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Review

In vitro susceptibility of chromoblastomycosis agents to antifungal drugs: A systematic review

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ABSTRACT

Chromoblastomycosis (CBM) is a chronic granulomatous mycosis caused by dematiaceous fungi that affects cutaneous and subcutaneous tissues. The standard antifungal drug for treatment is itraconazole, followed by terbinafine. However, cure rates vary from 15% to 80% when these drugs are used as monotherapy. A systematic review of the in vitro susceptibility of CBM agents to antifungal drugs, alone and in combination, was conducted using the Cochrane methodology. Forty-seven search terms were included in the PICOS method of searching electronic databases. The search resulted in 35 studies, of which 8 evaluated antifungal drugs in combination. Based on minimum inhibitory concentrations (MICs), posaconazole, terbinafine, itraconazole and voriconazole were, in descending order, the most effective antifungal drugs against CBM agents in vitro. In drug combination studies, only terbinafine–voriconazole and itraconazole–caspofungin showed 100% synergy for *Fonsecaea pedrosoi*, *Exophiala jeanselmei* and *Phialophora verrucosa*. However, none of the combinations studied showed antagonism.

Contents

1. Introduction

Chromoblastomycosis (CBM) is a chronic granulomatous disease that affects cutaneous and subcutaneous tissues and is caused by seven genera of dematiaceous fungi, including *Fonsecaea*, *Phialophora*, *Rhinocladiella*, *Cladophialophora*, *Exophiala*, *Veronaea* and *Cyphellophora* [1–13]. The latter two genera have been recently described and only one species of each were reported, namely *Veronaea botryosa* [14] and *Cyphellophora ludovingensis* [15].

The distribution of CBM is cosmopolitan, being more frequent in subtropical and tropical regions [16,17]. This mycosis is included in the list of neglected tropical diseases [17,18]. Transmission of the infection occurs by traumatic inoculation of fungus into the skin [16,17]. The clinical manifestations of CBM resemble a cauliflower as there is production of verrucous lesions with crusts and nodules [16,17]. Currently the disease is classified into six clinical types, namely nodular, tumoral, verrucous, plaque, cicatricial and

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Table 1
Minimum inhibitory concentrations (MICs) obtained from studies that performed antifungal susceptibility testing against chromoblastomycosis agents.

Protocol	CFU/mL ($\times 10^4$)	*n*	Parameter	MIC (μg/mL)														Mol. biol.[a]	Ref.
				TRB	VCZ	PCZ	KET	ITR	AMB	FLU	ISZ	5FC	CAS	AFG	MFG	NAT	RVZ	MCZ	
Fonsecaea spp.																			
M38-A		10	Range					0.5–1							16–64				[52]
M38-A2[b]	1.5–4	44	Range	0.031–0.25	0.5–16		0.063–1	0.063–0.5	0.5–16										[41]
Fonsecaea pedrosoi																			
M38-A2[b]	1.5–4	1		2	2	0.25		0.5	2	>64			8	8					x	[2]
M38-A2	0.4–5	10	MIC_{50}	0.015	0.125	0.125		0.03	4										x	[9]
M38-A[c]	0.4–5	11	Range				4–16													[32]
M38-P		1			0.03		0.03	0.25	1	8										[35]
M38-A[d]	1–5	8	GM		0.35	0.32		0.19		22.62							0.22			[48]
M38-A[c]	0.4	4	Range		0.125–0.5				2–4					0.5–4						[51]
M38-A	1–3	1			0.2			0.025	3.1	19	0.39									[30]
M38-A2		8	Range	0.256–4.10	0.064–0.128			0.25–0.5	2–32											[31]
M38-A	2.5	11	Range	0.02–0.5				0.25–0.63	0.75–4											[40]
Fonsecaea compacta																			
M38-A2[c]	0.4–5	1					8													[32]
M38-A	1–3	1			0.2			0.1	3.1	33	0.39									[30]
Fonsecaea monophora																			
M38-A2		1		0.125	1			0.125	2										x	[3]
M38-A	0.4–5	1		0.125	0.0625			1											x	[12]
M38-A2[b]	1.5–4	1		2	1	0.063		0.125	2	64			8	4					x	[2]
M38-A	0.4–5	1		0.125	0.5			1											x	[8]
M38-A		3	Range	0.0313–0.0625															x	[10]
M38-A[d]	0.5–5	18	MIC_{50}	0.125				0.0313											x	[11]
Cladophialophora carrionii																			
M38-A2		81	MIC_{50}	0.031	0.125	0.016		0.031	2	32	0.125		2		0.25				x	[4]
M38-A2	0.4–5	29	MIC_{50}	0.03	0.5	0.125		0.125	4										x	[6]
M38-A	1–3	1			0.013			<0.004	0.78	2.9	0.013									[30]
M38-A2[c]	0.4-5	4	Range				4													[32]
M38-A[d]	1–5	17	GM		0.21	0.21		0.18		26.09							0.30			[48]
M38-A[d]	0.1–0.5	35	Range	0.0039–0.06	0.015–0.5			0.007–0.5	4–32	0.5–32		0.06–8								[36]
M38-A	2.5	22	Range	0.01–0.13				0.05–1	1–8											[40]
M38-A2[b]	1.5–4	6	Range	0.125–1	0.5–8		0.063–0.5	0.063–0.125	1–8											[41]
Phialophora verrucosa																			
M38-A2[d]	2.5	31	MIC_{50}	0.125	0.25	0.125		0.5	4	32		16	8		8				x	[37]
M38-A2	0.1	1						3.12												[47]
M38-A2[c]	0.4–5	4	Range				4													[32]
M38-A[c]	0.4	5	Range		0.125–1				2–4					0.03–0.25						[51]

