Acta Biologica Hungarica 68(3), pp. 334–344 (2017)
DOI: 10.1556/018.68.2017.3.10

IN VITRO ACTIVITY OF CALCIUM CHANNEL BLOCKERS IN COMBINATION WITH CONVENTIONAL ANTIFUNGAL AGENTS AGAINST CLINICALLY IMPORTANT FILAMENTOUS FUNGI

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(Received: March 13, 2017; accepted: April 18, 2017)

Despite the current therapeutic options, filamentous fungal infections are associated with high mortality rate especially in immunocompromised patients. In order to find a new potential therapeutic approach, the *in vitro* inhibitory effect of two antiarrhythmic agents, diltiazem and verapamil hydrochloride were tested against different clinical isolates of ascomycetous and mucoralean filamentous fungi. The *in vitro* combinations of these non-antifungal drugs with azole and polyene antifungal agents were also examined. Susceptibility tests were carried out using the broth microdilution method according to the instructions of the Clinical and Laboratory Standards Institute document M38-A2. Checkerboard microdilution assay was used to assess the interactions between antifungal and non-antifungal drugs. Compared to antifungal agents, diltiazem and verapamil hydrochloride exerted a relatively low antifungal activity with high minimal inhibitory concentration values (853–2731 µg/ml). Although in combination they could increase the antifungal activity of amphotericin B, itraconazole and voriconazole. Indifferent and synergistic interactions were registered in 33 and 17 cases, respectively. Antagonistic interactions were not revealed between the investigated compounds. However, the observed high MICs suggest that these agents could not be considered as alternative systemic antifungal agents.

Keywords: Diltiazem hydrochloride – verapamil hydrochloride – antifungal activity – drug combinations – synergistic interaction

INTRODUCTION

Filamentous fungi could be responsible for severe, opportunistic, life-threatening infections, especially among immunocompromised organ transplant and cancer patients [18]. Although the genus *Aspergillus* still remains the most common cause of invasive mould infections, non-*Aspergillus* moulds, such as *Fusarium* and *Scedosporium* species and members of the order Mucorales are also reported as emerging human pathogens in recent years [6, 19, 26]. Conventional antifungal drugs

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0236-5383/$ 20.00

Table 1

Literature overview of the antifungal properties of diltiazem and verapamil hydrochloride

Drug	Class	Therapeutic plasma level (ng/ml)	Traditional application	Microorganism	Antifungal effect	Reference
VHC	phenyl-alkylamine	60–300	Hypertension Supraventricular arrhythmias Ischemic heart diseases	*Aspergillus* spp., *Candida* spp.	MIC range: 10–50 mg/ml	[12,13]
				Aspergillus fumigatus	Inactive in the tested range: MIC>640 µg/ml. No interaction with ITC	[2]
				Candida albicans	Increased the sensitivity to FLC. MICs decreased from 5.5 to 0.83 µg/ml	[4]
				Candida albicans	Increased the antifungal activity of ketoconazole *in vitro*, found synergistic effect between them	[14]
				Candida spp.	Efficiently reversed the resistance to FLC	[23]
				Aspergillus parasiticus	Inhibited the aflatoxin production (>490 µg/ml). Growth inhibition was not observed	[24]
				Candida albicans	Inhibited the biofilm formation both alone and in combination with FLC	[27]
				Candida albicans	Inhibited the hyphal development (≥10 µg/ml), adhesion and gastrointestinal colonization	[28]
				Candida albicans	Inhibited the oxidative stress response	[29]
DHC	benzo-thiazepine	40–200	Angina pectoris Hypertension	*Candida albicans*	Increased the sensitivity to FLC. MICs decreased from 5.5 to 0.83 µg/ml	[4, 21, 25]
				Aspergillus parasiticus	Inhibited the aflatoxin production (>450 µg/ml). Growth inhibition was not observed	[24]

VHC – verapamil hydrochloride; MIC – minimal inhibitory concentration; ITC – itraconazole; FLC – fluconazole; DHC – diltiazem hydrochloride.

applied in clinical practice often have limited activity against these pathogens [7]. Moreover, their long-term use may cause severe adverse effects in the patients. According to the recent studies, more than 1.6 million people die of a serious fungal infection each year despite the currently available antifungal treatments [9]. These facts underline the importance of developing novel, safely applicable antifungal therapeutic strategies.

