

Expression of candidate ivermectin resistance genes in the scabies mite, *Sarcoptes scabiei*

Kate E Mounsey^{1*}, Deborah C Holt¹, James McCarthy², Bart J Currie¹ and Shelley F Walton¹

¹Tropical & Emerging Infectious Diseases Division, Menzies School of Health Research, Darwin, NT Australia, ²Queensland Institute of Medical Research, Brisbane, QLD, Australia

Background

Scabies is caused by the burrowing infestation of the ectoparasitic mite *Sarcoptes scabiei*. The disease is a significant health problem in indigenous populations worldwide, and also affects companion animals and livestock.

Oral ivermectin (IVM) is increasingly used to treat ordinary scabies, and is the drug of choice in northern Australia for hyper-infested (crusted) scabies. Reports of ivermectin resistance in crusted scabies raise concerns regarding the sustainability of this relatively new drug for scabies. In light of increasing ivermectin use, and its likely incorporation into community mass treatment programs, it is critical to define mechanisms of ivermectin resistance in scabies mites.

Ivermectin resistance may potentially be conferred by over-expression of efflux transporters or detoxification enzymes, or by alteration to ligand-gated chloride channel drug targets.

We applied qRT-PCR to scabies mites to measure expression levels of candidate ivermectin resistance genes. These included a novel chloride channel gene and representative members of the glutathione S-transferase and ABC transporter gene families.



Fig 1: Female *S. scabiei* var. *hominis* mite



Fig 2: Crusted scabies: infected hyper-keratotic crusts preceding fatal sepsis



Fig 3 & 4: Severe sarcoptic mange in a puppy from an NT community, and a wombat from southern Australia



Methods

Live scabies mites collected from crusted scabies patients

Total RNA extracted from pooled mite samples (TRIzol)

Reverse transcription (sensiscript RT, random primers)

qPCR (SYBR green amplification of 8 target genes in parallel with B-actin)

Data analysis (relative quantification, Pfaffl method, REST[®])

	Total Mites	Pooled Samples
Larvae	73	8
Nymph	92	11
Males	153	16
Females	95	11
Mixed	140	12
TOTAL	553	
Untreated	243	
IVM treated	310	

Up-regulation of scabies mite MRP and GST genes is associated with ivermectin exposure

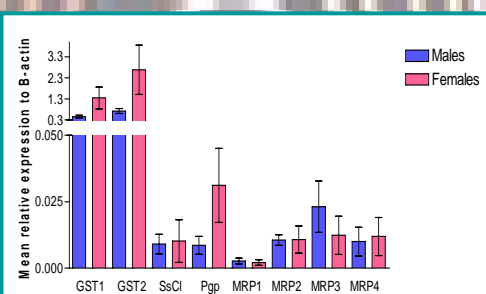


Fig 5: Relative expression of candidate ivermectin resistance genes in adult male and female *S. scabiei* var. *hominis*. GSTs were highly expressed in all developmental stages. Conversely, expression of SsCl and ABC transporter genes was low.

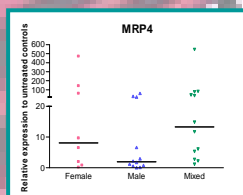
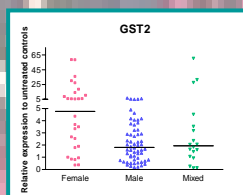


Fig 6 & 7: Normalised expression in mites collected post IVM treatment, relative to untreated controls. Scatter plot shows each sample comparison, median observations are indicated by the horizontal line. Delta class GST2 and MRP4 were up-regulated in all life-stages, and most dramatically in female mites.

Gene	Median fold upregulation	P (REST)
GST1 Mu type Glutathione S-transferase	1.2	0.86
GST2 Delta type Glutathione S-transferase	2.6	0.18
SsCl pH gated chloride channel	2.3	0.12
P-gp P-glycoprotein	0.4	0.35
MRP1 Multidrug resistance protein 1	1.1	0.95
MRP2 Multidrug resistance protein 2	0.6	0.97
MRP3 Multidrug resistance protein 3	0.2	0.17
MRP4 Multidrug resistance protein 4	6.3	0.03*

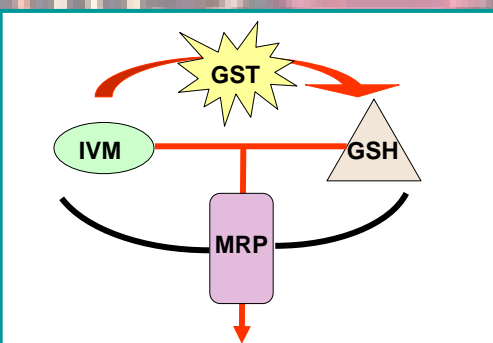


Fig 8: In other organisms many MRPs are glutathione conjugate transporters. Is it possible that MRPs and GSTs work together in the detoxification and extrusion of ivermectin?

Implications

For the first time, a qRT-PCR assay has been developed for scabies mites. Molecular studies on this parasite have been traditionally limited due to insufficient genetic material. The potential involvement of GST and MRP proteins highlights a previously unexplored mechanism of ivermectin resistance. These approaches are giving us new insights into scabies mite biology and the basis of emerging ivermectin resistance. This may assist in overcoming the many of the current difficulties in monitoring treatment efficacy and allow more sensitive methods for monitoring emerging resistance in the community.

Acknowledgements

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