Table 1 (*Continued*)

Protocol	CFU/mL ($\times 10^4$)	*n*	Parameter	MIC (μg/mL)														Mol. biol.[a]	Ref.
				TRB	VCZ	PCZ	KET	ITR	AMB	FLU	ISZ	5FC	CAS	AFG	MFG	NAT	RVZ	MCZ	
M38-A	2.5	20	Range	0.02–0.5				0.25–2	2–4										[40]
M38-A2[b]	1.5–4	6	Range	0.063–0.125	4–8		0.5–2	0.125–0.25	4–8										[41]
Exophiala spp.																			
M38-A2		50	GM	0.19	0.18	0.06		0.11	0.6					6.28		2.69			[33]
M38-A[c]	1–4	3	Range			≤0.015		0.03	0.25–0.5										[43]
Exophiala dermatitidis																			
M38-A[c]	1–4	27	MIC_{50}		0.125	0.03		0.5	0.5										x [43]
M38-A2[c]	0.4–3.1	20	MIC_{50}		0.25	0.063		0.125	0.25				4						x [44]
M38-A2		5	Range		0.25–0.5	0.25–0.5		1	1										x [45]
M38-A2[e]	1	43	MIC_{50}	0.015	0.06			0.06	1			1							x [38]
M38-A2		18	Range		0.25–0.5	0.25–1		0.5–1	0.5–1										x [46]
M38-A2[c]	0.4–3.1	81	MIC_{50}		0.25	0.063		0.125	0.25	16	0.5		4	2					x [42]
M38-A2		9	GM	0.15	0.13	0.15		0.23	0.54					>8		2.94			x [33]
M38-A	1–3	2	Range		0.2–0.39			0.05–0.1	0.2–0.39	22–26	0.39–1.56								[30]
M38-p[b]		1		0.03	0.125			0.06	0.5									0.06	[34]
Exophiala jeanselmei																			
M38-A[c]	1–4	8	MIC_{50}		0.125	≤0.015		0.03	0.5										x [43]
M38-A2		1			0.25	0.06		0.125	1					>8		2			x [33]
M38-A2[c]	0.4–5	1					4												[32]
M38-A[c]	0.4	5	Range		0.5–2				2–16					0.125–2					[51]
M38-A	1–3	2	Range		0.2–0.78			0.0063–0.05	0.2–0.39	18–20	0.2–3.1								[30]
M38-A2		6	Range	0.512–1.02	0.064–0.512			0.25–8	1–16										[31]
Exophiala oligosperma																			
M38-A2		11	GM	0.22	0.22	0.06		0.09	0.88					6.62		3.52			x [33]
Exophiala spinifera																			
M38-A[c]	1–4	8	MIC_{50}		0.25	≤0.015		0.03	0.5										x [43]
M38-A2[e]	1	4	GM	0.02	0.06			0.05				0.35							x [38]
M38-p[e]		23	GM	0.26	0.3			0.05	0.7	55.05		3.44							x [39]
M38-A2		3	GM	0.5	0.25	0.04		0.12	0.39					>8		2			x [33]
M38-p[e]		10	Range					0.031–0.125	0.25–2			2-32							[49]
M38-p[d]	0.5–4.5	9	Range	0.031–1	0.031–0.5			0.031–0.125	0.5–2	16–128		0.5–64							[50]
M38-A	1–3	1			0.39			0.013	1.56	36	0.78								[30]
M38-p[b]		9	Range	0.03–0.125	0.03–0.25			0.03–0.5	0.125–1									0.125–1	[34]
M38-A2[b]	1.5–4	2	Range	2	8–16		0.5–1	0.25	4										[41]
Exophiala xenobiotica																			
M38-A[c]	1–4	39	MIC_{50}		0.125	≤0.015		0.03	0.25										x [43]
M38-A2		9	GM	0.1	0.21	0.07		0.13	0.79					4.32		3.42			x [33]