Based on previous studies, several non-antifungal medications possess secondary antifungal activity *in vitro*. Calcium channel blockers (CCBs), which are commonly used as antiarrhythmic drugs, block the Ca^{2+} influx influencing the calmodulin system of the cell, which modulates metabolism and growth [1]. Verapamil hydrochloride (VHC) is a phenylalkylamine CCB, exerts inhibitory effect on *Candida* and *Aspergillus* species (Table 1) [2, 28, 29]. Diltiazem hydrochloride (DHC) belongs to the benzothiazepine class of CCBs and it is able to increase the sensitivity of *Candida albicans* to fluconazole (FLC) (Table 1) [4]. These non-antifungal drugs, as monotherapeutic agents or in combination with conventional antifungals could serve as a potential basis for a novel therapeutic approach.

Considering the above-mentioned arguments, the objectives of the present work were (i) to examine and compare the *in vitro* antifungal effect of two CCBs (i.e., DHC and VHC) and conventional antifungal drugs (i.e., amphotericin B, AmB; FLC; itraconzole, ITC; ketoconazole, KTC; terbinafine, TRB; and voriconazole, VRC), and (ii) to investigate their *in vitro* combinations against clinical isolates of ascomycetous and mucoralean fungi.

MATERIALS AND METHODS

Strains

Ten ascomycetous and mucoralean fungal strains from different human infections were involved in the present study: *Aspergillus fumigatus* (Szeged Microbiology Collection, Szeged, Hungary; SZMC 2394 from keratitis), a member of the *Fusarium solani* species complex (SZMC 11412 from keratitis), *Scedosporium aurantiacum* (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CBS 136046 from lung infection), *Scedosporium boydii* (CBS 120157 from lung infection), *Trichoderma longibrachiatum* (Devonian Botanic Garden, University of Alberta Herbarium and Microfungus Collection, Edmonton, Alberta, Canada; UAMH 7955 from sinus infection), *Lichtheimia corymbifera* (SZMC 95033 from lung infection), *Rhizopus microsporus* var. *rhizopodiformis* (CBS 102277 from rhinocerebral infection), *Rhizomucor miehei* (CBS 360.92 from kidney and liver infection), *Rhizopus oryzae* (CBS 146.90 from soft palate infection), and *Rhizomucor pusillus* (Swiss Federal Institute of Technology Culture Collection, Zurich, Switzerland; ETH M4920 from tracheal discharge). All the isolates were maintained on malt extract agar (MEA, Biolab) slants.

Susceptibility testing

The *in vitro* minimal inhibitory concentration (MIC) values were determined using the broth microdilution method following the guidelines described in the CLSI M38-A2 document [5].

The antifungal effect of two CCBs (i.e., DHC and VHC [Sigma-Aldrich]) and seven clinically used antifungal agents (i.e., AmB [Medispec Pharmaceuticals Pvt. Ltd], FLC [Molekula Ltd.], CLT, ITC, KTC, TRB [Sigma-Aldrich], and VRC [Pfizer Inc.]) was investigated and compared. Stock solutions of non-antifungal agents were prepared in sterile distilled water, while antifungal agents were dissolved in the solvents recommended by CLSI M38-A2 document [5]. Further dilutions were prepared in the testing medium, RPMI-1640 buffered to pH 7.0 with 0.165 mol/l 3-[N-morpholino] propanesulfonic acid (Sigma-Aldrich). The final concentration ranges were 128–4096 µg/ml for CCBs and 0.25–128 µg/ml for antifungal drugs. Considering the speeds of germination and growth, microtiter plates of mucoralean fungi were evaluated after 24 hours, whilst *Aspergillus*, *Fusarium* and *Trichoderma* strains after 48 hours, and *Scedosporium* strains after 72 hours of incubation at 37 °C. Results were read using a microplate reader in well-scanning mode (SPECTROstar Nano, Germany). Untreated control samples served as growth controls and we take their absorbance (OD_{620}) as 100%. MIC was defined as the lowest concentration of the tested compound that totally inhibited the growth of the fungus on the basis of the OD_{620} values as compared to the untreated control.

