In vitro Post-Antifungal Effect of Posaconazole and Its Impact on Adhesion-Related Traits and Hemolysin Production of Oral *Candida dubliniensis* Isolates

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Significance of the Study

- The post-antifungal effect, adhesion, and hemolysin production are virulent attributes of *Candida* isolates.
- Posaconazole is an antifungal agent used in the treatment of candidiasis.
- Posaconazole induced a post-antifungal effect on *Candida dubliniensis* isolates.
- Posaconazole also suppressed the adhesion attributes and production of hemolysin of *Candida dubliniensis* isolates.

Keywords

Adhesion · *Candida dubliniensis* · Hemolysin · Posaconazole · Post-antifungal effect

Abstract

Objective: Candidal adherence to denture acrylic surfaces (DAS) and oral buccal epithelial cells (BEC), formation of candidal germ tubes (GT), candidal cell surface hydrophobicity (CSH), and hemolysin production are important pathogenic traits of *Candida*. The antifungal drug-induced post-antifungal effect (PAFE) also impacts the virulence of *Candida*. *Candida dubliniensis* isolates are associated with the causation of oral candidiasis which could be managed with posaconazole. Thus far there is no evidence on posaconazole-induced PAFE and its impact on adhesion-related attributes and production of hemolysin by *C. dubliniensis* isolates.

Hence, the PAFE, adhesion to DAS and BEC, formation of GT, CSH, and hemolysin production of 20 oral *C. dubliniensis* isolates after brief exposure to posaconazole was ascertained.

Materials and Methods: The PAFE, adherence to DAS and BEC, formation of GT, candidal CSH, and hemolysin production were investigated by hitherto described in vitro assays.

Results: The mean PAFE (h) induced by posaconazole on *C. dubliniensis* isolates was 1.66. Exposure to posaconazole suppressed the ability of *C. dubliniensis* to adhere to DAS, BEC, formation of candidal GT, candidal CSH and to produce hemolysin by a reduction of 44, 33, 34, 36, and 15% ($p < 0.005$ to $p < 0.001$), respectively. **Conclusion:** Exposure of *C. dubliniensis* isolates to posaconazole for a brief period induced an antimycotic impact by subduing its growth in addition to suppressing pathogenic adherence-associated attributes, as well as production of hemolysin.

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Introduction

In immunologically compromised patients as well as in other patient groups, *Candida dubliniensis* has been clinically implicated as an important pathogen. For example, in patients infected with human immunodeficiency virus (HIV), this pathogen has been largely associated with oral candidiasis. Moreover, in patients with *Candida*-induced denture stomatitis, *C. dubliniensis* was one of the predominant pathogens recovered [1]. This association between *Candida*-attributed denture stomatitis suggests an important role for *C. dubliniensis* in the establishment and persistence of denture stomatitis [1]. This microbe has also been recovered from the oral niches of patients with diabetes and cystic fibrosis patients [2, 3]. Cases of *C. dubliniensis* fungemia have been reported which is suggestive of dissemination of this species to other ecological niches as well [4]. Furthermore, cases of multifocal osteomyelitis, leptomenigeal disease, spondylodiscitis, and endocarditis have also been linked to *C. dubliniensis* [5–8]. Interestingly, resistance of *C. dubliniensis* isolates to azoles (i.e., voriconazole, itraconazole, and fluconazole) has also been reported, emphasizing its clinical importance in both oral and systemic candidal infection [9–11].

