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SHORT COMMUNICATION

Antifungal activities of diphenyl diselenide and ebselen against echinocandin-susceptible and -resistant strains of *Candida parapsilosis*

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SUMMARY

We evaluated the in vitro antifungal activity of diphenyl diselenide and ebselen against echinocandin-susceptible and -resistant strains of *Candida parapsilosis* using the broth microdilution method. Diphenyl diselenide (MIC range =1-8 µg/mL) and ebselen (MIC range =0.25-4 µg/mL) showed *in vitro* activity against echinocandin-susceptible isolates. However, ebselen also showed the highest antifungal activity against echinocandin-resistant strains (MIC range =0.06-4 µg/mL). This study demonstrated that the antifungal potential of diphenyl diselenide and ebselen deserves further investigation using in vivo experimental protocols.

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C. parapsilosis is the second most common agent of fungal infection in South American, Mediterranean, and Asian countries (Guinea, 2014; Montagna *et al.,* 2014; Wu *et al.,* 2014; Doi *et al.,* 2016). However, it naturally requires higher concentrations of echinocandins for treatment than other species, and has additionally been reported to develop resistance against echinocandins after continuous treatment (Moudgal *et al.,* 2005). In order to overcome the concern of fungal resistance, *in vitro* evaluation of new candidates is required. The organoselenium compounds diphenyl diselenide ($[PhSe]_2$) and ebselen deserve attention because their antifungal activity has been scarcely tested, but the reported results were encouraging. As far as we know, the effect of $(PhSe)_2$ and ebselen against echinocandin-resistant *C. parapsilosis* has not been previously demonstrated. This study aims to evaluate the *in vitro* activity of two organoselenium compounds, $(PhSe)_2$ and ebselen, against echinocandin-susceptible and echinocandin-resistant *C. parapsilosis* isolates.

We studied four groups of *C. parapsilosis* strains: the first included thirty clinical echinocandin-susceptible (ES) isolates obtained from the Mycological Research Laboratory (LAPEMI) of the Federal University of Santa Maria, Brazil. These isolates were identified by standard methods (Kurtzman and Fell, 1998) and molecular methods (Tavanti *et al.,* 2005; Tavanti *et al.,* 2007). The echinocandin-resistant (ER) group included three subgroups:

a) anidulafungin-resistant strains (AR) (n=14);
b) caspofungin-resistant strains (CR) (n=19);
c) micafungin-resistant strains (MR) (n=18) - all of which were obtained from susceptible isolates by exposing them to increasing concentrations of echinocandins using the *in vitro* method described by Fekete-Forgács *et al.* (Fekete-Forgacs *et al.,* 2000).

Diphenyl diselenide ($[PhSe]_2$) was synthesised according to the method described by Paulmier (Paulmier, 1986). Ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]-one) was synthesised according to the method of Engman and Hallberg (Lars and Anders, 1989). Spectral analysis of the ^1H NMR and ^{13}C NMR were in accordance with the assigned structure. The chemical purity of the compounds (99.9%) was determined by gas chromatography/high-performance liquid chromatography (GC/HPLC). Susceptibility tests were performed according to the CLSI M27-A3 microdilution method and were interpreted by the M27-S4 document (CLSI, 2008; CLSI, 2012). *C. parapsilosis* strain ATCC 22019 and *C. krusei* strain ATCC 6258 were used as quality controls. All assays were performed in triplicate.

The results of the tests of the *in vitro* susceptibility of *C. parapsilosis* isolates to $(PhSe)_2$ and ebselen are described in Table 1. Based on susceptibility parameters (MIC range, MIC_{50}, MIC_{90}, and geometric mean), the ES *C. parapsilosis* group was very susceptible to ebselen, showing an MIC_{90} of 1 µg/mL and a geometric mean (GM) of 0.54 µg/mL. For inhibition, the MR group required higher concentrations of ebselen, with an MIC_{90} of 2 µg/mL and a geometric mean of 0.70 µg/mL. The AR and CR groups showed an MIC_{90} of 0.5 and 2 µg/mL and a geometric mean of 0.26 and 0.33 µg/mL, respectively.

Susceptibility tests of the ES group to $(PhSe)_2$ showed an MIC_{90} of 8 µg/mL and a geometric mean of 2 µg/mL. On

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Table 1 - *Susceptibility (μg/mL) in vitro of echinocandin-susceptible and -resistant strains of Candida parapsilosis to diphenyl diselenide and ebselen.*

Agents	*Group of isolates (n)*	*MIC echinocandins (μg/mL)*	*Range*	*Geometric Mean*	*MIC50 (μg/mL)*	*MIC90 (μg/mL)*
Diphenyl Diselenide	ES (30)	≤2	1-8	2	2	8
	AR (14)	≥8	16-64	49.96	64	64
	CR (19)	≥8	16-64	46.08	64	64
	MR (18)	≥8	32-64	61.58	64	64
Ebselen	ES (30)	≤2	0.25-4	0.54	0.5	1
	AR (14)	≥8	0.06-4	0.26	0.25	0.5
	CR (19)	≥8	0.06-4	0.33	0.25	2
	MR (18)	≥8	0.06-4	0.7	0.5	2

MIC50 = Minimal inhibitory concentration for 50% of strains;
MIC90 = Minimal inhibitory concentration for 90% of strains.

the contrary, most strains of the AR, CR, and MR groups were less susceptible to $(PhSe)_2$ than ebselen, as demonstrated by an MIC_{90} of 64μg/mL and geometric means varying from 46.08 to 61.58 μg/mL.

