Comparison of in vitro susceptibility of different azoles agents against dermatophytes

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Short report

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Abstract

The research on antifungal resistance in dermatophytoses lags behind that on systemic mycose. Lack of datas of antifungal susceptibility testing in dermatophytoses is one reason. 121 clinical dermatophytes isolates were tested against 6 azole antifungal agents according to the Clinical and Laboratory Standards Institute (CLSI) method. Geometric mean MIC of all isolates were in increasing order: isavuconazole (GM 0.06 mg/L), posaconazole (GM 0.10 mg/L), itraconazole (GM 0.22 mg/L), voriconazole (GM 0.32 mg/L), ketoconazole (GM 0.40 mg/L), fluconazole (GM 10.18 mg/L).

Introduction

Dermatophytes as a group of filamentous fungi can cause dermatophytes infection which is the most common fungal infectious disease, affecting 20–25% of the world population[1]. Dermatophytes have a global distribution, especially in warm and humid environments such as tropical and subtropical regions[2]. Shenzhen city is located in Guangdong province, near the border with Hong Kong, which belongs to the subtropical zone enjoying a humid monsoon climate. However, none of studies have been done focused on in vitro antifungal susceptibility testing against dermatophytes in Shenzhen. Due to the lack of standardized drug susceptibility testing data, the research on antifungal resistance in dermatophytoses lags behind that on systemic mycose[3]. Besides, infectious diseases caused by dermatophytes are considered as an important public health problem for the reasons of prevalence, long-term therapy and difficult eradication of recurrent chronic infection.

Today topical and systemic antifungal agents are used to treat dermatophytes infection, such as the allylamines, azoles, polyenes and ciclopirox[4]. Griseofulvin (GRI) was introduced in 1950s used for dermatophytoses and then transcended to ketoconazole introduced in 1980s. Fluconazole, terbinafine and itraconazole came into use over the next decade which have been the mainstay of treatment for dermatophytes infection[5]. Although traditional azole antifungal drugs of dermatophytes infection are generally susceptible to most dermatophytes in vivo and in vitro, treatment is a big challenge, because frequent relapses and failures are observed due to fungal resistance and recalcitrance[3]. Therefore, new antifungal agents are needed in order to improve safety and efficacy. Third generation azoles have shown antifungal activities but clinical use has been sparsely reported which is mainly limited in settings of severe infections associated with underlying immunodeficiency[6]. In vitro susceptibility testing would be useful for selecting the most suitable antifungal agents. To date, the Clinical and Laboratory Standards Institute document M38-A2 method (CLSI M38-A2) plays an important role in determining the minimum inhibitory concentration (MICs) for dermatophyte (Trichophyton, Microsporum, and Epidermophyton spp.)[7].

Methods

Clinical samples

A total of 121 clinical isolates of dermatophytes were collected from the teaching hospital in Shenzhen, with clinically diagnosed and laboratory confirmed (by microscopic examination, culture test and internal transcribed spacer sequence) dermatophytes strains were used in this study. Of the 121 fungal isolates, 96 (79.3%) were Trichophyton rubrum, 7 (5.8%) were Microsporum canis, 6 (5.0%) were Microsporum gypseum, 6 (5.0%) were Trichophyton interdigitale, 4 (3.3%) were Epidermophyton floccosum, 1 (0.8%) were Trichophyton violaceum, 1 (0.8%) were Trichophyton tonsurans.

In vitro susceptibility testing

In vitro susceptibility testing was performed as the guidelines in documents M38-A2 of the Clinical and Laboratory Standards Institute [8]. The antifungal agents used were fluconazole, itraconazole, ketoconazole, voriconazole, posaconazole, isavuconazole. All antifungal agents were obtained as reagent-grade powders provided by the manufacturers (Meilunbio). These drugs were easily applied to clinical cases. Apart from fluconazole, all drugs were dissolved in 10% DMSO. Fluconazole was dissolved in sterile distilled water. All drugs were diluted with RPMI 1640 growth media (Gibco, USA) buffered (pH 7.0) with 0.165 mol/L morpholinepropanesulphonic acid (MOPS; BBI Life Sciences, China). Fluconazole were tested at concentrations from 0.125 to 64 mg/L. Itraconazole, ketoconazole, voriconazole, posaconazole and isavuconazole were at concentrations from 0.03 to 16 mg/L. Flat-bottomed microdilution trays containing 100 μL of serial dilutions of the antifungal agents were inoculated

