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Original Article

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Activity of isavuconazole and other triazole derivatives against clinical isolates of *Aspergillus fumigatus**

Aktywność izawukonazolu oraz innych pochodnych triazolowych wobec klinicznych izolatów *Aspergillus fumigatus*

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Aspergillus fumigatus is the most frequent pathogen of the genus *Asperillus*, which is highly susceptible to triazole derivatives, especially to isavuconazole and voriconazole. Many countries face a growing problem of infections due to *A. fumigatus* showing acquired resistance to one or several triazoles. In medical centres, monitoring the susceptibility of isolated *Aspergillus* spp. is recommended.

Aim: The aim of this study was to collect and test triazole susceptibility of *Aspergillus fumigatus* obtained from clinical samples, which were investigated in diagnostic laboratories located in Wrocław, Warszawa and Ruda Śląska (Poland). In addition, 5 resistant *A. fumigatus* strains with TR34/L98H mutation were included.

Material/Methods: The microdilution method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was applied to test susceptibility to isavuconazole (ISV), voriconazole (VOR), posaconazole (POS) and itraconazole (ITR).

Results: During a period of 24 months, a total number of 75 *A. fumigatus* isolates were collected. Most of the strains were obtained from lower respiratory tract specimens (58/75; 77%), from patients

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hospitalized on pulmonology (41%) or intensive care and surgery units (29%). No isolate resistant to ISV or other triazoles was found. The minimal inhibitory concentration (MIC) value of ISV ranged from 0.125 to 1 mg/L (mean 0.4 ±0.15 mg/L) in triazole susceptible isolates, whereas among triazole-resistant strains, three showed a MIC of 8 mg/L and two had a MIC of 4 mg/L.

Conclusions: *A. fumigatus* isolates carrying the mutation TR34/L98H are cross-resistant to ISV. The acquired resistance is very rare in our region (0-4%), which supports of use of triazole derivatives (VOR, ISV) in the therapy of aspergillosis.

Keywords: **isavuconazole • aspergillosis • Aspergillus fumigatus • resistance to triazoles**

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INTRODUCTION

Isavuconazole (ISV) is the newest triazole derivative characterized by a broad spectrum of antifungal activity, covering many common (*Aspergillus* spp., *Mucor* spp., *Candida* spp.) and rare (e.g. *Trichosporon* spp., *Saccharomyces* spp.) mould and yeast pathogens [4]. As for many other antimicrobials, the effectiveness of ISV is species-dependent. Among the genus *Asperillus*, the most susceptible species are *Aspergillus fumigatus*, *A. lentulus, A. nidulans* and *A. terreus* whereas the highest isavuconazole minimal inhibitory concentrations (MICs) were reported in *A. niger* and *A. tubingiensis* [2, 4]. Unfortunately, ISV activity does not usually affect *A. fumigatus* strains that have acquired resistance to triazoles, which is an increasing problem in modern medicine. The prevalence of resistant isolates varies between geographical regions and medical centres, ranging from 0 to 26% [13]. The majority of such isolates exhibit TR34/L98H mutation (34-bp tandem repeats in the promoter region of *cyp*51A gene and with leucine-to-histidine substitution in Cyp51A protein) resulting in cross-resistance to all triazoles in medical use. Other mechanisms, *e.g.,* G138C, Y431C, G434C, lead to multi-resistance, or resistance to some triazole derivatives, *e.g.*, strains with G54 mutation display elevated MIC to posaconazole (POS) and itraconazole (ITR), but maintain susceptibility to ISV and voriconazole [4, 7]. Similarly to other infections, in the case of invasive aspergillosis, the culture of fungi from medically relevant specimens (*e.g.*, biopsy) and the subsequent determination of their susceptibility to antifungals are essential to implement the targeted therapy. Unfortunately, in at-risk populations, we face many problems, *e.g.*, difficulties in obtaining biopsy samples, irrelevant results of biomarkers in the case of prior antifungal prophylaxis, *etc*. A definitive diagnosis is often impossible, which supports the need to apply strategies of empiric and pre-emptive therapies.