Table 1 (*Continued*)

Protocol	CFU/mL ($\times 10^4$)	*n*	Parameter	MIC (μg/mL)															Mol. biol.[a]	Ref.
				TRB	VCZ	PCZ	KET	ITR	AMB	FLU	ISZ	5FC	CAS	AFG	MFG	NAT	RVZ	MCZ		
Rhinocladiella similis																				
M38-A2[d]	2.5	1		0.5	2	0.5	2	1	8										x	[7]
Rhinocladiella aquaspersa																				
M38-A2	0.4–5	1		0.06	1	0.25		0.125	2										x	[5]
M38-A2[b]	0.5–4	3	Range		2	0.063–0.125		0.063–0.125	1–2	32–64	2		8	1					x	[1]
M38-A2[c]	0.4–5	2	Range				4–8													[32]
M38-A2[b]	1.5–4	2	Range	0.125–0.25	2–4		1–2	0.125–0.5	4											[41]

TRB, terbinafine; VCZ, voriconazole; PCZ, posaconazole; KET, ketoconazole; ITR, itraconazole; AMB, amphotericin B; FLU, fluconazole; ISZ, isavuconazole; 5FC, 5-fluorocytosine; CAS, caspofungin; AFG, anidulafungin; MFG, micafungin; NAT, natamycin; RVZ, ravuconazole; MCZ, miconazole; MIC_{50}, concentration inhibiting the growth of 50% of the isolates, GM, geometric mean.

[a] In studies marked with an 'x', the micro-organism was identified using molecular biology tools.

[b] 530 nm, 0.15–0.17 absorbance or 68–71% transmittance.

[c] 530 nm, 0.09–0.13 absorbance or 80–82% transmittance.

[d] Neubauer counting chamber.

[e] 405 nm.

lymphatic dissemination [17]. All forms can cause severe complications to the affected individual if not treated in the initial stages, including social isolation and incapacity to work [7,17]. Histologically, the infection is characterised by muriform cells [5–7,9,17], also called sclerotic cells [11,16,17], which represent the invasive form of the causative fungus [17].

Identification of the species was, for many years, based only on macroscopic and microscopic characteristics of the fungus. Identifying the species based on genetic sequencing of internal transcribed spacer or other DNA regions [7,10] provided more accurate identification of the causative agent and may allow directing the most appropriate therapeutic resource if the susceptibility profile of the species differs among the antifungal drugs used for treatment. Treatment comprises antifungal therapy, surgical excision and cryotherapy, however relapse and treatment failure are common due to the chronic nature of the disease [2,3,8,12,17]. Itraconazole (ITR) is the drug of choice for CBM treatment, followed by terbinafine (TRB) [17]. Cure rates for monotherapy with ITR or TRB vary from 15% to 80% [17–26]. Other antifungal options include ketoconazole (KET), posaconazole (PCZ), voriconazole (VCZ), amphotericin B (AMB) and 5-fluorocytosine (5FC). In the current study, a systematic review was performed to compare the susceptibility of CBM agents to antifungal drugs, alone and in combination.

