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Acta Veterinaria-Beograd 2017, 67 (2), 137-152
UDK: 615.284.03
DOI: 10.1515/acve-2017-0013

Review article

A BRIEF REVIEW ON THE MODE OF ACTION OF ANTINEMATODAL DRUGS

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(Received 01 May, Accepted 24 May 2017)

Anthelmintics are some of the most widely used drugs in veterinary medicine. Here we review the mechanism of action of these compounds on nematode parasites. Included are the older classes of compounds; the benzimidazoles, cholinergic agonists and macrocyclic lactones. We also consider newer anthelmintics, including emodepside, derquantel and tribendimidine. In the absence of vaccines for most parasite species, control of nematode parasites will continue to rely on anthelmintic drugs. As a consequence, vigilance in detecting drug resistance in parasite populations is required. Since resistance development appears almost inevitable, there is a continued and pressing need to fully understand the mode of action of these compounds. It is also necessary to identify new drug targets and drugs for the continued effective control of nematode parasites.

Key words: anthelmintic, parasite, benzimidazoles, avermectins, cholinergic, emodepside, derquantel

INTRODUCTION

Anthelmintics are drugs that are used to treat infections caused by parasitic worms (helminths) [1]. There are three major groups of helminths namely: nematodes (roundworms), trematodes (flukes) and cestodes (tapeworms). These groups of helminths are divided into two phyla; nematodes (roundworms) and platyhelminths (trematodes and cestodes) [2]. Anthelmintics either kill worms or cause their expulsion from the body, without causing any significant damage to the host [3]. Although there is a high prevalence of parasitic worms, the progress of anthelmintic drug discovery and development by pharmaceutical companies has been slow over the years. One contributing factor is that the majority of those suffering from helminth infections live in developing nations who lack the resources to support a profitable drug market [4]. Development of new anthelmintics is limited by high costs and modest global markets for antiparasitic drugs and chemicals. The cost of development of a new

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drug is estimated at US $400 million for livestock use, and more than US $800 million for human use. The global market for antiparasitic drugs and chemicals are estimated at US $12 billion for plant pathogens, $11 billion for livestock and companion animals, and $0.5 for human health [5-8]. Many anthelmintic drugs used to treat humans were first developed and marketed as veterinary drugs [9-11]. There are only a few classes of anthelmintics including; benzimidazoles, imidazothiazoles, tetrahydopyrimidines, macrocyclic lactones, amino-acetonitrile derivatives, spiroindoles and cyclooctadepsipeptides. Here we review the mode of action of several classes of drug used to treat infections with parasitic nematodes.

Benzimidazoles (BZs)

Thiabendazole was the first benzimidazole anthelmintic agent produced. Since the introduction of thiabendazole in 1961, a number of benzimidazoles with improved efficacy and extended spectrum of action have been developed [12]. These include mebendazole, albendazole and flubendazole (Figure 1). The initial mode of action of benzimidazoles was thought to be inhibition of various parasite metabolic enzymes including fumarate reductase and malate dehydrogenase [13,14]. However, it is now established that benzimidazoles selectively bind with high affinity to parasite β-tubulin and inhibit microtubule polymerization. This results in the destruction of cell structure and consequent death of the parasite [15].

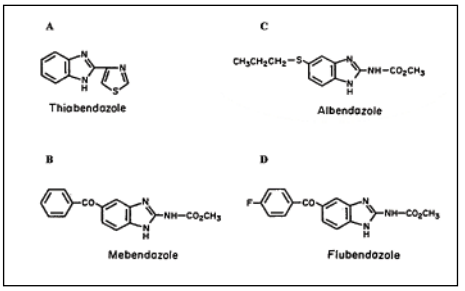

Figure 1. Chemical structures of thiabendazole **(A)**, mebendazole **(B),** albendazole **(C)** and flubendazole **(D)**. From [12].

