high-pressure microinjection device. The resulting transgenic worms were then screened for YFP expression using a fluorescent microscope. Worms expressing YFP in the muscle of the head or posterior were identified as positive controls.

**Whole-cell recording**. Several techniques were used to study the electrical properties of the muscle cells in *B. malayi*. Whole-cell recordings were obtained from microfilariae and adult worms, whereas single-cell recordings were obtained from the isolated muscle cells. Whole-cell recordings were obtained from microfilariae using the whole-cell patch-clamp technique (Stern and Tauc, 1984). Single-cell recordings were obtained using the bath solution containing 120 mM KCl, 5 mM MgCl2, 5 mM TRIS, 0.25 mM CaCl2, 4 mM NaATP, 5 mM EGTA, and 36 mM sucrose without calcium (120 mM KCl, 20 mM KOH, 4 mM MgCl2, 5 mM NaATP, 5.2 mM EGTA, and 36 mM sucrose). The pH for both solutions was adjusted to 7.2 with KOH and the osmolarity maintained at ~315–330 mOsm. Pipettes with resistances of 3–5 MΩ were used. A 1 cm region near the tip of the electrode was covered with Sygard® to reduce background noise and improve frequency responses. Giga ohm seals were formed before breaking the membrane with suction. The preparation was continuously perfused in bath solution at 2 ml/min. The current signal was amplified by an Axopatch 200B amplifier (Molecular Devices, CA, USA) filtered at 2 kHz (three-pole Bessel filter), sampled at 25 kHz, and digitized with a Digidata 1440A (Molecular Devices, CA, USA).

**RNA extraction and cDNA synthesis**. Microfilariae and adult worms were snap frozen and crushed into fine powder in a 1.5 ml micro-centrifuge tube using Kimble® Kontes® Pellet Pestle® (Fisher Scientific, USA). Total RNA was extracted using TRIzol® Reagent (Life Technologies, USA) according to the manufacturer’s instructions. About 1 µg of total RNA was used to synthesize cDNA using SuperScript® VILO™ Master Mix (Life Technologies, USA). cDNA was later used to perform PCR reactions.

**Synthesis and delivery of dsRNA**. dsRNA was synthesized as explained in refs. 13, 19. Target and non-target T7 promoter labeled primers were amplified using the primers trp-2f, trp-2r, and trp-2r (1 µl, 300 nM). The highly motile microfilariae were used as templates for electrophysiology recordings and the rest was snap frozen in liquid nitrogen and stored at −80 °C for transcript analysis by qPCR.

**Analysis of transcript levels**. cDNA from dsRNA-treated worms were amplified using the following target and reference gene (Bma gapdh) primers: trp-2f, trp-2r, gon-2f1, gon-2r1, ced-11f1, ced-11r1, osm-9f, osm-9r, SSK5F, and SSK5R (Molecular Probes, USA). cDNA was later used to perform PCR reactions. The relative quantification of target gene knockdown was estimated by the ΔΔCt method.
electrophysiology recordings, blinding was not possible as operator had to have the knowledge of the specimen to test the drugs and randomization was irrelevant considering our experimental design as described above.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All data generated or analyzed during this study are included in this published article and its supplementary files. Any remaining data can be obtained from the corresponding author upon reasonable request.

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**References**

1. Ramahia, K. & E. Ottesen, E. Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. *PloS Negl. Trop. Dis.* 8, e3319 (2014).

2. Hawking, F. Recent advance in the chemotherapy of tropical diseases. *East Afr. Med. J.* 24, 375 (1947).

3. Geary, T. G. et al. Unresolved issues in anhelimthic pharmacology for helminthiasis of humans. *Int. J. Parasitol.* 40, 1–13 (2009).

4. Bird, A. C., El-Sheikh, H., Anderson, J. & Fuglsang, H. Changes in visual function and in the posterior segment of the eye during treatment of onchoecerciasis with diethylcarbamazine citrate. *Br. J. Ophthalmon*. 64, 191–200 (1980).

