DOI: 10.2478/10004-1254-63-2012-2236

Scientific paper

SUSCEPTIBILITY TO CASPOFUNGIN AND FLUCONAZOLE AND *ALS1/ALS3* GENE EXPRESSION IN BIOFILM AND DISPERSAL CELLS OF *CANDIDA ALBICANS**

Helena BUJDÁKOVÁ, Naďa KULKOVÁ, and Lucia ČERNÁKOVÁ

Comenius University, Faculty of Natural Sciences, Department of Microbiology and Virology, Bratislava, Slovakia

Received in May 2012
CrossChecked in August 2012
Accepted in November 2012

The biofilm of *Candida albicans* has been implicated as a source of bloodstream infections. Dispersal cells, as the final biofilm stage, are responsible for its spread. The aim of this study was to compare the susceptibility of biofilm and dispersal cells *vs.* planktonic cells (overnight liquid culture) of *C. albicans* to caspofungin (CAS) and fluconazole (FLU) when the drugs were added: i) at the beginning of the experiment; ii) after 1.5 h (adherence stage); iii) after 24 h (early mature biofilm). The findings were evaluated after 48 h (mature biofilm) using the XTT reduction assay. Later administration of the drug increased biofilm sessile minimal inhibitory concentration ($SMIC_{80}$) of both FLU and CAS from 1 μg mL^{-1} to over 64 μg mL^{-1} and from 0.125 μg mL^{-1} to over 16 μg mL^{-1}, respectively. Susceptibility of dispersal cells also decreased with time of administration.

We also determined the expression of the *Als1* and *Als3* genes in 48-h sessile biofilm and dispersal cells of *C. albicans* SC5314 and compared it to planktonic cells. The expression was normalised to the standard *Act1* gene in every condition tested. Quantitative real-time PCR revealed a strong up-regulation of the *Als1* gene in the dispersal cells but not in biofilm and high expression of the *Als3* gene in both biofilm and dispersal cells. High expression of both *Als1* and *Als3* genes supports the hypothesis that dispersal cells pose a high-risk of infection.

KEY WORDS: Act1, *bloodstream infections, MIC, PCR, XTT reduction assay*

Microbial biofilm is a well-organised community of one or several microbial species (1). Biofilms have recently been associated with many infections, especially in patients using medical devices such as catheters, tracheal tubes, and joint implants (2, 3). The yeasts of the genus *Candida* are the fourth most common cause of nosocomial bloodstream infections (4). It has already been suggested that biofilms formed on foreign bodies can be a serious source of such infections (2). Therapy can be a problem because biofilm is usually refractory to conventional therapy due to resistance or reduced uptake of antifungals through the extracellular matrix formed by mature biofilm (5-7). Recent *in vitro* and *in vivo* studies (8-11) point to echinocandins caspofungin (CAS) and anidulafungin as effective agents against planktonic cells and biofilms formed by *C. albicans*.

Biofilm develops in four major stages: adherence stage, initial formation (pre-mature biofilm), maturation

* The subject of this article has partly been presented at the International Symposium "Power of Fungi and Mycotoxins in Health and Disease" held in Primošten, Croatia, from 19 to 22 October 2011.

(forming of extracellular matrix) and, cell dispersion (12, 13). While the first three stages of biofilm formation have been studied in detail (5, 12, 13), research on dispersal cells is still scarce. These cells are released from mature biofilm into the blood stream and can cause infection and/or start a new biofilm elsewhere. Dispersion can be triggered by changes in the concentration of carbon sources or growth conditions (14), but little is known about gene regulation (15). During the development of *C. albicans* biofilm, many specific genes are expressed. *C. albicans* adhesins Als1 and Als3 play an important role and are highly expressed in *in vitro* (16) as well as *in vivo* biofilms (17, 18). While the *Als1* gene is expressed in the earlier stages of biofilm formation (19), the *Als3* gene is mainly up-regulated in the mature biofilm (11). However, this is not a rule, as the expression of either gene can be significantly affected by cultivation conditions (11, 20).

