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In Vitro Analysis of Finasteride Activity against *Candida albicans* Urinary Biofilm Formation and Filamentation

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Candida albicans **is the 3rd most common cause of catheter-associated urinary tract infections, with a strong propensity to form drug-resistant catheter-related biofilms. Due to the limited efficacy of available antifungals against biofilms, drug repurposing has been investigated in order to identify novel agents with activities against fungal biofilms. Finasteride is a 5-α-reductase inhibitor commonly used for the treatment of benign prostatic hyperplasia, with activity against human type II and III isoenzymes. We analyzed the *Candida* Genome Database and identified a *C. albicans* homolog of type III 5-α-reductase, Dfg10p, which shares 27% sequence identity and 41% similarity to the human type III 5-α-reductase. Thus, we investigated finasteride for activity against *C. albicans* urinary biofilms, alone and in combination with amphotericin B or fluconazole. Finasteride alone was highly effective in the prevention of *C. albicans* biofilm formation at doses of ≥16 mg/liter and the treatment of preformed biofilms at doses of ≥128 mg/liter. In biofilm checkerboard analyses, finasteride exhibited synergistic activity in the prevention of biofilm formation in a combination of 4 mg/liter finasteride with 2 mg/liter fluconazole. Finasteride inhibited filamentation, thus suggesting a potential mechanism of action. These results indicate that finasteride alone is highly active in the prevention of *C. albicans* urinary biofilms *in vitro* and has synergistic activity in combination with fluconazole. Further investigation of the clinical utility of finasteride in the prevention of urinary candidiasis is warranted.**

Candida albicans, while normally a human commensal organism, can become an opportunistic pathogen in an immunocompromised host or when mucosal barriers are disrupted. *C. albicans* is the leading cause of invasive fungal infections in the United States, and it is particularly prevalent in catheter-related urinary tract infections (1–6). *C. albicans* readily forms biofilms, which are characterized by resistance to standard antifungal therapy and host immune responses (1, 2), enabling the colonization of mucosal surfaces, with the potential for subsequent invasion and dissemination. *C. albicans* also forms biofilms on catheters and medical devices, which are difficult to eradicate unless the device is removed (1–7). Consequently, these clinical issues have driven the search for novel antifungal therapies directed against biofilm-related *C. albicans* infections, particularly when device removal or replacement is undesirable or high risk.

A variety of antifungal agents have been evaluated for their *in vitro* activities against *C. albicans* biofilms, including supratherapeutic concentrations of antifungals used for systemic infections, such as echinocandins, polyenes, and azoles, as well as a wide range of other agents (7–9). However, resistance to azoles and echinocandins has been well documented in *Candida* species, and amphotericin B (AMB) is limited by substantial toxicity. These shortcomings have spurred an investigation for new antifungal agents (10–12). There is considerable interest in repurposing FDA-approved drugs for potential antifungal activity, which may be more readily employed in a clinical setting. In addition, it has been proposed that targeting essential fungal cell processes alone may be inadequate. Rather, inhibiting virulence-related factors responsible for causing disease (such as filamentation and biofilm formation) has been suggested to have potential advantages in the treatment of invasive fungal infections (10).

Several recent studies have screened repurposed or alternative agents for their antifungal activity against virulence-related phenotypes in *C. albicans*. These studies have reported the discovery of nonazole compounds (11, 13), immunosuppressive drugs (14), tunicamycin (15), and farnesol (16), among others. Additionally, numerous studies have evaluated repurposed agents as part of antifungal lock solutions against *C. albicans* biofilms, including ethanol (17, 18), heparin (9), doxycycline (19), chitosan (20), EDTA (21), and tigecycline (8). In our laboratory, we routinely study alternative compounds individually and in combination with standard antifungal drugs, and finasteride is one of several promising agents that we have selected to study in detailed analyses due to preliminary observations of its inhibition of *C. albicans* filamentation in liquid medium and of biofilm formation.

Finasteride (FIN) inhibits the type II and type III isoenzymes of human 5-α-reductase (22–24), which converts testosterone to 5-α-dihydrotestosterone, the primary androgen responsible for overgrowth of the prostate, and it aids in the treatment of urinary obstruction caused by benign prostatic hypertrophy. Using the National Center for Biotechnology Information (NCBI) Protein Basic Local Alignment Search Tool (BLASTp), we identified a homologous protein for human type III 5-α-reductase as a potential therapeutic target in the yeast *Saccharomyces cerevisiae* (26% homology) and in *C. albicans* (37% homology). A recent study using an animal model demonstrated that FIN had an antibacterial effect on chronic bacterial prostatitis by reducing the number of bacterial urinary isolates of animals after 4 weeks of FIN therapy