Combination tests

Interactions were investigated between CCBs and AmB, ITC, and VRC using the checkerboard microdilution method [8]. Interactions between VHC and AmB were not tested, since according to the drug information leaflet provided by the manufacturer, the co-administration of these two drugs should be avoided. The final concentration ranges of each drug were chosen based on the MIC data obtained by the antifungal susceptibility tests. Fractional inhibitory concentration index (FICI) values were calculated to describe the interactions between the compounds as described previously [11]. Synergism was defined as $\text{FICI} \leq 0.5$, indifference as $0.5 < \text{FICI} \leq 4$, and antagonism was defined when FICI was >4 [22].

RESULTS

Susceptibility testing

Results of the susceptibility tests are presented in Table 2. In general, the MICs of DHC and VHC were quite high, but mucoralean isolates (MIC range: 853–2048 µg/ml) proved to be slightly more susceptible to these non-antifungal drugs than the

Table 2

In vitro antifungal susceptibility of the investigated filamentous fungal isolates

Strain	Source	MIC values of non-antifungal and antifungal agents (µg/ml)[a]								
		DHC	VHC	AmB	CLT	FLC	ITC	KTC	TRB	VRC
Ascomycetous fungi										
A. fumigatus, SZMC 2394	human keratitis	2731	2731	2	16	>128	2	13	8	16
F. solani, SZMC 11412	human keratitis	2048	2048	8	16	>128	>128	24	>128	53
S. aurantiacum, CBS 136046	human invasive lung infection	2048	1024	128	<0.25	64	128	2	>128	16
S. boydii, CBS 120157	human lung infection	2048	2048	64	1	>128	32	2	>128	16
T. longibrachiatum, UAMH 7955	human sinus infection	1707	2048	2	>128	>128	>128	8	27	53
Mucoralean fungi										
L. corymbifera, SZMC 95033	human lung infection	2048	2048	0.09	<0.25	>128	2	0.8	0.7	171
R. microsporus var. *rhizopodiformis,* CBS 102277	human rhinocerebral infection	2048	1024	1.33	<0.25	>128	5	1.5	0.3	107
R. miehei, CBS 360.92	human kidney and liver infection	1024	853	0.04	<0.25	>128	2	0.5	4	128
R. oryzae, CBS 146.90	human soft palate infection	1707	1024	0.17	64	>128	8	2	>128	85
R. pusillus, ETH M4920	human tracheal discharge	1024	1024	0.05	<0.25	>128	3	1.3	4	85

[a] MIC – minimal inhibitory concentration; DHC – diltiazem hydrochloride; VHC – verapamil hydrochloride; AmB – amphotericin B; CLT – clotrimazole; FLC – fluconazole; ITC – itraconazole; KTC – ketoconazole; TRB – terbinafine; VRC – voriconazole.

