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Review

Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions

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ABSTRACT

Anthelmintic resistance has a great impact on livestock production systems worldwide, is an emerging concern in companion animal medicine, and represents a threat to our ongoing ability to control human soil-transmitted helminths. The Consortium for Anthelmintic Resistance and Susceptibility (CARS) provides a forum for scientists to meet and discuss the latest developments in the search for molecular markers of anthelmintic resistance. Such markers are important for detecting drug resistant worm populations, and indicating the likely impact of the resistance on drug efficacy. The molecular basis of resistance is also important for understanding how anthelmintics work, and how drug resistant populations arise. Changes to target receptors, drug efflux and other biological processes can be involved. This paper reports on the CARS group meeting held in August 2013 in Perth, Australia. The latest knowledge on the development of molecular markers for resistance to each of the principal classes of anthelmintics is reviewed. The molecular basis of resistance is best understood for the benzimidazole group of compounds, and we examine recent work to translate this knowledge into useful diagnostics for field use. We examine recent candidate-gene and whole-genome approaches to understanding anthelmintic resistance and identify markers. We also look at drug transporters in terms of providing both useful markers for resistance, as well as opportunities to overcome resistance through the targeting of the transporters themselves with inhibitors. Finally, we describe the tools available for the application of the newest high-throughput sequencing technologies to the study of anthelmintic resistance.

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Contents

1. Introduction

The Consortium for Anthelmintic Resistance and Susceptibility (CARS) met for the fifth time on the 25th of August, 2013 in Perth, Australia. The group meets every two years to discuss the latest research aiming to elucidate mechanisms of resistance to anthelmintic drugs. The CARS group aims to promote research on anthelmintic resistance, with a view towards the development of molecular markers for anthelmintic resistance diagnosis, and to assist in the development of new drugs. The meeting was attended by approximately 90 delegates drawn from universities, government primary industry departments, national research institutes (medical and veterinary), animal health companies, and veterinary service providers.

Anthelmintic resistance has great impact on sheep and cattle production systems worldwide (Kaplan, 2004; Sutherland and Leathwick, 2011), while the impact on equine health is also increasing (von Samson-Himmelstjerna, 2012). In addition, there is some concern that resistance may arise in soil-transmitted helminth parasites infecting humans (Vercruysse et al., 2011), and there is evidence of ivermectin resistance emerging already in *Onchocerca volvulus* (Osei-Atweneboana et al., 2011) and praziquantel resistance has been reported in *Schistosoma haematobium* (reviewed in Wang et al., 2012). Resistance also has an impact on parasite control in companion animals; for example, resistance to nicotinic agonist anthelmintics has been reported in canine hookworms (Kopp et al., 2007), and there is some evidence that resistance to macrocyclic lactones is emerging in canine heartworms (Geary et al., 2011). Clearly, there is a significant need for better management of drug use in helminth control. If means existed to reliably identify the presence of parasite populations resistant to specific drugs, improvements in drug-use decisions could be made to avoid ineffective treatment and, hence, slow the selection for resistance. Sensitive molecular diagnostics are an attractive option for providing the basis for such drug-use decisions.

Research on resistance markers is not only useful for developing diagnostic tools, but can also help increase our understanding of drug effects: for example, the interaction of drugs with their molecular targets. Also, an understanding of drug resistance mechanisms, can assist in revealing the nature of the interactions of anthelmintics with parasite defensive systems, including drug efflux pumps (e.g. P-glycoprotein, P-gp) and detoxification enzymes (e.g. cytochrome P450). The development of resistance markers is, therefore, seen as an enabling science which will help counter the impact of anthelmintic resistance in multiple ways; providing new drug targets, new synergistic or combination drug preparations, and a better understanding of how parasites evolve to cope with any potential xenobiotic, including the next generation of anthelmintic drugs.

The last review paper produced by the CARS group was in 2011, covering the 2009 meeting (Beech et al., 2011), and hence the present review represents a timely 'state of play' document describing the most recent research into anthelmintic resistance mechanisms and molecular markers. The CARS2013 meeting covered the latest research on anthelmintic resistance against each of the main drug classes, focusing on the development of markers for resistance to each class. For drugs where resistance has not yet been reported (the cyclooctadepsipeptides, e.g. emodepside) or has only recently been reported (monepantel- Scott et al., 2013), the talks described research on the drug's mode of action, revealing that changes at the receptor site(s) are likely resistance mechanisms. Drug transporters (e.g. P-glycoproteins, P-gps) were examined from two perspectives, firstly, in terms of their potential use as markers for resistance, and secondly, analysis of the potential to use P-gp inhibitors as synergists to overcome transporter-mediated anthelmintic resistance. The meeting focused both on candidate gene-based (Fig. 1) and worm genetics and genomics-based approaches to elucidate resistance mechanisms. Finally, the meeting was presented with an update on the genomic resources available to researchers studying molecular aspects of nematode and trematode biology.

2. Macrocyclic lactones (MLs): the target site

The biological targets for MLs are glutamate-gated chloride ion channel receptors (GluClRs) expressed in the neurons and muscle cells of nematodes (Cully et al., 1994). ML drugs irreversibly activate these channels, thereby inhibiting neuronal activity and muscle contractility, and thus inducing flaccid paralysis and death. MLs also activate other ligand-gated ion channel receptors, namely the γ-aminobutyric acid (GABA) and glycine (Gly) receptors, however, this activation requires much higher drug concentrations than required for the GluClRs (Adelsberger et al., 2000), and hence the GluClRs are considered to be the principal target for this class of anthelmintics.