Anthelmintic drugs*

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Abstract

***C. elegans* is sensitive to the majority of anthelmintic drugs that are used against parasitic worm infections of humans and livestock. This has provided the opportunity to use molecular genetic techniques in the worm for mode of action studies. These approaches continue to be of considerable value to the field of parasitology. In addition, there are numerous examples of anthelmintic drugs providing exceptionally useful pharmacological tools to delineate fundamental aspects of cell signalling in *C. elegans.* This has primarily been achieved through the use of anthelmintics in forward genetic screens followed by the mapping and characterization of genes that confer altered susceptibility to the drug. Less fruitful so far, but nonetheless useful, has been the direct use of *C. elegans* for anthelmintic discovery programmes. In this brief review we provide an introduction to the use of *C. elegans* as a 'model parasite', outline the actions of the main classes of anthelmintics, and highlight approaches that have been of particular value.**

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1. Introduction to anthelmintics

Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections (http://www.who.int/wormcontrol/statistics/). Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes.

Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery or therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines. There is thus a pitifully small repertoire of chemotherapeutic agents available for treatment (see Table 1). In some respects, this situation has been exacerbated by the remarkable success of ivermectin over the last twenty years (Geary, 2005), which has decreased motivation for anthelmintic drug discovery programmes (Geary, Sangster and Thompson, 1999). This prompts concern, as anthelmintic resistance has been widely reported in livestock and it may also only be a matter of time before this phenomenon occurs in parasites of humans.

Table 1. Key drugs registered for the treatment of parasitic worms in humans.

Schistosomiasis (blood fluke)	**Intestinal round worms**
Antimonials	Piperazine
Metrifonate	Benzimidazoles
Oxamnaquine	Morantel
Praziquantel	Pyrantel
	Levamisole
Cestodiasis (tape worm)	Avermectins and milbemycins
Niclosamide	Closantel (and halogenated salicylamides)
Benzimidazoles	Emodepside
Praziquantel	
Fasciolasis (liver fluke)	**Filariasis (tissue round worms)**
Praziquantel	Diethylcarbmazine
Closantel	Suramin
(and halogenated salicylamides)	Ivermectin

Broad spectrum anthelmintics are effective against parasitic flat worms and nematodes. However, the majority of drugs are more limited in their action, e.g., praziquantel, a drug used in the treatment of schistosomiasis and thought to act by disrupting calcium homeostasis (Greenberg, 2005), has no activity against nematodes (see Table 1). For the purpose of this review we will focus on drugs used in human and veterinary medicine to treat parasitic nematode infection.

2. Is *C. elegans* a model 'parasite'?

Parasitic nematodes are not an ideal laboratory animal for many reasons, not least of which is the problem with maintaining sometimes complex life-cycles. Thus *C. elegans* has routinely been exploited as a more 'user-friendly' model system that is also highly tractable to molecular genetic techniques. However, the life-style of the free-living worm *C. elegans* is very different to that of the parasites, and therefore parasitologists have given careful thought to its relevance (Geary and Thompson, 2001). In terms of overall 'body plan' there is no doubt that all the species in the phylum Nematoda exhibit similarity despite their very different habitats. More detailed

consideration has been given to a comparison of the genetics, e.g., the arrangement of the genome, synteny and homology between specific genes (Mitreva et al., 2005). Overall, there would seem to be considerable molecular diversity between the different species in the phylum. It is probably safe to conclude that *C. elegans* is no more dissimilar to parasitic nematodes than each individual species of parasite is to another. Using *C. elegans* permits the application of powerful molecular genetic approaches and it has been extensively, and successfully, exploited as a model system to define molecular components of signalling pathways that underpin nematode physiology.