Imidazothiazoles

Imidazothiazoles act as nicotinic acetylcholine receptor (nAChR) agonists. They bind to nAChRs on body wall muscles, causing spastic paralysis of the worm, and hence, its expulsion from the host [16]. Tetramisole (Figure 2), an aminothiazol derivative, was the first member of this class of anthelmintics, and constitutes a racemic mixture of 50% L- or S- and D- or R-isomers [17-19]. The L-isomer was later demonstrated to be more potent than the racemic mixture or the D-isomer [20,21]. Consequently, the D-isomer was removed from the racemic mixture and this led to the development of the L-isomer as levamisole. The detailed mode of action of levamisole, the only existing drug in this class, has been carefully studied at the single-channel level in nematode body wall muscles [22-24]. Robertson and Martin [24], showed using the

patch-clamp technique that at the single-channel level in *A. suum* muscles, levamisole (1 – 90 μM concentrations) causes activation of cation-selective channels, in addition to voltage-sensitive open channel-block and desensitization. The mean open-times for single-channel currents activated by levamisole were 0.80 – 2.85 ms and the conductance levels were 19 – 46 pS, with a mean of 32.9 ± 1.23 pS. This corresponded to the levamisole-sensitive, L-subtype nAChR with a channel conductance of 35 pS, as revealed by Qian *et al.* [22]. Robertson *et al.* [23], later revealed the presence of a similar nAChR subtype in levamisole-sensitive *O. dentatum* muscle patches which was absent in the levamisole-resistant muscle patches. In subsequent oocyte expression studies, the reconstituted *O. dentatum* L-subtype nAChR (UNC-29, UNC-38, UNC-63 and ACR-8) was preferentially sensitive to levamisole and also had a single-channel conductance of ~35 pS [25]. Levamisole not only causes spastic paralysis but it also stimulates egg-laying in wild-type *C. elegans* [26].

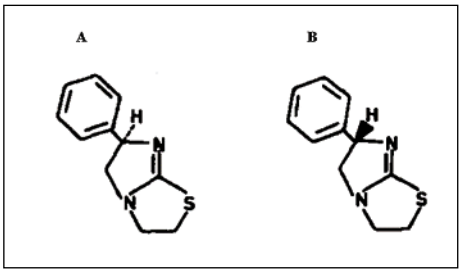

Figure 2. Chemical structures of R (+)-tetramisole **(A)** and S (-)-tetramisole (levamisole) **(B).** From [21].

Tetrahydropyrimidines

Tetrahydropyrimidines share a similar mode of action to imidazothiazoles and are commonly grouped together as nicotinic agonists [27,28]. Examples of this anthelmintic drug class include pyrantel, oxantel and morantel (Figure 3). Pyrantel is an imidazothiazole-derived tetrahydropyrimidine that was discovered in 1966 as an anthelmintic agent with broad spectrum activity against roundworms and hookworms in domestic animals [29,30]. Pyrantel however lacks activity against whipworms [31]. Studies on the mode of action of pyrantel at the single-channel level identified the L-subtype nAChR in *A. suum* as also preferentially activated by pyrantel [32]. Pyrantel, like levamisole, also causes open channel-block [33]. Although not characterized at the single-channel level, the *O. dentatum* nAChR receptor subunits UNC-29, UNC-38 and UNC-63 reconstitute a pyrantel/tribendimidine- but not levamisole-sensitive nAChR subtype in *X. laevis* oocytes [25]. The search for an agent with activity against whipworms led to the development of oxantel, an m-oxyphenol derivative of pyrantel [31]. Contrary to pyrantel, oxantel preferentially activates the N-subtype nAChRs in *A. suum* [34]. Oxantel, like levamisole and pyrantel, also causes open channel-block in *A. suum* [35]. Morantel is a methyl ester analog of pyrantel which also targets the L-subtype nAChR in *A. suum* [36,37]. At the single-channel level, morantel causes the activation and block of this receptor subtype [38]. Recently, morantel was shown to act

as an agonist of the nAChR subtype comprising ACR-26/ACR-27 subunits from *H. contortus* or *Parascaris equorum* expressed in *X. laevis* oocytes [39]. In oocyte expression studies, morantel was seen to cause a non-competitive voltage-sensitive open channel block of the newly characterized *A. suum* ACR-16 receptor [40].

