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Diphenyl diselenide alone and in combination with itraconazole against *Sporothrix schenckii* s.str. and *Sporothrix globosa*

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Abstract

We evaluated the in vitro susceptibility of *Sporothrix schenckii* s.str. and *Sporothrix globosa* to diphenyl diselenide $(PhSe)_2$ alone and in association with itraconazole (ITZ). Eight clinical isolates were tested in microdilution and checkerboard assays. $(PhSe)_2$ alone inhibited all isolates in concentration ≤ 8 µg/mL and was effective in killing one *S. schenckii* isolate. Inhibitory and fungicidal beneficial effects in its interaction with ITZ were shown against 87.5% (7/8) and 50% (4/8) of the isolates tested, respectively. Our study demonstrates the in vitro antifungal activity of $(PhSe)_2$ against two pathogenic *Sporothrix* species, suggesting studies of in vivo applications are warranted.

Keyword Antifungal activity · Sporotrichosis · Organoselenium compound · Subcutaneous mycoses

Sporotrichosis is the main subcutaneous mycosis in several countries, caused by *Sporothrix* spp. [1]. *Sporothrix schenckii* sensu stricto (s.str.), *Sporothrix globosa*, and *Sporothrix brasiliensis* are the most clinically relevant species [1]. *S. brasiliensis* is related to zoonotic transmission, and largely restricted to Brazil, where it is a public health problem, occurring as outbreaks and epidemics [2, 3]. On the other hand, *S. schenckii* s.str. and *S. globosa* are acquired by injuries with plant debris worldwide [1]. *S. globosa* is a worldwide species, particularly a problem in Asia, causing several outbreaks [4]. *S. schenckii* s.str. is the most common species, with universal geographical distribution, with higher prevalence in Central and North America, and the western part of South Africa [1].

The first-line treatment against sporotrichosis, itraconazole (ITZ), has adverse effects associated with prolonged therapy necessary for the clinical cure of the disease [5]. In addition, absorption and interactions drug-drug are problematic and therapeutic failures have been reported [6, 7]. Difficulties associated with ITZ treatment, the few alternative antifungal drugs available and their high cost, worsen the epidemiological situation in endemic areas [5, 7].

Considering what was mentioned before, searches for alternative therapies are urgent. In this way, the organoselenium compound diphenyl diselenide $(PhSe)_2$ has shown potential antifungal activity against several pathogenic fungi [8, 9]. Given that a promising antifungal activity of $(PhSe)_2$ alone and in combination with ITZ against the zoonotic species *S. brasiliensis* has been described [10], this study evaluated the in vitroactivity of $(PhSe)_2$ against *S. schenckii* s.str. and *S. globosa*, as well as its interaction with ITZ.

Microdilution assay was performed using ITZ obtained commercially (Copervet®, Brazil) and $(PhSe)_2$ obtained as described previously [8, 10] through a research collaboration with the Chemistry Department of the *Universidade Federal de Santa Maria* (UFSM), Brazil. Eight clinical isolates from human patients were included, six *S. schenckii* s.str. (4 ITZ in vitro resistant (Minimal Inhibitory Concentration—MIC ≥ 2 µg/mL); 2 ITZ in vitro susceptible (MIC

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˂ 2 µg/mL)) and two *S. globosa* (2 ITZ in vitro resistant (MIC ≥ 2 µg/mL) [11]. All isolates were previously identified to species level by molecular methods (PCR species-specific, PCR–RFLP, and/or sequencing) [12, 13]. All of them were stored frozen at − 20 °C in brain heart infusion (BHI) broth with glycerol during a period ranging from 1 month to 11 years in the fungal collection of Mycology Laboratory of the *Faculdade de Medicina* (FAMED)—*Universidade Federal do Rio Grande* (FURG). This study is part of a project approved by the Ethics Committee in Health Area (CEP /FURG: 234/2018).

