Antiparasitic and Antibacterial Drug Discovery

From Molecular Targets to Drug Candidates

Edited by
Paul M. Selzer
Antiparasitic and Antibacterial Drug Discovery

Edited by
Paul M. Selzer
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Light microscopic image of the helminth *Schistosoma mansoni*—with a male hosting a female in the *canalis gynaecophorus*: courtesy of Dr. Conor R. Caffrey, University of California San Francisco, USA.

Scanning electron microscopic image of the gram-negative bacteria *Mannheimia haemolytica*: courtesy of Prof. Dr. Lothar H. Wieler, Freie Universität Berlin, Dr. Heike Kaspar, and Dr. Christoph Schaudinn, Robert Koch Institut Berlin, Germany. The chemical structure is taken from chapter 19 authored by Thorsten Meyer *et al.*, figure 19.9.

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Foreword

It is ironic that three decades ago infectious diseases were viewed as a problem of the past. Malaria and tuberculosis were going to be eradicated, effective vaccines were available for major childhood infections, and an armamentarium of antibiotics was available for common community and hospital-acquired infections. Young physicians were advised not to enter infectious disease specialties because they were becoming irrelevant. The AIDS epidemic was the first wakeup call that infectious diseases would again become a major global health problem. Drug-resistant malaria and tuberculosis are now almost ubiquitous and new and emerging infectious diseases are almost a weekly staple of the popular press. Indeed the need for new drugs for infectious diseases has never been greater. Global industry and global travel means that formerly exotic diseases can rapidly establish themselves at any port of entry. Effective vaccines against the most prevalent infectious diseases like AIDS and malaria have proven difficult to develop. Multidrug-resistant organisms are an issue in any clinical setting. This publication provides a window on new approaches to drug discovery and development targeting infectious diseases. Fortunately, technology and training in new methodologies of drug discovery have expanded rapidly in the past 10 years. The challenge is how to effectively apply this technology to the thorny problems of global infections and to maintain a drug development pipeline for infectious diseases in light of the immense cost now associated with bringing new drugs to market.

San Francisco, USA
November 2008

James H. McKerrow
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Preface

In the age of antibiotics, vaccines, and drugs, we might be lulled into a sense of complacency regarding infectious diseases and that there is “a cure for everything”. This sense of security is maintained at our peril, however. One has only to consider the growing devastation caused by such big-name diseases as influenza, HIV-AIDS, tuberculosis, and malaria to see that the struggle to treat and control infectious diseases is truly titanic and indeed becoming more perilous with the ever-evolving development and spread of drug resistance compounded by the greater freedom and speed of movement of goods, animals, and people. Aside from the recently perceived security threat to the health and business structures of the developed world caused by these and a plethora of other infectious disease, billions living in developing countries must endure the daily struggle of diseases. In contrast to most human health-related pharmaceutical companies, academic institutions, veterinary science, and animal health companies remain very much focused on infectious diseases, including those caused by bacteria and parasites. As illustrated in Figure 1, the animal health sector remains profitable, and thankfully so, as history has shown that therapies produced in this sector often prove invaluable for treatment of similar infectious diseases of humans – the application of anthelmintics being a case in point.

The improved understanding of the resilience of disease-causing agents to therapies, their expanding disease menace in the era of “globalization,” and the balance provided by the opportunities for cross-sector exchange of ideas and applications spurred the preparation of this book. Also, the book serves to highlight the importance and visibility of drug discovery efforts for infectious diseases of both animals and humans.

Though it is not possible to address every aspect, disease, or approach within a single volume, this book sets forth a series of case studies and review articles that focus on bacterial and parasitic diseases in order to showcase how scientists in the different disciplines strive to move drug discovery forward. The contributing authors are experts drawn from drug discovery units of the pharmaceutical industry, academia, and nonprofit organizations in an effort to offer a global and balanced insight into the issues and problems at stake and their possible solutions.

Writing this has been a rewarding task for everybody involved. My heartfelt thanks go to the contributing authors for their excellent work performed within a short timeframe. In addition, I am grateful to Intervet/Schering-Plough Animal Health and its Drug Discovery Unit for their unreserved support, inspiration, and motivation.
Figure 1  The world animal health market based on data from 2006. The first row depicts the proportion of antiparasitics and antiinfectives in the whole animal health market. Rows two and three represent the antiparasitics and antiinfectives market, respectively. From left to right the individual proportions are broken down according to regional sales (ROW = rest of world), sales per animal species, and sales per chemical class. The area of the individual pie charts is not size-adjusted. Original data were derived by Wood Mackenzie and kindly provided by Linda Franken-Horspool, International Marketing, Intervet/Schering-Plough Animal Health.

during the preparation of this book. I also thank the members of Intervet’s BioChemInformatics Unit for their excellent technical backing and team spirit. Finally, I am very grateful to Ms Simone Maus-Gilbert for her outstanding editorial assistance.

