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Short Communication

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In vitro susceptibility of *Scedosporium* isolates to N-acetyl-L-cysteine alone and in combination with conventional antifungal agents

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Abstract

In recent years, *Scedosporium* species have been more commonly recognized from severe, difficult-to-treat human infections, such as upper respiratory tract and pulmonary infections. To select an appropriate therapeutic approach for these infections is challenging, because of the commonly observed resistance of the causative agents to several antifungal drugs. Therefore, to find a novel strategy for the treatment of pulmonary *Scedosporium* infections the *in vitro* antifungal effect of a mucolytic agent, N-acetyl-L-cysteine and its *in vitro* combinations with conventional antifungals were investigated. Synergistic and indifferent interactions were registered in 23 and 13 cases, respectively. Antagonism was not revealed between the compounds.

Key words: *Scedosporium* spp., pulmonary infection, N-acetyl-L-cysteine, antifungal activity, drug combinations, synergistic interaction.

Introduction

Members of the genus *Scedosporium* are known to cause localized infections in immunocompetent hosts or invasive mycoses in immunocompromised patients. *Scedosporium apiospermum* and related species may form fungus balls in patients with previous or underlying cavitary lung diseases (e.g., tuberculosis); they are frequent colonizers of the airways of cystic fibrosis patients and can cause *Scedosporium* pneumonia in otherwise healthy hosts.[1,2] As their symptoms and clinical manifestations can be similar to aspergillosis, the real incidence and clinical importance of these pathogens may be underestimated.[2] In a recent survey involving fungal samples from 29 hospitals of Spain, *Scedosporium* species proved to be the second most frequently isolated filamentous fungi after Aspergilli.[3] Furthermore, their poor susceptibility to clinically used antifungals makes the *Scedosporium* infections difficult to treat.[1] Therefore, finding new agents with a better antifungal activity against these species is urgently needed.

N-acetyl-L-cysteine (NAC) has an excellent antioxidant activity and it is a commonly used mucolytic drug to treat acute infections of the respiratory tract.[4] The *in vitro* inhibitory effect of NAC has been previously proven against certain agriculturally and medically important filamentous fungal pathogens.[5,6]

In this study, we evaluated the *in vitro* antifungal effect of NAC against nine *Scedosporium* isolates and its combination with conventional antifungal agents.

Materials and methods

Pseudallescheria angusta (CBS 254.72 from sewage), *Pseudallescheria ellipsoidea* (CBS 301.79 from dung), *Scedosporium boydii* (previously known as *Pseudallescheria boydii*, CBS 120157, CBS 117410, CBS 117432 from human lung, soil, and sputum, respectively), and *Scedosporium aurantiacum* (CBS 136046, CBS 136047, CBS 136049, CBS 116910 from human lung, soil, soil and wound exudate, respectively) were involved in this study. Susceptibility tests were performed in accordance with the slightly modified instructions of the CLSI M38-A2 broth microdilution method,[7] in triplicates. Modifications related to stock solution and inoculum preparation were detailed previously.[8] The final drug concentrations in the tests ranged from 64 to 1024 μg ml^{-1}. In some cases, where the MICs of NAC (Sigma-Aldrich, USA) could not be determined in this concentration range, further higher concentrations (2048-8192 μg ml^{-1}) were also tested.

Drug interactions were investigated between NAC and four conventional antifungal agents (i.e., amphotericin B, AMB; caspofungin, CSP; terbinafine, TRB; and voriconazole, VRC) representing a polyene, an echinocandin, an allylamine, and an azole antimycotic, using the checkerboard microdilution method.[9] Serial twofold dilutions were prepared in a final concentration range of 64–4096 μg ml^{-1} for NAC, and 0.125-128 μg ml^{-1} for antifungal drugs. Fractional inhibitory concentration indexes (FICI) were calculated as described before.[10]

Results

Susceptibility of clinical *Scedosporium* spp. to NAC and its combination with antifungal drugs has not been investigated before. This paper provides the first MIC dataset about it. All the MIC values of NAC were in the range of 1024–8192 μg ml^{-1} (Table 1). Environmental isolates proved to be relatively less susceptible to NAC with a MIC range of 1024–8192 μg ml^{-1} compared to clinical isolates (MIC range: 1024–2048 μg ml^{-1}). Our results are comparable with previously reported data against other fungal species. The complete growth inhibition of Mucoralean fungi by cysteine and its derivatives was observed at a concentration of 10 mmol l^{-1},[6] which means approx. 1200–2000 μg ml^{-1}. In another study, the MICs of NAC against *Aspergillus* and *Fusarium* spp. were in the range of 6000–25000 μg ml^{-1}.[5] In contrast, in case of *Scedosporium* spp., we observed a lower MIC range.

Results of combination tests are summarized in Table 1. The MIC range of NAC alone reduced in the combination tests to 64–2048 μg ml^{-1} by AMB, to 64–1024 μg ml^{-1} by CSP, 128–2048 μg ml^{-1} by TRB, and 64–512 μg ml^{-1} by VRC. A more prominent decrease was detected in the MICs of antifungal agents when they were combined with NAC. When applied alone, the MIC ranges of AMB, CSP, TRB, VRC were 8–128 μg ml^{-1}, 32–64 μg ml^{-1}, 128 μg ml^{-1}, and 8–64 μg ml^{-1}, respectively. While, in combination with NAC the MIC ranges of AMB, CSP, TRB, and VRC could be decreased to 0.125–64 μg ml^{-1}, 0.125–16 μg ml^{-1}, 0.5–128 μg ml^{-1}, and 0.125–64 μg ml^{-1}, respectively. Between NAC and CSP, synergy was detected at seven isolates. While at NAC+AMB and NAC+TRB combinations, synergy was observed against six out of the nine strains. Between NAC and VRC, synergism was observed in four cases (Table 1). Antagonism was not detected between the investigated drugs.