The recent ESCMID-ECMM-ERS guideline recommends either VOR or ISV in the targeted therapy for invasive pulmonary aspergillosis due to azole-susceptible *A. fumigatus* and *A. terreus* strains [12]. Liposomal amphotericin B or a combination of VOR and echinocandin are used in cases of infection due to triazole-resistant *Aspergillus* strains. The guideline emphasizes the role of local epidemiological data in choosing antifungal management, especially in cases of infections without documented etiology. When the rate of environmental triazole-resistance is lower than 10%, no changes in the primary therapy (ISV/VOR) are recommended. When the percent of resistance exceeds 10, liposomal amphotericin B or a combination of voriconazole and echinocandin should be considered. For this reason, performing periodical surveillance studies relying on testing all clinical *Aspergillus* spp. isolates with a reference microdilution method is a task of prime importance [12].

Currently, there are only a few studies on the distribution of resistant *A. fumigatus* in Central and Eastern Europe. In one such study, we reported about 4% prevalence of triazole-resistant strains that were positive for TR34/L98H mutation [8, 9]. The present study aims to continue this topic. We investigated triazole-susceptibility of 75 *A. fumigatus* clinical isolates collected in 2015-2017 from three geographical locations in Poland. In addition to traditional anti-mold derivatives, ITR, VOR, POS, susceptibility to the new drug, isavuconazole, was investigated.

MATERIAL AND METHODS

Study description

Three diagnostic laboratories located in 3 cities consented to participate in the study: Warszawa (Department of Microbiology, Central Clinical Hospital, Medical Univer-

sity of Warsaw), Wrocław (Microbiology Laboratory of Diagnostyka Sp. z o.o.), and Ruda Śląska (Analytical and Microbiological Laboratory of Ruda Śląska, KORLAB) and the Research Mycological Laboratory at the Department of Pharmaceutical Microbiology and Parasitology, Wroclaw Medical University (Poland). The study was conducted for 24 months, from September 2015 to August 2017. The participating laboratories were asked to send all *Aspergillus fumigatus* isolates obtained from all kinds of clinical samples to the Research Mycological Laboratory in Wrocław. The laboratories provided either primary cultures on Sabouraud's agar plates, or swabs filled with fungal spores obtained by touching up to 5 colonies of tested isolate. In the mycological laboratory, the isolates were subcultures on Sabouraud agar, identified on the basis of classical mycological methods (morphology, ability to growth in 42°C) to the species complex level and tested for their susceptibility to triazole derivatives. As a control, *Candida krusei* ATCC 6258 and five azole-resistant *A. fumigatus* strains with TR34/L98H mutation were used (Strain No: 15/5287, 21/2708, 24/1473, 13 (CBS 133436), 55) [8, 9].

Determination of Minimal Inhibitory Concentration

Susceptibility tests were performed with the use of a microdilution method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [1]. The serial dilutions (0.03-32 mg/L) of antifungals, itraconazole (ITR), voriconazole (VOR), posaconazole (POS) and isavuconazole (ISV), were prepared on microplates using the medium RPMI1640 2x buffered with MOPS (3-(N-Morpholino) propanesulfonic acid, 4-Morpholine propane sulfonic acid). The plates were stored until use at -80°C. For testing, 3-day-old fungal cultures on Sabouraud agar slants were used. The water suspensions of fungal spores (1-2.5 × 10^5 CFU/mL) were dispersed on the microplates with antimycotics. After incubation (48 h, 35°C) the MIC was read as the lowest drug concentration that resulted in 100% inhibition of fungal growth. The results were interpreted according to EUCAST Breakpoint Tables for Interpretation of MICs, Version 9.0 [6]. Strains with MIC values of >2 mg/L for ITR and VOR, >1 mg/L for ISV, and >0.25 mg/L for POS, were regarded as resistant, whereas MIC values of ≤1 mg/L for ITR, VOR, ISV or ≤0.12 mg/L for POS were regarded as susceptible.

