Inhibitory effect of farnesol on biofilm formation by *Candida tropicalis*

1Zibafar E., 1Hashemi SJ., 1Zaini F., 2Zeraati H., 1Rezaie S., 1Kordbacheh P.

1Department of Medical Parasitology & Mycology, 2Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Iran.

Received 21 Apr 2008; Revised 28 Sept 2008; Accepted 12 Oct 2008

**ABSTRACT**

**Background:** Candidiasis associated with indwelling medical devices is especially problematic since they can act as substrates for biofilm growth which are highly resistant to antifungal drugs. Farnesol is a quorum-sensing molecule that inhibits filamentation and biofilm formation in *Candida albicans*. Since in recent years *Candida tropicalis* have been reported as an important and common non-albicans *Candida* species with high drug resistance pattern, the inhibitory effect of farnesol on biofilm formation by *Candida tropicalis* was evaluated.

**Methods:** Five *Candida tropicalis* strains were treated with different concentration of farnesol (0, 30 and 300 µM) after 0, 1 and 4 hrs of adherence and then they were maintained under biofilm formation condition in polystyrene, 96-well microtiter plates at 37°C for 48 hrs. Biofilm formation was measured by a semiquantitative colorimetric technique based on reduction assay of 2,3- bis -2H-tetrazolium- 5- carboxanilide (XTT).

**Results:** The results indicated that the initial adherence time had no effect on biofilm formation and low concentration of farnesol (30 µM) could not inhibit biofilm formation. However the presence of non-adherent cells increased biofilm formation significantly and the high concentration of farnesol (300 µM) could inhibit biofilm formation.

**Conclusion:** Results of this study showed that the high concentration of farnesol could inhibit biofilm formation and may be used as an adjuvant in prevention and in therapeutic strategies with antifungal drugs.

**Keywords:** Farnesol, Biofilm, *Candida tropicalis*, Azole

**INTRODUCTION**

Biofilms are colonies of microbial cells wrapped in a self-product organic polymeric matrix which are formed in response to attachment to a surface and are highly resistant to antimicrobial drugs (1, 2). They are often performed in indwelling medical devices (IMD) (1). Although majority of implant infections are caused by gram-positive bacteria, such as *staphylococci*, infections due to gram-negative bacteria and fungi are more serious (3). Transplantation procedures, immuno-suppression, chronic use of indwelling devices, and prolonged staying in intensive care unit have increased the prevalence of fungal disease. The most commonly fungi associated with such diseases are *Candida* species, notably *Candida albicans*, which causes both superficial and systemic diseases. Even with antifungal therapy, mortality of patients with invasive candidiasis may be up to 40% (4).

Farnesol one of the extracellular compounds released by *C. albicans* is a quorum-sensing molecule that inhibits conversion of yeast to *Candida* mycelium which is essential for initiation of infection and biofilm formation on catheter surface (5).

*Non-albicans Candida* species such as *C. tropicalis, C. parapsilosis, C. krusei, C. lipolytica, C. dubliniensis* also form biofilm on IMD surfaces (1). These species cause infections of higher complication and death rates compared to those resulting from *Candida albicans* (6). Among *non-albicans Candida* species, the prevalence of *C. tropicalis* is about 30% and it is an important colonizing fungus causing hematogenous disseminated Candidiasis (7, 8). *Candida tropicalis* is the second or third agent of candidemia especially in patients with neutropenia, leukemia, lymphoma and diabetes (9).

To our knowledge there are only few studies about inhibitory effects of farnesol on biofilm formation by *C. albicans, C. parapsilosis* and *C. dubliniensis* (10-12). Since farnesol production differs significantly between *Candida* spp. (13) and because *C. tropicalis* have been noted as an important and common *non-albicans Candida* species (7) with the low susceptibility to azole

Correspondence: pkordbacheh@tums.ac.ir
isolates in recent years (14), in these study inhibitory effects of farnesol on biofilm formation by Candida tropicalis was evaluated. To our knowledge there has been no documented study in Iran in this area of investigation.

MATERIAL AND METHODS

Organisms
C. tropicalis strain ATCC 750 and four other strains which obtained from patients referred to Medical Mycology Laboratory, School of Public Health, Tehran University of Medical Sciences were used in this study.