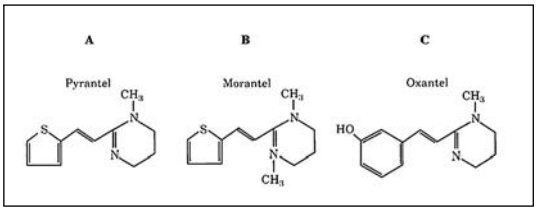

Figure 3. Chemical structures of pyrantel **(A)**, morantel **(B)** and oxantel **(C)**. From [28].

Macrocyclic lactones (MLs)

Macrocyclic lactones (avermectins and milbemycins) are a group of chemical compounds derived from soil microorganisms of the genus *Streptomyces* [41-43]. MLs were introduced in the 1980s as antiparasitic agents with broad spectrum activity against nematodes and arthropods [44,45]. Examples of commercially available avermectins are ivermectin, abamectin, doramectin and selamectin, while milbemycin oxime and moxidectin, are examples of commercially available milbemycins (Figure 4). MLs are selective agonists of glutamate-gated chloride channels (GluCls) which are present in neurons and pharyngeal muscles of nematodes and arthropods, but absent in humans. ML activation of GluCls inhibits movement and pharyngeal pumping [46,47]. In addition to GluCl effects, the avermectins also act as antagonists of 4-aminobutyric acid (GABA) and nicotinic receptors expressed on somatic muscle cells of parasitic nematodes [48-50]. Ivermectin, the first member of the avermectins, although originally developed as a veterinary drug, was later approved for use in humans for the control of onchocerciasis and lymphatic filariasis [9-11,51]. Also, ivermectin was shown to act as an irreversible agonist of recombinant human glycine receptors at higher concentrations (>0.3 μM), but at lower concentrations (30 nM), it acted as a positive allosteric modulator [52]. Ivermectin showed a similar positive allosteric modulation effect on the vertebrate neuronal α7 nicotinic acetycholine receptor [53].

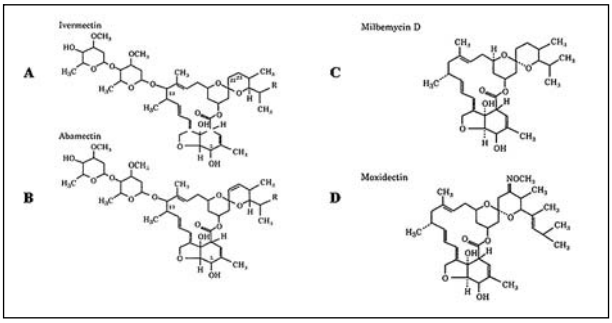

Figure 4. Chemical structures of ivermectin **(A)**, abamectin **(B),** milbemycin D **(C)** and moxidectin **(D)**. From [28].

Amino-acetonitrile derivatives (AADs)

The AADs are a new class of synthetic anthelmintics with broad spectrum activity against nematodes that are resistant to the benzimidazoles, imidazothiazoles and macrocyclic lactones [54-56]. Monepantel, also known as AAD 1556, is the first member of this class to be developed for the control of a broad range of parasitic nemtaodes in sheep (Figure 5) [56]. Genetic screens of *C. elegans* identified ACR-23, which belongs to the nematode-specific DEG-3 subfamily of nAChRs, as the target of AADs [55]. Further studies on the mode of action of the AADs have led to the confirmation of ACR-23 as the principal target for monepantel in *C. elegans*, as well as the identification of other DEG-3-like nAChR target genes; *H. contortus monepantel-1* (*Hco-mptl-1*, formerly *Hc-acr-23*), *Hco-des-2*, *Hco-deg-3* and *C. elegans acr-20* [57-60]. Baur *et al.*, [57], also demonstrated that at low concentrations (<1 nM), monepantel acts as a positive allosteric modulator of *H. contortus* MPTL-1 and *C. elegans* ACR-20 receptors, and at high concentrations (>0.1 μM), it acts as a direct agonist of these receptors. In a different study, monepantel by itself did not activate *H. contortus* DEG-3/DES-2 receptors expressed in *X. laevis* oocytes, but did cause a potentiation in the receptors' current responses when co-applied with choline [61].