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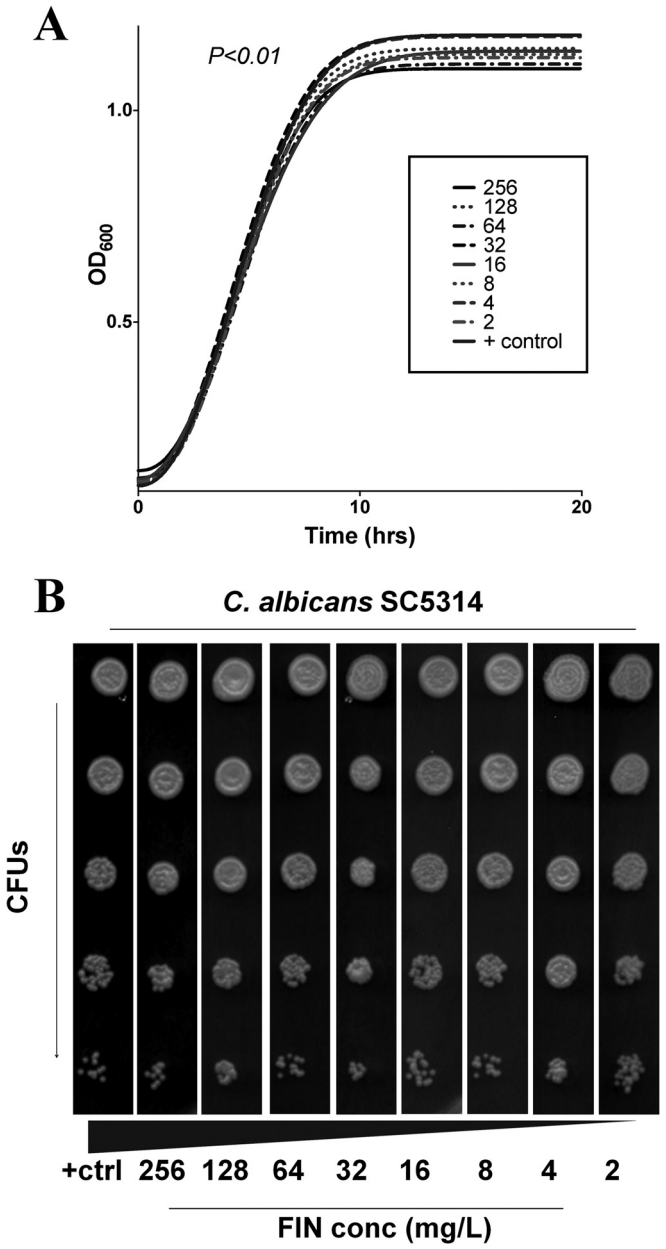

FIG 1 (A) Planktonic growth of *C. albicans* in urine medium. OD readings were taken every 15 min for 30 h at 30°C. Decreasing concentrations of 256 to 2 mg/liter FIN were tested for growth, with a starting OD_{600} of 0.1. No significant difference was observed in planktonic growth when FIN was added to the medium at decreasing concentrations. (B) Effect on *C. albicans* planktonic growth with increasing concentrations of FIN. Concentrations of 1×10^8, 2×10^7, 4×10^6, 8×10^5, 1.6×10^5, 3.2×10^4, 6.4×10^3, and 1.28×10^2 CFU were spotted on YPD containing FIN at concentrations of 265 to 2 mg/liter with a drug-free control. Each CFU concentration was replicated four times using a V&P Scientific Multi-Blot replicator. The plates were incubated for 2 days at 30°C. +ctrl, positive control.

(25). However, no studies have evaluated the efficacy of FIN against fungal infections, and in particular, urinary fungal biofilms. Therefore, the aim of this study was to evaluate the efficacy of FIN (alone and in combination with fluconazole [FLC] and AMB) for the prevention and treatment of *C. albicans* biofilms.

MATERIALS AND METHODS

Strains and reagents. Five wild-type *C. albicans* strains were selected for this study: the laboratory reference strain SC5314 (26) and four clinical urinary isolates (18) (gifts of L. Massie, University of New Mexico). The *C. albicans* strains were grown and maintained at 30°C in YPD (1% yeast extract, 2% peptone, 2% glucose). For the biofilm formation and susceptibility studies, overnight cultures were resuspended at a density of 1.0×10^9 cells/liter in urine medium (0.086% calcium chloride dihydrate, 0.68% magnesium chloride, 0.46% sodium chloride, 0.23% sodium sulfate anhydrous, 0.074% sodium citrate dihydrate, 0.002% sodium oxalate, 0.28% potassium phosphate monobasic, 0.08% potassium chloride, 0.1% ammonium chloride, 2.5% urea, 0.11% creatinine, 0.03% yeast nitrogen base without amino acids, 0.004% complete synthetic medium without uracil, 8% dextrose) and buffered to pH 7.0 with 165 mM morpholinepropanesulfonic acid (MOPS).