ascomycetous isolates (MIC range: 1024–2731 µg/ml). Differences in the susceptibility to antifungal drugs were also observed between the two groups: while VRC was more effective against ascomycetous fungi, mucoralean fungi proved to be more susceptible to AmB, ITC and KTC. Against the investigated Ascomycetes, KTC proved to be the most effective antifungal drug (MIC range: 2–24 µg/ml), followed by VRC (MIC range: 16–53 µg/ml). MICs of ITC were generally high, but *A. fumigatus* SZMC 2394 (MIC: 2 µg/ml) was susceptible to it. AmB and KTC proved to be the most effective antifungals against mucoralean isolates with MICs ranging between 0.04–1.33 µg/ml and 0.5–2 µg/ml, respectively. The growth of mucoralean fungi was also inhibited by low concentrations of CLT (MICs < 0.25 µg/ml) and TRB (MIC range: 0.3–4 µg/ml), except the case of *R. oryzae* CBS 146.90, where the MICs of 64 µg/ml for CLT and > 28 µg/ml for TRB were recorded. With one exception (*S. aurantiacum* CBS 136046, MIC: 64 µg/ml), FLC was ineffective against all isolates in the investigated concentration range. Summarizing, conventional antifungal drugs proved to be more effective than CCBs.

Combination tests

The results of the combination tests are summarized in Tables 3 and 4. Compared to the single use, the relatively high MIC values (853–2731 µg/ml) of CCBs decreased or remained the same in the combination tests (MIC range: 32–> 2048 µg/ml). Antagonistic interactions were not detected between the investigated compounds. Synergistic and indifferent interactions were revealed in 17 and 33 cases, respectively. Between VHC and VRC, and DHC and VRC only indifferent interactions were observed; while the interactions of CCBs with ITC were synergistic in most cases. Between AmB and DHC no interactions were revealed against all mucoralean isolates, while against ascomycetous fungi synergistic and indifferent interactions were registered in three and two cases, respectively.

DISCUSSION

The antifungal effect of CCBs has been investigated previously against *Candida* and *Aspergillus* species, but data on its effect against other human pathogenic ascomycetous and mucoralean fungi are not reported in the literature (Table 1). Basically, our results are in agreement with these reports: relative high concentrations of DHC and VHC inhibited the growth of the investigated fungal strains, MICs were between 853 and 2731 µg/ml (Table 2). These values are much higher than their therapeutically available plasma levels (Table 1). Khalaf et al. [12] observed a much broader and higher MIC range (10,000–50,000 µg/ml) for *Aspergillus* and *Candida* strains. Afeltra et al. [2] reported that VHC was inactive against *Aspergillus fumigatus* (MIC > 640 µg/ml). In another study, *Aspergillus parasiticus* also proved to be resistant to VHC and DHC, but their applied concentrations (<490 µg/ml and <450 µg/ml,

Table 3

In vitro antifungal activity of diltiazem hydrochloride in combination with conventional antifungal agents against ascomycetous and mucoralean fungal strains

Isolate	Antifungal agent/MIC values (µg/ml)[a]				FICI[b]	Interaction[c]
	$\text{DHC}_{\text{alone}}$	$\text{DHC}_{\text{in combination}}$	$\text{AmB}_{\text{alone}}$	$\text{AmB}_{\text{in combination}}$		
Ascomycetous fungi						
A. fumigatus	2731	2048	2	2	**1.75**	NI
F. solani	2048	256	8	2	**0.38**	S
S. aurantiacum	2048	512	128	16	**0.38**	S
	2048	256	128	32	**0.38**	S
S. boydii	2048	32	64	8	**0.14**	S
T. longibrachiatum	1707	1024	2	0.5	**0.85**	NI
Mucoralean fungi						
L. corymbifera	2048	2048	0.09	0.015	**1.17**	NI
R. microsporus	2048	1024	1.3	0.25	**0.69**	NI
R. miehei	1024	128	0.04	0.06	**1.63**	NI
R. oryzae	1707	256	0.17	0.25	**1.62**	NI
R. pusillus	1024	2048	0.05	0.001	**2.02**	NI