2. Methods

The systematic review was performed according to the Cochrane methodology and the research question was constructed using the PICOS strategy [27] (Supplementary Table S1 in the online version at DOI: 10.1016/j.jgar.2018.09.010). Data collection was performed in October 2017 and the literature was searched using PubMed, LILACS and SciELO databases. The search terms were composed of 47 keywords. A flow chart of the literature search and selection of studies is shown in Supplementary Fig. S1 in the online version at DOI: 10.1016/j.jgar.2018.09.010. Inclusion criteria were studies related to CBM and that used antifungal susceptibility tests based on standard approved protocols for filamentous fungi such as the Clinical and Laboratory Standards (CLSI) methods M38-P (1998); M38-A (2002) and M38-A2 (2008). Exclusion criteria were studies related to other diseases and/or that used standard protocols to assess the susceptibility of yeasts; such as those from the CLSI M27-P (1992) and M27-A (1997) adapted for filamentous fungi; and Etest.

Susceptibility of the CBM agents to the antifungal drugs was compared based on the minimum inhibitory concentration (MIC), which is defined as the lowest concentration of drug capable of inhibiting visible growth of a micro-organism [28]. For echinocandins, guidelines for antifungal susceptibility tests recommend using the minimum effective concentration (MEC), which is defined as the lowest concentration of drug to produce hyphal morphological changes [29]. However, not all studies provided MEC results. Therefore, for comparison purposes, MIC results for echinocandins were analysed. Importantly, when comparing in vitro with in vivo results, there are still no breakpoints established to analyse resistance and antifungal susceptibility for CBM agents. Therefore, the breakpoints used were based on: (i) those proposed in CLSI document M38-A2 [29]; (ii) the results obtained by Yamazaki et al. [30], which considered samples susceptible to fluconazole (FLU) at an MIC of $\leq$8 μg/mL and resistant at an MIC of $\geq$64 μg/mL, and susceptible to isavuconazole (ISZ) at an MIC of $\leq$1 μg/mL and resistant at an MIC of $\geq$4 μg/mL; and (iii) the results of Gonzáles et al. [5,6], which considered MIC $\geq$ 4 μg/mL as resistant, 2 μg/mL as intermediate susceptible and <1 μg/mL as susceptible to TRB, ITR, PCZ, VCZ and AMB.

Studies that evaluated combinations of antifungal drugs were analysed based on the fractional inhibitory concentration index (FICI), which is calculated using the formula FICI = (MIC_A in combination/MIC_A alone) + (MIC_B in combination/MIC_B alone). Interactions were interpreted as synergistic at FICI $\leq$ 0.5, indifferent at FICI > 0.5 to $\leq$4, and antagonistic at FICI > 4.

3. Results and discussion

A total of 35 studies met the entry criteria (Table 1). Analysing the MIC ranges of the studies, it was possible to trace the susceptibility profile of the CBM agents to antifungal drugs (Table 2). ITR, VCZ and AMB were evaluated against all CBM agents with exception of the species *C. ludovingensis* and *V.*

Table 2
Interpretation of the susceptibility of chromoblastomycosis agents to antifungal drugs.

