Journal of Antimicrobial Chemotherapy (2009) **63**, 337–339
doi:10.1093/jac/dkn488
Advance Access publication 26 November 2008

JAC

Curcumin as a promising antifungal of clinical interest

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Received 19 August 2008; returned 21 October 2008; revised 28 October 2008; accepted 5 November 2008

***Objectives*: The antifungal activity of curcumin was evaluated against 23 fungi strains and its *in vitro* inhibitory effect on the adhesion of *Candida* species to human buccal epithelial cells (BEC) was also investigated.**

***Methods*: The antifungal susceptibility was evaluated by broth microdilution assay following the CLSI (formerly the NCCLS) guidelines. The inhibitory effect of curcumin on the cell adhesion was performed with *Candida* species and BEC.**

***Results*: *Paracoccidioides brasiliensis* isolates were the most susceptible to curcumin while the growth of *Aspergillus* isolates was not affected. Curcumin was much more efficient than fluconazole in inhibiting the adhesion of *Candida* species to BEC, particularly those strains isolated from the buccal mucosa of AIDS patients.**

***Conclusions*: The lack of antifungal compounds with reduced side effects highlights the importance of studying natural products for this purpose. Curcumin was a more potent antifungal than fluconazole against *P. brasiliensis*, the causal agent of the neglected disease paracoccidioidomycosis. Curcumin dramatically inhibited the adhesion of *Candida* species isolated from AIDS patients to BEC, demonstrating that curcumin is a promising lead compound that warrants further investigation into its therapeutical use in immunocompromised patients.**

Keywords: antifungal activity, adhesion, MIC, natural products

Introduction

Fungal infections have increased significantly, contributing to the cause of morbidity and mortality. The increase in antimicrobial resistance and populations of patients at some risk, in conjunction with the restricted number of commercially available antifungal drugs that still present many side effects, are the cause for this problem.[1,2] These limitations emphasize the need to develop new and more effective antifungal agents. Natural products are attractive prototypes for this purpose due to their broad spectrum of biological activities.[3]

Curcumin is a yellow–orange polyphenol compound produced by the rhizome of *Curcuma longa* plants, which is widely used as a spice in Asian cooking. This compound has been shown to possess a wide range of pharmacological activities,[4] where antifungal activity was assessed by experiments done with crude extracts of *C. longa*.

This work focused on the evaluation of curcumin antifungal activity against 23 fungi strains of clinical interest as well as its ability to inhibit the adhesion of *Candida* spp. to human buccal epithelial cells (BEC).

Materials and methods

All chemicals used in this study were obtained from Sigma, unless otherwise stated.

Twenty-three fungi strains, which included *Candida* spp., *Cryptococcus neoformans*, *Sporothrix schenckii*, *Paracoccidioides brasiliensis* and *Aspergillus* spp., were the subject of this study

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Table 1. MICs of curcumin that completely inhibited the growth of human-pathogenic fungi

Fungi	MIC (mg/L)	
	curcumin	fluconazole[a]
C. albicans ATCC 18804	64	2
C. tropicalis ATCC 750	256	2
Candida krusei ATCC 20298	256	32
C. parapsilosis ATCC 20019	>256	1
Candida glabrata ATCC 2001	>256	1
C. dubliniensis (Cd22)[b]	32	0.5
C. dubliniensis (Cd28)[b]	32	0.5
C. neoformans ATCC 32608	32	8
S. schenckii ATCC 10212	32	64
P. brasiliensis MG05[b]	0.5	16
P. brasiliensis Pb01[b]	8	4
P. brasiliensis Pb18[b]	2	8
P. brasiliensis 608[b]	32	4
P. brasiliensis 17[b]	8	16
P. brasiliensis MG04[b]	4	4
P. brasiliensis B339[b]	2	8
Aspergillus fumigatus ATCC 16913	>256	64
Aspergillus nomius ATCC 15546	>256	>64
Aspergillus flavus IMI190443	>256	>64
Aspergillus fumigatus[b]	>256	64
Aspergillus tamarii[b]	>256	>64
Aspergillus terreus[b]	>256	>64
Aspergillus clavatus[b]	>256	64

[a]Positive control.
[b]Clinical isolate.