Effect of finasteride on *C. albicans* growth. The growth studies were performed by diluting an overnight culture of *C. albicans* SC5314 in fresh urine medium to a final optical density at 600 nm (OD_{600}) of 0.1 with FIN (Sigma Chemical Co.) at a $\log_2$ concentration range of 2 to 256 mg/liter, with drug-free positive controls. The experimental cultures were incubated at 30°C, and the optical densities were read every hour for 24 h using the BioTek microplate automated plate reader (Thermo Scientific Co.). Additionally, the overnight cultures were diluted in 96-well microtiter plates and spotted into YPD agar plates with and without FIN. The plates were incubated for 24 h at 30°C, and growth was recorded by photography after incubation.

Biofilm formation and susceptibility assays. The antifungal activity of FIN in the prevention and treatment of biofilms was assessed using the XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] reduction assay (27), as previously described, but it was modified to include urine medium. In brief, for studies on the prevention of biofilm formation, planktonic cells from an overnight culture were resuspended at a final density of 1.0×10^9 cells/liter in urine medium containing increasing concentrations of FIN and incubated for 24 h at 37°C. For the studies on the treatment of mature biofilms, the cells were incubated in urine medium in a 96-well microplate to allow the formation of mature biofilms for 24 h of incubation at 37°C. Next, the wells were gently washed and the biofilms were incubated for another 24 h at 37°C with increasing $\log_2$ concentrations of FIN (2, 4, 8, 16, 32, 64, 128, and 256 mg/liter in urine medium). For both the prevention and treatment of biofilm studies, the metabolic activity within the biofilms was measured using the XTT reduction assay. The experiments were performed independently two times (biological replicates), each time in quadruplicate (technical replicates).

The effects of FIN in combination with fluconazole (FLC) (Sigma Chemical Co.) and amphotericin B (AMB) (Sigma Chemical Co.) on *C. albicans* SC5314 biofilms in urine medium were studied using checker-

TABLE 1 FIN biofilm sMICs for *C. albicans* isolates

C. albicans strain	Prevention sMICs (mg/liter)[a]		Treatment sMICs (mg/liter)	
	MIC_{90}	MIC_{50}	$sMIC_{90}$	$sMIC_{50}$
SC5314	128	16	>256	128
42379	>256	>256	>256	>256
53264	128	16	>256	256
UI3	256	64	>256	>256
UI5	>256	32	>256	>256
UI6	>256	128	>256	>256
UI8	>256	64	>256	>256

[a] Sessile MIC_{50} ($sMIC_{50}$) and $sMIC_{90}$ values were defined as the lowest concentrations of FIN that inhibited 50% XTT reduction (50% biofilm metabolic activity) and 90% XTT reduction (10% biofilm metabolic activity) for the prevention of biofilm formation or treatment of mature biofilms, respectively.

