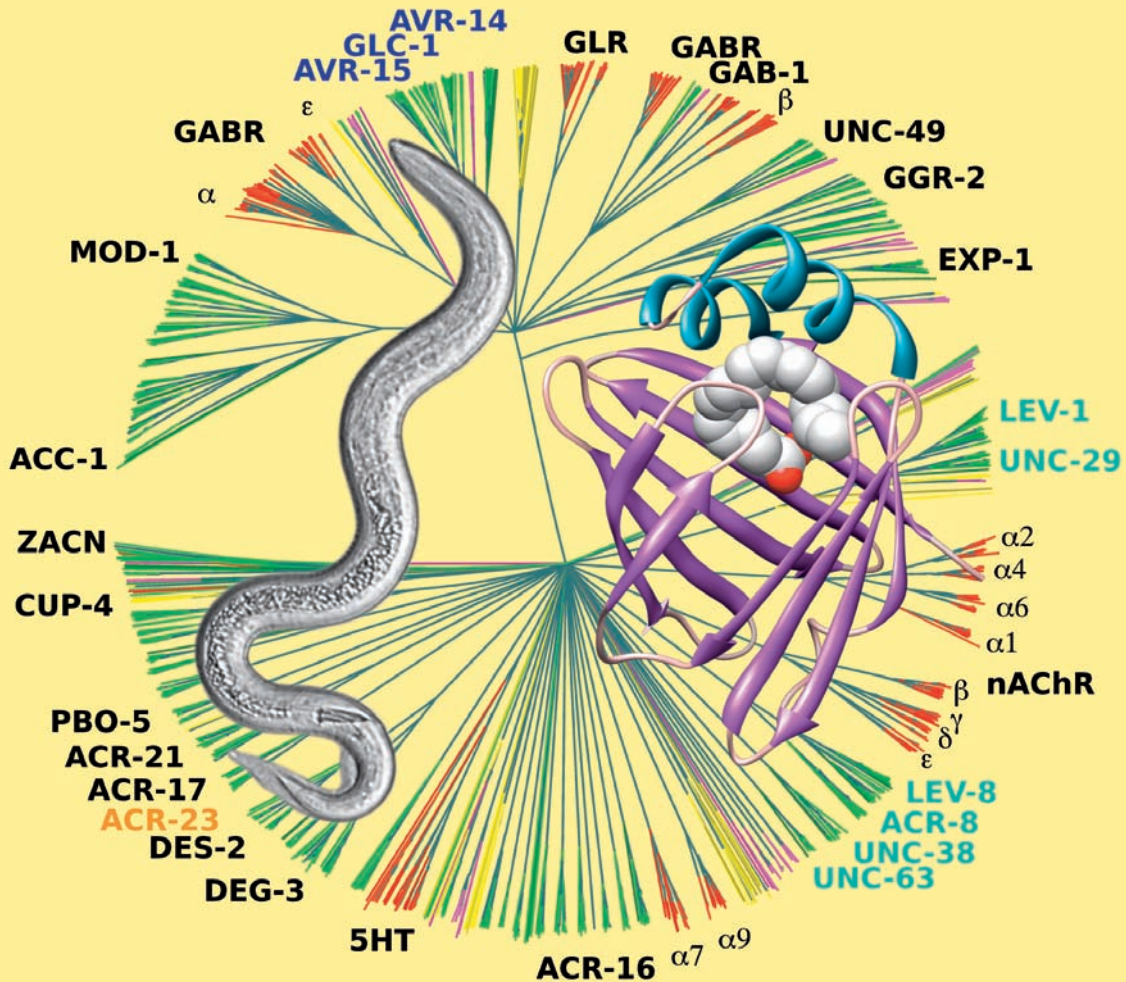




Parasitic Helminths

Targets, Screens, Drugs and Vaccines

Edited by Conor R. Caffrey



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Parasitic Helminths

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Parasitic Helminths

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Cover

The cover depicts a phylogenetic tree based on the ligand binding regions of putative ligand-gated ion channel genes. Nematode, platyhelminth, insect and vertebrate sequences are shown in shades of green, yellow, purple and red, respectively. Some *C. elegans* and human subunits are indicated and the labels for proteins involved in drug susceptibility to levamisole, monopantel and ivermectin are colored in cyan, orange and blue, respectively (see Rufener *et al.*, PLoS Pathogens (2010) 6(9):e1001091; courtesy of R. Kaminsky; see Chapter 17 for details). Left inset: freshly hatched *Ascaridia galli* larva (courtesy of M. Uphoff, Intervet Innovation GmbH, MSD AH; see Chapter 9 for details). Right inset: ribbon representation of the crystal structure of the *Schistosoma mansoni* Sm14 fatty acid binding protein in complex with arachidonic acid that is shown in a space fill representation (PDB 1VYG). The image was prepared by R. Marhöfer, Intervet Innovation GmbH, MSD AH and based on an original adapted from Angelucci *et al.*, Biochemistry (2004) 43:13000-13011 that was kindly provided by M. Tendler (see Chapter 26 for details).

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Foreword to *Parasitic Helminths: Targets, Screens, Drugs and Vaccines*

Peter Hotez

The last decade has witnessed a renewed interest in neglected diseases caused by parasitic helminths, especially for the high prevalence gastrointestinal nematode infections, filarial infections, schistosomiasis, food-borne trematodiasis and larval cestode infections. A number of factors have contributed to this resurgent interest in helminthic infections as global health threats:

- 1) There is new information suggesting that parasitic helminthiasis are the most common causes of infection among the “bottom billion”, *i.e.*, the 1.4 billion world’s poorest people who live below the World Bank poverty level in developing countries of Asia, Africa, and the Americas. The major helminthiasis include 600–800 million people with one or more soil-transmitted helminth infection, 400–600 million with schistosomiasis, more than 100 million people filarial infections and tens of millions with food-borne trematode infections.
- 2) Additional studies have revealed that some of the most prevalent parasitic helminths may increase susceptibility to the “big three” diseases, *i.e.*, HIV/AIDS, malaria and tuberculosis or exacerbate the morbidities of the big three diseases.