Indeed, it could be argued that the most relevant consideration for using *C. elegans* as a model to study anthelmintics is the comparative physiology and pharmacology of the Nematoda. Most anthelmintics target the neuromusculature and therefore similarities here become of particular importance. The wiring diagram for the neuromuscular system is similar between *C. elegans* and the parasitic nematode *Ascaris suum* (Angstadt, Donmoyer and Stretton, 1989) as are the major neurotransmitters; acetylcholine in the excitatory motorneurones (Johnson and Stretton, 1985), GABA in the inhibitory motorneurones (Johnson and Stretton, 1987) and glutamate providing input onto the motorneurones (Davis, 1998). A striking feature of the pharmacology of the nematode nervous system is the abundance of neuropeptides. The majority, conceivably all, of *C. elegans* neurones express neuropeptides along with their classical neurotransmitters (for review see Li, 2005). Many of these peptides have potent effects in parasitic nematodes, specifically on musculature which regulates vital nematode processes including feeding, locomotion and egg-laying (Brownlee, Holden-Dye and Walker, 2000). Further evidence for an important functional role for peptidergic signalling has been provided by analysis of *C. elegans* mutants. In these studies mutants for the enzymes involved in the processing of neuropeptides, the proconvertase *egl-3* (Kass et al., 2001) and carboxypeptidase *egl-21* (Jacob and Kaplan, 2003), have been shown to be severely depleted in neuropeptide content (Husson et al., 2006) and to have altered behaviours consistent with the idea that neuropeptides have modulatory roles within the neural networks that direct a range of important worm behaviours.

Neuropeptides are present throughout the animal phyla and also subserve pivotal roles in the nervous systems of the mammalian hosts of parasitic nematodes. However, neuropeptides, unlike the small molecule ‘classical’ transmitters e.g. acetylcholine, have diverged considerably in structure during evolution and this presents the promise that drugs targeting peptidergic signalling in nematodes may have low mammalian toxicity. There has thus been considerable interest in the prospect of developing peptidomimetics as a novel approach for nematode control. A comparative analysis of the neuropeptide content for a range of nematodes has been comprehensively reviewed (McVeigh et al., 2005) and may become an important consideration if this strategy is to be pursued.

3. Approaches for the study of anthelmintics in *C. elegans*

There is a large body of literature describing the study of bioactive compounds in *C. elegans* and the proposal to use it for the study of anthelmintics precedes the publication of the *C. elegans* genome sequence by nearly 20 years. Rand and Johnson (1995) coined the term ‘genetic pharmacology’ to describe this approach. These studies generally hinge on the ability of a drug to elicit a significant, ideally quantifiable, change in the worm's growth, development, metabolism, and/or behaviour. Pharmacokinetic considerations include the method and duration of drug exposure. For the vast majority of experiments the anthelmintics are applied to intact *C. elegans*. There are thus two ways in which the drug can gain access to target tissues, namely by ingestion or by diffusion across the cuticle. In this regard it should be noted that for many drugs the cuticle presents a significant permeability barrier. Thus the lipophilicity of drugs has a strong bearing on the concentration that is achieved in target tissues following external application. It is not uncommon for polar drugs to be applied at a concentration 1000 fold higher than their predicted affinity for the target. It may be possible to ameliorate this problem to some extent by employing animals that have a compromised cuticle (Gravato-Nobre et al., 2005). Once the effect of a particular drug on *C. elegans* has been defined, two different strategies may be adopted to investigate the molecular basis for its biological activity. The first follows a hypothesis led approach in which strains with mutations in genes of known function are tested for altered sensitivity to the drug. The alternate strategy is to conduct a forward genetic screen. This is a powerful and objective approach that provides novel insight into the signalling pathways that mediate anthelmintic action. Often the impact of these studies extends beyond the interests of parasitologists and into the broader context of cellular and molecular neuroscience. This is because the vast majority of anthelmintics exert their effects in the neuromuscular system and key transduction molecules in the nervous system are highly conserved across the phyla from worm to human. Thus anthelmintic resistance screens can promote the discovery and characterisation of genes that have important roles in neurotransmission. An excellent example of the utility of this approach is provided by genetic screens employing the organophosphate cholinesterase inhibitors, in particular aldicarb. The detail of these studies is beyond the scope of this review as organophosphates are not widely used as anthelmintics. (Toxicity limits their use to highly regulated applications for plant parasitic nematodes). However, aldicarb screens provide an excellent

example of ‘genetic pharmacology’ and the interested reader is directed to Nguyen et al., 1995; Miller et al., 1996; and Seiburth et al., 2005.

4. Classes of anthelmintic drugs

Anthelmintics are separated into classes on the basis of similar chemical structure and mode of action. There are only a few main classes and each is briefly discussed in turn below. For the most part, information on the physiological and pharmacological actions of anthelmintics has been obtained from studies on the large parasitic nematode *A. suum*. *C. elegans*, on the other hand, has been valuable in defining molecular targets.