with 100 µL of the fungal suspensions (adjusted with blood cell counting plate) to give a final inoculum size of (1–5) × 10^3 colony – forming units (CFU)/mL for dermatophytes. The MICs were determined by visual inspection following incubation in a humid atmosphere at 35°C for 4–5 days. MIC end points were defined as the lowest antifungal concentration that showed approximately 80% growth inhibition compared to the growth in the control well (antifungal-free medium). All of the MICs were repeated twice. The reference strains Candida parapsilosis ATCC 22019, Trichophyton rubrum ATCC-MYA-4438 and Trichophyton interdigitale ATCC-MYA-4439 were included for quality control and their MICs were within established ranges. The geometric mean MICs, ranges and the MICs at which 50% and 90% of the strains were inhibited, MIC_{50} and MIC_{90}, respectively, were calculated for all the isolates tested.

Results And Discussion

This study compared six azole antifungal agents including fluconazole, itraconazole, ketoconazole, voriconazole, posaconazole, isavuconazole against dermatophytes. 121 clinical isolates of dermatophytes including Trichophyton rubrum, Microsporum canis, Microsporum gypseum, Trichophyton interdigitale, Epidermophyton floccosum, Trichophyton violaceum and Trichophyton tonsurans. MIC range, GM MIC, MIC_{50}, MIC_{90} of fluconazole, itraconazole, ketoconazole, voriconazole, posaconazole and isavuconazole for dermatophytes by the microdilution methods are shown in Table 1. The MICs of 121 dermatophytes ranged between 0.125 ~ 64 mg/L for fluconazole, 0.03 ~ 2 mg/L for itraconazole, 0.06 ~ 2 mg/L for ketoconazole, 0.03 ~ 2 mg/L for voriconazole, 0.06 ~ 1 mg/L for posaconazole and 0.03 ~ 2 mg/L for isavuconazole.

Table 1

Minimum inhibitory concentrations (MICs) of the six antifungal agents against 121 strains of dermatophyte (μg/mL)

strains		fluconazole	itraconazole	ketoconazole	voriconazole	posaconazole	isavuconazole
Trichophyton rubrum(96)	Range	1 ~ 64	0.06 ~ 2	0.25 ~ 2	0.125 ~ 0.5	0.06 ~ 0.125	0.03 ~ 0.06
	GM	14.15	0.24	0.47	0.39	0.08	0.04
	MIC_{50}	16	0.5	0.5	0.5	0.06	0.03
	MIC_{90}	32	1	1	0.5	0.06 ~ 0.125	0.06
Microsporum canis(n = 7)	Range	0.125 ~ 2	0.06 ~ 0.125	0.06 ~ 0.5	0.06 ~ 0.125	0.06 ~ 0.125	0.06 ~ 2
	GM	0.41	0.08	0.14	0.08	0.08	0.25
	MIC_{50}	0.25	0.06	0.125	0.06	0.06	0.125
	MIC_{90}	1	0.125	0.25	0.125	0.06 ~ 0.125	2
Microsporum gypseum(n = 6)	Range	8 ~ 16	0.06 ~ 0.125	0.125 ~ 2	0.03 ~ 0.06	0.06 ~ 0.125	0.03 ~ 0.25
	GM	11.31	0.10	0.50	0.04	0.08	0.10
	MIC_{50}	8	0.125	0.25	0.03	0.06	0.06
	MIC_{90}	16	0.125	1	0.06	0.125	0.25
Trichophyton interdigitale(n = 6)	Range	1 ~ 4	0.03 ~ 0.5	0.125 ~ 1	0.25 ~ 2	0.25 ~ 1	0.25 ~ 0.5
	GM	1.59	0.12	0.35	0.63	0.45	0.40
	MIC_{50}	2	0.125	0.5	0.5	0.5	0.5
	MIC_{90}	4	0.25	0.5	2	0.5	0.5
Epidermophyton floccosum(n = 4)	Range	32 ~ 64	1 ~ 2	0.125 ~ 0.06	0.25 ~ 0.5	0.5 ~ 1	0.5 ~ 1
	GM	38.06	1.19	0.10	0.42	0.71	0.71
Trichophyton violaceum(n = 1)	Range	0.125	0.03	0.25	0.5	0.25	0.125
Trichophyton tonsurans(n = 1)	Range	16	0.5	0.125	0.125	0.5	0.125
Total(n = 121)	Range	0.125 ~ 64	0.03 ~ 2	0.06 ~ 2	0.03 ~ 2	0.06 ~ 1	0.03 ~ 2
	GM	10.18	0.22	0.40	0.32	0.10	0.06
	MIC_{50}	16	0.125	0.5	0.5	0.06	0.06
	MIC_{90}	32	0.125	1	0.5	0.125	0.125