Isolate	Antifungal agent/MIC values (µg/ml)[a]				FICI[b]	Interaction[c]
	$\text{DHC}_{\text{alone}}$	$\text{DHC}_{\text{in combination}}$	$\text{VRC}_{\text{alone}}$	$\text{VRC}_{\text{in combination}}$		
Ascomycetous fungi						
A. fumigatus	2731	2048	16	4	**1.00**	NI
F. solani	2048	2048	53	4	**1.08**	NI
S. aurantiacum	2048	2048	16	1	**1.06**	NI
	2048	128	16	16	**1.06**	NI
S. boydii	2048	128	16	8	**0.56**	NI
T. longibrachiatum	1707	128	53	32	**0.68**	NI
Mucoralean fungi						
L. corymbifera	2048	2048	171	256	**2.50**	NI
R. microsporus	2048	2048	107	8	**1.07**	NI
R. miehei	1024	1024	128	8	**1.06**	NI
R. oryzae	1707	2048	85	8	**1.29**	NI
R. pusillus	1024	2048	85	8	**2.09**	NI

Table 3 (cont.)

Isolate	Antifungal agent/MIC values (µg/ml)[a]				FICI[b]	Interaction[c]
	DHC_{alone}	$DHC_{in\ combination}$	ITC_{alone}	$ITC_{in\ combination}$		
Ascomycetous fungi						
A. fumigatus	2731	128	2	0.5	**0.30**	S
F. solani	2048	>2048	>128	>128	**>0.50**	NI
S. aurantiacum	2048	128	128	8	**0.13**	S
S. boydii	2048	128	32	2	**0.13**	S
T. longibrachiatum	1707	1024	>128	1	**>0.50**	NI
Mucoralean fungi						
L. corymbifera	2048	128	2	0.25	**0.19**	S
R. microsporus	2048	2048	5	0.03	**1.01**	NI
R. miehei	1024	128	2	0.5	**0.38**	S
R. oryzae	1707	128	8	1	**0.20**	S
R. pusillus	1024	128	3	0.25	**0.21**	S

[a] MIC – minimum inhibitory concentration; DHC_{alone}, AmB_{alone}, ITC_{alone} and VRC_{alone} – mean MICs of diltiazem hydrochloride, amphotericin B, itraconazole, and voriconazole, respectively, when applied alone; $DHC_{in\ combination}$, $AmB_{in\ combination}$, $ITC_{in\ combination}$, and $VRC_{in\ combination}$, mean MICs of diltiazem hydrochloride, amphotericin B, itraconazole, and voriconazole, respectively, when applied in combination.

[b] FICI – fractional inhibitory concentration index.

[c] S – synergism (FICI≤0.5); NI – no interaction (0.5<FICI≤4) [22].

respectively) could inhibit the aflatoxin production of this strain [24]. VHC inhibited the adhesion, gastrointestinal colonization and the oxidative stress response of *C. albicans* and significantly decreased the hyphal development in a concentration of ≥10 µg/ml [28, 29].

Our findings on the interactions between CCBs and conventional antifungal drugs are comparable to previously reported observations. We proved that DHC and VHC could interact synergistically with azoles and polyene antifungals (Tables 3, 4). While in these tests the MICs of non-antifungal drugs were still beyond their *in vivo* achievable plasma concentrations, the MICs of AmB, ITC and VRC could be decreased to their therapeutic plasma levels [3, 10, 16].

Krajewska-Kułak and Niczyporuk [14] reported that VHC and other CCBs increased the antifungal activity of ketoconazole against *C. albicans* strains *in vitro* and found synergistic effect between them. Afeltra et al. [2] observed no interaction between VHC and ITC against *A. fumigatus*. The sensitivity of *C. albicans* to FLC was increased dramatically in the presence of DHC and VHC [4]. Other calcium channel antagonists were tested by Liu et al. [17] against FLC-resistant *Candida* strains. All the CCBs exhibited no antifungal activity with MICs >512 µg/ml,

Table 4

In vitro antifungal activity of verapamil hydrochloride in combination with conventional antifungal agents against ascomycetous and mucoralean fungal strains