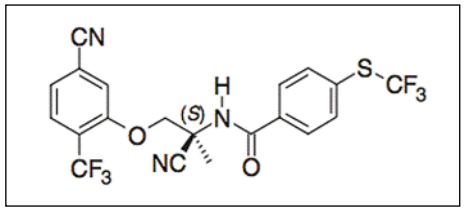

Figure 5. Chemical structure of monepantel. From [56].

Spiroindoles

Derquantel (2-deoxy-paraherquamide or PNU-141962) is the first semi-synthetic member of this new class of anthelmintics (Figure 6) [62,63]. Derquantel which is also the first commercial member of the spiroindoles, was introduced in 2010 for use in combination with the macrocyclic lactone, abamectin, under the trade name STARTECT®, for the control of parasitic nematodes in sheep. Derquantel acts as an antagonist of nAChRs to cause flaccid paralysis which results in the expulsion of parasites from the host [64]. The combination of derquantel and abamectin has an excellent broad spectrum efficacy against several parasitic nematodes in sheep, including those resistant to benzimidazoles, levamisole and macrocyclic lactones [63,65]. The efficacy of the derquantel and abamectin combination has also been shown in muscle contraction and electrophysiological studies on *A. suum* muscle flaps. Derquantel or abamectin alone inhibited responses to acetylcholine, and the inhibition was greater when a combination of derquantel and abamectin was used, producing a synergistic (greater than additive) effect [50]. Also, the derquantel and abamectin combination was shown to produce a greater inhibition of acetylcholine- or pyantel-induced current responses from expressed pyrantel/tribendimidine *O. dentatum* receptors compared to

derquantel or abamectin alone [48]. The introduction of combination anthelmintics provides a useful tool to increase anthelmintic drug efficacy, overcome resistance to other anthelmintic classes and delay resistance development [66,67].

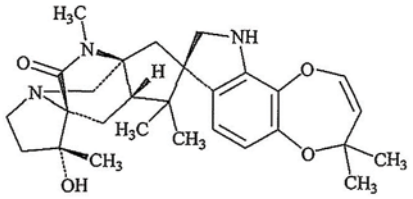

Figure 6. Chemical structure of derquantel. From [63].

Cyclooctadepsipeptides

Cyclooctadepsipeptides were discovered in the early 1990s. In 1992, PF1022A, the parent compound, was isolated from the fungus, *Mycelia sterilia*, which grows on the leaves of the plant, *Camellia japonica* [68]. PF1022A is made up of four N-methyl-L-leucine, two D-lactate and two D-phenyllactate residues that are arranged as a cyclic octadepsipeptide with an alternating L-D-L configuration (Figure 7) [69]. Emodepside, formerly PF1022-221 and BAY 44-4400, is a semisynthetic derivative of PF1022A, produced by attaching a morpholine ring at the para position of the two D-phenyllactic acids [70]. This modification resulted in improved pharmacokinetic properties. The anthelmintic potential of PF1022A and emodepside has been reported in numerous *in vitro* and *in vivo* studies [71-76]. Interestingly, PF1022A and emodepside have a broad spectrum of activity against several nematode species including those that are resistant to benzimidazoles, levamisole and ivermectin [77]. This indicated that the mode of action of the cyclooctadepsipeptides is different and that this class of anthelmintics possess 'resistance-busting' properties.

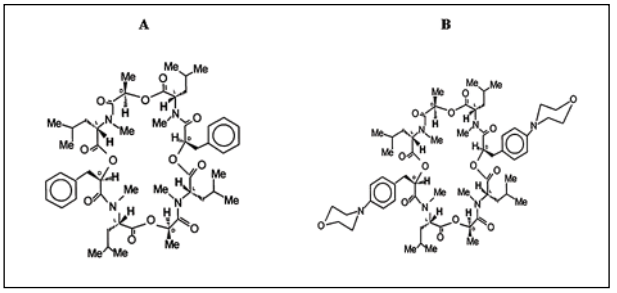

Figure 7. Chemical structures of PF1022A **(A)** and emodepside **(B)**. From [69].