4.1. Piperazine

Piperazine was first used as an anthelmintic in the 1950s and it is still the active constituent of over the counter remedies for thread worm infection in children. Its mode of action has primarily been studied in *A. suum*. There is surprisingly no literature on its action in *C. elegans* though there is no indication that it acts differently from its effects in *A. suum*. In *A. suum* it acts as a weak GABA-mimetic and causes a flaccid, reversible paralysis of body wall muscle. Single channel recordings provide evidence that it is a low efficacy, partial agonist at GABA-gated chloride channels (Martin, 1985).

4.2. Benzimidazoles

The first of this class, thiabendazole, was discovered in 1961 and subsequently a number of further benzamidazoles were introduced as broad spectrum anthelmintics. There is an extensive literature on these compounds reporting a number of different biochemical effects. Nonetheless, it is clear that their anthelmintic efficacy is due to their ability to compromise the cytoskeleton through a selective interaction with β-tubulin (Borgers and de Nollin, 1975; for review see Lacey, 1990). The effects of benzimidazoles on *C. elegans*, which include impaired locomotion, reproduction and a detrimental effect on oocytes, are consistent with disruption of processes requiring integral microtubules. The sensitivity of *C. elegans* to benzimidazoles is mediated by a single gene, *ben-1*, which encodes β-tubulin (Driscoll et al., 1989). This has provided a platform to investigate the molecular basis of benzimidazole resistance in parasitic nematodes. It has been noted that benzimidazole resistance in *Haemonchus contortus* seems to be associated with the presence of specific alleles for β-tubulin in the drug resistant isolates (Kwa et al., 1994). Whether or not a specific β-tubulin isoform could confer resistance to the drug was tested by experiments which showed that the sensitivity of *C. elegans ben-1* mutants to benzimidazole can be rescued by expressing a *H. contortus* allele of β-tubulin from benzimidazole susceptible isolates but cannot be rescued by the allele present in the resistant isolates (Kwa et al., 1995). This unequivocally demonstrated that a single amino acid sustitution, Y for F, in β-tubulin, can confer anthelmintic resistance. This is the first elegant example of a 'model hopping' approach in which the genetic tractability of *C. elegans* is directly exploited to define gene function in a parasitic worm.

4.3. Levamisole, pyrantel and morantel

These anthelmintics are nicotinic receptor agonists (Aceves et al., 1970; Aubry et al., 1970) and elicit spastic muscle paralysis due to prolonged activation of the excitatory nicotinic acetylcholine (nACh) receptors on body wall muscle. Their precise mode of action has been carefully studied at the single-channel level on the body wall muscle preparation of *A. suum* (see Martin et al., 2005 for review). Pharmacological analysis has provided evidence for subtypes of nACh receptor (Qian et al., 2006), an N-type (preferentially activated by nicotine), a B-type (preferentially activated by bephenium) and an L-type (preferentially activated by levamisole and associated with levamisole resistance). Levamisole, and related compounds, also cause spastic paralysis and egg-laying in *C. elegans*. Indeed, recordings from *C. elegans* body wall muscle using levamisole and nicotine as agonists have provided further evidence that there are muscle subtypes of nACh receptor and that these subtypes have different nACh receptor subunit compositions. At least four subunits, *unc-38*, *unc-29*, *unc-63* and *lev-1* contribute to the levamisole receptor (Culetto et al., 2004; for further information on the nACh receptor subunit family see Rand, 2007). Thus, these anthelmintics are providing pharmacological tools to dissect subtypes and stoichiometries of native nematode nicotinic receptors.

Perhaps more importantly, levamisole has been extremely productive in forward genetic screens. In the earliest studies tetramisole was used (Brenner, 1974) and later this was replaced by the more active isomer, levamisole (Lewis et al., 1980). These screens have provided a resource of mutants that have been used over the last two decades to assign function to genes expressed at the neuromuscular junction. Some of these are nACh receptor

subunits, but others interestingly are not and serve to either regulate nicotinic receptors or muscle function (see Table 2).

Table 2. A summary of gene function for *C. elegans* levamisole resistant mutants.