The inoculum was standardized according to the M38A-2 protocol from the Clinical and Laboratory Standards Institute [14]. In all tests, the inoculum concentration was confirmed by pour plate and colony counting. Microdilution assay was performed in 96-well microplates, following the CLSI M38A-2 protocol [14]. $(PhSe)_2$ and ITZ were diluted in dimethyl sulfoxide (DMSO) and tested in twofold dilutions, 0.25–16 µg/mL and 0.015–8 µg/mL, respectively. Microdilution results were read visually in 72 h at 35 °C to determine the MIC, defined as the lowest concentration able to inhibit 100% of the visible fungal growth. The minimal fungicidal concentration (MFC), considered the minimum concentration of drug resulting in killing ≥ 99% of the inoculum, was evaluated by plating 50 µL of each well without visual growth on Sabouraud dextrose agar, and enumerating any colonies.

Drug interaction between $(PhSe)_2$ and ITZ was evaluated by checkerboard assay in 96-well microplates according to a previous protocol [15]. Microplates were incubated for 72 h at 35 °C for visual reading of MICs. Two internal control strains (*Candida* spp. – ATCC CP90018 and *S. brasiliensis* – M745) [10] were used in all tests. Testing was performed in duplicate. The fractional inhibitory concentration index (FICi) was determined by the equation: FICi = (MICa in combination/MICa tested alone) + (MICb in combination/MICb tested alone), where the MICa is the MIC values obtained with $(PhSe)_2$ and the MICb is the MIC values obtained with ITZ. The values of MFC were used with the same equation to calculate the fractional fungicidal concentration index (FFCi). Interactions in either FICi or FFCi were classified as strong synergism when values resulted < 0.5, weak synergism when 0.5 to < 1, additive when 1 to < 2, indifferent when 2, and antagonistic when > 2.

$(PhSe)_2$ alone showed inhibitory activity against *S. schenckii* s.str. and *S. globosa* at concentrations of 4–8 µg/mL and 8 µg/mL, respectively. In 87.5% (7/8) of the isolates tested, a positive interaction between $(PhSe)_2$ and ITZ was observed, being 43% (3/7) strong synergism and 57% (4/7) weak synergism. Furthermore, 50% (3/6) of the in vitro resistant isolates to ITZ (MIC values ≥ 2 µg/mL) became susceptible to the drug in its association with $(PhSe)_2$ (MIC values ˂ 0.5 µg/mL) [11]. No antagonistic reactions occurred (Table 1).

Fungicidal activity of $(PhSe)_2$ alone was observed against 83% (5/6) of the *S. schenckii* s.str. isolates tested, and no *S. globosa* isolates. However, the interaction between $(PhSe)_2$ and ITZ was effective in improving this fungicidal activity against 50% of the isolates tested (3 *S. schenckii* s.str. and 1 *S. globosa*). No fungicidal antagonism was observed (Table 2).

Our study shows the in vitro activity of $(PhSe)_2$ against *Sporothrix* sapronotic species in similar concentrations (4–8 µg/mL) to that described for *S. brasiliensis* (4–32 µg/mL) [10]. $(PhSe)_2$ probably acts in fungal cells as a pro-oxidant factor, in contrast to melanin layers found at the fungal cell wall, which have antioxidant properties [16]. Studies have shown that melanin interferes with amphotericin B and terbinafine activity against *Sporothrix* spp. [17–19]. However, melanin of *Sporothrix* species (*S. brasiliensis*,

Table 1 Results of the minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICi) of diphenyl diselenide alone and in combination with itraconazole against *Sporothrix schenckii* sensu stricto and *Sporothrix globosa*

Sporothrix species	FURG ID	MIC*		MIC* combination			
		ITZ	$(PhSe)_2$	ITZ	$(PhSe)_2$	FICi	IN**
S. schenckii sensu stricto	6	> 8	4	0.25	2	≤ 0.515	WS
	23	> 8	4	4	1	≤ 0.281	SS
	768	0.25	8	0.125	1	0.625	WS
	1917	> 8	4	0.5	1	≤ 0.281	SS
	2657	> 8	4	0.5	1	≤ 0.281	SS
	3947	0.25	8	0.125	1	0.625	WS
S. globosa	3765	> 8	8	4	4	≤ 0.75	WS
	5823	> 8	8	> 8	8	2	IND