Isolate	Antifungal agent/MIC values (µg/ml)[a]				FICI[b]	Interaction[c]
	$\mathrm{VHC}_{\mathrm{alone}}$	$\mathrm{VHC}_{\mathrm{in\ combination}}$	$\mathrm{VRC}_{\mathrm{alone}}$	$\mathrm{VRC}_{\mathrm{in\ combination}}$		
Ascomycetous fungi						
A. fumigatus	2731	2048	16	4	**1.00**	NI
F. solani	2048	2048	53	4	**1.08**	NI
S. aurantiacum	1024	128	16	8	**0.63**	NI
S. boydii	2048	128	16	8	**0.56**	NI
T. longibrachiatum	2048	128	53	32	**0.67**	NI
Mucoralean fungi						
L. corymbifera	2048	2048	171	8	**1.05**	NI
R. microsporus	1024	1024	107	8	**1.07**	NI
R. miehei	853	256	128	64	**0.80**	NI
R. oryzae	1024	1024	85	8	**1.09**	NI
R. pusillus	1024	1024	85	8	**1.09**	NI
Isolate	Antifungal agent/MIC values (µg/ml)[a]				FICI[b]	Interaction[c]
	$\mathrm{VHC}_{\mathrm{alone}}$	$\mathrm{VHC}_{\mathrm{in\ combination}}$	$\mathrm{ITC}_{\mathrm{alone}}$	$\mathrm{ITC}_{\mathrm{alone}}$		
Ascomycetous fungi						
A. fumigatus	2731	128	2	0.5	**0.30**	S
F. solani	2048	2048	>128	>128	**2.00**	NI
S. aurantiacum	2048	256	128	16	**0.25**	S
S. boydii	2048	128	32	2	**0.13**	S
T. longibrachiatum	2048	1024	>128	4	**>0.50**	NI
Mucoralean fungi						
L. corymbifera	2048	128	2	0.125	**0.13**	S
R. microsporus	1024	128	5	1	**0.33**	S
R. miehei	853	64	2	0.25	**0.20**	S
R. oryzae	2048	1024	8	0.25	**0.53**	NI
R. pusillus	1024	64	3	0.125	**0.10**	S

[a] MIC – minimum inhibitory concentration; $\mathrm{VHC}_{\mathrm{alone}}$, $\mathrm{AmB}_{\mathrm{alone}}$, $\mathrm{ITC}_{\mathrm{alone}}$, and $\mathrm{VRC}_{\mathrm{alone}}$ – mean MICs of verapamil hydrochloride, amphotericin B, itraconazole, and voriconazole, respectively, when applied alone; $\mathrm{VHC}_{\mathrm{in\ combination}}$, $\mathrm{AmB}_{\mathrm{in\ combination}}$, $\mathrm{ITC}_{\mathrm{in\ combination}}$, and $\mathrm{VRC}_{\mathrm{in\ combination}}$, mean MICs of verapamil hydrochloride, amphotericin B, itraconazole, and voriconazole, respectively, when applied in combination.

[b] FICI – fractional inhibitory concentration index.

[c] S – synergism ($\mathrm{FICI} \leq 0.5$); NI – no interaction ($0.5 < \mathrm{FICI} \leq 4$) [22].

although, in combination with FLC, strong synergistic interactions were revealed. Moreover, Pina-Vaz et al. [23] observed that FLC resistance of *Candida* strains could be efficiently reverted by the application of VHC.

As CCBs affect all eukaryotic cells, their potential clinical use as antifungal agents must be clarified by further studies. The influence of DHC and VHC on human peritoneal polymorphonuclear cells and monocytes were investigated by Levy et al. [15]. The authors reported that these CCBs significantly reduced the bactericidal and fungicidal activity of phagocytic cells *in vitro*, however *in vivo* this effect was not observed. In addition to this, CCBs are both substrates and inhibitors of the cytochrome P450 family CYP3A4. Their co-administration with other drugs that share the CYP3A4 pathway (e.g. azoles) may alter the pharmacokinetic properties and increase the plasma levels of both drugs [20]. Optimal therapeutic drug-level monitoring and dosage adjustments may also be necessary during therapy to avoid serious side effects.