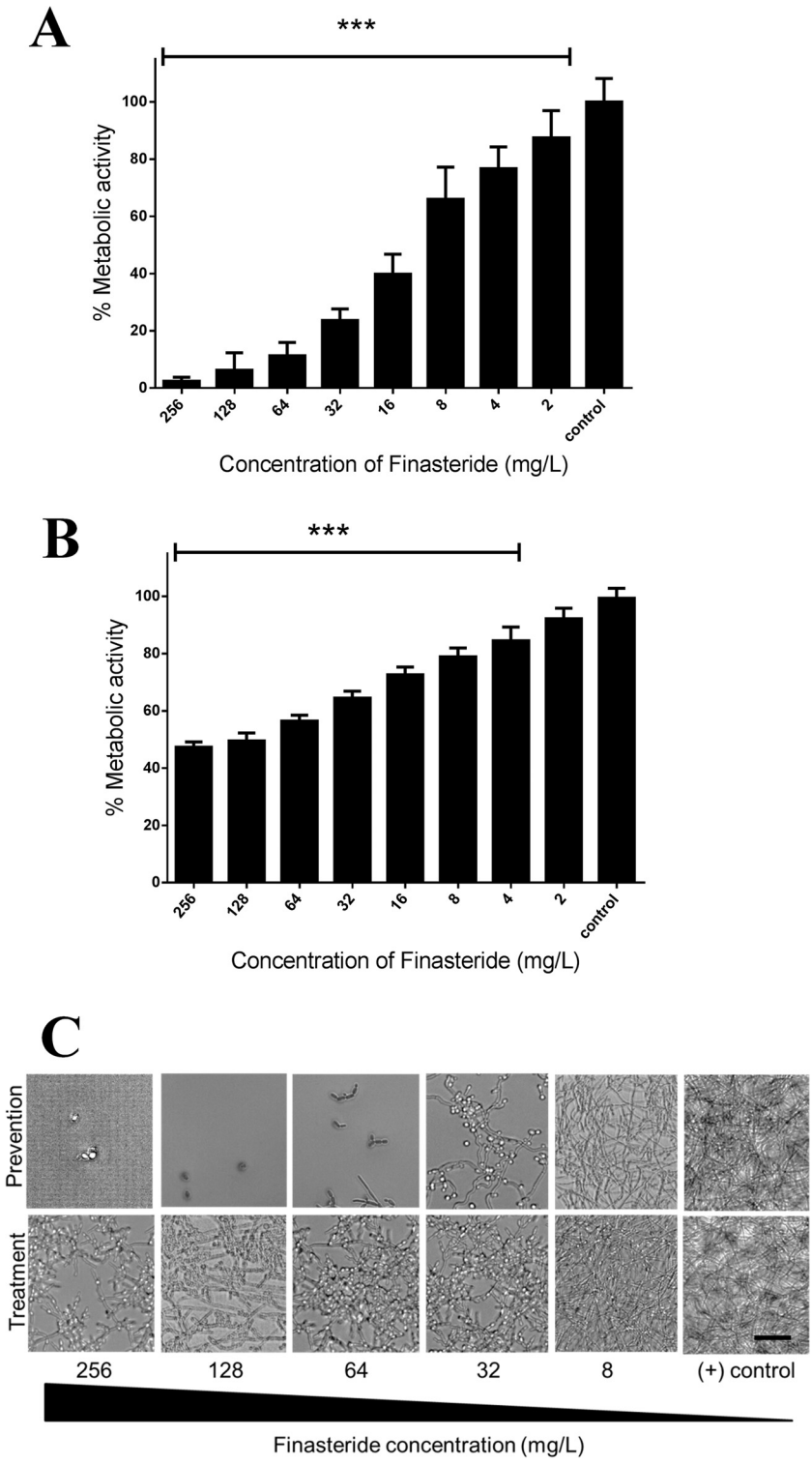

FIG 2 (A) *In vitro* effect of FIN against formation (prevention) of *C. albicans* biofilms. Planktonic cells were incubated in urine medium containing FIN at different concentrations (from 2 mg/liter to 256 mg/liter), and biofilms were allowed to form for 24 h. Metabolic activity was then assessed using the XTT assay. (B) *In vitro* effect of FIN against mature *C. albicans* biofilms. Mature (preformed) biofilms were treated with FIN at different concentrations (from 2 mg/liter to 256 mg/liter), and metabolic activity was assessed using the XTT assay. Drug-free biofilm wells containing urine medium only were used as controls. ***, significant difference ($P < 0.05$) in the reduction in biofilm metabolic activity with FIN compared to that with the growth control (wells containing only medium and biofilms). (C) Representative light microscopy images of *C. albicans* strain SC5314 biofilms. Visual differences in the biofilm architectures are apparent at four different FIN concentrations (32, 64, 128, and 256 mg/liter). The pictures were taken using a ×40 power field. Bar, 10 μm.

board assays. Additionally, we tested FIN in combination with FLC with three clinical isolates (*C. albicans* UI3, UI5, and UI6) using checkerboard assays. The checkerboard assays were performed independently in duplicate for each drug combination (FIN with FLC and FIN with AMB). FIN was added at increasing $\log_2$ concentrations (0.25 to 128 mg/liter) along the horizontal axis, while the antifungal, either FLC ($\log_2$ concentrations, 4 to 256 mg/liter) or AMB ($\log_2$ concentrations, 0.008 to 0.5 mg/liter), was added at increasing concentrations along the vertical axis. The positive controls (cells and urine medium only), negative controls (urine medium only), and serial concentrations of each agent alone were present on the microtiter plate. The checkerboard plates were incubated for 24 h at 37°C. Biofilm metabolic activity was measured using the XTT reduction assay. The sessile MIC_{90} and sessile MIC_{50}, or the concentrations at which 10% and 50% of biofilm metabolic activity were detected, respectively, were utilized in the calculations of fractional inhibitory concentrations (28).

The FIN and AMB stocks were dissolved in dimethyl sulfoxide (DMSO), whereas FLC was dissolved in urine medium. We have observed that concentrations of <4% of DMSO in both RPMI 1640 and urine media do not result in a significant change in biofilm metabolic activity (data not shown), and the highest concentration of DMSO that was present in the checkerboard assays was 2%.