Gene	Function	Reference
unc-38[1]	α-like nACh receptor subunit	Fleming et al., 1997
unc-29[1]	Non α-like nACh receptor subunit	
lev-1[2]	Non α-like nACh receptor subunit	
unc-63[1]	α-like nACh receptor subunit	Culetto et al., 2004
unc-22[2]	Twitchin	Moerman et al., 1988
unc-50[1]	Transmembrane protein required for receptor trafficking.	Bessereau[3]
unc-68[2]	Ryanodine receptor	Maryon et al., 1996
lev-9[2]	Putative secreted protein required for levamisole nACh receptor clustering	Bessereau[3]
lev-11[2]	Tropomyosin	Kagawa et al., 1995
lev-8 (acr-13)[2]	α-like nACh receptor subunit	Towers et al., 2005
lev-10[2]	CUB/LDL protein; receptor clustering	Gally et al., 2004
ric-3	Required for maturation of nACh receptor	Halevi et al., 2002
Isolated and identified as levamisole resistant by [1]Brenner (1974), [2]Lewis et al. (1980). [3]Unpublished data, see www.wormbase.org		

4.4. Paraherquamide

Paraherquamide A and marcfortine A are both members of the oxindole alkaloid family, originally isolated from *Penicillium paraherquei* and *Penicillium roqueforti*, respectively (Zinser et al., 2002). Marcfortine A was found to be active against *C. elegans* in a high throughput screen (Lee et al., 2002). A specific, high affinity binding site for paraherquamide has been identified in a membrane preparation isolated from *C. elegans*, with an apparent Kd of 263nM (Schaeffer et al., 1992). Paraherquamide and its derivative, 2-deoxy-paraherquamide, induce flaccid paralysis in parasitic nematodes, *in vitro*. Pharmacological analysis of the effects of these drugs on acetylcholine-stimulated body wall muscle contractions in *A. suum* muscle strips *in vitro* has shown that they act as typical competitive antagonists, shifting the concentration-response curves to the right in a parallel fashion (Robertson et al., 2002). These drugs have no apparent direct effect on *A. suum* body wall muscle tension or membrane potential (Lee et al., 2002). Paraherquamide also blocks the actions of other nicotinic agonists, but not equipotently (Zinser et al., 2002; Robertson et al., 2002). Interestingly, this antagonist seems to distinguish nicotinic receptor subtypes on the muscle and has a greater affinity for the receptors mediating the response to levamisole and pyrantel, than the receptors that mediate the response to nicotine. One might therefore expect that paraherquamide would be an effective antagonist of the levamisole-selective receptor on *C. elegans* body wall muscle. Importantly, the mode of action of this class of anthelmintics differs from the more established drugs that interfere with cholinergic transmission, e.g., levamisole, in that they act as competitive antagonists rather than cholinomimetics. The use of paraherquamide in forward genetic screens has not yet been reported but could potentially generate interesting new mutants. As it is a competitive inhibitor of the body wall nACh receptor it would be predicted that mutations that increase transmitter release should confer resistance. Thus a forward genetic screen might reveal further negative regulators of neurotransmitter release.

4.5. Ivermectin (macrocylic lactones and milbemycins)

Ivermectin was introduced as an anthelmintic in the 1980s by Merck. It is a semi-synthetic derivative of avermectin which is a large macrocyclic lactone fermentation product of the micro-organism *Streptomyces avermitilis*. It is remarkably potent (~1nM) and persistent in its effect and its discovery enthused other companies to invest in the development of ivermectin analogues which include moxidectin, milbemycin oxime, doramectin,

selamectin, abamectin and eprinomectin. Here *C. elegans* played a role as it was employed in a screen for further macrocyclic lactones with ivermectin-like activity (Haber et al., 1991).