- 3) According to some estimates the major parasitic helminth infections together cause a disease burden measured in disability adjusted life years that may rival or even exceed the big three conditions, while additional information indicates that these helminthiasis may actually cause poverty through their deleterious effects on child growth and cognitive development, pregnancy outcome and agricultural worker productivity.

The global health community has responded to this public health threat by expanding efforts directed at mass drug administration (MDA). For example, using either diethylcarbamazine citrate or ivermectin together with albendazole, lymphatic filariasis (LF) has been eliminated as a public health problem in more than 20 countries, while through annual treatments with ivermectin, onchocerciasis has been eliminated in Senegal and Mali and may soon be eliminated from the Americas. Simultaneously, large scale financial support from the United States Agency for International Development (USAID), the British Department for International Development (DFID) and the non-profit Global Network for Neglected Tropical

Diseases has facilitated combining LF and onchocerciasis MDA efforts with MDA for soil-transmitted helminth infections and schistosomiasis to create “rapid impact” packages of anthelmintic interventions in national programs of helminth control in more than a dozen African countries, in addition to selected countries in Asia, Latin America and the Caribbean.

The promise of MDA for parasitic helminth infections has generated excitement among the international community that it might be possible to one day eliminate several helminthiases globally thereby achieving successes on this front that cannot yet be imagined for any of the big three diseases. However, there are warning signs that MDA with currently available drugs might fail to achieve such expectations: 1) high rates of mebendazole drug failure have been reported for hookworm infection caused by *Necator americanus* and trichuriasis, *i.e.*, two of the helminth infections with the greatest prevalence; 2) there is the looming specter of benzimidazole drug resistance among gastrointestinal nematodes of humans as has already occurred for nematode parasites of livestock, and 3) it has been shown that high rates of post-treatment re-infection occur for most of the major soil-transmitted helminth infections, schistosomiasis, and opisthorchiasis and other food-borne trematode infections.

Such concerns highlight the urgent need to develop and maintain a pipeline of new anthelmintic drugs in addition to anthelmintic vaccines to prevent infection or re-infection. Sadly, there is a glaring disconnect between the urgency for research and development (R&D) for new anthelmintic products and the global R&D budget for helminthiases. According to the global health think tank, *Policy Cures*, less than \$100 million annually is spent on R&D for all human helminthiases compared to more than \$3 billion spent annually on R&D for all the other neglected infections, including the big three diseases.