Definitions. The fractional inhibitory concentrations (FICs) (28, 29) were calculated for each combination of FIN and antifungal agent. The FIC value is calculated by dividing the MIC_{90} or MIC_{50} of one drug when used in combination by the MIC_{90} or MIC_{50} of the same drug when used alone (e.g., FIC of drug A = [drug $A_{combination}$]/[drug A_{alone}]). The summation of each FIC value, the FIC index (FICI) (e.g., FIC of drug A plus the FIC of drug B), determines whether the combination of drugs produces an antagonistic, synergistic, or indifferent effect. The combination is considered synergistic when the FICI is ≤0.5, indifferent when the FICI is >0.5 to ≤4, and antagonistic when the FICI is >4. When the highest concentration of drug tested did not produce a 90 or 50% decrease in metabolic activity, the highest concentration of the drug was used in place of the MIC_{90} or MIC_{50} to calculate the FICI, according to published methods (29–32). These concentrations are preceded by ">".

Light microscopy of biofilms. Light micrographs of the biofilms were acquired using an inverted microscope (Micromaster digital inverted microscope with Infinity optics; Fisher Scientific) and data acquisition software (Micron 2.0.0; Westover Scientific).

Statistical analyses. The metabolic activities of the treatment groups and controls were compared using one-way analysis of variance (ANOVA), followed by the *post hoc* or Tukey comparison posttest. The differences between groups were considered significant at a P value of <0.05. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

Filamentation assays. Filamentation was assayed using solid and liquid media. Briefly, an overnight culture of *C. albicans* SC5314 was spotted onto YPD supplemented with 10% fetal calf serum (FCS) plates with increasing concentrations of FIN (8, 32, 64, and 128 mg/liter). The same FIN concentrations were used in hypha-inducing Spider plates (1% nutrient broth, 1% D-mannitol, 0.2% dipotassium phosphate, 2% agar [pH 7.2]). Filamentation was visualized after 2 and 5 days of incubation at 37°C and compared to that of a FIN-free control. For the liquid filamentation assays, fresh inocula of the overnight cultures of *C. albicans* SC5314 were incubated in 5 ml of RPMI 1640 medium, with FIN concentrations of 256, 128, and 64 mg/liter for 5 h before observation. Filamentation was observed at 2, 6, and 8 h and compared to that of a FIN-free control. A Zeiss Axio Imager M1 system was used to capture images using light microscopy, and rendering was completed using the AxioVision 4.7 software (Zeiss).

RESULTS

Finasteride does not affect planktonic growth of *C. albicans*. The antifungal activity of FIN against planktonic *C. albicans* SC53214 was studied using two approaches: (i) the measurement of optical density in liquid medium and (ii) an assessment of serial dilutions of spotted colonies after 24 h of incubation. Regardless of the concentration used, FIN had no activity against *C. albicans* planktonic growth in liquid culture (Fig. 1A) or on solid agar (Fig. 1B).

TABLE 2 Antifungal activity of FIN in combination with FLC and AMB against *C. albicans* SC5314 biofilms

Biofilm target	Agent[a]	$sMIC_{90}$ (mg/liter) of each agent[b]: Alone	In combination	FICI (combination)	Outcome[c]
Prevention	FLC	>256	4	0.27	Synergism
	FIN	>128	32		
	AMB	>0.50	0.50	2.00	Indifference
	FIN	>128	128		
Treatment	FLC	>256	256	2.00	Indifference
	FIN	>128	128		
	AMB	>0.50	>0.50	2.00	Indifference
	FIN	>128	>128		

[a] FLC, fluconazole; AMB, amphotericin B; FIN, finasteride.
[b] The sessile minimum inhibitory concentrations indicated correspond to the inhibition of 90% of metabolic activity ($sMIC_{90}$). The inhibition of 90% metabolic activity was not detected in the highest concentration of each agent tested alone; therefore, the sMIC90 of antifungals alone are indicated with the sign ">" before their respective concentrations.
[c] The outcome represents the interpretation of the FICI values considering the following criteria: synergism, FICI of <0.5; indifference, FICI of >0.5 to <4; and antagonism, FICI of >4.

Finasteride inhibits *C. albicans* biofilms. FIN was tested for activity against mature preformed biofilms and for the prevention of biofilm formation. FIN alone was tested against five *C. albicans* strains, including the wild-type reference strain SC5314 and four clinical urinary isolates (previously designated UI3, UI5, UI6, and UI8) (33). Table 1 lists the FIN sessile MICs (sessile MIC_{50} [$sMIC_{50}$] and sessile MIC_{90} [$sMIC_{90}$]) against all five *C. albicans* strains for the prevention and treatment of biofilm formation. The $sMIC_{50}$s and $sMIC_{90}$s varied depending on the strain. For reference strain SC5314, the $sMIC_{50}$ of biofilm prevention was 16 mg/liter, and the $sMIC_{90}$ was 128 mg/liter FIN. In addition to calculating the sMIC values for strain SC5413, statistical analysis was performed to determine the FIN concentrations that significantly decreased metabolic activity within the biofilms (Fig. 2). For the prevention of biofilm formation, FIN concentrations of ≥2 mg/liter caused a significant decrease ($P < 0.05$) in biofilm metabolic activity compared to that in the untreated control (Fig. 1A). For the treatment of mature biofilms, statistically significant differences in metabolic activity were observed at FIN concentrations of ≥4 mg/liter (Fig. 1B).