(Table 1). Dimorphic fungi (*S. schenckii* and *P. brasiliensis*) were employed as the yeast form.

Analysis of *in vitro* susceptibility was performed by broth microdilution assay following the CLSI (formerly the NCCLS) guidelines for yeasts and filamentous fungi.[5,6] Curcumin was tested in the range of 0.5–256 mg/L and fluconazole (0.06–64 mg/L; Pfizer, São Paulo, Brazil) was included as a positive control. The MIC values correspond to the lowest concentrations that did not allow for the detection of any visual fungal growth.

The adhesion assay was carried out according to Lyon and de Resende.[7] Briefly, *Candida* spp. isolates were exposed to curcumin at its MIC value for 1 h, and were then incubated with BEC for another 1 h. Assay with *Candida parapsilosis* was carried out with 256 mg/L curcumin since its MIC value was not determined. The number of yeast cells that adhered to BEC was quantified by light microscopy (×400 magnification) from 50 randomly chosen BEC.

Results and discussion

The MICs of curcumin that completely abolished the growth of fungi strains are shown in Table 1. *P. brasiliensis* isolates were the most susceptible to curcumin. Curcumin was 32-fold more potent than fluconazole in the inhibition of *P. brasiliensis* MG05 growth. Fluconazole was also 4-fold less potent than curcumin in inhibiting the growth of *P. brasiliensis* Pb18 and B339. The curcumin effect on *P. brasiliensis* 17 was roughly the same as that of fluconazole, while *P. brasiliensis* Pb01 and 608 strains were more susceptible to fluconazole (Table 1).

Even though the greatest antifungal activity of curcumin was against *P. brasiliensis* isolates, promising results were also achieved for this compound against other fungi species. For instance, curcumin was twice as potent as fluconazole in the growth inhibition of the opportunistic yeast *S. schenckii* (Table 1). *S. schenckii* promotes infections of hosts with predisposing conditions, which includes alcoholics, diabetics, transplant recipients, and patients with haematological malignancies, chronic obstructive pulmonary disease, long-term treatment with corticosteroids and AIDS.[8]

Curcumin (32 mg/L) was able to inhibit the growth of *C. neoformans* and the clinical isolates of *Candida dubliniensis* Cd22 and Cd28. Non-*albicans Candida* species are emerging as colonizers and pathogens causing nosocomial fungal bloodstream infections.[9] *Candida albicans* was the most susceptible to curcumin among the *Candida* species studied (Table 1). The growth of the remaining fungi isolates was only affected by curcumin at concentrations ≥256 mg/L.

Curcumin was used to further explore its ability to prevent the adhesion of *Candida* species to BEC. These experiments were performed with curcumin at its MIC values. Curcumin was able to inhibit the adhesion to BEC of all the *Candida* species studied, being more potent than the commercial antifungal fluconazole (Figure 1a). *C. dubliniensis* Cd22 and Cd28 had the most significant reduction in adhesion to BEC (63% and 74%, respectively) in the presence of curcumin. The curcumin effect on all clinical isolates was 2.5–6.0-fold higher than that of fluconazole. Figure 1(b–d) shows the representative images of *C. dubliniensis* Cd28 adhesion to BEC. These strains were isolated from the oral cavities of AIDS patients at the Santa Maria University Hospital, RS, Brazil. Other *C. dubliniensis* strains were reported to be recovered from HIV-infected and AIDS patients under fluconazole treatment for oropharyngeal candidiasis, suggesting that this commercial antifungal was either selecting resistant *Candida* isolates or inducing cell adhesion.[10] The adhesion of microorganisms to host mucosal surfaces is a prerequisite for colonization and infection.[7] Our results indicate that curcumin is a promising lead compound for the design of new antifungal agents capable of inhibiting the adhesion of *C. dubliniensis*. The adhesion of *Candida tropicalis* to BEC was inhibited by 55% in the presence of curcumin while the inhibition caused by fluconazole accounted for only 13%. Curcumin was 2.5-fold more potent than fluconazole at inhibiting the adhesion of *C. albicans* or *C. parapsilosis* to BEC (Figure 1a).