This volume summarizes the work of dedicated investigators in the medical and veterinary fields who are applying the latest technologies to discover the next generation of anthelmintic drugs and vaccines. Despite the difficulty in working with parasitic helminths in the laboratory, these investigators are overcoming significant hurdles in the study of the world’s most important helminths affecting more than a billion people worldwide and countless livestock.

Their work is leading to a new generation of advances and represents the best in science and in the pursuit of humanitarian goals.

Peter Hotez MD PhD is Dean of the National School of Tropical Medicine and Professor of Pediatrics and Molecular Virology & Microbiology, Texas Children’s Hospital and Baylor College of Medicine, Houston, Texas, USA

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Preface

Parasitic helminths continue to plague the lives of billions of people, and those of farm and domestic animals. Their capacity to persist in the environment is infuriating, costly (health-wise and economically), and fascinating depending on one's perspective as a livestock farmer, medical provider, or research scientist. For the animal health industry, the intrinsic capacity of helminths to resist drug pressure drives the never-ending quest to bring new anthelmintic drugs to market. In recent years, we have seen the fruits of that industry with the registration of new drugs containing emodepside, monepantel, and derquantel. These drugs and other compounds in the pipeline are critical not just for staying "one up" on resistant parasites of animals, but also for their potential to cross-over to human medicine, as has occurred with earlier anthelmintics that have been of immeasurable value to improving global health. That contribution becomes all the more relevant today given the increasing concerns over the continued efficacy of many first-generation anthelmintic drugs relied upon to treat human helminthiases, not least the benzimidazoles and the "wonder drug" ivermectin, and the serious implications for public health should these drugs fail.

This volume is intended to showcase the state-of-the-art in the fields of drug and vaccine development for parasitic helminths as well as draw attention to the challenges associated with bringing such products to market. The book is Volume 3 in the series *Drug Discovery in Infectious Diseases* and expands on some of the themes raised in Volume 1, *Antiparasitic and Antibacterial Drug Discovery: From Molecular Targets to Drug Candidates*. Contributions from the animal health industry figure prominently with a focus on the discovery and development of new chemical entities. Importantly, however, the book also covers the increasingly relevant contribution of academia, not just in its traditional strengths of identifying new drug targets or understanding how drug resistance arises, but also in the ways and means of preclinical and translational drug discovery through highly collaborative and interdisciplinary research. Indeed, this exciting movement into the traditional domain of the pharmaceutical industry can be viewed as a natural consequence of the central importance and success of academia in the public-private consortia that currently maintain dynamic drug development portfolios for other global parasitic diseases such as malaria and the trypanosomatid diseases. The creativity and productivity of academic scientists are highlighted in the many chapters covering

the development and expansion of genomics and functional genomic tools, and the application of automated screening technologies to prosecute anthelmintic drug discovery with rigor.

Finally, this volume discusses the need for, and the particular difficulties associated with, developing anthelmintic vaccines for both humans and animals – for many the “holy grail” in providing the tool (including in combination with chemotherapy) to ultimately control and, hopefully, eliminate helminth diseases. Great progress has been made in identifying a number of candidates with proven efficacy in target animal species or that are now entering human trials, thanks in part to the establishment of the necessary national and transnational institutional infrastructures.

To all of the authors, my sincere thanks for their time, insights, and patience in contributing to an important collection of on-topic discussions. My thanks also to the book series editor, Paul M. Selzer of Intervet Innovation GmbH, and to my colleagues at the Sandler Center for Drug Discovery at the University of California San Francisco for their constructive input.