GM: geometric mean; 2. MIC_{50}: MIC required to inhibit the growth of 50% of the test strains; 3. MIC_{90}: MIC required to inhibit the growth of 90% of the test strains.

Itraconazole (GM 0.22 mg/L) was one of the most effective antifungal agents against 121 dermatophytes strains comparing to ketoconazole (GM 0.40 mg/L) and fluconazole (GM 10.18 mg/L) and the findings confirmed those of previous studies[9–13]. Three newer triazoles voriconazole (GM 0.32 mg/L), posaconazole (GM 0.10 mg/L) and isavuconazole (GM 0.06 mg/L) as novel broad-spectrum triazole agents showing potent in vitro activities against the dermatophytes and were more active than ketoconazole and fluconazole tested. But in vitro antifungal activity of itraconazole (0.22 mg/L) is better than voriconazole (0.32 mg/L). Voriconazole[14]is a derivative of fluconazole with improved antifungal activity and enhanced potency against fungal 14α-dermethylase, which has been approved by the Food and Drug Administration for treating acute invasive aspergillosis and serious fungal infections caused by Scedosporium apiospermum and Fusarium spp. However, it also showed in vitro activity against dermatophyte isolates[15] in previous studies. Similarly, we investigated voriconazole (GM, 0.32 mg/L) offered good activity against all dermatophytes strains we tested, while it has the strongest antifungal activity against to Microsporum gypseum (GM 0.04 mg/L). Posaconazole (GM 0.10 mg/L) is a new antifungal triazole with potent activity against filamentous fungi that cause systemic infections. Barchiesi et al[16] firstly investigated its activity against clinical isolates of dermatophytes in vitro. They compared the antifungal activities between posaconazole (MIC Range 0.015–4.0 mg/L, MIC_{50} 0.5 mg/L, MIC_{90} –4 mg/L) and itraconazole(MIC Range 0.06–4.0 mg/L, MIC_{50} 1 mg/L, MIC_{90} –4 mg/L) and found posaconazole showed higher antifungal activity against dermatophytes. Isavuconazolium (BAL8557) was specifically designed as a water-soluble prodrug of the azole derivative isavuconazole (BAL4815) [17]. Yamazaki et al confirmed the antifungal spectrum of isavuconazole, firstly showing which has activity against clinical isolates of Japanese origin as well as against isolates from North America and Europe. Deng et al tested 111 clinical Trichophyton rubrum isolates against 6 azole antifungal agents including isavuconazole [18]. The MICs of Trichophyton rubrum against to isavuconazole tested by Deng et al (GM 0.13 mg/L) were higher than we investigated (GM 0.04 mg/L). Furthermore, we also studied other six dermatophytes clinical strains including Microsporum canis (GM 0.25 mg/L), Microsporum gypseum (GM 0.10 mg/L), Trichophyton interdigitale (GM 0.40 mg/L), Epidermophyton floccosum (GM 0.71 mg/L), Trichophyton violaceum (GM 0.125 mg/L) and Trichophyton tonsurans (GM 0.125 mg/L) in vitro antifungal activities against isavuconazole, which were not conducted in previous studies.

In conclusion, voriconazole, posaconazole and isavuconazole were shown good potent antifungal activities against dermatophytes in vitro including Trichophyton rubrum, Microsporum canis, Microsporum gypseum, Trichophyton interdigitale, Trichophyton violaceum and Trichophyton tonsurans collected from the teaching hospital in Shenzhen city, southern China. So it provides an

alternative antifungal agents against dermatophytes infection. But we must noted that the correlation between in vitro results and clinical outcomes of dermatophytosis cases is still needed to be established, and we therefore caution against extrapolating these results to clinical situations without additional testing of a larger sample of dermatophytes.