Biofilm Formation
According to Ramage et al experience (10), C. tropicalis strains were maintained on Sabouraud dextrose agar (Difco) plates and after 48 hrs subcultured onto yeast peptone dextrose (YPD) agar plates (1% [W/V], yeast extract (Oxoid); 2% [W/V], peptone (BioLife); 2% [W/V], dextrose (Merck); 2% [W/V], agar agar (BBL)). After 48 hrs one loop of colonies were transferred to 20 ml YPD broth in 250-ml Erlenmeyer flasks and incubated overnight in an orbital shaker (100 rpm) at 30°C under aerobic condition. All C. tropicalis strains were grown in the budding-yeast phase under this condition. Yeast cells were harvested and washed twice in sterile phosphate-buffered saline (PBS) (0.8% [W/V], sodium chloride (Merck); 0.02% [W/V], KH 2 PO 4 (Merck); 0.31% [W/V], Na 2 HPO 4·12H 2 O (Merck); 0.02% [W/V], potassium chloride (Panreac); pH 7.4). Then they were resuspended in RPMI 1640 supplemented with L-glutamine (Gibco) and buffered with morpholinopropanesulfonic acid (MOPS) (Merck) and adjusted to 1.0×10^6 cells/ml after counting with a hemocytometer.

Farnesol (Trans-Trans Sigma Chemical Co.) was as a 3 M stock solution and then diluted to obtain a 30 mM working stock solution in 100% (V/V) methanol. Working stock solution was used for the preparation of 300 μM and 30 μM concentrations of farnesol in RPMI 1640. All experiments were performed in presterilized, polystyrene, flat-bottom, 96-well microtiter plates (Nunc).

In this study for each biofilm formation, five different groups were considered. The first, as control group consisted of three wells containing 0, 30 and 300 μM of farnesol. The other groups named as H0A, H0B, H1 and H4 and consisted of three wells containing 100 μl of 1.0×10^6 cells/ml and 100 μl of farnesol solution (0, 30 and 300 μM) which were added after 0, 0, 1, and 4 hrs respectively.

For H0A and H0B groups, standardized cell suspensions were seeded into sterile 0.5 ml microtubes (TrefCleer, Switzerland) and double strength of different concentration of farnesol also were added into these sterile tubes at the same time to obtain 0, 30, 300 μM as final concentration of farnesol and both of them were transferred into wells of microtiter plates. After one (in H0A group) and four hrs (in H0B group) the medium was aspirated, and non adherent cells were removed by washing of the preparations thoroughly three times in sterile PBS. Then farnesol solution was added at different concentrations (0, 30, and 300 μM) to the adherent cells. However in H1 and H4 groups since farnesol was added to the aspirated and pre-washed wells, single strength of farnesol solutions were used (0, 30, and 300 μM).

Biofilm formation assay
A semiquantitative measure of biofilm formation was assayed by using a 2,3-bis-(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay. XTT (Sigma Chemical Co.) was prepared as a saturated solution at a concentration of 0.5 mg/ml in Ringer’s lactate. This solution was filter sterilized through a 0.22-μm-pore-size filter, divided into aliquots, and then stored at -70°C. Prior to each assay, an aliquot of the XTT stock solution was thawed, and treated with menadione sodium bisulfite (10 mM prepared in Distilled Water; Sigma Chemical Co.) to obtain a final concentration of 1 μM of menadione. A 100-μl aliquot of XTT-menadione was then added to each pre-washed biofilm and wells to measure background XTT levels. The plates were then incubated in dark for 2 hrs at 37°C, and the colorimetric change at 490 nm (a reflection of the metabolic activity of the biofilm) was measured with a microtiter plate reader (Titertekplus-MS2, reader, UK) (10).