Studies on the mode of action suggest that emodepside targets the calcium-activated potassium channel (SLO-1), there is also evidence for the involvement of the latrophilin (LAT-1) receptor [78-80]. Mutagenesis screens in *C. elegans* revealed a lack of sensitivity of *slo-1* null mutants to emodepside's inhibitory effects on locomotion

and feeding. Inhibition of locomotion was achieved via the action of emodepside on SLO-1 expressed in body wall muscles or neurons, whereas inhibition of feeding was achieved via the action of emodepside on SLO-1 expressed in neurons but not muscle [81]. RNAi studies implicated a role for LAT-1 in mediating emodepside's inhibitory effect on pharyngeal pumping in the pharynx [81,82]. Guest *et al.* [81], showed *C. elegans lat-1* null mutants had an estimated five-fold reduction in sensitivity to emodepside. These studies suggest that the inhibitory effect of emodepside on feeding is both SLO-1 and latrophilin-dependent. However, emodepside treatment inhibited locomotion in both wild type and *lat-1:lat-2* null *C. elegans*, implying that the inhibitory effect of emodepside on locomotion is latrophilin-independent [81]. The sensitivity of nematode SLO-1 channels to calcium is different from that of insects and humans [83].

Tribendimidine

Tribendimidine is a symmetrical diamidine derivative of amidantel (Figure 8) [84]. It was developed in the mid 1980s by the National Institute of Parasitic diseases in Shanghai, China, as a broad spectrum anthelmintic drug [85]. In 2004, tribendimidine was approved by the Chinese Food and Drug Administration for treatment of helminth infections in humans [86]. It is the only new anthelmintic drug that has been approved for human use within the past 3 decades [87]. Tribendimidine has been shown in laboratory and clinical studies to have a broad spectrum of activity against several nematode, trematode and cestode species [84,88-92] . The activity of tribendimidine against 20 helminth parasites has been documented [93]. Earlier studies on the mode of action of tribendimidine in the nematode model *C. elegans* demonstrated that tribendimidine acts as an agonist of the L-subtype nAChR in this species, similar to levamisole and pyrantel [94]. Parasitic nematodes however, show different nAChR subtype selectivity from *C. elegans*, and this varies across nematode species. Buxton *et al.* [25], showed in oocyte expression studies that tribendimidine, just like pyrantel, is more selective for the reconstituted pyrantel/tribendimidine nAChR subtype comprising of UNC-29, UNC-38 and UNC-63 subunits from *O. dentatum* and had little or no effect on the levamisole-sensitive subtype. In *A. suum*, the action of tribendimidine is pharmacologically similar to that of bephenium rather than levamisole, leading to the conclusion that tribendimidine selectively acts on the bephenium-sensitive, B-nAChR

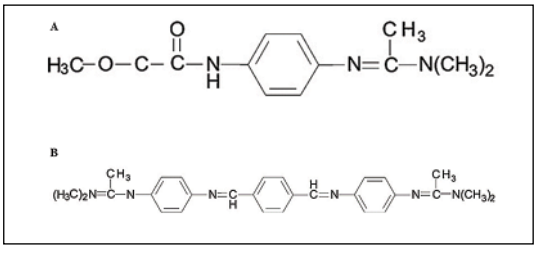

Figure 8. Chemical structures of amidantel **(A)** and tribendimidine **(B)**. From [84].

subtype, not the L-subtype nAChR in *Ascaris* [95]. Robertson *et al.* (2015), further showed tribendimidine to cause a more potent inhibition of migration of *O. dentatum* levamisole-resistant larvae (LEVR) than levamisole-sensitive larvae (SENS). Thus, confirming their hypothesis that unlike in *C. elegans*, tribendimidine does not act on the L-subtype nAChR in parasitic nematodes.

Anthelmintic resistance

In broad terms, anthelmintic resistance is referred to as the decline in the efficacy of an anthelmintic drug in a population of parasites that were once susceptible to the drug. The repeated and improper use of currently available anthelmintics has led to the development of resistance in numerous veterinary parasite species worldwide, with increasing concerns that this may extend to human parasites [96-98]. Since anthelmintics within each drug class act in a similar manner, resistance to one anthelmintic in a given drug class is likely to be accompanied by resistance to other anthelmintics of that same class (side resistance). There is also the likelihood for the development of cross resistance from anthelmintics of one drug class to those of another, if the two drug classes share similar targets [99]. Hence, the widespread occurrence of resistance across the majority of anthelmintic drug classes (Table 1). Sadly, the onset of anthelmintic resistance development can be rapid, thiabendazole resistance occurred 3 years after its introduction to the market [100].

