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Note

In vitro interaction between glabridin and voriconazole against *Aspergillus fumigatus* isolates

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ABSTRACT

Background: Voriconazole (VRC) is widely recommended as the first-line therapy for invasive aspergillosis. However, surveillance studies have demonstrated that there is an increase in the frequency of azole resistance among *Aspergillus fumigates* isolates. In recent years, more studies on effective synergisms between natural agents and antifungal drugs have been published.
Aims: To evaluate the synergistic antifungal effect of glabridin (Gla) and VRC against *A. fumigatus* isolates.
Methods: Potential interactions between Gla and VRC were studied by using a microdilution checkerboard method based on the CLSI reference technique. To assess the interaction of drugs the fractional inhibitory concentration index (FICI) was calculated based on the Loewe Additivity model.
Results: The minimum inhibitory concentrations (MIC) obtained with Gla alone were relatively high (MIC_{50} 16 μg/ml). However, our results showed synergistic interaction between Gla and VRC against *A. fumigatus* strains, with FICI range values between 0.15 and 0.5.
Conclusions: Synergistic activity of Gla and VRC against both VRC-sensitive and -resistant *A. fumigatus* isolates may lead to design new antifungal agents, especially for inhibiting those azole-resistant strains.

Interacción *in vitro* entre la glabridina y el voriconazol frente a aislamientos de *Aspergillus fumigatus*

Palabras clave:
Glabridina
Voriconazol
Aspergillus fumigatus
Sinergia

RESUMEN

Antecedentes: El voriconazole (VRC) es ampliamente recomendado como terapia de primera línea en la aspergilosis invasiva. Sin embargo, los estudios de vigilancia muestran un aumento en la frecuencia de resistencia a los azoles en aislamientos de *Aspergillus fumigatus*. En los últimos años se han ampliado los estudios sobre agentes naturales que establecen sinergias efectivas con medicamentos antimicóticos.
Objetivos: En el presente estudio se investigó el potencial efecto antifúngico sinérgico de la glabridina (Gla) con el VRC frente a aislamientos de *A. fumigatus*.
Métodos: Se estudió la interacción de Gla y VRC mediante un método de microdilución basado en la técnica de referencia CLSI. Para evaluar la interacción entre ambos compuestos se calculó el índice de concentración inhibitoria fraccional (FICI) en base al modelo de aditividad de Loewe.
Resultados: Las concentraciones mínimas inhibitorias (MIC) obtenidas con Gla fueron relativamente altas (MIC_{50} 16 μg/ml). Sin embargo, nuestros resultados mostraron interacciones sinérgicas entre Gla y VRC con valores de rango FICI entre 0,15 y 0,5 contra los aislamientos de *A. fumigatus*.

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Conclusiones: La actividad sinérgica de Gla y VRC observada contra aislamientos de *A. fumigatus* sensibles y resistentes a VRC podría encaminarse al diseño de nuevos agentes antifúngicos, especialmente para combatir aquellos aislamientos resistentes a los azoles.

The prevalence of azole-resistant isolates of *Aspergillus fumigatus* in Iran has increased remarkably from 3.3% to 6.6% in comparison with previous epidemiological researches.[6] This issue, which is associated with high rates of treatment failure, has become a major medical concern.[8,9] In recent years, studies on natural agents with effective synergisms with antifungal drugs have increased, and natural products are overall more studied in an attempt to find in them anticancer, antioxidant, anti-inflammatory and anti-microbial properties. Glabridin (Gla) is an isoflavonoid, the main component of the root extract of *Glycyrrhiza glabra* (Licorice plant).[1] Several reports have shown the antifungal activity of Gla against some filamentous fungi, different *Candida* species and *Cryptococcus neoformans*.[1,3] However, no reports were found about any potential synergism effect of Gla and voriconazole (VRC). In the present study, the synergistic antifungal action of Gla and VRC against both VRC-resistant and -susceptible *A. fumigatus* isolates was studied.

