Mapping the interaction of the *Candida albicans* multidrug resistance ABC protein Cdr1 with pump inhibitor clorgyline that stimulates ATPase activity

M. Niimi^{1,2}, K. Niimi², S. Saiwan¹, S. Assavachin¹, A. Chindamporn¹, R.D. Cannon² and E. Lamping²

¹Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and ²Sir John Walsh Research Institute, University of Otago, Dunedin, New Zealand

INTRODUCTION

The overexpression of ATP-binding cassette (ABC) transporters such as *Candida albicans* Cdr1 is a major cause of antifungal drug resistance in clinical isolates. An attractive solution for overcoming antifungal drug resistance is combination drug therapy of existing antifungals with efflux pump inhibitors. The antidepressant, monoamine oxidase A inhibitor, clorgyline (CLO) is one such broad-spectrum fungal efflux pump inhibitor. The objective of this study was to investigate how clorgyline interacts with fungal efflux pumps.

RESULTS

Figure 1. CLO stimulates the ATPase activities of *Saccharomyces cerevisiae* Pdr5 (A) and *C. albicans* Cdr1 (B).

Figure 2. Disk diffusion assays (10 µg CLO/disk) of *S. cerevisiae* strains overexpressing wild-type (wt) *CDR1* or naturally generated, CLO-resistant, *CDR1* suppressor mutants.

Figure 3. CLO resistant suppressor mutations do not affect expression or localization of Cdr1. The top panel shows confocal microscopy of *S. cerevisiae* AD overexpressing wt *CDR1* or CLO-resistant *CDR1* suppressor mutants tagged with green fluorescent protein (GFP). Lower panel: Coomassie blue-stained SDS-PAGE and in-gel fluorescence of 10 µg plasma membranes isolated from the indicated strains. ADΔ and the ATPase-deficient *CDR1*-K901M mutant are negative controls.

Table 1. Drug susceptibilities of ADΔ, AD/CDR1 and CLO resistant AD/CDR1 suppressor mutants.

Strain*	Minimum inhibitory concentrations (mg/l)							
	FLC	CTZ	VRC	KTC	ITC	CHX	R6G	CLO
ADΔ	2	<0.125	<0.0625	0.5	<0.013	0.125	<1	256
AD/CaCDR1	512	64	16	64	>32	4	256	256
-F556C	>512	64	16	64	>32	4	512	256
-F556L	128	32	2	16	16	1	512	256
-F608I	512	32	8	64	16	1	512	256
-V616F	128	8	4	16	2	2	512	256
-T671P/G915C	256	8	4	32	16	4	512	256
-Q1244E	512	64	16	64	>32	4	512	256

Figure 4. CLO-resistant *CDR1* mutants exhibit increased R6G efflux pump activities but with reduced CLO responsiveness. R6G efflux was measured after 5 min incubation with 20 mM glucose.

Figure 5. CLO-dependent Cdr1 ATPase activity of AD/CDR1 and CLO-resistant AD/CDR1 suppressor mutants.

Figure 6. CLO-resistant Cdr1 suppressor mutations locate in the centre (F556C/L) of transmembrane segment 2 (green), the centre (F608I) and top (V616F) of TMS3 (turquoise), the top (T671P) of TMS5 (orange) and the bottom of TMS8 (Q1244E; grey). (A) is a model view of Cdr1 from the top and (B) is a side-on view of Cdr1.

CONCLUSION

(1) CLO is the first described stimulator of the ATPase activity of fungal PDR transporters.

(2) CLO possibly interacts directly with Cdr1, and it may inhibit drug efflux by changing the kinetics of the transporter.

(3) All CLO-resistant mutations were found in highly conserved residues of Cdr1. The mutants had altered transport function, and the Cdr1 ATPase activities responded poorly to CLO when compared with wild type Cdr1.

(4) Our discovery opens promising new avenues for drug discovery and the fight against fungal drug resistance.

Acknowledgements: We thank Michele Bonus and Prof. Holger Gholke for creating the homology model of Cdr1. This research was supported by a grant from the National Research Council of Thailand. CONTACTS: Masakazu Niimi (masa.niimi@otago.ac.nz) and Erwin Lamping (erwin.lamping@otago.ac.nz).