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ORIGINAL ARTICLE / ARTICLE ORIGINAL

In vitro susceptibility of clinically important zygomycetes to combinations of amphotericin B and suramin

Sensibilité in vitro de zygomycètes d'importance clinique à l'association d'amphotéricine B et de suramine

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Summary The *in vitro* antifungal activity of suramin (25—300 μg/ml) and its combinations with amphotericin B (0.024—12.5 μg/ml) against 15 zygomycetes isolates representing nine clinically important genera (*Absidia*, *Micromucor*, *Mortierella*, *Mycocladus*, *Mucor*, *Rhizomucor*, *Rhizopus*, *Saksenaea* and *Syncephalastrum*) were tested. The investigated compounds acted antagonistically, synergistically and/or additively on the growth when a strain was sensitive to both compounds or resistant to suramin and sensitive to amphotericin B. At the same time, synergistic interactions were detected if strains were insensitive to both agents. In the case, when a fungus was sensitive to suramin but resistant to amphotericin B, inhibitory effect of only the suramin was measured.

Résumé Dans cette étude, nous examinons l'effet antifongique in vitro de la suramine (25—300 μg/ml) et celui de son association avec l'amphotéricine B (0,024—12,5 μg/ml), sur 15 isolats de zygomycètes, qui representent neuf genres d'importance clinique (*Absidia*, *Micromucor*, *Mortierella*, *Mycocladus*, *Mucor*, *Rhizomucor*, *Rhizopus*, *Saksenaea* et *Syncephalastrum*). Nous avons examiné l'effet inhibiteur de la suramine sur la multiplication en combinaison avec l'amphotéricine B. Les composants examinés ont exercé leur influence d'une manière antagonique, synergique et/ou additive lorsqu'une souche était sensible aux deux components ou résistante à la suramine et sensible à l'amphotéricine B ; en même temps on a pu constater une interaction synergique lorsque la souche était résistante aux deux agents.

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Dans le cas où le champignon était sensible à la suramine et résistant à l'amphotéricine B, seul a été mesuré l'effet inhibiteur de la suramine.

Introduction

The incidence of zygomycosis (opportunistic fungal infections caused by the members of the class Zygomycetes) has increased during recent years, especially among imunocompromised hosts [3,24,26,32]. Actually, it is one of the most frequent mycotic diseases caused by non-*Aspergillus* moulds [1,20,23,28,29]. *Rhizopus*, *Mucor*, *Absidia*, *Rhizomucor*, *Apophysomyces*, *Cunninghamella*, *Cokeromyces*, *Saksenaea* and *Syncephalastrum* spp. have been confirmed as potential opportunistic human pathogens while *Mortierella* spp. (e.g. *Mortierella wolfii*) are considered as true animal pathogens [3,6,7,20,23,24]. These fungi have a substantial intrinsic resistance to most of the widely used antifungal drugs (e.g. most azoles), and also show high *in vitro* MIC values for a variety of other agents [1,27]. The most efficient antifungal agent for the treatment of such infections is amphotericin B (AMB) and its lipid complexes [29]; however, these are quite toxic and may have serious side effects [9,30]. Combined application of AMB with other effective antifungal agents would be advantageous as a basis of a less toxic therapy. Therefore, there is a substantial interest for drugs, which can act synergistically with AMB, and allow decreasing its therapeutic concentration.

Suramin, a hexasulfonated naphtylurea, was originally applied as an antiparasitic agent against sleeping sickness, furthermore, as an antitumor drug, based on its anti-proliferative properties [31]. Suramin inhibits several enzyme activities (e.g. reverse transcriptase, mitochondrial oxidative enzymes and protein kinase C isoforms) as well as binding of some growth factors to their receptors [31]. In a recent study, the antifungal effect of 100 μg/ml suramin against Zygomycetes was tested on isolates of eight clinically important genera. Strains of four species (*Absidia glauca*, *Mucor racemosus*, *Rhizomucor miehei* and *Rhizomucor pusillus*) proved to be sensitive to suramin in microtitre plate assay. In that work, ability of suramin to co-operate with the non-antifungal drug fluvastatin, which has antifungal activity, was also revealed. It was demonstrated that suramin acts synergistically or additively with fluvastatin exhibiting a potent antifungal activity against several Zygomycetes [8].

The aim of the present study was to investigate the effect of *in vitro* combinations of AMB and suramin against clinically important Zygomycetes.

Materials and methods

Strains and media

Effects of AMB and suramin were tested on 15 fungal isolates (some of them derived from clinical sources), representing nine different genera (Table 1). Isolates were maintained on malt extract slants (ME; 0.5% malt extract, 0.5% yeast extract, 0.5% glucose, 1.0% KH_2PO_4, 1.5% agar) at 4 °C. Inhibition tests of sporangiospore germination and hyphal growth were performed in RPMI 1640 medium (Sigma, with L-glutamine, without sodium bicarbonate, powder, buffered, with 0.165 M morpholinepropanesulfonic acid at pH = 7.0.) based on the recommendation of CLSI M38-A method [19].

Table 1 Investigated strains and their sources.
Les souches utilisées et leur origine.

Species	Strain	Clinical source
Absidia glauca	SzMC 11072	No
Micromucor ramanniana	NRRL 5844	No
Mortierella wolfii	NRLL 28640	No
Mucor circinelloides f. *lusitanicus*	CBS 277.49 (+)	No
Mucor hiemalis f. *luteus*	NRRL 3632	No
Mucor mucedo	WRL CN(M)12034	No
Mucor racemosus	WRL CN(M)304	No
Mycocladus corymbifera	SzMC 95033	Human lung infection
Rhizomucor miehei	CBS 360.92	Human mycosis
Rhizomucor pusillus	ETH M4920	Human tracheal discharge
Rhizopus microsporus var. *rhizopodiformis*	CBS 102.277	Human rhinocerebral infection
Rhizopus oryzae	CBS 146.90	Human palatum molle infection
Rhizopus schipperae	CBS 138.95	Bronchial washing of a myeloma patient
Saksenaea vasiformis	FSU 870	No
Sycephalastrum racemosum	SzMC 2011	No

CBS: Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; ETHM: Swiss Federal Institute of Technology Culture Collection, Zurich, Switzerland; FSU: Fungal Reference Centre University of Jena, Jena, Germany; NRRL: Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois USA; SzMC: Szeged Microbial Collection, University of Szeged, Szeged, Hungary; WRL CN: Wellcome Bacterial Collection, Beckham, Great Britain.