Schwabenheim, Germany

Paul M. Selzer

November 2008
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Part One
Drug Discovery Approaches
Target Identification and Mechanism-Based Screening for Anthelmintics: Application of Veterinary Antiparasitic Research Programs to Search for New Antiparasitic Drugs for Human Indications

Timothy G. Geary, Debra J. Woods, Tracey Williams, and Solomon Nwaka

Abstract

Anthelmintic discovery in the veterinary pharmaceutical industry has succeeded only through screening synthetic compounds and fermentation products against whole parasites in culture or in host animals. Following trends in the parent, and much larger, human pharmaceutical industry, many programs have been developed in the past 20 years to exploit mechanism-based screening strategies for the identification of new leads in this therapeutic area. This strategy relies on the robust identification of parasite proteins as targets for chemotherapeutic intervention and their subsequent validation. Expanding access to sequenced genomes of parasitic nematodes will facilitate identification of genes that encode putative drug targets. Of particular relevance will be those that are shared among nematodes of veterinary and human importance. These targets offer the best chance for finding new molecules with potential utility in both arenas and provide an opportunity for collaboration and synergy between the two sectors. Validation of these gene products as drug targets will require advances in functional genomics methods for parasites. Expanded capacities for parasite-based physiological and biochemical experiments are also likely to be needed. While mechanism-based approaches remain an attractive alternative to organism-based strategies for broad-spectrum anthelmintic discovery, proof-of-concept for the platform is still needed.

Introduction

Screening for antiparasitic drugs as a scientific exercise can be traced to the early work of Ehrlich, who screened a collection of synthetic dyes for trypanocidal activity in mice with the aim of allowing the importation of European horses and
cattle into the African colonies of Germany prior to 1900 (see Refs. [1, 2]). This was perhaps the first example of a screen of a collection of chemicals for any therapeutic indication; Ehrlich’s efforts at drug discovery seem to have begun with a veterinary parasite as target, but led to the introduction of the first anti-infective drugs for use in humans. Thus, the process by which drugs introduced into veterinary practice for parasite control were adopted for use in humans has a long history.

The motivation to discover “modern” antiparasitic drugs for the animal health industry can be traced to the introduction of sulfaquinoxaline for the prevention of mortality and morbidity due to poultry coccidiosis in the late 1940s, phenothiazine (1930s) and piperazine (1950s) as veterinary anthelmintics, and the chlorinated hydrocarbons and organophosphates as ectoparasiticides in the 1940s and 1950s. Diethylcarbamazine was discovered as an agent for use in human filariasis within the same time-frame, being developed for veterinary practice for heartworm prevention some time later. It is important to note that all these drugs were first used in humans – not necessarily for parasites – prior to being adopted for veterinary use. Their utility for controlling parasites in animals paved the way for the institution of systematic screening of chemical collections for new synthetic antiparasitic drugs for veterinary application. Their use in clinical settings proved that chemotherapeutic control of parasites which plagued livestock and poultry was economically rewarding for the manufacturer, the veterinarian, and the farmer.

The general flow of antiparasitic drugs from human to veterinary application (Table 1.1) reversed over time. For anthelmintics, the reversal began with the discovery of thiabendazole for veterinary medicine in the 1960s (Table 1.2), which was later introduced for treatment of various gastrointestinal (GI) nematodes in humans. This pattern was repeated for the nicotinic cholinergic agonists (pyrantel, levamisole), the second generation benzimidazoles, particularly albendazole and mebendazole, and ivermectin (and, potentially, related macrocyclic lactones). In contrast, antiprotozoal drugs have not moved as easily between the sectors (Table 1.2) or continue to flow in the opposite direction, a situation that primarily reflects the

<table>
<thead>
<tr>
<th>Drug</th>
<th>Human use</th>
<th>Veterinary use</th>
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<tbody>
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<td>Heartworm prophylaxis</td>
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<tr>
<td>Arsenicals</td>
<td>Trypanosomiasis, onchocerciasis</td>
<td>Heartworm therapy</td>
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<td>Theileriosis</td>
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<tr>
<td>Halofuginone</td>
<td>Malaria</td>
<td>Poultry coccidiosis</td>
</tr>
</tbody>
</table>
differences in the major species of protozoal pathogens of animals compared to humans (see below).