Post-antifungal effect (PAFE) is the suppression of fungal growth which occurs consequent to brief exposure of fungi to antifungal drugs. Pathogenic traits relating to candidal adherence and *Candida* enzyme production have also been shown to be modified by limited exposure to drugs with anti-*Candida* pharmacodynamics [12]. Adhesion of *Candida* to the host mucosa and oral prosthesis is an important initial factor that facilitates microbial colonization and subsequent candidal infection in the oral cavity [12]. *Candida* has been shown to attach to host mucous membranes, resulting in *Candida*-associated mucosal diseases [12]. Therefore, candidal adherence to denture prosthesis and to epithelial cells in the niches of the oral cavity is a vital initial step in the pathogenicity of oral candidal infection [1, 12]. Additionally, germ tubes (GT) of *Candida* facilitate *Candida* adhesion to mucosal cells and even impart resistance to phagocytosis [13]. Hyphal intrusions of *Candida* stimulate candidal aggregation by bridging adjoining hyphal elements, thereby conveying a large consignment of *Candida* into intimate interaction with the epithelium [13]. Apart from the above underlying biological forces related to adhesion, cell surface hydrophobicity (CSH) of *Candida* is a non-biological attribute facilitating adherence of *Candida* particularly to inert surfaces such as acrylic denture surfaces [12, 14, 15].

Therefore, *Candida* which are hydrophobic are believed to be more pathogenic than their hydrophilic counterparts; positive correlations between adherence to denture acrylic surfaces (DAS) and oral buccal epithelial cells (BEC) with CSH of *Candida* have also been reported [14, 15]. Moreover, in addition to these adhesion-related traits, production of candidal enzymes such as hemolysin also augments the virulent ability of *Candida* aiding its pathogenesis. Secretion of candidal hemolysin and ensuing lysis of erythrocytes facilitates candidal hyphal invasion [16, 17]. It also facilitates the release of hemoglobin, which is thereafter utilized by the microbe as a source of iron [17]. Hemoglobin is also an important attribute triggering an alternative channel needed for *Candida* infections to disseminate in the host [18]. For instance, hemoglobin could facilitate candidal adherence to numerous host proteins and also support *Candida* to consume exogenous heme or hemoglobin to obtain iron and yield cytoprotective molecules [18]. Such interactions between *Candida* and hemoglobin seem to be a unique acclimatization of this microbe to be pathogenic in the human host [18].

Posaconazole is a fairly new second-generation azole antifungal drug. It is derived from itraconazole, by replacing the chlorine substituents in the phenyl ring in itraconazole with fluorine and also by hydroxylating the triazole side chain of itraconazole, which in turn improves the potency and spectrum of activity of posaconazole [19]. Hence, in treating candidal infection caused by *C. dubliniensis* isolates, posaconazole can be used as an alternate drug, particularly with the increased emergence of drug resistance to pharmaceutical agents such as the azoles (i.e., fluconazole, voriconazole, itraconazole) and 5-fluorocytosine (a DNA analogue); where *C. dubliniensis* isolates resistant to 5-fluorocytosine have been identified in Kuwait and in the Middle-East region [20, 21].

Taking into account the abovementioned background, and the finding of *C. dubliniensis* oral isolates having the highest prevalence among non-*albicans* species of *Candida* in Kuwait [22], the emphasis of the current study was to study the PAFE of posaconazole on oral *C. dubliniensis* isolates. Furthermore, the effect of this exposure to posaconazole on candidal adherence to DAS and to BEC, candidal GT formation and candidal CSH as well as hemolysin production was also determined. Additionally, as *C. dubliniensis* are phenotypically similar to *Candida albicans*, for the purpose of comparison of results between these two species, oral isolates of *C. albicans* acquired from Kuwait were also studied.

Materials and Methods

Organisms

Twenty oral isolates of *C. dubliniensis* and *C. albicans*, previously identified and stored as stock cultures were used in the study [22]. These *Candida* isolates had been previously verified by GT formation and their colony characteristics using CHROMagar *Candida* medium (Becton Dickinson and Company, Sparks, MD, USA). In addition, profiles of carbohydrate assimilation were obtained using the VITEK 2 *Candida* identification system (BioMérieux, France). In the same study [22], their identification was further confirmed by means of semi-nested PCR amplification of internally transcribed spacer (ITS)-2 region of rDNA followed by direct DNA sequencing of the ITS region of rDNA. These isolates, obtained from Kuwait, have been used in several previous studies [23–26].