In conclusion, the *in vitro* sensitivity of both ascomycetous and mucoralean fungi to azoles and AmB could be increased with the addition of DHC and VHC in the testing media. However, their observed high MIC values and low therapeutic plasma level suggest that these agents could not be administered systemically. A possible limitation of our study is that one isolate per species was investigated only, however antifungal susceptibility might vary among different isolates of the same species.

ACKNOWLEDGEMENTS

L.G. is supported by the Postdoctoral Excellence Programme (PD 120808) of the Hungarian National Research, Development and Innovation Office (NKFI Office). The authors extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for the support in the frame of the International Scientific Partnership Program (ISPP). This work was also connected to the project GINOP-2.3.2-15-2016-00012.

REFERENCES

1. Afeltra, J., Verweij, P. E. (2003) Antifungal activity of non-antifungal drugs. *Eur. J. Clin. Microbiol. Infect. Dis. 22,* 397–407.
2. Afeltra, J., Vitale, R. G., Mouton, J. W., Verweij, P. E. (2004) Potent synergistic *in vitro* interaction between nonantimicrobial membrane-active compounds and itraconazole against clinical isolates of *Aspergillus fumigatus* resistant to itraconazole. *Antimicrob. Agents Chemother. 48,* 1335–1343.
3. Ashbee, H. R., Barnes, R. A., Johnson, E. M., Richardson, M. D., Gorton, R., Hope, W. W. (2014) Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology. *J. Antimicrob. Chemother. 69,* 1162–1176.
4. Bulatova, N. R., Darwish, R. M. (2008) Effect of chemosensitizers on minimum inhibitory concentrations of fluconazole in *Candida albicans*. *Med. Princ. Pract. 17,* 117–121.
5. CLSI (2008) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved Standard – Second Edition. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne.
6. Crabol, Y., Lortholary, O. (2014) Invasive mold infections in solid organ transplant recipients. *Scientifica 2014,* 821969.