Statistical analyses
Overall in this study 25 independent biofilms were performed for 5 strains and the optical density (OD) of control group was considered as baseline which was exerted to other groups. After standardization of the biofilm formation test, normality distribution of data were analyzed with Kolmogorov-Smirnov test and it was shown that all of the data had normal distributions in significant level α=0.05. Therefore for comparison of the means, One-Way Anova test and post Hoc (LSD test) were used. SPSS version 11.5 was used as statistical software and significant level was α=0.05.
RESULTS

In this study the mean OD in H0A group increased from 0.25 ± 0.08 in the absence of farnesol to 0.27 ± 0.12 when the farnesol concentration was 30 µM and then it was decreased to 0.12 ± 0.07 in 300 µM farnesol solution (Table 1). H0B group Study showed that the mean OD increased from 0.26 ± 0.09 in the absence of farnesol to 0.27 ± 0.11 in 30 µM farnesol solution and then it decreased to 0.14 ± 0.08 in 300 µM farnesol solution (Table 1). In H1 group it was found that the mean (OD) decreased from 0.27 ± 0.10 in the absence of farnesol to 0.24 ± 0.14 in 30 µM of farnesol and then it decreased to 0.12 ± 0.08 in 300 µM farnesol solution (Table 1). One-Way Anova test showed this change in biofilm formation was significant for different concentration of farnesol (P<0.001) and Post Hoc (LSD Test) showed that this significant change was related to 300 µM farnesol solution (Fig. 1).

A comparison of H0A and H0B groups showed that the mean OD was 0.21 ± 0.11 in H0A and 0.23 ± 0.11 in H0B (Table 1) and the difference with Paired Sample T-Test was significant (Fig. 2) but with splitting data due to different concentration of farnesol (0, 30, 300 µM farnesol) in these two groups it was shown that the difference was significant only in 300 µM farnesol concentration (Fig. 3).

DISCUSSION

In Recent years, there has been an increase in frequency of invasive candidiasis as an opportunistic infection in human being (15) and high morbidity and mortality of disseminated candidiasis have been one of the most important problems (4). A survey of nosocomial blood stream infections of more than 24,000 cases in 49 US hospitals from 1995 to 2002 showed that 9.5% of these infections were caused by Candida species (1). Even with antifungal therapy, high mortality of patients with invasive candidiasis is notable (40%) (4). Although Candida albicans is the most frequently isolated specie,
autocrine and paracrine

by:

this study were almost

survival, dissemination

rs,

sis,

and these

arnesol to

-adjusted data were analyzed. Therefore

other groups to standardize the test and then

considered as baseline (blank) and exerted

Student's t test compared to control

In

independent biofilm were formed for each strain.

Candida

similar to

phenomenon which helps

biofilm formation by

Candida

spreading

population of biofilm to prevent overgrowth and

stimulation. So it is

phenomenon is

cells under the control cell signaling

Overall b

antifungal drugs

Candida

species with less susceptibility to

afungal drugs have been increased drastically

(9, 16).

Overall biofilm is an organized community of
cells under the control cell signaling and these
cells of microbial biofilms release chemical
compounds such as farnesol to regulate the
number of microbial population. This
phenomenon is similar to communication of the
cells of a tissue by autocrine and paracrine
stimulation. So it is essential for the whole
population of biofilm to prevent overgrowth and
spreading yeast cells far from initial biofilms (10).
Biofilm formation by Candida species is a
phenomenon which helps survival, dissemination
and resistance of infection to drug (4). Around 2-
24% of all candidemia are caused by Candida
tropicalis resistance pattern of this species to drug
is notable (15).