All reagents (ITR, VOR, POS, DMSO, RPMI 1640 medium, MOPS buffer) were obtained from Sigma-Aldrich Life Science and ISV was kindly provided by Basilea Pharmaceutica Ltd. (Basel, Switzerland).

The correlation between MICs of particular triazole derivatives was analyzed with the use of Spearman's test (Statistica v. 12 software, StatSoft Poland).

RESULTS

A total number of 75 *A. fumigatus* SC isolates were collected. The majority of strains were isolated from lower respiratory tract specimens (58/75; 77%), followed by ear (6/75; 8%) and wound swabs (5/75; 6.6%). The patients were usually hospitalized in pulmonology (41%) or intensive care and surgery units (29%) (Table 1). No isolate resistant to ISV or other triazoles was found in the examined collection of microorganisms. The MIC value of ISV ranged from 0.125 to 1 mg/L (mean 0.4 ±0.15 mg/L). Among previously detected triazole-resistant strains, three (No. 13, 21/2708, 24/1473) showed a MIC of 8 mg/L and two (No 55 and 15/5287) had a MIC of 4 mg/L. The mean values and ranges of MIC of ITR, VOR, and POS were as follows: 0.18 (0.06-0.5), 0.38 (0.03-1), and 0.044 (0.015-0.06) mg/L (Table 2). Within susceptible isolates, the MIC values of ISV and VOR were either the same (53/75, 70.6%) or differed by one (ISV higher in 13/75, 17% and lower in 7/75, 9%) or two dilutions (two strains with a MIC of ISV two dilutions higher than that of VOR). Among resistant strains, the MICs of ISV (4 and 8 mg/L) were always higher than those of VOR (one dilution in 2 strains and two dilutions in 3 strains). Compared to ITR, the MICs of ISV were ×2 higher in 38 isolates, ×4 higher in 23, the same in 12, and ×8 higher in two. In the resistant strains MIC of ITR was always higher (×4 or ×8) than the MIC of ISV. The relationship between MIC values obtained for all tested strains (triazole-susceptible and -resistant), calculated for any pair of antifungals, was statistically significant, and was the strongest between ISV and VOR (Table 3).

DISCUSSION

Isavuconazole (Cresemba®) was approved by the European Medicines Agency in 2015 for the treatment of invasive aspergillosis and mucormycosis. However, it is not currently marketed in many European countries, including Poland. The necessity for prolonged antifungal therapy as well as the use of triazoles in agriculture pose a risk of selecting strains cross-resistant to several or all triazoles. For this reason, it is necessary to perform epidemiological surveys that test the antifungal potential of all mould-active triazoles. In our previous, retrospective analysis we tested the susceptibility of *A. fumigatus* isolates to older triazoles (ITR, VOR, POS) collected during the period of 2009-2015 [8, 9]. Continuing this study, we wanted to have a view on the current distribution of triazole-resistant *A. fumigatus* in clinical patients. Previously, we found 5 (4%) triazole-resistant isolates with TR34/L98H mutation, but this time all tested isolates were triazole-susceptible. The highest MIC value for ISV was 1 mg/L and did not exceed the values of epidemiological cutoff values (ECVs) [5, 7] and the EUCAST clinical breakpoint (Version 9.0, valid from 2018-02-12) [6]. Similarly to other authors, we observed high correlation between the MIC of ISV and VOR [11]. The range of MIC obtained in our study (0.125-1 mg/L) was lower than reported in some other studies for a wild population of *A. fumigatus.* As an example, among 211 strains of *A. fumigatus sensu stricto* tested by Astvad et al. [2], a high proportion of isolates with WT sequence of the *cyp*51 gene showed a MIC of 2 mg/L, and this