In this study, we observed the *in vitro* antifungal activity of $(PhSe)_2$ and ebselen against ES *C. parapsilosis*, with MIC ranges (GM) of 2-8 μg/mL (2.0) and 0.5-1 μg/mL (0.54), respectively. Our results partially confirm the antifungal properties of these compounds previously noted in other studies, where $(PhSe)_2$ exhibited antifungal activity toward selected strains of *Candida albicans*, *C. glabrata*, *C. dubliniensis, Fusarium* spp., *Aspergillus* spp., and the oomycete *Pythium insidiosum* (Loreto *et al.,* 2011; Loreto *et al.,* 2012; Denardi *et al.,* 2013; Rosseti *et al.,* 2015). Resembling $(PhSe)_2$, the synthetic organoselenium compound ebselen has also shown potential antifungal activity against *Saccharomyces cerevisiae*, *Candida albicans*, *Cryptococcus neoformans*, *A. niger*, *Microsporum gypseum*, *P. chrysogenum*, and *Penicillium citrinum* (Soteropoulos *et al.,* 2000; Wojtowicz *et al.,* 2004; Moreira Rosa *et al.,* 2005; Chan *et al.,* 2007; Billack *et al.,* 2009). Previous experimental studies observed that subcutaneous or oral administration of $(PhSe)_2$ had no acute lethal toxic effects in rodents (Meotti *et al.,* 2003; Luchese *et al.,* 2007; Wilhelm *et al.,* 2009; Nogueira and Rocha, 2011). For ER strains (groups AR, CR, and MR), only ebselen showed effective antifungal activity. Billack *et al.* (2009) observed the potent *in vitro* activity of ebselen against fluconazole-resistant strains of *C. albicans*. To date, the efficacy of $(PhSe)_2$ and ebselen on echinocandin-resistant fungi remains unknown.

Diphenyl diselenide is a simple, stable, and highly lipophilic organoselenium compound that is widely used as an intermediate in organic synthesis. The biological mechanism of the antifungal activity of $(PhSe)_2$ and ebselen involves their interactions with the sulfhydryl groups of biomolecules present in fungal cells (Mugesh *et al.,* 2001; Wojtowicz *et al.,* 2004). Moreira Rosa *et al.* (2005) showed that *in vitro* assays of $(PhSe)_2$ interact non-enzymatically with the thiol group of glutathione, and according to Wojtowicz *et al.* (2003), the portion Se-Se is capable of covalently interacting with these groups. Similarly, the biological mechanism of the antifungal activity of ebselen involves their interactions of selenenamide the Se-N moiety with sulfhydryl groups of biomolecules present in the living cells (Parnham and Graf, 1991; Mugesh *et al.,* 2001). Probably the observed differences in activity to the diselenide and ebselen resulted from the different polarity and shape of these compound molecules. According to Rosseti *et al.* (2015), $(PhSe)_2$ can decrease both the growth and biofilm formation of *C. albicans* through mechanisms involving an increase in reactive oxygen species (ROS) production and membrane permeability. The ROS can promote damage to DNA, proteins, and cell membranes, leading to cell death (Imlay, 2003). The $(PhSe)_2$ can act as a pro-oxidant in yeasts by reducing the levels of cellular glutathione (GSH), which plays an important role in the antioxidant defence of the cell (Moreira Rosa *et al.,* 2005). The antifungal properties of ebselen appear to be partly related to its ability to inhibit the fungal plasma membrane H^+-ATPase (Pma1p) (Soteropoulos *et al.,* 2000; Chan *et al.,* 2007), an enzyme used by yeast to establish proton gradients across the plasma membrane and to maintain a proper intracellular pH (Serrano *et al.,* 1986; Monk and Perlin, 1994). Interference with the function of H^+ATPase in fungi by antagonists will lead to cell death. Thus, use of the plasma membrane H^+ATPase as a molecular target for antifungal drug therapy is an attractive possibility, provided that inhibition of the enzyme activity correlates with the cessation of cell growth. The antifungal activity of ebselen could be due to its ability to interact with the sulfhydryl group of one or more L-cysteine residues within Pma1p that are critical for H^+ transport (Chan *et al.,* 2007). Billack *et al.* (2009) suggested that ebselen may serve as a useful agent in the treatment of infections caused by fluconazole-resistant fungi due at least in part to inhibition of Pma1p, while Monk *et al.* (1993) previously demonstrated that the inhibition of *C. albicans* growth was correlated with the inhibition of the H^+ATPase of this organism.

In conclusion, our findings demonstrated that $(PhSe)_2$ and ebselen exhibit *in vitro* antifungal activity towards *C. parapsilosis*, highlighting that ebselen's ability to inhibit the growth of echinocandin-resistant strains makes it a significant candidate for a potential antifungal agent for future experimental studies.

List of abbreviations:

$[PhSe]_2$: diphenyl diselenide; ES: echinocandin-susceptible; LAPEMI: Mycological Research Laboratory; ER: echinocandin-resistant; AR: anidulafungin-resistant; CR: caspofungin-resistant; MR: micafungin-resistant; GC/

HPLC: gas chromatography/high-performance liquid chromatography; ROS: reactive oxygen species; GSH: cellular glutathione; GM: Geometric Mean; MIC_{50}: Minimal inhibitory concentration for 50% of strains; MIC_{90}: Minimal inhibitory concentration for 90% of strains.

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