The clinical isolate UI3 required 256 mg/liter FIN to produce ≤10% metabolic activity, whereas UI5, UI6, and UI8 required FIN concentrations of >256 mg/liter to prevent the equivalent metabolic activity. The FIN concentrations that produced ≤50% metabolic activity varied between each strain (Table 1). For the treatment of mature preformed biofilms, all strains had $sMIC_{90}$s and $sMIC_{50}$s of >256 mg/liter, except for SC5314, which had an $sMIC_{50}$ of 128 mg/liter.

The antifungal effect of FIN against reference strain SC5314

TABLE 3 Antifungal activity of FIN in combination with FLC and AMB against *C. albicans* SC5314 biofilms

Biofilm target	Agent[a]	$sMIC_{50}$ (mg/liter) of each agent[b]: Alone	In combination	FICI (combination)	Outcome[c]
Prevention	FLC	64	2	0.06	Synergism
	FIN	128	4		
	AMB	0.125	0.125	1.50	Indifference
	FIN	128	64		
Treatment	FLC	64	64	2.00	Indifference
	FIN	128	128		
	AMB	0.25	0.25	1.50	Indifference
	FIN	>128	64		

[a] FLC, fluconazole; AMB, amphotericin B; FIN, finasteride.
[b] The sessile minimum inhibitory concentrations indicated correspond to the inhibition of 50% metabolic activity ($sMIC_{50}$).
[c] The outcome represents the interpretation of the FICI values considering the following criteria: synergism, FICI of <0.5; indifference, FICI of >0.5 to <4; and antagonism, FICI of >4.

was also assessed using light microscopy. We observed a clear dose-dependent reduction in biofilm mass for the prevention and treatment of biofilms when FIN was added at concentrations of ≥8 mg/liter (Fig. 2C).

Checkerboard assays of finasteride combined with fluconazole or amphotericin B. Biofilm checkerboard assays were used to study the effect of FIN in combination with FLC or AMB in the prevention of biofilm formation and treatment of mature biofilms using reference strain SC5314. The results of the checkerboard assays for the urinary isolates are shown in Tables 2 ($sMIC_{90}$) and 3 ($sMIC_{50}$). The FLC and FIN $sMIC_{90}$s for the prevention and treatment of biofilms remained constant at concentrations of >256 mg/liter FLC and >128 mg/liter FIN when the agents were used alone. For the prevention of biofilm formation when the agents were used in combination at concentrations of 4 mg/liter FLC and 32 mg/liter FIN, the fractional inhibitory concentration index (FICI) was 0.27 ($sMIC_{90}$), meeting the criteria for a synergistic effect. The association of FIN and FLC had an indifferent effect in the treatment of mature biofilms. For instance, the calculated FICI was 2.00 when FLC and FIN were combined at the highest concentrations tested ($sMIC_{90}$s, 256 mg/liter and 128 mg/liter, respectively). For the treatment and prevention of biofilm formation, the calculated $sMIC_{90}$s for AMB and FIN when used alone were >0.50 mg/liter and >128 mg/liter, respectively. When AMB and FIN were combined and both the $sMIC_{90}$ and $sMIC_{50}$ criteria were selected for the FICI calculations, an indifferent effect was observed for the treatment and prevention of biofilms. We observed a synergistic effect in UI3 when FIN was used in combination with FLC (16 and 4 mg/liter, respectively) for the prevention of biofilm formation (Table 4), and an indifferent effect was observed for UI5 and UI6 for both the treatment and prevention of biofilms (Tables 4 and 5).

Effects of finasteride on filamentation. On solid agar, FIN appeared to inhibit filamentation in all five *C. albicans* strains. We then investigated whether this was a dose-dependent phenomenon using two different approaches: visualization of filamentous morphology in spotted plates and microscopic observation in liquid medium (Fig. 3). When SC5314 was spotted onto fetal calf serum and Spider agar plates, hyphal structures were not grossly evident after the 2nd day of incubation; to observe this effect in detail, we next used light microscopy to assess the differences in hyphal formation (Fig. 3A). The extent of filamentation was greatly reduced when FIN was added to the plates at concentrations of ≥8 mg/liter. When the plates were incubated for 5 days, the filamentation defect was more pronounced at concentrations of ≥32 mg/liter than at lower doses (Fig. 3B).