Species	TRB[a]	VCZ[a]	PCZ[a]	KET[b]	ITR[a]	AMB[a]	FLU[c]	ISZ[c]	5FC[b]	CAS[b]	AFG[b]	MFG[b]	RVZ[b]
Fonsecaea spp.	S	S, I, R		S	S, I	S, I, R						R	
Fonsecaea pedrosoi	S, I, R	S, I	S	S, I, R	S	S, I, R	S, I, R	S		R	S, I, R		S
Fonsecaea compacta		S		I	S	I	I	S					
Fonsecaea monophora	S, I	S, I	S		S, I	I	R			R	R		
Cladophialophora carrionii	S, I	S, I, R	S	S, I	S, I	S, I, R	S, I	S	S	S, I, R		S, I, R	S
Phialophora verrucosa	S	S, I, R	S	S, I	S, I	I, R	I		S, I, R	I, R	S	S, I, R	
Exophiala spp.	S	S	S		S	S					S, I, R		
Exophiala dermatitidis	S	S	S, I		S, I	S, I	I	S, I	S, I, R	S, I, R	S, I, R		
Exophiala jeanselmei	S, I	S, I	S	I	S, I, R	S, I, R	I	S, I			S, I, R		
Exophiala oligosperma	S	S	S		S	S					S, I, R		
Exophiala spinifera	S, I	S, R	S	S	S	S, I, R	I, R	S	S, I, R		R		
Exophiala xenobiotica	S	S	S		S	S					S, I, R		
Rhinocladiella similis	S	I	S	I	I	R							
Rhinocladiella aquaspersa	S	I, R	S	S, I	S	I, R	I, R	I		R	S		

TRB, terbinafine; VCZ, voriconazole; PCZ, posaconazole; KET, ketoconazole; ITR, itraconazole; AMB, amphotericin B; FLU, fluconazole; ISZ, isavuconazole; 5FC, 5-fluorocytosine; CAS, caspofungin; AFG, anidulafungin; MFG, micafungin; RVZ, ravuconazole; S, susceptible; I, intermediate susceptible; R, resistant; MIC, minimum inhibitory concentration.

[a] Interpretation was based on data from Gonzáles et al. [5,6]: MIC ≥ 4 μg/mL as resistant, 2 μg/mL as intermediate susceptible and <1 μg/mL as susceptible.

[b] Interpretation was based on data from Clinical and Laboratory Standards Institute (CLSI) M38-A2 protocol [29].

[c] Interpretation was based on data from Yamazaki et al. [30]: susceptible to FLU at MIC ≤ 8 μg/mL and resistant at MIC ≥ 64 μg/mL; and susceptible to ISZ at MIC ≤ 1 μg/mL and resistant at MIC ≥ 4 μg/mL.

botryosa. All tested strains showed susceptibility or intermediate susceptibility to PCZ, ISZ and ravuconazole (RVZ), although these drugs have not been tested against all species involved in the infection. The same was found for TRB and KET except for the presence of resistant strains of *Fonsecaea pedrosoi* [31,32], and for ITR to which only *Exophiala jeanselmei* showed resistance [31].

It is important to note that some antifungal drugs have been poorly studied against CBM agents, such as miconazole (MCZ) and natamycin (NAT), with only one study reporting the antifungal activity for each drug [33,34]. Despite MCZ and NAT not having established breakpoints for CBM agents, reduced susceptibility of *Exophiala* spp. was found to these drugs [33,34].

Filamentous fungi generally exhibit low susceptibility to the antifungals 5FC and FLU, with MICs >64 μg/mL [29]. This was also observed in CBM studies that evaluated FLU in which only a few samples of *F. pedrosoi* and *Cladophialophora carrionii* showed susceptibility to this drug [30,35,36], and those that evaluated 5FC showing the presence of resistance among *Phialophora verrucosa*, *Exophiala spinifera* and *Exophiala dermatitidis* strains [37–39].

High MICs were observed for caspofungin (CAS), anidulafungin and micafungin (MFG) (Table 2), which highlights the lack of in vitro activity of echinocandins against CBM agents when drugs of this class are used alone. The CLSI M38-A2 protocol [29] recommends using MEC values as they are more consistent and reproducible than MICs. Notwithstanding, as not all studies provided this information, in the current study MIC values were used for comparison purposes, although the results might not be different if MEC values were used.