Table 1. The origin of *Aspergillus fumigatus* isolates under study

Strain source		Number of isolates				
		Ruda Śląska	Warszawa	Wrocław	Bydgoszcz	Total
Clinical Specimen	Sputum/respiratory secretion/ BAL	13	17	26	2	58
	Biopsy	0	1	2		3
	Ear swab	1	3	2		6
	Wound	1	0	4		5
	Other[1]			3		3
Hospital ward	Pulmonology	7	9	15		31
	ICU & Surgery	3	4	13	2	22
	Haematology	0	3	2		5
	Ambulatory	4	3	2		9
	Other[2]	1	2	5		8

[1] - urine, stool, pharyngeal swab, [2] - gastroenterology, nephrology, geriatric unit

Table 2. The distribution of MICs of isavuconazole and other triazoles in clinical and resistant *Aspergillus fumigatus* isolates

Triazole derivative	Number of isolates/ MIC [mg/L]										
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	32
ISV				2	32	37	4		2	3	
VOR		1		1	40	29	4	4	1		
ITR			3	41	28	3					5
POS	4	31	40		1	4					

Table 3. The relationship between the MIC values of tested triazoles obtained for *Aspergillus fumigatus* S.C. isolates

Antifungals pars	Number of isolates	R	p
ISV/VOR	80	0.649128	0.000000
ISV/ITR	80	0.348182	0.001552
ISV/POS	80	0.448539	0.000030
VOR/ITR	80	0.434814	0.000056
VOR/POS	80	0.456130	0.000021
POS/ITR	80	0.436214	0.000052

R - Spearman's correlation coefficient; $p<0.05$ is regarded as statistically significant

value was established as ECV. Recently, Pfaller et al. [10] compared *in vitro* activity of isovuconazole and isavuconazonium sulfate, which is a prodrug administrated intravenously and orally. The MICs obtained for this prodrug were up to two dilutions higher than for ISV. The authors stated that due to some kind of mistake in their previous studies on isavuconazonium, probably a sulfate was used, which may have caused the excessive results [5]. We need to emphasize that the powder tested in this study was an active ISV provided by a producer (Basilea Pharmaceutica Ltd. Basel, Switzerland).

The present study has several limitations. First, despite the 2-year study period and the inclusion of three medical centers, which performed up to a thousand of mycological examinations monthly, the number of collected isolates was not large enough for a comprehensive analysis (75 isolates). As an optimistic explanation, we may state that the colonization/infection with *Aspergillus* spp. is rather rare in our hospitals. In fact, the low number of clinical samples with a positive culture of *Aspergillus* spp. may also be connected to problems evolving from diagnostic procedures. As an example, in many

hospitals, screening tests directed at mycoses are rarely performed and do not cover all patients at risk, *e.g.* patients with bronchiectasis [3]. In the presented group, 3 isolates originated from patients with invasive aspergillosis and 5 from patients with aspergilloma. Unfortunately, we were not able to collect detailed clinical data for all cases under study, *e.g.*, diagnosis (aspergillosis/colonization), previous exposure to antifungals, applied antifungal therapy, or patients' outcome.

Taking into account high susceptibility to triazoles, we refrain from performing molecular tests. Identifying the isolates using classical morphological criteria without performing genetic or proteomic analysis, we have to be aware of the possibility of overlooking cryptic species. However, even if such species were present, they were highly susceptible to triazoles. In summary, the current and previous surveys [8, 13] on the susceptibility of *A. fumigatus* to triazoles revealed that acquired resistance is rare in our region (0-4%), which supports the use of triazole derivatives (VOR, ISV) in the therapy of aspergillosis. Due to cross-resistance, ISV is not an appropriate drug for *A. fumigatus* isolates carrying TR34/L98H mutation even in ISV-naïve patients. The rarity of aspergillosis and frequent false-negative results of clinical cultures should obligate diagnostic laboratories to preserve clinically relevant mold isolates.

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