March 2012
San Francisco

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Part One

Targets

1

Ligand-Gated Ion Channels as Targets for Anthelmintic Drugs: Past, Current, and Future Perspectives

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Abstract

Ligand-gated ion channels (LGIC) are targets for anthelmintic drugs used in human health and veterinary applications. Given the diverse physiological roles of LGICs in neuromuscular function, the nervous system, and elsewhere, it is not surprising that random chemical screening programs often identify drug candidates targeting this superfamily of transmembrane proteins. Such leads provide the basis for further chemical optimization, resulting in important commercial products. Currently, members of three LGIC families are known to be targeted by anthelmintics. These include the nicotinic acetylcholine receptors gating cation channels, glutamate-gated chloride channels, and γ -aminobutyric acid-gated chloride channels. The recent impact of genomics on model invertebrates and parasitic species has been far-reaching, leading to the description of new helminth LGIC families. Among the current challenges for anthelmintic drug discovery are the assessment of newly discovered LGICs as viable targets (validation) and circumventing resistance when exploring further the well-established targets. Recombinant expression of helminth LGICs is not always straightforward. However, new developments in the understanding of LGIC chaperones and automated screening technologies may hold promise for target validation and chemical library screening on whole organisms or *ex vivo* preparations. Here, we describe LGIC targets for the current anthelmintics of commercial importance and discuss the potential impact of that knowledge on screening for new compounds. In addition, we discuss some new technologies for anthelmintic drug hunting, aimed at the discovery of novel treatments to control veterinary parasites and some neglected human diseases.

Introduction

Anthelmintic drugs are central to combating many human and veterinary disorders. One in four of the world's population is infected with a parasitic roundworm or nematode, with infestation being particularly severe in tropical and subtropical regions. The consequent debilitating effects on the workforce and the compounding

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risk of other pathogenic infections represents a considerable social and economic burden. If we add to that a very high level of roundworm infestation among the world's farmed animals, and the devastating impact of trematode parasites in man and animals, then the need for adequate helminth control is transparent [1, 2].

The veterinary economic burden is reflected in the scale of the global animal health drug market (approximately US\$11 billion/annum) [3]. The human health antiparasitic drug market is around US\$0.5 billion/annum. However, it costs around US\$40 million to develop a new drug that controls livestock nematodes, whereas it can cost US\$800 million for a new drug for human use. Understandably, the cost barrier has limited progress, but the size of the global markets for antiparasitic drugs and chemicals make their pursuit of commercial interest as well as an important human and animal health priority.

Exciting new developments in research on vaccines targeting helminth parasites are underway and these, undoubtedly, will make important contributions in the future. However, at present, chemical approaches to helminth control predominate. For example, the world's three top-selling veterinary antiparasitic drugs (imidacloprid, fipronil, and ivermectin) and several others such as selamectin, levamisole, pyrantel, morantel, tribendimidine, piperazine, and amino-acetonitrile derivatives (AADs) act on Cys-loop ligand-gated ion channels (LGICs). These transmembrane receptor molecules facilitate the fast actions of neurotransmitter chemicals at nerve-nerve synapses and neuromuscular junctions (NMJs) in invertebrates. Often they offer rapid control of the pathogen. Much of our current knowledge of these important drug targets stems from the genetic model organism and free-living nematode, *Caenorhabditis elegans*, which possesses the most extensive known superfamily of Cys-loop LGICs, consisting of 102 subunit-encoding genes [4]. They include cation-permeable channels gated by acetylcholine (ACh) and γ -aminobutyric acid (GABA) as well as anion-selective channels gated by ACh, GABA, glutamate, 5-hydroxytryptamine (5-HT), dopamine, and tyramine [5–7]. Less than half of the genes in the *C. elegans* Cys-loop LGIC superfamily have been functionally characterized.

Unfortunately, many of the anthelmintic drugs in current use are under threat (Table 1.1). Important compounds such as ivermectin, which have given excellent service, are at the end of their patent life. Repeated use of effective chemicals leads to the development of pathogen resistance. Indeed, multidrug resistance against the three major classes of anthelmintics including macrocyclic lactones, which target glutamate-gated chloride channels (GluCl_s), has become a global problem for the treatment of gastrointestinal nematode parasites of farm animals [8–10]. The increasing development costs and poor return from conventional screening approaches are also problematic. Together, these factors bring a sense of urgency to the development of new, effective anthelmintics.

The life of a patent has always been finite, but as the time from discovery to market becomes protracted and the bar is raised for new, safer molecules with improved specifications on toxicity and environmental residues, the task of discovery becomes more difficult. The introduction of generic forms of a drug has the potential to lower the cost of treatment and make it available more widely, although this positive benefit