The aim of this study was to see whether susceptibility of a biofilm to antifungal drugs CAS and fluconazole (FLC) is time-dependent and whether dispersal cells follow the susceptibility/resistance characteristics of biofilm or of planktonic cells. In addition, we wanted to see whether the *Als1* and *Als3* genes are up-regulated in dispersal cells.

MATERIALS AND METHODS

Strain characterisation, preparation of the yeast suspension and MIC_{80} determination

For this study, we used the standard strain *C. albicans* SC5314, as it is frequently used for wild-type control in systematic sequencing projects and for deriving laboratory strains. The original strain is a clinical isolate and it is virulent in mouse systemic infection (21). We also included two clinical isolates of *C. albicans*, 16755/1 and 21922/1, isolated from central venous catheters (CVC). The strains were cultivated on Yeast Extract Peptone Dextrose (YPD) plates (1 % yeast extract, 1 % mycological peptone and 1 % D-glucose, supplemented with 2 % agar, Applichem, Germany) at 28 °C for 24 h before use. A large loop of cells was transferred to 20 mL of Yeast Nitrogen Base broth (YNB, Sigma-Aldrich, USA), containing 0.9 % D-glucose. After incubation with shaking at 37 °C for 16 h, the planktonic cells were centrifuged and washed twice with 1 mL of phosphate-buffered saline (PBS, pH 7.2), vortexed and centrifuged at 5000 *g* for 5 min. The cells were then re-suspended in 1.0 mL of YNB broth and the suspension counted in a haemocytometer and prepared to a final inoculum concentration of 10^6 cells mL^{-1}. Susceptibility of FLC (0.125 μg mL^{-1} to 64 μg mL^{-1}, Pfizer, USA), and CAS (0.03 μg mL^{-1} to 16 μg mL^{-1}, Merck Sharp & Dohme, USA) was tested using the broth microdilution method according to the Clinical Laboratory Standard Institute (CLSI) M27-A3 protocol (22) in an RPMI-1640 medium (Sigma-Aldrich, USA) supplemented with 2 % (w/v) D-glucose and buffered with 0.165 mol L^{-1} morpholinepropanesulphonic acid (MOPS; Sigma-Aldrich, USA), pH 7.0. Results were determined as minimal drug concentration that inhibited growth by 80 % compared to the growth of strains without antifungal agents (MIC_{80}). All tests were performed in five parallels.

Biofilm formation and collection of non-adhered and dispersal cells

The biofilm was prepared according to the protocol of Li et al. (23). From each strain, 100 μL of cell suspension (10^6 cells mL^{-1}) prepared in YNB containing 0.9 % D-glucose was transferred into polystyrene, 96-well microplates (flat bottom; Sarstedt, Germany) and incubated at 37 °C for 1.5 h to allow for the cells to attach. The medium with non-adhered cells was removed and the wells were gently washed twice with 150 μL of sterile PBS. For mature biofilm to develop, 100 μL of fresh YNB medium with 0.9 % D-glucose was added and the plates were incubated at 37 °C. After 48 h, media containing dispersal cells were discarded and the wells were washed three times with 200 μL of sterile PBS. Biofilm was estimated using the viability test with XTT [2,3-bis(2-methoxy-4-nitro-5-sulphenyl)-2H-tetrazolium-5-carboxanilide] sodium salt (Sigma-Aldrich, USA). The colorimetric reaction was determined using a microplate reader (OD_{490}, MRX Microtiter plate absorbance reader, Dynex Technologies, USA) after 3 hour incubation at 37 °C in the dark. Three independent experiments were performed with each strain cultivated in three parallel wells. YNB medium containing no inoculum (no biofilm) was used as control. The results were expressed as average ± standard deviation (SD).