### Potential for Veterinary → Human Transfer of new Antiparasitic Drugs

Like most of the pharmaceutical industry, animal health companies underwent a considerable reduction in abundance over the past 20 years from mergers and acquisitions [3, 4]. This led to a net reduction in investment in antiparasitic drug discovery, with a consequent focus of efforts on the most profitable sectors of the animal health market [5]. As a result, priorities for veterinary parasite control now diverge more extensively from those of human medicine. A summary of current emphasis on types of parasites targeted for drug discovery for human versus veterinary applications is shown in Box 1.1. It is worth noting in this context that there may be a

<table>
<thead>
<tr>
<th>Drug</th>
<th>Veterinary indication</th>
<th>Human indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazoles</td>
<td>Anthelmintic/antiprotozoal</td>
<td>Nematodes, protozoa</td>
</tr>
<tr>
<td>Pyrantel/levamisole</td>
<td>GI Nematodes, Lungworms</td>
<td>Nematodes</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>Cestodes</td>
<td>Schistosomiasis</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>Heartworm prophylaxis</td>
<td>Filarias</td>
</tr>
<tr>
<td>Nitazoxanide</td>
<td>Sarcocystis in horses</td>
<td>Protozoa, nematodes</td>
</tr>
<tr>
<td>Moxidectin</td>
<td>Nematodes, ectoparasites</td>
<td>Onchocerciasis (in development)</td>
</tr>
<tr>
<td>Emodepside</td>
<td>Nematodes</td>
<td>Onchocerciasis (investigation)</td>
</tr>
</tbody>
</table>

### Box 1.1: Areas of synergism/overlap based on current trends in discovery investment

| Apicomplexan protozoa: | human ↑, veterinary ↓ |
| Kinetoplastids:        | human ↑, veterinary ↓ |
| *Giardia/ameba/Cryptosporidium*: | human ↓, veterinary ↓ |
| Trematodes:            | human ↔, veterinary ↔ |
| Filarial nematodes:    | human ↑, veterinary ↔ |
| GI nematodes:          | human ↓, veterinary ↑ |

This box illustrates the potential for flow of compounds in each direction as discovery efforts continue.

↑: relatively high interest and activity in discovering new drugs.
↔: modest interest/activity.
↓: minimal or declining interest/activity.
resumption of drug transfer for parasites from the human to the veterinary side in the future. This situation may benefit both areas, as described below.

**Protozoan Parasites**

A renaissance has occurred in the attention of public and private funders to the discovery of new drugs for protozoal parasites that infect humans. The primary targets for chemotherapy include the Apicomplexan malaria parasites (Plasmodium spp.), kinetoplastids such as Leishmania spp., Trypanosoma brucei and T. cruzi, Entamoeba histolytica, Giardia lamblia, Toxoplasma gondii, Trichomonas vaginalis, and Cryptosporidium parvum. Based on prevalence and pathogenicity, these drug discovery efforts are considerably weighted to malaria and the kinetoplastids [6–12]. In contrast, the primary protozoal target for veterinary medicine is a distinct group of Apicomplexans, the Eimeria spp. of poultry, with additional interest in phylogenetically related parasites (Neospora caninum, Sarcocystis neurona) and in Giardia spp. and Cryptosporidium spp. [3]. However, dedicated antiprotozoal discovery programs are no longer common in the animal health pharmaceutical industry (vaccine discovery is more prevalent at this time), and so future drugs for these infections will likely flow from human to veterinary use. Current work in this area on the human side is heavily focused on mechanism-based, as opposed to whole-organism, high-throughout screening. The extent of target overlap is likely to be reasonably good across the human/veterinary species divide, though target choice in the human-focused projects does not routinely include an assessment of relevance for parasite species of strictly veterinary importance. Inclusion of this factor as a criterion for prioritization could provide a for-profit component that would appeal to potential animal health partners, with benefits similar to those anticipated in the anthelmintic arena (see below).

**Ectoparasites**

Indications for the use of ectoparasiticides in human medicine are far fewer than for veterinary clientele, which in turn is a much smaller market than agricultural applications. The flow of these compounds has typically been from agriculture to animal health to human applications, with the exception of DDT, which was first developed for use in humans. The use of ivermectin for the treatment of head lice and scabies is an example of an ectoparasiticide developed for animals being adopted for humans. However, the current economic driving force for this arena is so small that discovery programs in animal health sectors typically do not include a component that addresses possible human uses. From the human medical perspective, the temporally limited (as opposed to chronic) use of these products and the relatively low number of infestations in the West make the cost–benefit analysis in terms of registration unrewarding. This situation may change if head lice and scabies develop more extreme resistance to available ectoparasiticides, including ivermectin.
Trematodes