Declarations

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Authors' contributions

Jie Liu: Responsible for performing *in vitro* susceptibility testing against dermatophytes and manuscript writing. Lanting Liu: Responsible for collecting clinical samples. Xiaoyun Liu: Responsible for identification the clinical isolates of dermatophytes. Bo Yu: Responsible for collecting the manuscript. Xiaoping Hu: Responsible for conceiving the idea, data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study does not involve animals or clinical trials in humans.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008;51 Suppl 4:2-15.

2. Kemna ME, Elewski BE. A U.S. epidemiologic survey of superficial fungal diseases. J Am Acad Dermatol. 1996;35:539-5

3. Khurana A, Sardana K, Chowdhary A. Antifungal resistance in dermatophytes: recent trends and therapeutic implications. Fungal Genet Biol. 2019;132:

4. Gupta AK, Cooper EA. Update in antifungal therapy of dermatophytosis. Mycopathologia. 2008;166:353-3

5. Sardana K, Arora P, Mahajan K. Intracutaneous pharmacokinetics of oral antifungals and their relevance in recalcitrant cutaneous dermatophytosis: time to revisit basics. Indian J Dermatol Venereol Leprol. 2017;83:730-73

6. Jachiet M, Lanternier F, Rybojad M, Bagot M, Ibrahim L, Casanova JL, et al. Posaconazole treatment of extensive skin and nail dermatophytosis due to autosomal recessive deficiency of CARD9. JAMA Dermatol. 2015;151:192-19

7. Toukabri N, Corpologno S, Bougnoux ME, El ED, Sadfi-Zouaoui N, Simonetti G. *In vitro* biofilms and antifungal susceptibility of dermatophyte and non-dermatophyte moulds involved in foot mycosis. Mycoses. 2018;61:79-87.

8. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA. 2008.

9. Sun L, Wan Z, Li R, Yu J. *In vitro* activities of six antifungal agents and their combinations against Chaetomium spp. J Med Microbiol. 2019;68:1042-104

10. Rezaei-Matehkolaei A, Khodavaisy S, Alshahni MM, Tamura T, Satoh K, Abastabar M, et al. *In vitr*o antifungal activity of novel triazole efinaconazole and five comparators against dermatophyte i Antimicrob Agents Chemother. 2018;62.

11. Badali H, Mohammadi R, Mashedi O, de Hoog GS, Meis JF. *In vitro* susceptibility patterns of clinically important Trichophyton and Epidermophyton species against nine antifungal drugs. Mycoses. 2015;58:303-30

12. Silva LB, de Oliveira DBC, Da Silva BV, de Souza RA, Da Silva PR, Ferreira-Paim K,et al. Identification and antifungal susceptibility of fungi isolated from dermatomycoses. Journal of the European Academy of Dermatology and Venereology. 2014;28:633-6

13. Gupta AK, Kohli Y, Batra R. *In vitro* activities of posaconazole, ravuconazole, terbinafine, itraconazole and fluconazole against dermatophyte, yeast and non-dermatophyte species. Med Mycol. 2005;43:179-1

14. Pearson MM, Rogers PD, Cleary JD, Chapman SW. Voriconazole: a new triazole antifungal agent. Ann Pharmacother. 2003;37:420-4

15. Wildfeuer A, Seidl HP, Paule I, Haberreiter A. In vitro activity of voriconazole against yeasts, moulds and dermatophytes in comparison with fluconazole, amphotericin B and griseofulvin. Arzneimittelforschung. 1997;47:1257-12

16. Barchiesi F, Arzeni D, Camiletti V, Simonetti O, Cellini A, Offidani AM, et al. *In vitro* activity of posaconazole against clinical isolates of dermatophytes. J Clin Microbiol. 2001;39:4208-420

17. Ohwada J, Tsukazaki M, Hayase T, Oikawa N, Isshiki Y, Fukuda H, et al. Design, synthesis and antifungal activity of a novel water soluble prodrug of antifungal triazole. Bioorg Med Chem Lett. 2003;13:191-19

18. Deng S, Zhang C, Seyedmousavi S, Zhu S, Tan X, Wen Y, et al. Comparison of the *in vitro* activities of newer triazoles and established antifungal agents against Trichophyton rubrum. Antimicrob Agents Chemother. 2015;59:4312-431