The number of strains and independent biofilm
which were formed in this study were almost
similar to those of previous studies (10-12). Five
Candida tropicalis strains used in this study and 5
independent biofilm were formed for each strain.
In the previous study data were tested with
Student's t test compared to control group (10) but
in this study the mean OD of control group was
considered as baseline (blank) and exerted to
other groups to standardize the test and then these
adjusted data were analyzed. Therefore, this study
consisted of 4 groups: H0A, H0B, H1 and H4.
Since in H0A and H0B groups, cell suspension
and farnesol were added at the same time, farnesol
was used in double strength, after one hrs (in
H0A) and 4 hrs (in H0B), medium was aspirated
and farnesol solutions were added again in single
strength to pre-washed wells to obtain similar
condition to the other groups. It was hypothesized
that non adherent cells can bias. In H0B a three-
hrs delay in washing the wells, increased biofilm
formation drastically in this study, whereas it was
not noted in previous studies (10-12).
In the previous study the decrease of OD from 0
30 µM of farnesol concentration at 0, 1 and 24
hrs was significant and the initial adherence time,
prior to the addition of farnesol (0, 1, 2, 4 and 24
h), was important in the ability of farnesol to
inhibit biofilm formation except for 24-h biofilms
in which treated biofilms with 30 and 300 µM
farnesol were noticeably lower than those of the
untreated controls. This was explained by the
presence of high concentration of farnesol which
could inhibit newly budded yeast cells which were
formed in mature biofilms (10). In another report,
the initial adherence time (0 and 2 h) was
effective only in 3 of 4 phenotypes of C.parapsilosis
(11) and In another study for both C.albicans and C. dubliniensis, as the initial
adherence time increased (from 0 to 24) the effect
of farnesol on biofilm development diminished and
minimal visible defect was noted on 24-h

<table>
<thead>
<tr>
<th>Group</th>
<th>Farnesol Concentration (µM)</th>
<th>N</th>
<th>Mean OD</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Farnesol</td>
<td>25</td>
<td>0.24508</td>
<td>0.081340</td>
</tr>
<tr>
<td>H0A</td>
<td>30</td>
<td>25</td>
<td>0.26592</td>
<td>0.119244</td>
</tr>
<tr>
<td>H0B</td>
<td>300</td>
<td>25</td>
<td>0.11908</td>
<td>0.067272</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>0.21003</td>
<td>0.11759</td>
</tr>
<tr>
<td></td>
<td>No Farnesol</td>
<td>25</td>
<td>0.25896</td>
<td>0.087247</td>
</tr>
<tr>
<td>H0B</td>
<td>30</td>
<td>25</td>
<td>0.27288</td>
<td>0.106609</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>25</td>
<td>0.14380</td>
<td>0.077653</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>0.22521</td>
<td>0.107248</td>
</tr>
<tr>
<td></td>
<td>No Farnesol</td>
<td>25</td>
<td>0.27044</td>
<td>0.099674</td>
</tr>
<tr>
<td>H1</td>
<td>30</td>
<td>25</td>
<td>0.24400</td>
<td>0.141228</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>25</td>
<td>0.12052</td>
<td>0.075666</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>0.21165</td>
<td>0.125994</td>
</tr>
<tr>
<td></td>
<td>No Farnesol</td>
<td>25</td>
<td>0.21740</td>
<td>0.113837</td>
</tr>
<tr>
<td>H4</td>
<td>30</td>
<td>25</td>
<td>0.24032</td>
<td>0.121359</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>25</td>
<td>0.12840</td>
<td>0.098629</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>0.19537</td>
<td>0.120400</td>
</tr>
</tbody>
</table>

Table 1. Standardized mean of optical density (OD) according to different concentrations of Farnesol in study groups.
performed biofilms (12) but in this study in HOA and HOB and H4 groups the mean OD (biofilm formation) increased from 0 to 30 µM concentration of farnesol. Although it was not statistically significant but it can be considered in future studies and it may be due to difference between Candida strains that low concentration of farnesol is unable to inhibit biofilm formation in these strains. Differences in biofilm formation and inhibitory effect of farnesol are shown among different phenotypes of Candida parapsilosis (11). In this study the initial adherence time (0, 1 and 4 hrs) was not effective for inhibition of biofilm formation. While potential ability of farnesol as an adjuvant in prevention and therapeutic strategies with antimicrobial drugs has been reported (12, 17) the doses of farnesol as an adjuvant with antifungal drugs to inhibit biofilm formation by Candida species should be determined. Overall in this study the inhibitory effect of farnesol was related to its concentration and similar to result of other studies, farnesol at concentration of 300 µM reduced biofilm formation decisively (10-12).

ACKNOWLEDGMENTS
This research has been supported by Tehran University of Medical Sciences & Health Services grant 3882. No: 132/8491. Date: 11/26/2006. We are grateful to Professor Koichi Makimura (Institute of Medical Mycology & Genomic Research Center, Teikyo University, Tokyo, Japan) for providing C. tropicalis strain ATCC 750.

REFERENCES