Table 1. Anthelmintic resistance and mechanisms of resistance to the major anthelmintic drug classes. Modified from [101,107,108].

Anthelmintic class	Host	Year of initial approval	Year of first published report of resistance	Potential mechanism of resistance
Benzimidazoles				
Thiabendazole	Sheep	1961	1964	Mutations in β-tubulin; Phe200Try, Phe167Try or Glu198Ala
	Horse	1962	1965	
Imidothiazoles-tetrahydropyrimidines				
Levamisole	Sheep	1970	1979	Changes in nicotinic acetylcholine receptors
Pyrantel	Horse	1974	1996	
Avermectin-mylbemicins				
Ivermectin	Sheep	1981	1988	Reduced sensitivity of GluCl/GABA receptors
	Horse	1983	2002	
Moxidectin	Sheep	1991	1995	
	Horse	1995	2003	

Reports of resistance to anthelmintics in various parts of the world have been well-documented [101-103]. In spite of the numerous reports of anthelmintic resistance, the mechanisms by which resistance occurs remain to be fully elucidated (Table 1).

Resistance mechanisms include: (i) mutation or deletion of one or more amino acids in the target genes, (ii) reduction in the number of receptors, (iii) decreased affinity of receptors for drugs, and (iv) absence of bioactivating enzymes [104-108]. Management practices can also delay or overcome anthelmintic resistance. Anthelmintic resistance can be delayed or overcome by: (i) identifying new drug targets with different pharmacological profiles from those of existing drugs, (ii) introducing new anthelmintics with different modes of action from those of existing anthelmintics, (iii) combination therapy, with members of the combination from different drug classes, (iv) rotating drugs with different modes of action between dosing seasons, and (v) keeping some parasites in untreated refugia [109-111]. A detailed understanding of the biochemical and genetic basis of anthelmintic action is therefore imperative as this will allow for the development of sensitive assays for early detection, and hence more efficient management of anthelmintic resistance.

DISCUSSION

It is interesting to note, that with the exception of the benzimidazoles, the majority of antinematodal drugs act on ion channel proteins in the parasite. Given the number and diversity of predicted channel types in the parasite, it would seem reasonable to focus on these proteins as new drug targets. The success of the macrocyclic lactones led to a hiatus in new drug development to treat nematode infections. Fortunately, the arrival of compounds such as emodepside and derquantel seems to indicate this hiatus is coming to an end. However, the well recognized phenomenon of drug resistance remains a concern. Resistance can be slowed, for example by leaving a refugia of sensitive parasites or by using drug combinations with multiple mechanisms of action. However, there remains a compelling need to discover new compounds with new modes of action in timely manner.

Acknowledgments

The authors would like to acknowledge funding from NIH R21AI121831-01 to APR, NIH R01AI047194 to RJM and the Schlumberger Foundation Faculty for the Future Program to MA.

Authors' contributions

MA, RJM & APR wrote the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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KRATAK PRIKAZ NAČINA DELOVANJA ANTINEMATODALNIH LEKOVA

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Anthelmintici su jedni od najčešće upotrebljavanih lekova u veterinarskoj medicini. U ovom radu su opisani mehanizmi delovanja nekih od ovih preparata na nematode. Ispitivanjima su obuhvaćeni neki od starijih preparata: benzimidazoli, holinergični agonisti i makrociklični laktoni. Takođe, ispitivani su noviji preparati uključujući emodepside, derquantel i tribandimidin. U odsustvu vakcina, kontrola većine vrsta parazita nematoda, nastaviće da se obavlja primenom anthelmintika. Kao posledica, neophodno je da se neprestano prati i uočava mogućnost stvaranja rezistentnih populacija parazita. Pošto je razvoj rezistencije skoro neizostavan, postoji stalna i jaka potreba da se u potpunosti razume način delovanja ovih preparata. Isto tako, neophodno je da se identifikuju novi ciljevi lekova kao i novi lekovi, a sa ciljem stalne i efikasne kontrole nematoda.