Both VRC-susceptible ($n = 58$) and VRC-resistant ($n = 4$) *A. fumigatus* strains were used in this study. All the strains are maintained in the culture collection of the Invasive Fungi Research Center (Sari, Iran) and have been previously identified by sequencing the beta-tubulin gene. Antifungal susceptibility testing against VRC was previously performed according to Clinical and Laboratory Standards Institute (CLSI) M38 3rd edition document,[10] and the minimum inhibitory concentrations (MICs) were compared with CLSI breakpoints. The MIC of Gla for each isolate was determined adapting the conditions in the CLSI document. The reference drug VRC (Pfizer Central Research, US), and Gla (Sigma, Taufkirchen, Germany) were obtained as reagent-grade powders to perform the CLSI microdilution assays. The potential interaction between Gla and VRC was checked by using a microdilution checkerboard method based on the CLSI reference technique, with 96-well microtiter plates.[7] The experiments were run twice and in duplicate. To assess the interaction of both drugs, the fractional inhibitory concentration index (FICI) was calculated based on the Loewe Additivity model.[7]

Table 1 shows the results of the in vitro antifungal susceptibility profiles of the 62 isolates of *A. fumigatus* both for Gla and VRC. According to the aforementioned CLSI M38 document (VRC resistance >2 μg/ml), four *A. fumigatus* isolates were classified as resistant. Excluding the resistant strains, MICs for VRC ranged in the interval 0.5–0.063 μg/ml. The MIC values of Gla for all strains ranged from 8 to 16 μg/ml. When checking VRC in the presence of several sub-inhibitory concentrations of Gla (8–0.063 μg/ml), the MIC values for sensible and resistant isolates when compared with VRC alone decreased from 0.063 to 0.016 μg/ml and from 4 to 0.5 μg/ml, respectively. The synergistic effect of Gla with VRC is shown in Table 1 (FICI: 0.25–0.5).

Nowadays, the increasing emergence of fungal resistance to common antifungal agents is one of the main problems in the field of medical mycology. There are few studies regarding the inhibitory effect of Gla on different fungi. Liu and colleagues examined Gla alone and in combination with fluconazole against different species of *Candida* and *Cryptococcus neoformans* in different ways. It was shown that Gla alone had an antifungal effect at high concentrations, but in low concentrations and combined with fluconazole there was an effective synergistic effect against drug-resistant *Candida albicans* and *Candida tropicalis*.[2] The synergistic activity of Gla and nystatin has also been reported against *C. albicans*.[3] To the best of our knowledge there are still no studies regarding the synergistic effect of Gla and VRC against fungi. In our study both VRC-susceptible and VRC-resistant isolates showed the same MICs for Gla, which suggests that the mechanism of action is independent from that of azoles. In previous studies, an increasing expression of two genes (*MCA1* and *NUC1)* involved in yeast apoptosis was observed when *C. albicans* and *Candida glabrata* cells were exposed to Gla.[4,5] These results point out that the synergism between Gla and VRC is the sum of apoptosis signaling and the ergosterol biosynthesis pathway targeting. However, the results need to be confirmed by in vivo studies of *Aspergillus* infection in animal models. The synergistic in vitro activity between Gla and VRC against both VRC-sensitive and -resistant *A. fumigatus* isolates may lead to design new antifungal agents to especially address *A. fumigatus* isolates resistance.

Table 1
Inhibitory effect of Gla and FICI results on *A. fumigatus* VRC-susceptible (S) and VRC-resistant (r) isolates .

Isolates	Number (*n*)	Antifungal agent	MIC (μg/ml)									MIC range	MIC90	GM*	Mode	Mechanism of resistant	FICI¥		
			16	8	4	2	1	0.5	0.25	0.125	0.062						≤0.5	>0.5–4	≥4
A. fumigatus	58 (S)	VRC**	0	0	0	1	0	3	36	0	18	0.062–2	0.25	0.1627	0.25	None	58	0	0
		Gla§	56	2	0	0	0	0	0	0	0	8–16	16	15.62	16				
	4 (R)	VRC	1	2	1	0	0	0	0	0	0	4–16	14	8	8	TR34/L98H (9)	4	0	0
		Gla	0	4	0	0	0	0	0	0	0	None	None	None	None				

* MIC geometric mean
** Voriconazole
§Glabridin
¥ Fractional inhibitory concentration index (synergy, FICI ≤0.5; antagonism, FICI >4; and no interaction, FICI >0.5–4)

Ethical approval

Not required.

Conflict of interest

Nothing to declare.

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