In liquid medium, the germination time and the extent of filament elongation were reduced at FIN concentrations of ≥32 mg/liter after 6 h of incubation (Fig. 3C).

TABLE 4 Antifungal activity of FIN in combination with FLC against three urinary isolates (*C. albicans* UI6, UI5, and UI6)

Biofilm target (strain)	Agent[a]	$sMIC_{50}$ (mg/liter) of each agent[b]: Alone	In combination	FICI (combination)	Outcome[c]
Prevention (UI6)	FLC	32	4	0.63	Indifference
	FIN	128	64		
Prevention (UI5)	FLC	>256	4	1.02	Indifference
	FIN	>128	>128		
Prevention (UI3)	FLC	>256	4	0.27	Synergism
	FIN	64	16		
Treatment (UI6)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		
Treatment (UI5)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		
Treatment (UI3)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		

[a] FLC, fluconazole; FIN, finasteride.
[b] The sessile minimum inhibitory concentrations indicated correspond to the inhibition of 50% metabolic activity ($sMIC_{50}$).
[c] The outcome represents the interpretation of the FICI values considering the following criteria: synergism, FICI of <0.5; indifference, FICI of >0.5 to <4; and antagonism, FICI of >4.

TABLE 5 Antifungal activity of FIN in combination with FLC against three urinary isolates (*C. albicans* UI6, UI5, and UI6)

Biofilm target (strain)	Agent[a]	$sMIC_{90}$ (mg/liter) of each agent[b]:		FICI (combination)	Outcome[c]
		Alone	In combination		
Prevention (UI6)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		
Prevention (UI5)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		
Prevention (UI3)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		
Treatment (UI6)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		
Treatment (UI5)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		
Treatment (UI3)	FLC	>256	>256	2.00	Indifference
	FIN	>128	>128		

[a] FLC, fluconazole; FIN, finasteride.
[b] The sessile minimum inhibitory concentrations indicated correspond to the inhibition of 90% metabolic activity ($sMIC_{90}$). The inhibition of 90% metabolic activity was not detected in the highest concentration of each agent tested alone; therefore, $sMIC_{90}$s of antifungals alone are indicated with the sign ">" before their respective concentrations.
[c] The outcome represents the interpretation of the FICI values considering the following criteria: synergism, FICI of <0.5; indifference, FICI of >0.5 to <4; and antagonism, FICI of >4.

DISCUSSION

Urinary candidiasis is an increasingly common in hospitalized patients (4, 5). Candiduria may present as asymptomatic colonization, urinary tract infection, or disseminated candidiasis (34). In most patients with asymptomatic candiduria, the removal of the source of colonization, such an indwelling urinary catheter, is sufficient. Symptomatic patients and asymptomatic patients at high risk of dissemination (e.g., immunocompromised patients) require antifungal therapy in addition to adequate source control. FLC and AMB are first-line treatment options for *Candida* urinary tract infections, since echinocandins and other azoles (itraconazole, voriconazole, and posaconazole) have limited excretion into the urine (35). With the increasing frequency of FLC-resistant *Candida* strains isolated in urine and the high rates of toxicity associated with AMB, there is a clear need for novel agents that effectively treat *Candida* urinary tract infections. Furthermore, there is an increasing need to identify agents that can prevent colonization, thus decreasing the likelihood of a disseminated infection in the future, without inducing resistance to first-line antifungal agents.

FIN is commonly used to treat benign prostatic hyperplasia, and recent studies have reported that it may have antibacterial activity (24, 25); however, to our knowledge, there have been no prior studies investigating the effectiveness of FIN against fungi, in particular, *C. albicans*. Our results demonstrate that FIN does not affect *C. albicans* planktonic growth. Furthermore, targeting virulence factors, such as filamentation and biofilm formation, is potentially advantageous considering that resistance to agents that target virulence instead of growth may be less likely to develop.

In this study, high FIN concentrations alone were effective in the prevention of mature biofilms. Interestingly, for the prevention of biofilms, FIN added to FLC produced a synergistic reduction in biofilm growth. However, the utility of oral finasteride alone for urinary candidiasis in the clinical setting is limited. The peak serum concentrations of FIN after the administration of single FIN doses of 5, 10, 20, 50, and 100 mg in healthy volunteers were 38 ± 7 μg/liter, 81 ± 13 μg/liter, 147 ± 19 μg/liter, 442 ± 136 μg/liter, and 835 ± 199 μg/liter, respectively. Thirty-nine percent of FIN is excreted through the urinary system in the form of active and inactive metabolites (22). However, it is possible that high doses of finasteride (i.e., 100 mg, which has been given safely in early dose safety studies) might lead to 40 μg of finasteride being excreted in the urine over a 24-h period (36). More likely, any clinical application of FIN would rely on it being used in combination with FLC.