MIC* expressed as µg/mL. IN**: < 0.5 strong synergism (SS); 0.5 to < 1 weak synergism (WS); 1 to < 2 additive (AD); 2 indifferent (IND); > 2 antagonism (AN)

FURG ID: Isolate identification of *Universidade Federal do Rio Grande*; $(PhSe)_2$: Diphenyl diselenide; ITZ: Itraconazole; MIC: Minimal inhibitory concentration (no visual growth); FICi: Fractional inhibitory concentration index; IN: Interpretation

Table 2 Results of the minimum fungicidal concentration (MFC) and fractional fungicidal concentration index (FFCi) of diphenyl diselenide alone and in combination with itraconazole against *Sporothrix schenckii* sensu stricto and *Sporothrix globosa*

Sporothrix species	FURG ID	MFC*		MFC* combination			
		ITZ	$(PhSe)_2$	ITZ	$(PhSe)_2$	FFCi	IN**
S. schenckii sensu stricto	6	> 8	> 16	> 8	> 16	2	IND
	23	> 8	4	> 8	4	2	IND
	768	> 8	16	0.5	8	≤ 0.53	WS
	1917	> 8	16	2	4	≤ 0.37	SS
	2657	> 8	16	> 8	16	2	IND
	3947	1	16	0.125	2	0.25	SS
S. globosa	3765	> 8	> 16	8	4	≤ 0.62	WS
	5823	> 8	> 16	> 8	> 16	2	IND

MFC* expressed as µg/mL. IN**: < 0.5 strong synergism (SS); 0.5 to < 1 weak synergism (WS); 1 to < 2 additive (AD); 2 indifferent (IND); > 2 antagonism (AN)

FURG ID: Isolate identification of *Universidade Federal do Rio Grande*; MFC: Minimal fungicidal concentration (kill ≥ 99%); $(PhSe)_2$: Diphenyl diselenide; ITZ: itraconazole; FFCi: Fractional fungicidal concentration index; IN: Interpretation

S. schenckii s.str., and *S. globosa*) [19] is not effective to overcome the fungal cell damage due to the $(PhSe)_2$ pro-oxidative activity at the concentrations tested.

The promising in vitro interaction between $(PhSe)_2$ and ITZ against *S. schenckii* s.str. and *S. globosa* is consistent with that reported for *S. brasiliensis* [10]. These data show the $(PhSe)_2$ potential to be used as an adjuvant with ITZ in the treatment of animal and human sporotrichosis. $(PhSe)_2$ could have other advantages as an adjuvant, considering that this organoselenium compound, when orally administrated, acts as a hepatic and gastric protector in the host, and thus could decrease the collateral effects of ITZ therapy [5, 20].

Our data are complementary to the previous studies with *S. brasiliensis* [10] in the demonstration of the in vitro antifungal activity of $(PhSe)_2$, alone and in combination, against other *Sporothrix* clinical species. Future in vivo assays exploring the effects of $(PhSe)_2$ and its antifungal interactions are necessary to confirm the role of this organoselenium compound as an adjuvant or as an alternative in sporotrichosis treatment.

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Author contribution All authors contributed to the study conception and design. Vanice Rodrigues Poester, Lívia Silveira Munhoz, and Melissa Orzechowski Xavier performed the study design. Cristina Wayne Nogueira and Gilson Rogério Zeni performed the development of the methods. Vanice Rodrigues Poester, Lívia Silveira Munhoz, and Melissa Orzechowski Xavier performed the result analysis. The first draft of the manuscript was written by Vanice Rodrigues Poester and Lívia Silveira Munhoz. Melissa Orzechowski Xavier, Cristina Wayne Nogueira, Gilson Rogério Zeni, and David A. Stevens performed critical correction. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Ethics approval All procedures performed in studies were in accordance with the ethical standards of the institution. The license for the development of the project was acquired by the University Ethics Committee (CEP/FURG) corresponding to appear Nº 234/2018.

Consent to participate Not applicable.

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Conflict of interest The authors declare no competing interests.

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