Ivermectin elicits a potent and persistent paralysis of nematode pharyngeal (Brownlee, Holden-Dye and Walker, 1997; Pemberton et al., 2001) and body wall musculature (Kass et al., 1980; Kass et al., 1982). It has been shown to interact with a range of ligand-gated ion channels including α7 nACh receptors (Krause et al., 1998), acetylcholine-gated chloride channels (Bokisch and Walker, 1986), GABA-gated chloride channels (Robertson, 1989; Holden-Dye and Walker, 1990), histamine-gated chloride channels (Zheng et al., 2002), glycine receptors (Shan et al., 2001) and P2X4 receptors (Khakh et al., 1999). However, it is its high affinity for nematode glutamate-gated chloride channels (GluCl) that correlates with its potent anthelmintic activity. This was defined by the team at Merck which succeeded in expression cloning GluClα and GluClβ ion channel subunits from *C. elegans* (Cully et al., 1994). Both subunits were expressed either singly, or together, in *Xenopus* oocytes. GluClα responds to micromolar ivermectin, but not to glutamate whilst GluClβ responds to glutamate but not ivermectin. Co-expression of GluClα and GluClβ yields a channel which responds to glutamate and is positively allosterically modulated by nanomolar ivermectin. Subsequently a small family of nematode genes encoding GluCl channels has been identified (see Yates, Portillo and Wolstenholme for review, 2003). The nomenclature is confusing as the same genes have been discovered by both homology screening approaches and from forward genetic screens for ivermectin resistance genes. Essentially there are four *C. elegans* genes encoding GluClα subunits, two of which are alternately spliced yielding the GluCl channels: GluClα1 encoded by *glc-1*; GluClα2A and B encoded by *avr-15*; GluClα3A and B encoded by *avr-14*; GluClα4 encoded by *glc-3*. There is just one GluClβ subunit encoded by *glc-2* and a further gene, *glc-4*, which is divergent from the genes encoding α and β subunits. Although the pharmacology of channels assembled from these GluCl subunits has been defined in heterologous expression systems, the important question of the subunit stoichiometry and pharmacology of the native channels is much more poorly defined. Further studies on *C. elegans* are providing a better understanding of this by delineating the expression pattern for GluCl subunits in the nervous system. For example, the pharyngeal muscle expresses *avr-15* and *glc-2*, (Dent, Davis and Avery, 1997; Laughton, Lunt and Wolstenholme, 1997). Thus it might be expected that GluClα2 and GluClβ subunits co-assemble to form a native ivermectin sensitive channel. Whether or not other subunits contribute to the functional receptor is not yet clear. However, the pharyngeal muscle of *avr-15* mutants does not respond to ivermectin (Dent, Davis and Avery, 1997; Pemberton et al., 2001) clearly indicating an involvement of GluClα2. An important point to note in terms of the site of anthelmintic action of ivermectin is that although the pharynxes of *avr-15* mutants are not inhibited by ivermectin, populations of *avr-15* mutants exposed to ivermectin are still paralyzed. Thus GluCl channels in the pharynx are not required for the paralytic effect. This may also be true for parasitic nematodes. For example, ivermectin has anthelmintic activity against *Ascaridia galli* and yet the pharynx of this species is not inhibited by the drug (Holden-Dye and Walker, 2006). In order to obtain a better understanding of the role of GluCl channels in mediating the paralytic actions of ivermectin it is probably more informative to consider their role in the motornervous system. Currently most information is available for *avr-14* and *avr-15*. These genes are expressed in the motor nervous system of *C. elegans* (Dent et al., 1997; Dent et al., 2000) and there is immunostaining for GluClα3A and B in motorneurones of the parasitic nematode *H. contortus*, (Portillo, Jagannathan, Wolstenholme 2003). One role of these GluCl channels in *C. elegans* involves regulation of the duration of forward movement, a well established glutamatergic-regulated behaviour (Brockie and Maricq, 2006). This function may be conserved between *C. elegans* and *H. contortus* as *H. contortus* GluClα3 subunits expressed in *C. elegans avr-14* mutants restore the wild type pattern of movement (Cook et al., 2006). It is most likely that the paralytic action of ivermectin derives from its potent activation of GluCl in the motornervous system of nematodes. However, the precise role of individual GluCl channels in mediating the effects of ivermectin on these circuits is yet to be established. The mechanism of resistance to ivermectin has also been studied in *C. elegans*. High level resistance is a complex phenomenon which requires mutations in at least three genes, namely in *glc-1*, *avr-14* and *avr-15*. Further genes, regulating membrane permeability (*osm-1*) and gap junctions (*unc-7* and *unc-9*), are also involved (Dent et al., 2000). Defining the role of GluCl mutations in conferring ivermectin resistance to parasitic nematodes in the field is a less tractable and more controversial problem (Wolstenholme et al., 2004).