Antifungal Agents and Media

Posaconazole (Vetranal®; Sigma-Aldrich Laborchemikalien GmbH, Darmstadt, Germany) was dissolved in dimethyl sulfoxide. Initially, the drug was made as a solution of 10,000 µg/mL and stored at –20 °C [23–26]. Afterwards it was suspended in RPMI 1640 medium containing L-glutamine and lacking sodium bicarbonate, buffered with 0.165 M MOPS (morpholino propanesulfonic acid), dissolved in 1 L of sterilized distilled water and adjusted to a pH of 7.2 and filter sterilized.

Determination of Minimum Inhibitory Concentration

Antifungal susceptibility values for posaconazole for *C. albicans* and *C. dubliniensis* isolates were determined by E test according to the manufacturer's recommendations (AB BIODISK, Solna, Sweden) as done in previous studies [23–26]. In brief, each of these *Candida* isolates were sub-cultured, then five isolated colonies were suspended in sterile saline, and optical density was adjusted to 0.5 McFarland standard to obtain a cell concentration of 1×10^6 to 5×10^6 cells/mL as done in previous studies [23–26]. This inoculum of *Candida* was then swabbed onto 150 mm diameter plates containing RPMI 1640 agar supplemented with 2% glucose and buffered with MOPS (0.165 M; pH 7.0) and allowed to dry for 10–15 min. The E test strips were then placed on these agar plates. After incubation for 24–48 h at 35 °C, the minimum inhibitory concentrations (MIC) were determined. The MIC for each tested isolate was the lowest concentration where the inhibition zone intersected the curve on the posaconazole antifungal strip. *C. albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019 reference strains were used for quality control of the susceptibility testing.

Preparation of Cell Suspension for in vitro Assays (i.e., PAFE, Adhesion to DAS, Adhesion to BEC, GT Formation, Relative CSH, and Hemolysin Production Assays)

Candida cells initially maintained on Sabouraud's dextrose agar (SDA) were freshly inoculated onto SDA plates prior to the experiment and incubated at 37 °C for 24 h. The resulting *Candida* were suspended into sterile PBS, and a 1.5 turbidity candidal cell suspension was made. 0.5 mL of this candidal suspension was poured into tubes containing 2 mL of RPMI (control) and 2 mL of RPMI/posaconazole (test), where the posaconazole concentration was thrice the MIC value. This resulted in 10^6 *Candida* cells mL⁻¹ suspension in the tubes [23–26]. Subsequently, the tubes were incubated for

60 min at 37 °C. Following this limited exposure to the drug, the removal of posaconazole was achieved by three cycles of dilution using sterile PBS followed by centrifugation at 3,000 g for 10 min. The resulting supernatant was decanted. The *Candida* pellets were resuspended in 10 mL of sterile PBS. This procedure has been successfully used in similar studies for the removal of antifungal drugs (i.e., polyenes, azoles, and echinocandins) and has been shown to reduce the drug concentration by 10,000-fold, eliminating any carry-over effect of posaconazole after its removal [23–26]. Viable counts (i.e., colony-forming units) of the test and the control were done following removal of posaconazole, and there was virtually no difference on the viable counts between the test and the control.

PAFE Assay

After removal of posaconazole, determination of the PAFE was established by an in vitro turbidity method using the equation $PAFE = T - C$ as done in similar PAFE determination studies [23–25] (T = time for the turbidity of the posaconazole-exposed *Candida* cell suspension to reach the optical density value of 0.05 at 520 nm; C = time for turbidity of posaconazole-free *Candida* cell suspension to reach the optical density value of 0.05 at 520 nm). Thus, $T - C$ indicates the duration by which posaconazole was able to suppress candidal growth following brief exposure to posaconazole (i.e., PAFE). For this purpose, as done in previous investigations [23–25], 1,600 µL of *Candida* cell suspensions were incubated at 37 °C after mixing with 2.4 mL of RPMI 1640, and the turbidity of this mixture was recorded every 15 min for 6 h; during this period both the test and control suspensions arrived at the optical density of 0.05, permitting the calculation of PAFE induced by posaconazole.