Among the 35 studies compiled in this review, 8 studies evaluated combinations of antifungal drugs against *Fonsecaea* spp. [1], *F. pedrosoi* [31], *Fonsecaea monophora* [3,8,11,12], *E. jeanselmei* [31], *P. verrucosa* [37] and *C. carrionii* [40]. Good results are expected for drugs that act at different points in the ergosterol biosynthesis pathway. For example, synergistic interactions were observed between TRB, which inhibits squalene epoxidation, and azoles, which inhibit 14α-demethylase activity. Moreover, TRB + VCZ showed 100% synergism against *F. pedrosoi* and *E. jeanselmei* [31]; the same result was observed for ITR + CAS against *P. verrucosa* [37] although this combination was only evaluated against one strain, without further studies using other CBM agents. For *Fonsecaea* spp., only the combination ITR + MFG was tested, with 50% synergism. For *C. carrionii*, the combinations TRB + VCZ, AMB + TRB and AMB + ITR were tested against one isolate, with no antagonism noted [40]. A low percentage of synergism was observed for the remaining combinations (Table 3).

ITR is the drug of choice to treat CBM and this is supported by its great activity against CBM agents in vitro. However, treatment with ITR in vivo is not always efficient [3]. For these cases, the ideal

Table 3
Fractional inhibitory concentration index (FICI) of *Fonsecaea* spp., *Fonsecaea pedrosoi*, *Fonsecaea monophora*, *Exophiala jeanselmei* and *Phialophora verrucosa* isolates.

Species (*n*)	FICI[a]						Reference
	TRB + AMB	TRB + VCZ	ITR + TRB	ITR + CAS	ITR + MFG	AMB + 5FC	
Fonsecaea spp. (10)					50%[a]		[1]
F. monophora (1)	0.27	0.37					[3]
F. monophora (1)		1	0.75				[12]
F. monophora (1)		0.75	0.75				[8]
F. monophora (18)			0.16–1.13 67%[a]				[11]
F. pedrosoi (8)	0.005–0.126 96.5%[a]	0.018–0.0645 100%[a]	0.127–0.25 75.9%[a]				[31]
E. jeanselmei (6)	0.033–0.127 96.5%[a]	0.017–0.0645 100%[a]	0.033–1.07 75.9%[a]				[31]
P. verrucosa (31)			0.245–2 25.8%[a]	0.125–0.5 100%[a]	<0.141–1.25 12.9%[a]	0.094–2 45.2%[a]	[37]

TRB, terbinafine; AMB, amphotericin B; VCZ, voriconazole; ITR, itraconazole; CAS, caspofungin; MFG, micafungin; 5FC, 5-fluorocytosine.

[a] Based on the FICI, interactions were interpreted as synergistic (FICI ≤ 0.5), indifferent (FICI >0.5 to ≤4) or antagonistic (FICI >4). Percentage of strains in which the combination of drugs was synergistic.

would be to perform antifungal susceptibility testing. This discrepancy between in vitro and in vivo results, besides the physiological and behavioural characteristics of the patient, is likely to be explained by the fact that in vitro tests are performed with spores and not with the parasitic form of CBM agents (sclerotic cells).

Different species of the same genus may present a distinct susceptibility profile to the same antifungal drug. However, all of them showed a good susceptibility profile to ITR and TRB, which are the drugs of choice for CBM treatment (Table 2). It is important to emphasise that only a limited number of strains showed resistance to ITR, TRB and VCZ, strains that were not identified by molecular methods [31,41]. When the comparison is limited to strains with molecular identification, no differences in susceptibility profile or resistant strains were observed (Table 1). Thus, species-level identification in clinical practice is not necessary, but it is important to compose epidemiological data. Antifungal activity is interesting to be determined in cases of resistance to the treatment of choice or in relapse cases in order to obtain the susceptibility profile and to direct the most appropriate treatment for the patient.

In conclusion, only the combinations TRB + VCZ and ITR + CAS showed 100% synergy for *F. pedrosoi*, *E. jeanselmei* and *P. verrucosa*. Despite only a limited number of studies analysing antifungal combinations for CBM agents, antagonism was not reported. Overall MIC results showed that PCZ, TRB, ITR and VCZ, in descending order, were the most active antifungal drugs against CBM agents in vitro. ISZ and RVZ showed good activity in vitro, but more studies need to be conducted. Molecular identification at species level is interesting to compose epidemiological data, but in clinical practice it is not necessary since the aetiological agents have a similar susceptibility profile, showing no resistance to the drugs of choice for CBM. Therefore, routine antifungal susceptibility testing may be more indicated in cases of resistance to standard treatment or relapse.

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None declared.

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