5. Hawking, F. & Laurie, W. Action of hetrazan on *A. suum* (1980).

6. Maizels, R. M. & Denham, D. A. Diethylcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. *Parasitology* 105, 49–560 (1992).

7. Peixoto, C. & Silva, B. Anti-inflammatory effects of diethylcarbamazine: a review. *Eur. J. Pharmacol.* 734, 35–41 (2014).

8. Kanasa-thasan, N., Douglas, J. G. & Kazura, J. W. Diethylcarbamazine inhibits endothelial and microfilarian prostanoitid metabolism in vitro. *Med. Biochem. Parasitol.* 49, 11–19 (1991).

9. Samje, M., Metuje, G., Mba, J., Nguesson, B. & Cho-Ngwa, F. In vitro anti-onchoecerca ochensi activities of extracts and chromatographic fractions of *Crasperispermum laurinum* and *Morinda lucida*. BMC Complement Alter Med. 14, 325 (2014).

10. Buxton, S. K., Neveu, C., Charvet, C. I., Robertson, A. P., & Martin, R. J. On the mode of action of emodepside: slow effects on membrane potential and voltage-activated currents in *Acarus suum*. *Br. J. Pharmacol.* 164, 453–470 (2011).

11. Rae, M. G., Hilton, J. & Sharkey, J. Putative TRP channel antagonists, SKF 10.

12. Buxton, S. K., Neveu, C., Charvet, C. I., Robertson, A. P., & Martin, R. J. On the mode of action of emodepside: slow effects on membrane potential and voltage-activated currents in *Acarus suum*. *Br. J. Pharmacol.* 164, 453–470 (2011).

13. Elokely, K. et al. Understanding TRPV1 activation by ligands: insights from *C. elegans* model of nicotine-dependent behavior: regulation by TRP-family channels. *Cell 127*, 621–633 (2006).

14. Park, S. K. et al. The anhelimthic drug praziquantel activates a schistosome transient receptor potential channel. *J. Biol. Chem.* 294, 18873–18880 (2019).

15. Santoni, G. et al. "Immuno-transient receptor potential ion channels": the role in monocyte- and macrophage-mediated inflammatory responses. *Front. Immunol.* 9, 1273 (2018).

16. Storey, B. et al. Utilization of computer processed high definition video imaging for measuring motility of microscopic nematode stages on a quantitative scale: "The Worminator". *Int. J. Parasitol. Drugs Drug Resist.* 4, 233–243 (2014).

17. Qian, H., Robertson, A. P., Powell-Coffman, J. A., & Martin, R. J. Levamisole resistance resolved at the single-channel level in *Caenorhabditis elegans*. *FASEB J.* 22, 3247–3254 (2008).

18. Richmond, J. E. & Jorgensen, E. M. One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. *Nat. Neurosci.* 2, 791–797 (1999).

19. Robertson, A. P., Buxton, S. K. & Martin, R. J. Whole-cell patch-clamp recording of nicotinic acetylcholine receptors in adult *Brugia malayi* muscle. *Parasitol. Int.* 62, 616–618 (2013).

20. McCoy, C. J. et al. RNA interference in adult *Acarus suum*—an opportunity for the development of a functional genomics platform that supports organism-, tissue- and cell-based biology in a nematode parasite. *Int. J. Parasitol.* 45, 673–678 (2015).

21. Paffil, M. W. A new mathematical model for. *Nucleic Acids Res.* 29, e45 (2001).

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**Author contributions**

S.V., S.S.K., A.P.R., and R.J.M. conceived the research idea and hypothesis; S.V. and S.S.K., performed research; S.V., S.S.K., A.P.R., and R.J.M. contributed new reagents/analytic tools; S.V., S.S.K., A.P.R., and R.J.M. analyzed and interpreted data; and S.V., S.S.K., A.P.R., and R.J.M. wrote, revised, and edited the manuscript.
Competing interests
The authors declare no competing interests.

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