Epigallocathechin-3-gallate Antimycotic and Azole Resistant Modulator Potencial Against Triazole-Resistant *Aspergillus fumigatus*

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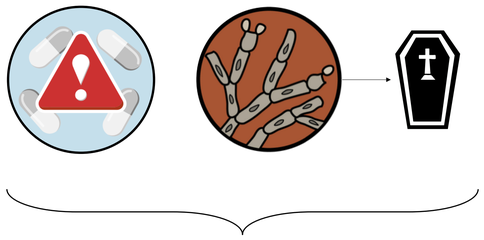
Introduction

Systemic–opportunistic fungal infections caused by azole-resistant fungi, including *Aspergillus fumigatus*, are emerging as an important cause of human disease with high associated morbimortality rates worldwide. The resistance to the limited arsenal of antifungal drugs is a serious concern, making it imperative to assess new compounds with antimicrobial properties and no cytotoxic effects, to be utilized as adjuvants in fungal therapy.



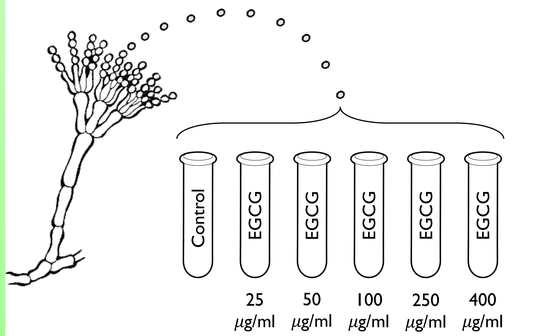
Green tea (*Camellia sinensis*)

Epigallocatechin-3-gallate (EGCG), the largest constituent of green tea, has demonstrated anti-infective properties in several *in vitro* studies however, information regarding EGCG antimycotic activity and synergistic interactions between EGCG and triazoles against *A. fumigatus* is scarce.

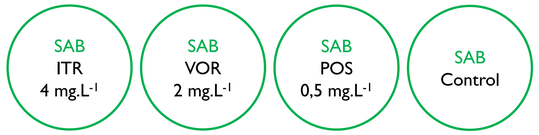
This work aimed to assess the antimycotic potential of EGCG against azole-resistant isolates of A. fumigatus, and the potential synergistic effects between EGCG and commonly used triazole antifungal drugs, namely itraconazole (ITR), voriconazole (VOR) and posaconazole (POS).

Methodology

Azole-resistant A. fumigatus isolates (N=4) collected during exposure assessment campaigns performed in the last ten years in Portugal and abroad, and previously characterized through conventional culture-based (agar-plate screening) and molecular (sequencing the calmodulin or β-tubulin genes for Aspergillus spp.) methods, were grown in MEA medium.



The obtained pure colonies were suspended in 1mL solutions with EGCG at final concentrations of 400 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml and with no EGCG at all, for control purposes.



Each suspension was then spread onto Sabouraud dextrose agar media supplemented with 4 mg.L^{-1} ITR, 2 mg.L^{-1} VOR or 0.5 mg.L^{-1} POS and with no supplementation, for screening of antifungal resistance (adapted from the EUCAST 2020 guidelines).

The inoculated plates were incubated at 25°C for 7 days.

Results

Table 1. Growth pattern of azole-resistant *A. fumigatus* isolates suspended in EGCG solutions and spread onto Sabouraud dextrose agar media supplemented with triaoles

Sample	EGCG (µg/ml)	W/o Triazole	2 mg.L^{-1} VOR	0.5 mg.L^{-1} POS	4 mg.L^{-1} ITR
A	0	+	+	-	-
	25	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
	250	-	-	-	-
	400	-	-	-	-
B	0	+	-	-	-
	25	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
	250	-	-	-	-
	400	-	-	-	-
C	0	+	-	-	-
	25	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
	250	-	-	-	-
	400	-	-	-	-
D	0	+	-	-	-
	25	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
	250	-	-	-	-
	400	-	-	-	-

After the incubation period, all resistant isolates grew on Sabouraud dextrose agar media with no azole supplementation.

The addition of 25 µg/ml of EGCG to the suspensions canceled the growth observed in Sabouraud dextrose agar media of the 4 isolates.

One isolate grew on Sabouraud dextrose agar media supplemented with 2 mg.L^{-1} VOR but showed no growth when 25 µg/ml of EGCG was added to the suspension.

Conclusion

These results build evidence towards antimycotic potential of EGCG against azole-resistant isolates of *A. fumigatus*.

Sample A growth pattern indicates sensitization of the azole-resistant isolate when EGCG and VOR were used together.

At last, this study suggests that EGCG is a potential therapeutic adjuvant for therapies against azole-resistant fungi.

Recommendations

We recommend increasing the sample size in further studies and evaluating EGCG potential against other fungal pathogens of clinical interest, such as dermatophytes.

Acknowledgements

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