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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Dracorhodin perchlorate inhibits biofilm formation and virulence factors of *Candida albicans*

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KEYWORDS
Candida albicans;
Biofilm;
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Summary
Objective. — The aim of this study was to investigate the antifungal activity of dracorhodin perchlorate (DP) against planktonic growth and virulence factors of *Candida albicans*.
Methods. — Microdilution method based on CLSI-M27-A3 was used to test the antifungal susceptibility of DP. The activity of DP against biofilm formation and development of *C. albicans* was quantified by XTT assay and visualized by confocal laser scanning microscope. The effect of DP on the morphological transition of *C. albicans* induced by four kinds of hyphal-inducing media at 37°C for 4 hours was observed under microscope. The rescue experiment by adding exogenous cAMP analog was performed to investigate the involvement of cAMP in the yeast to hyphal transition and biofilm formation of *C. albicans*. Egg yolk emulsion agar was used to determine the inhibition of DP on the phospholipase production of *C. albicans*. Human JEG-3 and HUVEC cell lines, as well as the nematode *Caenorhabditis elegans* was used to assess the toxicity of DP.
Results. — The minimum inhibitory concentration (MIC) of DP is 64 μM while the antifungal activity was fungistatic. As low as a concentration at 16 μM, DP could inhibit the yeast to hyphal transition in liquid RPMI-1640, Spider, GlcNAc and 10% FBS-containing Sabouroud Dextrose medium, as well as on the solid spider agar. Exogenous cAMP analog could rescue part of biofilm

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viability of *C. albicans*. DP could inhibit the production of phospholipase. The toxicity of DP against human cells and *C. elegans* is low.
Conclusion. — DP could inhibit the planktonic growth and virulent factors in multiple stages, such as yeast to hyphal transition, adhesion, biofilm formation and production of phospholipase of *C. albicans*.

Introduction

Candida albicans is the major opportunistic pathogenic fungus in human which could grow harmlessly on the skin, in the oral cavity, gastrointestinal tracts and urogenital tracts of healthy people as a commensal organism [1,2]. In the case of the dysbiosis of normal microbiota, damages to the gastrointestinal-blood barrier resulting from surgery or injury, implantation of medical devices and compromised immune defense, the risk of infection by *C. albicans* grows up [1—4]. Infections could be mucosa-associated or systematic, such as oral thrush, vaginitis and systematic infections [2,5]. This pathogenic fungus is the fourth causing agents for bloodstream infections and the annual infections caused by *C. albicans* was approximately 400 000 with a high mortality (about 40%), causing a loss of about 1.7 billion dollars every year [6,7]. The lack of antifungal drugs and the emergence of resistance present a pressing mission to develop new antifungal agents.

The capacity to form biofilms on medical devices such as catheters and prostheses is thought to be one of the virulence factors of *C. albicans*, imposing remarkable influence on the morbidity and mortality [8]. In comparison with their planktonic counterpart, the biofilms of *C. albicans* demonstrated decreased susceptibility to antifungal drugs such as azoles, which is manifold and complicate, involving upregulation of drug efflux pumps, increased cell density, the presence of matrix of exopolymeric substances (EPS) secreted by *C. albicans* and the existence of persister cells [9,10].

Dracorhodin perchlorate (DP) is a derivative of the flavonoid dracorhodin, which is isolated from the traditional Chinese medicine ''Dragon's blood'' (the exudates of the fruit of Daemonorops draco) [11]. DP has been shown anticancer activities against various tumors [12—16]. DP could also prevent and treat the renal fibrosis of diabetic nephropathy patients [17]. Recently DP was shown to suppress the virulence of methicillin-resistant *Staphylococcus aureus* (MRSA) strain USA300 by depressing the expression of the pore-forming α-toxin [18]. However, the antifungal activity of DP has never been evaluated. This study evaluates the antifungal activity of DP, and here we show DP inhibits growth, biofilm formation and virulence factors of *C. albicans* such as the yeast to hyphal transition and phospholipase production.

Materials and Methods

Strains and culture conditions

C. albicans strain SC5314 was used for its ability to form robust biofilms [19]. SC5314 was obtained from China General Microbiological Culture Collection Center and was stored at −80°C in yeast extract-peptone-dextrose medium (YPD, 1% yeast extract, 2% peptone, 2% dextrose in ddH_2O) supplemented with 20% glycerol. Before each assay, frozen stocks of SC5314 were subcultured twice on YPD agars at 35°C.

Chemicals

2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT), 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl-2H-tetrazoliumbromide (MTT) and Menadione (2-methyl-1, 4-naphthoquinone) were bought from Sigma-Aldrich (Shanghai, China). RPMI-1640 medium (without $NaHCO_3$ and with L-glutamine) was bought from Sigma and buffered with 0.165 mol/L morpholinepropanesulfonic acid (MOPS, from Sigma) to pH 7.0. Other agents were bought from Sangon Biotech except DP, which was bought from National Institutes of Food and Drug Control. DP was dissolved in DMSO at 20 mM as stock solution and stored at −20°C.

The effect of DP on the planktonic *C. albicans*

The antifungal activity of DP against planktonic *C. albicans* was determined by CLSI-M27-A3 [20]. Briefly, *C. albicans* was grown in YPD medium, and then cells were collected by centrifugation and diluted to 2×10^3 cells/mL in RPMI-1640 medium. An aliquot of 100 μL cell suspension was added into each well of 96-well plates before DP was added into wells to achieve various concentrations (2—128 μM). After incubation with DP or DMSO (<0.5%, control) for 24 h at 35°C, the viability of *C. albicans* cells in each well was determined by MTT reduction assay. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of drugs at which no visual growth of fungi was found. These assays were performed in triplicate and repeated for three times.

The colony forming units (CFU) of *C. albicans* in wells treated with MIC, 2MIC, 4MIC, 8MIC of DP was measured by taking 20 μL liquid from wells and plating on YPD agar. The minimal fungicidal concentration (MFC) was defined as the lowest concentration of DP at which the growth of *C. albicans* was not visible on the solid agar. The MFC/MIC ratio was used to judge whether DP had a fungistatic (MFC/MIC > 4) or fungicidal (MFC/MIC < 4) effect [21].

The time-killing curve of DP

To confirm the fungistatic effect of DP, a time-killing assay was performed as previously described [22]. *C. albicans* cells, which were grown overnight in YPD medium, were collected and inoculated into RPMI-1640 medium to get a suspension of 10^6 cells/mL. Four aliquots of *C. albicans* cells suspensions were made and supplemented with DP to

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