Adherence to DAS

For the adherence assay, acrylic strips were made as reported previously [15, 26]. Polymethyl methacrylate powder was spread over the surface of a 2.5 × 7.5 cm glass slide covered with a thin layer of aluminum foil. Onto this aluminum foil 1 mL of monomer liquid was poured and immediately another slide was placed over the polymerizing mixture. Using two binder clips, the two slides were then firmly clasped at both ends. After allowing to bench cure for 30 min, the binder clips were removed and the glass slides were separated. The resulting acrylic strips were cut into squares of 5 × 5 mm and were submerged in sterile distilled water for 1 week to allow excess monomer to seep out, and then washed in running water for 3 h. These acrylic strips were then disinfected by immersing in 70% alcohol and washed with sterile distilled water. To remove any contaminants from the surfaces, the acrylic strips were subjected to ultra-sonication for 20 min and rewashed in sterile distilled water and then dried.

The strips were placed vertically and kept in wells of sterilized, serological plates. 400 µL of candidal suspension was added to each well submerging the strip within the well. These plates were then incubated at 37 °C for 1 h with agitation at 120 revolutions/min. The strips were then washed thrice by dipping gently in sterile PBS, and then dried and stained with modified Gram stain without the counterstain. These strips were allowed to air-dry at room temperature and mounted on glass slides with glycerol and the attached *Candida* counted under a light microscope (×400 magnification). Adherent *Candida* cells in 20 fields of view for each strip (0.25 mm² per field) were counted, and the counts were expressed as *Candida* cells/mm².

BEC Adherence

Human BEC from 5 adults were acquired by gently rubbing the inner surface of their right and left buccal mucosa with sterile cotton swabs. This pool of human BEC were then added into tubes containing sterilized PBS. The pooled BEC suspension was washed in sterile PBS to detach any microbes and centrifuged for 10 min at 3,500 g. The resulting pellets of BEC (devoid of any microbes) were mixed with sterile PBS to obtain a BEC concentration of 1×10^5 cells/mL. For the adherence assay, 0.5 mL of *Candida* suspension (previously unexposed to posaconazole and briefly exposed to the drug) and 0.5 mL of the BEC suspension were mixed in sterilized plastic tubes and incubated for 1 h at 37 °C to facilitate candidal adherence to BEC. The resulting suspension was then diluted using 4 mL of sterile PBS. Thereafter, BEC were gathered onto polycarbonate filters (12 µm pore size). These polycarbonate filters were subjected to a gentle wash with sterile PBS to remove any *Candida* not attached to BEC. The filters were then positioned on a glass slide. After a period of 15 s, the filters were removed. The resulting cell preparations on these slides were dried and Gram-stained. Thereafter, using light microscopy (×400 magnification), the adherent *Candida* cells were quantified. BEC in 50 successive fields were observed to count the *Candida* cells adhered to BEC.

Quantification of Candidal GT-Forming Cells

For generation of candidal GT formation, a suspension of 250 µL of *Candida* (recovered following posaconazole removal and the unexposed control), was added to 1 mL of RPMI 1640 medium with L-glutamine and incubated for 90 min at 37 °C. Thereafter, these tubes were vortex mixed for 10 s and a drop of each cell suspension was placed on a Neubauer's hemocytometer chamber and covered with a cover slip for enumeration of GT formed. 300 *Candida* cells in adjacent fields were counted under ×40 magnification and the percentage of *Candida* cells forming GT was estimated. Only *Candida* cells with a GT, with no constriction at the junction between the cell and the elongation were counted. Clumped *Candida* cells and pseudo-hyphae-forming *Candida* cells were omitted [24, 25].