The idea behind combinational antifungal therapy is improving the antifungal effect and reducing the dosage of antifungals to avoid side-effects with the simultaneous application of two or more antifungal drugs. In our study, the MIC values of antifungals in combination with NAC could be decreased to their achievable plasma concentration in several cases.[11–14] The MICs of NAC were also decreased in the combination tests; the lowest MIC value (64 μg ml^{-1}) is still higher than its maximal plasma concentration, which is between 2.6–48.96 μg ml^{-1} depending on the dosage and the route of administration.[15–17] Apart from this, synergisms between antifungal agents and NAC suggest that it would be worthwhile to investigate the *in vivo* efficacy of these combinations, and to determine the clinical relevance of our results.

Conclusion

Although previous *in vitro* susceptibility data on the combinations of cysteine derivatives and conventional antifungal drugs are not available in the literature, another aspect of the co-administration was investigated and reported by Lee et al.[18] It was demonstrated that, in cancer or AIDS patients where itraconazole (ITC) metabolism is impaired due to the altered expression of cytochrome P450 (CYP), oral cysteine administration could restore the normal CYP and thus the ITC level, too.[18] This also supports that (beside the

Table 1. The combination test results of N-acetyl-L-cysteine and conventional antifungal drugs against clinical *Scedosporium* isolates based on the fractional inhibitory concentration index (FICI) values.

Isolate[a]	Mean MIC (μg/ml)[b]						Mean MIC (μg/ml)[b]					
	NAC_{alone}	NAC_{comb}	AMB_{alone}*	AMB_{comb}	FICI	Interaction[c]	NAC_{alone}	NAC_{comb}	TRB_{alone}*	TRB_{comb}	FICI	Interaction[c]
S. aurantiacum (CBS 136046)	2048	256	128	0.125	0.13	S	2048	128	128	1	0.07	S
S. aurantiacum (CBS 136047)	8192	1024	32	16	0.63	NI	8192	1024	128	64	0.63	NI
S. aurantiacum (CBS 136049)	8192	128	128	64	0.52	NI	8192	1024	128	64	0.63	NI
S. aurantiacum (CBS 116910)	1024	64	128	1	0.07	S	1024	64	128	8	0.13	S
S. boydii (CBS 120157)	1024	64	64	2	0.09	S	1024	64	128	2	0.08	S
S. boydii (CBS 117410)	8192	128	8	16	2.02	NI	8192	256	128	128	1.03	NI
S. boydii (CBS 117432)	1024	64	128	0.25	0.06	S	1024	64	128	2	0.08	S
P. angusta (CBS 254.72)	1024	64	128	8	0.13	S	1024	64	128	8	0.13	S
	1024	128	128	1	0.13	S	1024	128	128	0.5	0.13	S
P. ellipsoidea (CBS 301.79)	8192	2048	64	16	0.50	S	8192	1024	128	32	0.38	S
Isolate[a]	NAC_{alone}	NAC_{comb}	CSP_{alone}*	CSP_{comb}	FICI	Interaction[c]	NAC_{alone}	NAC_{comb}	VRC_{alone}*	VRC_{comb}	FICI	Interaction[c]
S. aurantiacum (CBS 136046)	2048	128	64	0.5	0.07	S	2048	128	32	0.5	0.08	S
S. aurantiacum (CBS 136047)	8192	2048	32	8	0.50	S	8192	64	16	32	2.01	NI
S. aurantiacum (CBS 136049)	8192	2048	32	16	0.75	NI	8192	64	32	32	1.01	NI
S. aurantiacum (CBS 116910)	1024	256	32	0.125	0.25	S	1024	512	8	16	2.50	NI
S. boydii (CBS 120157)	1024	128	32	0.5	0.14	S	1024	128	16	0.25	0.14	S
S. boydii (CBS 117410)	8192	1024	32	4	0.25	S	8192	64	64	64	1.01	NI
S. boydii (CBS 117432)	1024	128	64	1	0.14	S	1024	64	16	0.25	0.08	S
P. angusta (CBS 254.72)	1024	128	32	0.5	0.14	S	1024	256	32	0.125	0.25	S
P. ellipsoidea (CBS 301.79)	8192	128	32	16	0.52	NI	8192	64	16	16	1.01	NI

[a]**CBS**, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

[b]NAC_{alone}, AMB_{alone}, CSP_{alone}, TRB_{alone} and VRC_{alone}, mean MICs of N-acetyl-L-cysteine, amphotericin B, caspofungin, terbinafine and voriconazole, respectively, when applied alone; NAC_{comb}, AMB_{comb}, CSP_{comb}, TRB_{comb} and VRC_{comb}, mean MICs of N-acetyl-L-cysteine, amphotericin B, caspofungin, terbinafine and voriconazole, respectively, when applied in combination.

[c]**NI**, no interaction ($0.5 < FICI \leq 4$); **S**, synergism ($FICI \leq 0.5$).[10]

*The MICs of **AMB**, **CSP**, **TRB** and **VRC** were determined previously by our research group.[8]

direct inhibitory effect on fungi) NAC has other beneficial properties, which may improve the efficacy of a potential antifungal therapy and/or reduce the side effects caused by azoles. According to previous studies, NAC is able to increase the antioxidant capacity of the lung and enhance the antimicrobial activity of macrophages against *Candida* spp.[17,19] Furthermore, in combination with antifungal therapy, the administration of NAC alleviated oxidative stress and lung injury associated with invasive pulmonary aspergillosis in a neutropenic mice model.[20]

Our results together with the aforementioned considerations arise the need of further *in vivo* studies to clarify the efficacy and applicability of NAC in the treatment of pulmonary *Scedosporium* infections.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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