Our results also indicate that FIN significantly impairs filamentation. In the liquid filamentation assay, we observed a clear difference between the samples (control and FIN), with the control containing a dense network of long filaments. Similarly, the colonies spotted on plates showed a significant decrease in filamentation after 2 days of incubation at concentrations of ≥32 mg/liter, and the defect in filamentation was more evident after 5 days of incubation. The mechanism by which FIN inhibits filamentation remains undefined, and further studies are in progress in our laboratory.

The activity of FIN and FLC or AMB combinations varied according to the established criteria for MICs. When the $sMIC_{50}$ criteria were selected, the combination of agents for the prevention of biofilm formation (FIN-FLC) resulted in a synergistic effect. For treatment of mature biofilms, the combinations FIN-FLC and FIN-AMB resulted in indifference. The contrasting results observed in the combination therapy might be attributed to the different mechanisms of action of FLC and AMB. FLC indirectly inhibits ergosterol synthesis by the inhibition of microsomal cytochrome P450, whereas AMB interacts directly with ergosterol in the plasma membrane. Additionally, recent publications (37–42) have suggested that the antifungal activity of AMB might also be mediated by oxidative damage.

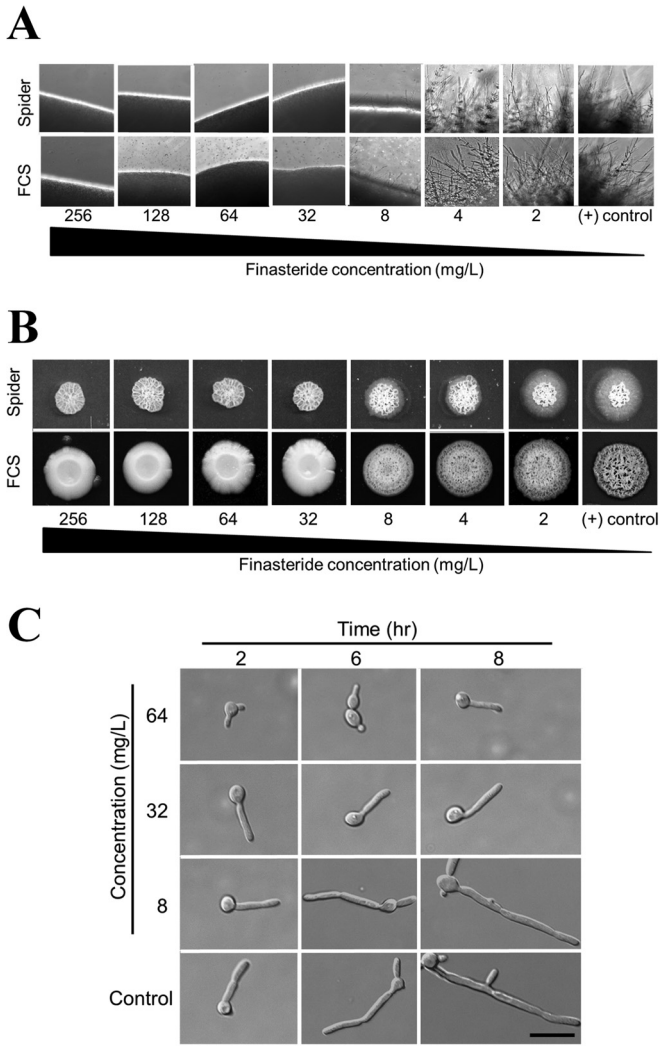

FIG 3 Filamentation of *C. albicans* on YPD plus FCS and Spider media containing decreasing concentrations of finasteride. SC5314 was plated with FIN concentrations of 256, 128, 64, 32, 28, 16, and 8 mg/liter, as well as a drug-free control. Plates were imaged after 2 days and observed under light microscopy with a ×40 objective (A) and again after 5 days of incubation at 37°C (B). (C) SC5314 was imaged in liquid RPMI with decreasing concentrations of FIN at 2, 6, and 8 h. Bar, 5 μm.

We demonstrated that FIN in combination with the traditional antifungal FLC has a synergistic effect on *C. albicans* SC5314 for the prevention of biofilm formation. The biofilm-specific antifungal efficacy of FIN, to our knowledge, has not been reported. To further investigate the target specificity of finasteride on *C. albicans* DFG10, as well as the role of DFG10 in filamentation and biofilm formation, we are constructing a relevant set of mutant strains (experiments currently in progress).

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S.A.L., A.A.C.-D., and L.L. conceived and designed the experiments. A.A.C.-D. and L.L. performed the experiments. and analyzed the data. S.A.L. contributed materials and analysis tools. A.A.C.-D., L.L. M.J., and C.J.W. wrote the paper.

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