Candidal CSH Assay

2.5 mL of a *Candida* cell suspension (posaconazole exposed as well as posaconazole-free controls) was poured into 20 mL sterilized glass test tubes, after which 0.5 mL of xylene was added into these glass tubes. These tubes were incubated for 10 min at 37 °C to allow the two layers to equilibrate, and then vortex-mixed for 30 s and incubated for an additional 30 min to allow the xylene layer to become distinct from the aqueous phase. The aqueous phase at the bottom of the tube was aspirated using a pipette and put into a sterilized test tube. Traces of xylene were eliminated by bubbling air through the suspension for 2 min. After vortex-mixing for 5 s, the turbidity of this mixture was measured at 520 nm. Candidal CSH was calculated as the reduction in percentage in the optical density value of the later suspension compared to the initial suspension (i.e., before adding xylene) [14, 15, 24, 25].

Hemolysin Production Assay

15 µL of the aforementioned posaconazole-exposed and -unexposed suspensions were spot-inoculated onto blood agar enriched with 3% glucose, which resulted in a circular inoculation site of approximately 10 mm in diameter. The plates were then incubated at 37 °C in 5% CO₂ for a period of 48 h. The diameter of the colony

Table 1. The PAFE (in hours) of 20 oral *C. dubliniensis* and *C. albicans* isolates following 1-hour exposure to posaconazole

<i>C. dubliniensis</i> isolate	PAFE	<i>C. albicans</i> isolate	PAFE
CD1	2.26	CA1	2.12
CD2	1.26	CA2	2.12
CD3	1.62	CA3	1.26
CD4	1.62	CA4	1.41
CD5	1.41	CA5	1.26
CD6	1.62	CA6	1.41
CD7	1.47	CA7	1.47
CD8	2.12	CA8	2.12
CD9	2.39	CA9	2.12
CD10	2.33	CA10	2.12
CD11	1.47	CA11	1.26
CD12	1.41	CA12	1.41
CD13	1.26	CA13	1.26
CD14	1.26	CA14	1.41
CD15	1.26	CA15	1.26
CD16	1.62	CA16	1.47
CD17	1.62	CA17	1.47
CD18	1.41	CA18	1.41
CD19	2.12	CA19	2.12
CD20	1.62	CA20	1.47
Mean	1.66	Mean	1.60
SEM	0.08	SEM	0.08

In calculating the PAFE, 1 = 60 min, 0.25 = 15 min, 0.50 = 30 min, and 0.75 = 45 min in the table. Hence, for isolate CD1 the PAFE of 2.26 equals 2 h and 15 min. All values indicate the mean of experiments done thrice in duplicate for each isolate.

(y) and the diameter of the colony plus the translucent zone due to hemolysis (x) were measured. The hemolytic activity (hemolytic index), which is the ratio of x/y, was determined as reported previously [16, 23, 25].

C. dubliniensis reference strain CD36 and *C. albicans* reference strain ATCC 90028 were included in all the abovementioned experiments. All experiments were repeated three times in duplicate.

Statistical Analysis

The data obtained from adherence to DAS, adherence to BEC, formation of GT, candidal CSH, and hemolysin assays were analyzed using paired sample *t* tests, with the group unexposed to posaconazole as the control and the group exposed to posaconazole as the test group. A *p* value of <0.05 was considered statistically significant.

Results

The MIC (µg/mL) of *C. dubliniensis* to posaconazole varied between 0.002 and 0.016. The mean PAFE on these isolates after a 1-h exposure to posaconazole was 1.66 h.

Table 2. Summary of adherence to BEC (*Candida* cells/50 BEC), adherence to DAS (*Candida* cells/mm²), mean percentage of GT-positive cells, candidal CSH, and hemolysin production (hemolytic index) of 20 oral *C. dubliniensis* and *C. albicans* isolates following brief exposure and subsequent removal of posaconazole