4.6. Emodepside (cyclodepsipeptides, PF1022A)

The cyclodepsipeptide molecule, emodepside, is a semi-synthetic derivative of PF1022A, a fermentation product obtained from the fungus, *Mycelia sterilia*, of *Camelia japonica*. Its discovery and anthelmintic activity has recently been reviewed (Harder, von Samson-Himmelstjerna, 2002). It is effective against isolates of parasites that are resistant to benzimidazole, levamisole and ivermectin indicating, importantly, that it has a novel mode of action. The molecule has pore-forming properties in planar lipids, however, this does not appear to be important in conferring its anthelmintic potency as an optical isomer of emodepside, with similar pore forming properties, does not have anthelmintic action. Thus it would appear that it may act through stereospecific binding to a receptor.

Studies in *A. suum* have highlighted muscle paralysis and point to a calcium- and potassium-dependent mechanism of action (Willson et al., 2003). A candidate receptor for the cyclodepsipeptides has been cloned from a *H. contortus* cDNA library by immunoscreening with an antibody to PF1022 A. This receptor, designated HC110R, has been expressed in HEK293 cells and shown to gate calcium flux in a PF1022 A-dependent manner (Saeger et al., 2001). It has homology to mammalian latrophilins, a class of G protein-coupled receptors which bind the neurotoxin, latrotoxin. Latrotoxin paralyses mammals by triggering neurotransmitter release, and thus the identification of latrophilin as an emodepside receptor raised the intriguing possibility that emodepside may cause paralysis of nematodes by stimulating excessive neurotransmitter release at neuromuscular sites.

C. elegans is very susceptible to the effects of emodepside at nanomolar concentrations (Bull et al., 2007). The effects include slowed development, inhibition of pharyngeal pumping, decreased locomotion (forward movement is most affected at low concentrations), and inhibition of egg-laying leading to 'bagging' in adult hermaphrodites. Thus *C. elegans* has provided an excellent model in which to define the molecular target, or targets, through which emodepside exerts its pleiotropic actions and indeed, to test whether latrophilins are involved.

There are two genes encoding candidate latrophilins in *C. elegans*, *lat-1* and *lat-2*. The role of these latrophilins in mediating the inhibitory effects of emodepside on feeding and locomotion has been investigated using RNAi and gene knockouts (Willson et al., 2004; Bull et al., 2007; Guest et al., 2007). The pharyngeal system of *lat-1* mutants show a reduced sensitivity to emodepside but their locomotor activity is inhibited in a similar fashion to wild-type animals (Guest et al., 2007). Emodepside does not exert its inhibitory effect on locomotion through the other latrophilin, LAT-2, as *lat-2* mutants respond just like wild-type animals. Nor is there redundancy of function between LAT-1 and LAT-2 in terms of the effect of emodepside on locomotion, as the double mutant *lat-2*,*lat-1* responds to emodepside in a similar fashion to the single mutant, *lat-1* (Guest et al., 2007). Clearly, emodepside has latrophilin-independent actions.

Recently, forward genetic screens have provided further insight into the mechanism through which emodepside exerts its inhibitory action on *C. elegans* muscle. A chemical mutagenesis screen of twenty thousand genomes for mutants that could move and propagate on micromolar emodepside recovered nine alleles of a single gene, *slo-1*. Strains carrying functional null alleles of *slo-1* are highly resistant to the inhibitory effects of emodepside on locomotion and feeding. Furthermore, strains carrying gain-of-function alleles of *slo-1* behave in a similar manner to emodepside-treated animals but are not themselves emodepside hypersensitive. These data strongly support the contention that emodepside activates a SLO-1-dependent pathway to bring about neuromuscular inhibition.

SLO-1 is a calcium-activated potassium channel (Wang et al. 2001) homologous to the mammalian BK channels. Thus the discovery of SLO-1 as an important effector for emodepside resonates with earlier work on *Ascaris* muscle which showed a calcium and potassium-dependent hyperpolarisation (Willson et al., 2003). This channel is highly conserved throughout the animal phyla and plays a pivotal role in regulating neuronal and muscle cell excitability (for review, Salkoff et al., 2006). In *C. elegans* it is widely expressed in the nervous system and body wall muscle, but not in pharyngeal muscle. By expressing a wild-type copy of *slo-1* in specific subsets of cells in a *slo-1* null background it has been shown that emodepside can inhibit locomotor activity when SLO-1 is present in neurones or body wall muscle but it can only inhibit feeding if SLO-1 is present in neurones (Guest et al., 2007). Earlier studies showed that mutations in a number of genes encoding synaptic proteins confer altered sensitivity to the effect of emodepside on feeding consistent with the idea that it acts in the neuronal network to disrupt rhythmic feeding behaviour (Willson et al., 2004).