These considerations suggest that the primary influence of animal health drug discovery research on human medicine will continue to be in the anthelmintic arena. More specifically, this will be largely restricted to drugs that primarily affect nematodes. The only flatworm of economic significance in veterinary medicine is the liver fluke, *Fasciola hepatica*. This parasite is important in some areas, but is not enough of a production problem in livestock to warrant dedicated screening in most animal health companies, even though resistance is emerging to the best available drug, triclabendazole (which is not even registered in the USA). Although *F. hepatica* is a significant human pathogen in some regions, it has not proven to be sufficiently prevalent to elicit a dedicated discovery effort for it. Instead, work on flatworms in the human sector focuses on *Schistosoma* spp., currently controlled by a single drug (praziquantel). In the absence of rigorously documented cases of praziquantel resistant schistosomes, investment in new antischistosomal drug discovery has been somewhat limited compared to the efforts mounted against protozoa. This situation may be changing in light of the Helminth Drug Initiative recently developed by WHO/TDR [13], which aims to reinforce and advance screening for new antischistosomal drugs. As for protozoan parasites, this effort may discover compounds that can be adopted for use against liver flukes in veterinary medicine.

Nematodes

Further analysis of the impact of veterinary antiparasitic drug discovery programs will be restricted to nematocides. Historically, the discovery of nematocides for use in animals or humans was based on low-throughput systems that employed infected animals as the primary screen. These assays were labor-intensive, slow to attain the final read-out and used relatively large amounts of experimental compounds. Even so, it remains true that at least the prototype of every available anthelmintic class, including emodepside, the paraherquamides, and the newest class, the AADs, was discovered by screening in infected animals or worms in culture. Nonetheless, there has been a marked shift of strategy in the animal health industry to emphasize discovery programs based on targets, or high-throughput, mechanism-based screening [14].

The initial change from screening in infected animals to tests run on organisms in culture was motivated primarily by the need for animal health operations to fit into the evolving discovery paradigms adopted by their parent companies. This meant a marked reduction in the amount of chemicals used in a screen (to adapt to new parameters for compound synthesis in medicinal chemistry programs) as well as a reduction in animal use and in labor costs associated with screening. In addition, there were concerns that in *vivo* screens might fail to detect truly interesting actives that are false negatives due to insufficient potency or pharmaceutical inadequacy.
Unfortunately, screening against parasites or the free-living species Caenorhabditis elegans in culture, while vastly increasing throughput and radically diminishing the amount of compound needed for primary screening, was not very successful in revealing new anthelmintic templates. Indeed, these procedures led to a very high rate of false positives, as many compounds were noted to kill nematodes in culture, but very few (almost none) were subsequently found to be active in infected animal models. As resources were typically insufficient to permit experiments designed to determine why in vitro actives routinely failed in vivo, improvements in the screening stream designed to reduce the incidence of false positives were not possible. A new approach was clearly needed, and it was incompatible with standard industry practices to return to the era of screening in infected animals. In keeping with drug discovery for human medicine, mechanism-based approaches came into vogue [14].

The drive to move from organism to target-based screens was based on several factors. One factor was the motivation to capitalize on biology-based intellectual property (IP); screens using infected animals or organisms in culture barred few competitors from an area and can only exploit chemistry-based IP. Mechanism-based screens can restrict the discovery activities of competitors by taking advantage of investments made in understanding the physiology and molecular pathology of diseases and infectious pathogens that dominate Western human medicine. In addition, advances in chemical technology, such as combinatorial chemistry, made it possible to synthesize thousands of molecules at a time in small amounts; organism-based screens were ill-equipped to test either the number or the small amount of available compounds. Advances in computational chemistry and structure-based drug design meant that the traditional whole-organism blind-screen approaches became seen as intellectually unchallenging and out of step with the times. Finally, whole-organism approaches are labor-intensive compared to mechanism-based strategies; the incorporation of mechanism-based screening allowed a relatively small team of screeners to evaluate hundreds of thousands of compounds against multiple targets in a matter of weeks. The combination of vastly increased throughput with lowered labor costs made this an irresistible strategy, validated by expert scientific opinion. To date, however, it is undeniably true that the adoption of this overall strategy has not led to an increase in the number of new chemical entities registered for use in humans. For our topic, it is crucial to stress again that at least the prototype of all commercially available anthelmintics was discovered in whole-organism assays, despite at least two decades using the more modern approaches to discovery. What does this bode for the switch to more modern screening platforms? We can use the neuropeptide area as an example (see page 12).

Discovery Synergies

Discovery programs in animal health companies can contribute to the discovery and development of drugs for use in humans in several ways. The most obvious is the