Pathogenic attribute	Control (unexposed to posaconazole)	Test (exposed to posaconazole)	Mean percentage reduction and significance
<i>C. dubliniensis</i> isolates			
Adherence to BEC	203.24±2.78	136.67±2.58	32.76%; $p < 0.001$
Adherence to DAS	45.06±0.58	25.14±0.53	44.21%; $p < 0.001$
GT formation	24.74±0.61	16.38±0.40	33.79%; $p < 0.001$
Candidal CSH	17.54±0.20	11.30±0.13	35.58%; $p < 0.001$
Hemolysin production	1.60±0.008	1.36±0.008	15.00%; $p < 0.005$
<i>C. albicans</i> isolates			
Adherence to BEC	205.32±2.14	143.18±2.41	30.26%; $p < 0.001$
Adherence to DAS	46.74±0.76	26.44±0.57	43.44%; $p < 0.001$
GT formation	26.03±0.81	17.38±0.58	33.25%; $p < 0.001$
Candidal CSH	16.96±0.39	11.37±0.21	32.96%; $p < 0.001$
Hemolysin production	1.61±0.007	1.41±0.007	12.42%; $p < 0.005$

All values indicate the mean of experiments done thrice in duplicate for each isolate.

The MIC (µg/mL) of *C. albicans* to posaconazole ranged from 0.012 to 0.016. The posaconazole-induced mean PAFE on oral isolates of *C. albicans* was 1.60 h (Table 1).

The mean adherence of *C. dubliniensis* to DAS (cells/mm²) not exposed to posaconazole and following limited exposure to the drug was 45.06 and 25.14, respectively (44.21% reduction; $p < 0.001$). Likewise, these values for *C. albicans* isolates not exposed to posaconazole and after limited exposure to posaconazole were 46.74 and 26.44, respectively (43.44% reduction; $p < 0.001$; Table 2).

The mean adherence of *C. dubliniensis* isolates to BEC not exposed to posaconazole and following brief acquaintance to posaconazole was 203.24 and 136.67, respectively (32.76% reduction; $p < 0.001$). Similarly, these values for isolates of *C. albicans* not exposed to posaconazole and after brief exposure to posaconazole were 205.32 and 143.18, respectively (30.26% reduction; $p < 0.001$; Table 2).

The mean percentages of cells with GT not exposed to posaconazole and after transient exposure to this drug were 24.74 and 16.38, respectively (reduction of 33.80%; $p < 0.001$). These assessments for *C. albicans* isolates unexposed to posaconazole and following brief exposure to posaconazole were 26.03 and 17.38, respectively (reduction of 33.25%; $p < 0.001$; Table 2).

The mean CSH of *C. dubliniensis* isolates not exposed to posaconazole and after limited acquaintance to this drug was 17.54 and 11.30, respectively (reduction of 35.58%; $p < 0.001$). These values for isolates of *C. albicans* not exposed to posaconazole and after limited acquaintance

to posaconazole were 16.96 and 11.37, respectively (reduction of 32.96%; $p < 0.001$; Table 2).

Mean hemolysin production by unexposed *C. dubliniensis* isolates was 1.60. After brief exposure to posaconazole it was 1.36 (reduction of 15.00%; $p < 0.005$). Likewise, the mean hemolytic index by unexposed *C. albicans* isolates was 1.61, whereas following brief exposure to posaconazole it was 1.41 (reduction of 12.42%; $p < 0.005$; Table 2).

Discussion

C. dubliniensis is an opportunistic yeast implicated in oral candidiasis, while *C. albicans* is considered to be the most pathogenic of all *Candida* species responsible for oral candidiasis [12]. Adhesion of *Candida* to DAS and to BEC of the oral mucosa and the ability of *C. dubliniensis* and *C. albicans* isolates to produce GT are critical pathogenic attributes relating to their adherence, which are closely allied with all forms of oral candidiasis [1, 12, 13]. In addition to these biological adherence traits, candidal CSH is considered to be a non-biological physical attribute believed to facilitate adhesion of *Candida* [14, 15]. Another factor determining *Candida* virulence is the PAFE, which is the capability of *Candida* to recover and grow, following brief exposure to antifungal agents, where more susceptible and less virulent *Candida* will have a higher PAFE, whereas more resilient and pathogenic *Candida* will have a lower PAFE [12, 23, 24, 26].