The discovery of SLO-1 as a mediator of the inhibitory effects of emodepside on *C. elegans* is an important development in this field which in the light of ivermectin resistance has an urgent need for such breakthroughs. In the broader context, there is an emerging body of evidence indicating that BK channels such as SLO-1 may play a pivotal role in conferring sensitivity to neuroactive drugs, including ethanol and local anaesthetics (Davies et al., 2003; Hawasli et al., 2004) and in regulating the pattern of activity of neural networks (Salkoff et al., 2006). Thus, an additional and valuable facet of these studies is that emodepside provides a new tool to dissect the functional role of SLO-1 in neural networks using the power of molecular genetics in *C. elegans*.

4.7. Nitazoxanide

Nitazoxanide, a pyruvate ferredoxin oxidoreductase inhibitor, acts against a broad spectrum of protozoa and helminths that occur in the intestinal tract. It is currently used for the treatment of protozoal infections (and is therefore not listed in Table 1). The site of action of this compound has not been established in nematodes although

anaerobic electron transport enzymes may be a potential target (Gilles and Hoffman 2002). The effect of nitazoxanide has been examined on growth and development of *C. elegans* (Fonseca-Salamanca et al., 2003). After seven days culture, nitazoxanide 100 µM, only reduced population growth by 33%. In contrast mebendazole, 5µM, and albendazole, 1 µM, reduced growth by over 90%. Nitazoxanide, 100 µM, had no effect on either embryonation or hatching in *Heligmosomoides polygyrus.* Therefore the efficacy of this compound is relatively low compared to other anthelmintic agents.

5. The future

A particular challenge for companies engaged in anthelmintic research, not shared by its more wealthy cousin the pharmaceutical industry, is that for the anthelmintic market cost-effectiveness is a prime concern. Furthermore, for tropical medicine, anthelmintics must be used in mass chemotherapy programmes in regions where clinical support is sparse and therefore drugs need to be very well tolerated in humans. For the last twenty years a small repertoire of drugs, especially ivermectin, has addressed this need. Indeed, ivermectin has been hugely successful both in veterinary and tropical medicine and its use in Africa has transformed the lives of populations previously devastated by the tropical disease Onchocerciasis (river blindness).

The most pressing concern for the future is the emergence of resistance to all the currently used anthelmintics, including ivermectin. *C. elegans* was pivotal in defining the mode of action for the majority of these drugs and can also contribute to understanding mechanisms of resistance especially through the adoption of a 'model-hopping' approach whereby the functionality of parasite genes is assessed by using *C. elegans* as an expression system.

Of equal importance for the future is the use of *C. elegans* as a model system for drug discovery. It is to be hoped that this will provide new anthelmintics that have novel modes of action and thus will circumvent the problem of anthelmintic resistance. The advent of RNAi and high throughput techniques for analysis of *C. elegans* has led to renewed interest in drug discovery using chemical genetic screens (Behm et al., 2005; Jones, Buckingham and Sattelle, 2005). The molecular targets for the drugs that come out of these screens, or from other pipe-lines, can be defined using the powerful approach of forward genetics in *C. elegans* and may uncover new effectors for anthelmintic action. Recently advances have been made exploiting *C. elegans* for such mode of action studies and have highlighted a new effector, SLO-1, in the anthelmintic action of the latest drug to hit the market, emodepside (Profender).

More targeted approaches to anthelmintic drug discovery are also ongoing. These are providing fundamental information on nematode neurobiology with the aim of identifying signalling molecules that have pivotal roles in neural circuits that underpin vital behaviours. Here it will be interesting to see whether or not the ambition to target peptidergic signalling pathways translates into a marketable drug (Greenwood, Williams and Geary, 2005).

In conclusion, it is clear that *C. elegans* has, and will continue to prove an exceptionally tractable and informative model system for parasitic nematodes and mode of action studies for anthelmintics. The future holds the promise that *C. elegans* may also directly contribute to drug discovery. These efforts are of utmost importance in view of the increasing threat to live-stock and humans from anthelmintic resistant strains of parasite.

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