7. Denning, D. W., Bromley, M. J. (2015) How to bolster the antifungal pipeline. *Science 347,* 1414–1416.
8. Eliopoulos, G. M., Moellering, R. C. (1996) Antimicrobial combinations. In: Lorian, V. (ed.) *Antibiotics In Laboratory Medicine.* 4th Edition. The Williams and Wilkins Co., Baltimore, pp. 330–396.
9. GAFFI – Global Action Fund for Fungal Infections (2015) Report on activities for 2015. Available from: http://www.gaffi.org/official-documents-and-reports/. Accessed 21 July 2016.
10. Hamill, R. J. (2013) Amphotericin B formulations: a comparative review of efficacy and toxicity. *Drugs 73,* 919–934.
11. Johnson, M. D., MacDougall, C., Ostrosky-Zeichner, L., Perfect, J. R., Rex, J. H. (2004) Combination antifungal therapy. *Antimicrob. Agents Chemother. 48,* 693–715.
12. Khalaf, R. M., Jabir, H. B., Abbas, F. N. (2012) Investigation of the antifungal activity of some non-antifungal drugs in clinical isolates of otomycosis. *J. Thi-Qar. Sci. 3,* 31–39.
13. Köppel, C., Wagemann, A. (1991) Plasma level monitoring of D,L-verapamil and three of its metabolites by reversed-phase high-performance liquid chromatography. *J. Chromatogr. 570,* 229–234.
14. Krajewska-Kułak, E., Niczyporuk, W. (1993) Effects of the combination of ketoconazole and calcium channel antagonists against *Candida albicans in vitro*. *Arzneimittelforschung 43,* 782–783.
15. Levy, R., Dana, R., Gold, B., Alkan, M., Schlaeffer, F. (1991) Influence of calcium channel blockers on polymorphonuclear and monocyte bactericidal and fungicidal activity. *Isr. J. Med. Sci. 27,* 301–306.
16. Lewis, R. E. (2008) What is the "therapeutic range" for voriconazole? *Clin. Infect. Dis. 46,* 212–214.
17. Liu, S., Yue, L., Gu, W., Li, X., Zhang, L., Sun, S. (2016) Synergistic effect of fluconazole and calcium channel blockers against resistant *Candida albicans*. *PLoS ONE 11,* e0150859.
18. Low, C.-Y., Rotstein, C. (2011) Emerging fungal infections in immunocompromised patients. *F1000 Med. Rep. 3,* 14.
19. Mendoza, L., Vilela, R., Voelz, K., Ibrahim, A. S., Voigt, K., Lee, S. C. (2014) Human fungal pathogens of Mucorales and Entomophthorales. *Cold Spring Harb. Perspect. Med. 5,* a019562.
20. Methaneethorn, J., Chamnansua, M., Kaewdang, N., Lohitnavy, M. (2014) A pharmacokinetic drug–drug interaction model of simvastatin and verapamil in humans. *Conf. Proc. IEEE Eng. Med. Biol. Soc. 2014,* 5711–5714.
21. Monteiro, N., Silvestre, J., Gonçalves-Pereira, J., Tapadinhas, C., Mendes, V., Póvoa, P. (2013) Severe diltiazem poisoning treated with hyperinsulinaemia-euglycaemia and lipid emulsion. *Case Rep. Crit. Care 2013,* 138959.
22. Odds, F. C. (2003) Synergy, antagonism, and what the chequerboard puts between them. *J. Antimicrob. Chemother. 52,* 1.
23. Pina-Vaz, C., Rodrigues, A. G., Costa-de-Oliveira, S., Ricardo, E., Mårdh, P. A. (2005) Potent synergic effect between ibuprofen and azoles on *Candida* resulting from blockade of efflux pumps as determined by FUN-1 staining and flow cytometry. *J. Antimicrob. Chemother. 56,* 678–685.
24. Praveen, R. J., Subramanyam, C. (1999) Requirement of Ca^{2+} for aflatoxin production: inhibitory effect of Ca^{2+} channel blockers on aflatoxin production by *Aspergillus parasiticus* NRRL 2999. *Lett. Appl. Microbiol. 28,* 85–88.
25. Richards, D., Aronson, J., Reynolds, D. J., Coleman, J. (2011) *Oxford Handbook of Practical Drug Therapy.* 2nd Edition. Oxford University Press, Oxford.
26. Roilides, E., Dotis, J., Katragkou, A. (2007) *Fusarium* and *Scedosporium*: emerging fungal pathogens. In: Kavanagh, K. (ed.) *New Insights in Medical Mycology.* Springer, Dordrecht, pp. 267–285.
27. Yu, Q., Ding, X., Xu, N., Cheng, X., Qian, K., Zhang, B., Xing, L., Li, M. (2013) *In vitro* activity of verapamil alone and in combination with fluconazole or tunicamycin against *Candida albicans* biofilms. *Int. J. Antimicrob. Agents 41,* 179–182.
28. Yu, Q., Ding, X., Zhang, B., Xu, N., Jia, C., Mao, J., Zhang, B., Xing, L., Li, M. (2014) Inhibitory effect of verapamil on *Candida albicans* hyphal development, adhesion and gastrointestinal colonization. *FEMS Yeast Res. 14,* 633–641.
29. Yu, Q., Xiao, C., Zhang, K., Jia, C., Ding, X., Zhang, B., Wang, Y., Li, M. (2014) The calcium channel blocker verapamil inhibits oxidative stress response in *Candida albicans*. *Mycopathologia 177,* 167–177.