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SHORT COMMUNICATION/COURTE COMMUNICATION

Micafungin alone and in combination therapy with deferasirox against *Pythium insidiosum*

Micafungine seul et en association avec le déférasirox contre Pythium insidiosum

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Summary This study evaluated the in vitro and in vivo activity of micafungin alone and in combination with the iron chelator deferasirox against *Pythium insidiosum*. Micafungin showed a poor in vitro activity when it was used alone, but synergistic interactions were observed for 88.2% of the strains when the drug was combined with deferasirox. Smaller lesions were observed in infected rabbits receiving the combination therapy, although it favored disease dissemination to the lungs. The present results show that micafungin alone is ineffective against *P. insidiosum*, and the combination micafungin—deferasirox might have deleterious effects for the host.

MOTS CLÉS
Oomycète ;
Échinocandine ;
Chélateur du fer ;
Sensibilité

Résumé Cette étude a évalué in vitro et in vivo l'activité de la micafungine seule et en association avec le déférasirox, un nouveau chélateur du fer, contre *Pythium insidiosum*. MICA a montré une faible activité in vitro lorsqu'elle a été utilisée seule. Par contre, les interactions synergiques ont été observées pour 88,2 % des souches lorsque le médicament a été combiné avec déférasirox. Des lésions de petite taille ont été observées chez les lapins infectés recevant la thérapie d'association, malgré la diffusion de la maladie aux poumons. Les présents résultats

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montrent que, dans cette étude, la micafungine seule a été inefficace contre *P. insidiosum*, et la combinaison micafungine—déférasirox pourrait avoir des effets secondaires délétères pour l'hôte.

Introduction

Pythium insidiosum is an oomycete of the Kingdom Straminipila that infects wild and domestic animals, mainly in the subcutaneous and gastrointestinal presentations, and humans in one of the four presentations: vascular, cutaneous/subcutaneous, ocular and disseminated [13,12,7]. Vascular or disseminated pythiosis is the most severe clinical entity, and the patients usually have an underlying thalassemia-hemoglobinopathy syndrome and its major pathological change — iron overload [7]. In animals, a predisposing factor is yet to be discovered because recurrent infections are commonly observed in endemic areas [12].

It is a consensus that the successful management of pythiosis requires early recognition and prompt treatment [13,11]. The use of combination antifungal therapy in treating *P. insidiosum* infection has paved the way for new treatment strategies, including the use of drugs of different pharmaceutical classes [8—10]. The iron chelator deferasirox (DEF) was found to be highly active against mucorales and *Aspergillus fumigatus*, both as monotherapy or in combination therapy [4,5]. Against *P. insidiosum*, DEF has been found to have limited activity in vitro, although the treatment in rabbits improved anemia [16]. Therefore, we sought to evaluate the in vitro activity of the echinocandin micafungin (MICA) alone and in combination with DEF against 17 strains of *P. insidiosum* and in rabbits with experimental pythiosis.

Material and methods

Sixteen Brazilian *P. insidiosum* strains obtained from equines with pythiosis, and which were used in previous studies [8—10,16], and a standard strain (CBS 101555) were included in this experiment. MICA (Astellas Pharma, US) and DEF (Novartis Pharma AG, Switzerland) were dissolved in sterile distilled water and dimethyl sulfoxide, respectively, and serially diluted in RPMI 1640 broth containing l-glutamine and buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid. The range of drug concentrations for use in the checkerboard assay was of 0.5—128 mg/L for MICA and 0.39—100 mg/L for DEF.

The susceptibility of the *P. insidiosum* strains to the drugs was tested by microdilution, following the CLSI M38-A2 protocol [1]. The inoculum consisting of *P. insidiosum* zoospores was adjusted according to previous studies [8,9,16]. For DEF susceptibility tests, strains were starved of iron as described elsewhere [4], with exception that *P. insidiosum* isolates were grown in corn meal agar instead of Sabouraud dextrose agar. For each strain tested, a positive (inoculum diluted) and negative control (only RPMI) was performed. The interaction of the combination was evaluated by the checkerboard microdilution method [6]. The microplates were incubated at 37 °C for 24 h. For DEF alone and in combination with MICA, minimum inhibitory concentration (MIC) was defined as the lowest drug concentration at which there was 100% of inhibition of fungal growth by visual readings. The minimal fungicidal concentration (MFC) was determined by plating 100 μl from MIC wells with no growth onto corn meal agar plates, which were incubated at 37 °C for 72 h. For MICA alone, minimal effective concentration (MEC), defined as the lowest drug concentration at which morphological alterations of the hyphal cells are detected, was determined. The tests were carried out in duplicate. The same strains were tested again at the same conditions in another day. The interactions, based on the respective fractional inhibitory concentration index (FICI), were interpreted as the following: FICI $\leq$ 0.5, synergism; FICI $>$ 0.5 to $\leq$ 4, indifference; FICI $>$ 4, antagonism. FICIs were obtained using the formula FICI = (MIC of drug A in combination/MIC of drug A alone) + MIC of drug B in combination/MIC of drug B alone. Off-scale MICs were converted to the next higher dilution for calculation purposes.

For the in vivo tests, 15 3-month-old female New Zealand rabbits were divided into three groups of five animals. All the animals were inoculated with *P. insidiosum* zoospores (isolate LAPEMI 232) by the subcutaneous route as previously described [10]. Group 1 (placebo) received saline intraperitoneally. Animals in group 2 were treated with 2 mg/kg/day MICA by the intraperitoneal route. Animals in group 3 were treated with 15 mg/kg/day DEF dissolved in orange juice and administered by oral gavage, according to the manufacturer's instructions, and the same dosage of MICA used in group 2. Doses were based on previous studies with rabbits [16,3,15]. Treatments started at day 25 after zoospore inoculation (week 0) and lasted for 35 days (week 5). Inoculated rabbits were checked weekly by measuring the subcutaneous nodular area using a sliding caliper.

The areas of the lesions (cm^2) were found to have normal distribution and were submitted to analysis of variance and Tukey's test using a significance level of 5%. Differences among treatments were also tested for significance using linear, quadratic and cubic regression equations. The Ethical and Animal Welfare committee of the Universidade Federal de Santa Maria approved the methodology used in this experiment.

Results and discussion

The in vitro activities of MICA and DEF against *P. insidiosum* are shown in Table 1. All the isolates showed MICs above 128 mg/L for MICA, while MECs of 16 and 32 mg/L were observed for 70% and 30% of the isolates, respectively. MICs for DEF ranged from 12.5 to 50 mg/L, and the MFC was of 100 and 50 mg/L for 82 and 18% of the isolates, respectively. In general, individual drugs showed only weak antifungal activity or none. However, the combination of MICA and DEF