ABC TRANSPORT PROTEINS & DRUG RESISTANCE IN NEMATODES

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CERTIFICATE OF AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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ABSTRACT

Widespread resistance to chemotherapeutic agents is one of the biggest challenges facing human health and the agricultural industry, with resistance to all current anthelmintics now recorded. Understanding the development of drug resistance in parasitic nematodes is critical to prolonging the efficacy of current anthelmintics, developing markers for monitoring drug resistance and is beneficial in the design of new chemotherapeutic agents or targets. Multidrug resistance (MDR) is mediated by ATP-binding cassette (ABC) transport proteins including the multidrug resistance-associated proteins (MRPs) and P-glycoproteins, which confer resistance to structurally and functionally different drugs. This work characterizes the role of these proteins in drug resistance in nematodes.

Using the model nematode *Caenorhabditis elegans,* ivermectin resistant sublines were developed through step-wise exposure to increasing concentrations of ivermectin commencing with a non-toxic concentration of 1 ng/ml. Resistant strains displayed a MDR phenotype with cross-resistance not only to the related drug moxidectin, but also to other unrelated anthelmintics, levamisole, pyrantel and thiabendazole but not to albendazole. Resistance was stable after 3 months without ivermectin treatment.

Resistance to low levels of ivermectin (≤6 ng/ml) was associated with increased expression of *mrp-1, mrp-6* and *pgp-1* and decreased glutathione, while higher level resistance (10 ng/ml) was primarily associated with the increased expression of P-glycoproteins. This resistance to ivermectin was reversible by the co-administration of MRP, P-glycoprotein and glutathione synthesis inhibitors confirming the involvement of these proteins in resistance. To show the relevance of this model, homologues of MRPs were identified in the gastrointestinal parasitic nematode of ruminants *Haemonchus contortus*. Increased expression of several MRPs identified in *H. contortus* was found in ivermectin resistant isolates, supporting the relevance of the *C. elegans* model.

The interaction of ivermectin with human P-glycoprotein and MRP-1 was also examined. Ivermectin clearly inhibits the transport of P-glycoprotein substrates and can reverse resistance to both daunorubicin and taxol in resistant cells. An interaction with mammalian MRP-1 is less clear, with a 10-fold lower affinity. Therefore ivermectin and modulators of P-glycoprotein have the potential to interfere with the biodisposition and bioavailability of

anthelmintics within parasitic hosts as well as parasites. Overall, this work demonstrated that low doses of ivermectin can induce resistance in nematodes through the increased expression of multidrug resistance transport proteins, adding further complexity of the development of drug resistance, and demonstrating the multi-factorial nature of MDR.

JOURNAL PUBLICATIONS

James CE & Davey, MW (2009) Increased expression of ABC transport proteins is associated with ivermectin resistance in the model nematode Caenorhabditis elegans. *Int J Parasitol,* **39**: 213-220.

James CE & Davey, MW (2007) A rapid colorimetric assay for the quantitation of the viability of free-living larvae of nematodes in vitro. *Parasitol Res,* **101**(4): 975-80.

PRESENTATIONS

James CE, Davey MW (2008) Multidrug resistance: It's as easy as ABC. RNSH/UTS/USYD/KIMR Scientific Research Meeting, Gore Hill NSW, Australia

James CE, Prichard RK, Davey MW (2008) Multidrug resistance-associated proteins in drug-resistant nematodes. ARC/NHMRC Network for Parasitology/ASP Conference, Glenelg SA, Australia

James CE, Davey MW (2008) ABC transporter-mediated ivermectin resistance in the model nematode *Caenorhabditis elegans* confers cross-resistance to other anthelmintics. Australasian *C. elegans* Meeting, Kioloa NSW, Australia

James CE, Davey MW (2007) Drug-efflux mediated resistance to anthelmintics in nematodes. ARC/NHMRC Network for Parasitology/ASP Conference, Canberra ACT, Australia

James CE, Davey MW (2006) Transport proteins in drug resistant nematodes. AWI Postgraduate Conference, Perth WA, Australia

James CE, Witcombe D, Davey MW (2006) Transport proteins in drug resistant nematodes. International Congress of Parasitology, Glasgow Scotland, United Kingdom.

James CE, Witcombe D, Davey MW (2005) Transport proteins as drug targets in parasitic nematodes. AWI/Sheep CRC Postgraduate Conference, Noosa QLD, Australia

LIST OF ABBREVIATIONS

Abbreviation	Full name
aa	amino acid
AAD	amino-acetonitrile derivative
ABC	adenosine tri-phosphate binding cassette
ACh	acetylcholine
ATP	adenosine tri-phosphate
BLAST	basic local alignment search tool
bp	base pairs
BSA	bovine serum albumin
BSO	buthionine sulfoxamine
cDNA	complementary deoxyribonucleic acid
C_T	cycle threshold
DMSO	dimethyl-sulfoxide
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
dsRNA	double stranded RNA
DTNB	5,5'-dithio-bis (2-nitrobenzoic acid)
E	efficiency
ETDA	ethylene diamine tetra acetic acid
g	centrifugal force (gravity)
GABA	gamma-amino butyric acid
GluCl	glutamate-gated chloride channel
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	glutathione S-transferase
GS-X	glutathione-conjugate
h	hour
IC_{50}	inhibitory concentration (50 %)
IPTG	isopropylthiogalactopyranoside
IVR	ivermectin resistant
kb	kilobases

kDa	kilodaltons
L1	first larval stage
L2	second larval stage
L3	third larval stage
L4	fourth larval stage
LB	Luria Bertani Broth
LDA	larval development assay
LTC_4	leukotriene C_4
m	metre
M	molar
MDR	multidrug resistance
min	minute
ml	milliliter
MOPS	3-(N-morpholino)propane-sulphonic acid
mRNA	messenger ribonucleic acid
MRP	multidrug resistance associated protein
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
mw	molecular weight
nAChR	nicotinic acetyl choline receptor
NADPH	nicotinamide adenine dinucleotide phosphate
NBD	nucleotide binding domain
ng	nanogram
NGM	nematode growth medium
OC	degrees Celsius
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Pgp	P-glycoprotein
q	quantitative
RNA	ribonucleic acid
RNAi	RNA interference
ROX	6-carboxyl-X-rhodamine
rpm	revolutions per minute

RT-PCR	reverse transcriptase polymerase chain reaction
siRNA	short interfering ribonucleic acid
SNP	single nucleotide polymorphism
SSA	sulphosalicylic acid
TAE	tris-acetate-ethylene diamine tetra acetic acid
TFB	transformation buffer
Tm	melting temperature
TMD	transmembrane domain
TSH	trypanothione
UV	ultraviolet
γGCS	γ-glutamyl cysteine synthetase

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