To the best of our knowledge, this study reports for the first time the effect of curcumin on the growth and cell adhesion to BEC of fungi of clinical interest. Our *in vitro* results highlight the potential of curcumin as an effective antifungal against different *P. brasiliensis* strains, being much more potent than the commercial antifungal fluconazole. This natural product was also much more efficient than fluconazole in inhibiting the adhesion of *Candida* species to BEC, particularly those strains isolated from the buccal mucosa of AIDS patients.

Synthesis of curcumin analogues and evaluation of their antifungal activities are in progress in our laboratory.

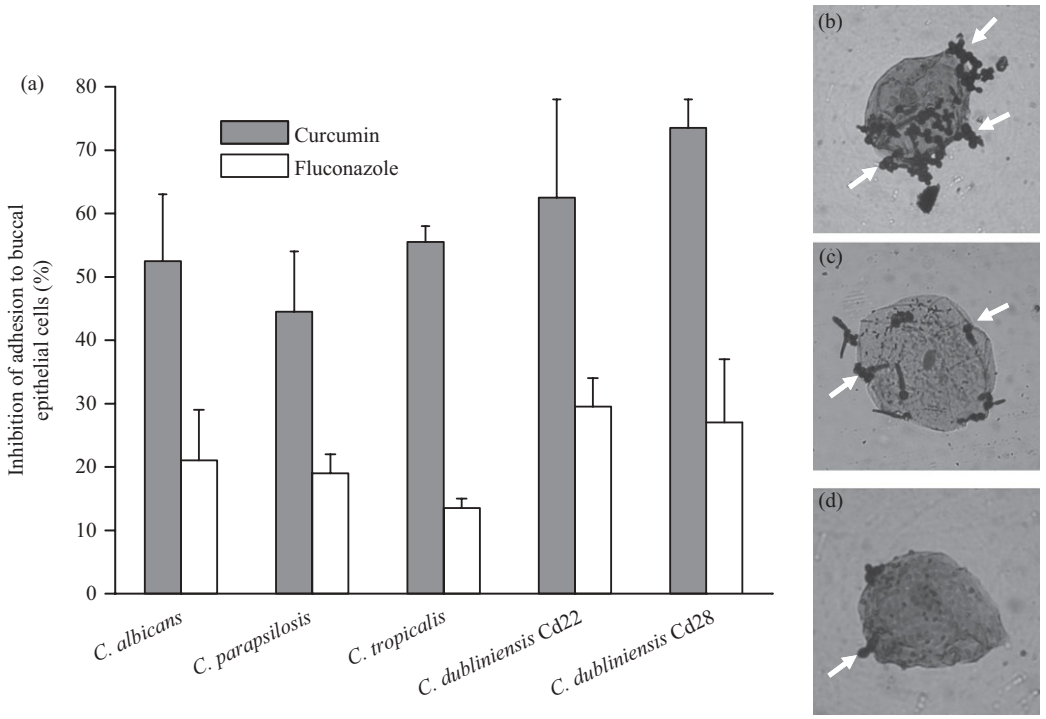

Figure 1. Effect of curcumin on the adhesion of *Candida* species to BEC. Percentage of cell adhesion inhibition (a). The results obtained with curcumin were significantly different from those obtained with fluconazole ($P < 0.008$; Kruskal–Wallis test). Micrography of BEC in the presence of untreated *C. dubliniensis* Cd28 (b), *C. dubliniensis* Cd28 pre-treated with 0.5 mg/L fluconazole (c) and *C. dubliniensis* Cd28 pre-treated with 32 mg/L curcumin (d). Arrows indicate fungal adhesion. (b), (c) and (d) correspond to images of cells at ×400 magnification.

Acknowledgements

We thank Dr Sydney Alves (Hospital Universitário Santa Maria, RS, Brazil) for kindly providing the isolates of *C. dubliniensis*.

Funding

This work was funded by FAPEMIG, CNPq and CAPES.

Transparency declarations

None to declare.

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