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=1-8µg/mL) and ebselen (MIC range=0.25-4µg/mL) showed in vitro activity against echinocandin-susceptible isolates. However, ebselen also showed the highest antifungal activity against echinocandin-resistant strains (MIC range=0.06-4µg/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) and, (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-Forgács 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 ¹³C 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, MIC₅₀,
MIC₉₀, and geometric mean), the ES C. parapsilosis group was very susceptible to ebselen, showing an MIC₉₀ of 1µg/mL and a geometric mean (GM) of 0.54µg/mL. For inhibition, the MR group required higher concentrations of ebselen, with an MIC₉₀ of 2µg/mL and a geometric mean of 0.70µg/mL. The AR and CR groups showed an MIC₉₀ of 0.5 and 2µg/mL and a geometric mean of 0.26 and 0.33µg/mL, respectively.

Susceptibility tests of the ES group to (PhSe)₂ showed an MIC₉₀ of 8µg/mL and a geometric mean of 2µg/mL. On the contrary, most strains of the AR, CR, and MR groups were less susceptible to (PhSe)₂ than ebselen, as demonstrated by an MIC₉₀ of 64µg/mL and geometric means varying from 46.08 to 61.58µg/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 2-8µg/mL (2.0) and 0.5-1µg/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 sulph hydryl 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 sulphydryl 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
REFERENCES


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

<table>
<thead>
<tr>
<th>Agents</th>
<th>Group of isolates</th>
<th>MIC isolates</th>
<th>MIC echinocandins</th>
<th>Range</th>
<th>Geometric Mean</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphenyl</td>
<td>ES (30)</td>
<td>≤ 2</td>
<td>1-8</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AR(14)</td>
<td>≥ 8</td>
<td>16-64</td>
<td>49.96</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>CR(19)</td>
<td>≥ 8</td>
<td>16-64</td>
<td>46.08</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>MR(18)</td>
<td>≥ 8</td>
<td>32-64</td>
<td>61.58</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Ebselen</td>
<td>ES (30)</td>
<td>≤ 2</td>
<td>0.25-4</td>
<td>0.54</td>
<td>0.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AR(14)</td>
<td>≥ 8</td>
<td>0.06-4</td>
<td>0.26</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>CR(19)</td>
<td>≥ 8</td>
<td>0.06-4</td>
<td>0.33</td>
<td>0.25</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MR(18)</td>
<td>≥ 8</td>
<td>0.06-4</td>
<td>0.7</td>
<td>0.5</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

MIC<sub>50</sub> = Minimal inhibitory concentration for 50% of strains;

MIC<sub>90</sub> = Minimal inhibitory concentration for 90% of strains.