The findings in this study reveal that posaconazole brought about a PAFE of nearly 1½ h on both *Candida* species investigated following 1-h exposure to posaconazole. This study also found a marked reduction in adherence to DAS and BEC, and formation of candidal GT of both *C. albicans* and *C. dubliniensis* oral isolates. These suppressive effects on growth and adhesion could be attributed to the mechanism of action of posaconazole on the candidal cell wall. Posaconazole inhibits fungal cytochrome P450-mediated 14- α lanosterol demethylation, which in turn blocks ergosterol synthesis [27]. The lack of ergosterol reduces the stability of the candidal cell membrane, the transport of nutrients, and the synthesis of chitin [28]. In addition, ergosterol depletion accompanied by a buildup of toxic methylated ergosterol precursors will bring about damage to the fungal cell membrane, increased permeability, and inhibition of fungal cell growth [29, 30]. Thus, it is reasonable to speculate that posaconazole-induced alterations in the structure of the *Candida* cell wall could disturb active multiplication and budding of *Candida*, thus attenuating candidal growth and resulting in a PAFE of approximately 1½ h, besides limiting candidal adherence to DAS and BEC, and formation of GT even after a limited exposure to posaconazole.

Phospholipids, lipopolysaccharides, proteins, lipoproteins, and fimbriae of the outer membrane are structures that augment microbial CSH [14, 15]. Thus, pharmaceutical agents which could modify these structures of microbes have been shown to reduce microbial CSH [23, 26]. In *Candida* in particular, it has been shown that candidal CSH correlates well with the fibril concentration in the exterior layer of the cell wall. Hence, the apparent decrease of CSH elicited by posaconazole on isolates of *C. albicans* and *C. dubliniensis* may also be attributed to the aforesaid mechanism of action of posaconazole on the cell wall of these two species [27, 30]. Hence, it is reasonable to suggest that by debilitating the candidal cell wall structure posaconazole could bring about a significant decrease in the CSH of the two *Candida* species.

Another well-recognized, putative virulent factor of *Candida* is the production of extracellular hemolysin [17, 18]. This study illustrates that exposure to posaconazole succeeds in suppressing hemolysin production of both *C. albicans* and *C. dubliniensis* isolates to a significant level. However, the precise pharmacodynamics by which posaconazole reduces the production of hemolysin by these two *Candida* species is not clear. Posaconazole acts by inhibiting fungal cytochrome P450-mediated 14- α lanosterol demethylation, which in turn blocks ergosterol synthesis, thus affecting the integrity of candidal cell wall

[27–30]. This may have an impact on hemolytic activity following exposure to posaconazole. Interestingly, hemolysin is also known to facilitate host invasion by *Candida*, particularly by aiding hyphal penetration of the mucosa. Thus, posaconazole could curtail dissemination of *Candida* within the host by reducing hemolysin production and formation of candidal GT as well. As disseminated *Candida* infections are becoming quite common [4–8, 12], posaconazole may have a negative impact on disease progression by reducing the aforesaid pathogenic attributes of *C. dubliniensis* and *C. albicans*.

Resistance to itraconazole, voriconazole, and fluconazole has been recognized previously in *C. dubliniensis* [9–11]. Resistance to 5-fluorocytosine has been recently documented in isolates obtained from the Middle-East region and Kuwait [20, 21]. Such candidal resistance signifies the need for identifying promising alternative antifungal agents, which may assist the management of candidiasis. These preliminary findings suggest that brief exposure to posaconazole induces a PAFE and also suppresses adherence to DAS and BEC as well as GT formation, relative CSH, and hemolysin production. This may further contribute to understanding the pharmacodynamics of posaconazole.

Conclusion

To the best of our knowledge, this investigation is the first to reveal the reduction of adhesion, hemolysin production, and the PAFE induced by posaconazole on oral *C. dubliniensis* and *C. albicans* isolates. However, further studies with sessile *Candida* isolates encased within biofilms, in contrast to planktonic isolates utilized in the present investigation, are needed to further enhance these in vitro findings.

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Disclosure Statement

The authors declare that there is no conflict of interest in relation to this study.

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