Dispersal cells were prepared according to the biofilm protocol with minor modifications. Briefly, polystyrene Petri dishes (10 cm in diameter, Sarstedt, Germany) were inoculated with 10^7 cells mL^{-1} in 20 mL of YNB supplemented with 0.9 % D-glucose

and cultivated at 37 °C for 90 min. After the adherence stage, media with non-adhered cells were removed and the collected cells were tested for MIC_{80} as described above. The adhered cells were washed three times with 20 mL of sterile PBS. For biofilm formation, 20 mL of fresh YNB medium was added and the plates were incubated at 37 °C for 24 h or 48 h. The dispersal cells from the supernatants of 24-hour and 48-hour sessile biofilm were collected and tested for MIC_{80} as described above.

Biofilm susceptibility testing

For this experiment, we combined the biofilm method and MIC_{80} determination according to the CLSI protocol, with some modifications. Biofilm was cultured in an RPMI-1640 medium with 0.165 mol L^{-1} M MOPS (pH 7.0) in 96-well polystyrene plates. FLC (0.125 μg mL^{-1} to 64 μg mL^{-1}) and CAS (0.03 μg mL^{-1} to 16 μg mL^{-1}) were added at three time points: 0 (the start of the experiment), 1.5 h (adherence stage), and 24 h (almost mature biofilm). All experiments were evaluated after 48 h (mature biofilm) using the above-described XTT reduction assay. The results were expressed as sessile MIC_{80} values ($SMIC_{80}$). Each assay was performed in triplicate and repeated in two independent experiments.

Quantitative Real-Time PCR (qRT-PCR)

C. albicans SC5314 planktonic cells (16-hour culture cultivated in an orbital shaker), 48-hour sessile biofilm, and 48-hour dispersal cells, all cultivated in YNB containing 0.9 % D-glucose, were used for the isolation of total RNA (RNeasy Mini kit, Qiagen, USA). Three microgrammes of RNA were then treated with DNase I (TaKaRa Bio, Japan) and used for cDNA synthesis using the TaqMan Reverse Transcription kit (Applied Biosystems, USA). Quantitative PCR was performed with SYBR® (Protocol) Maxima™ (Fermentas, Germany) according the manufacturer's instructions in ABI Prism 7900HT (Applied Biosystems, USA). The conditions were as follows; cycle 1 was repeated 40 times: 95 °C for 10 min, 95 °C for 15 s, 60 °C for 1 min and cycle 2 representing the dissociation level was performed once: 95 °C for 15 s, 60 °C for 15 s, 95 °C for 15 s. Data for the *Als1* and *Als3* genes were calculated and expressed as a fold regulation compared to the reference genes *Act1* and *Tef1* for each condition tested, using the standard curve quantification method (24). The expression data from the planktonic culture were used as reference samples. The primers for *Act1* (18), *Tef1, Als1,* and *Als3* have been described elsewhere (25). The qRT-PCR assay was performed independently in triplicate. Student's *t*-test was used for the statistical analyses. Results were considered to be statistically significant when $p<0.05$.

RESULTS

MIC_{80} to FLC in the standard strain was 0.5 μg mL^{-1} and in both clinical isolates 0.125 μg mL^{-1}. In turn, CAS's MIC_{80} for all strains was 0.03 μg mL^{-1}.

Table 1 shows biofilm formation in all strains. Biofilm was evaluated according to the following criteria: optical densities at 490 nm (OD_{490}) <0.20 was classified as a weak biofilm, OD_{490}=0.20 to 0.50 as a medium biofilm, and OD_{490} >0.50 as a strong biofilm. The standard *C. albicans* SC5314 strain formed a medium biofilm, isolate 16755/1 a strong biofilm, and isolate 21922/1 a weak biofilm.

Table 1 *Formation of biofilm determined by the XTT reduction assay*

***C. albicans* strains**	**Mean OD_{490}±SD**
SC 5314	0.248±0.034
16755/1	1.562±0.042
21922/1	0.041±0.025

OD_{490} = optical densities at 490 nm
SD - standard deviation

Table 2 shows susceptibilities of the biofilms formed in the presence of FLC and CAS added at different time points. Both the standard strain and clinical isolate 21922/1 (with a weak biofilm) remained susceptible to FLC when it was applied at the start of the experiment (time point 0) and after the adherence stage (time point 1.5 h). However, when added to early-mature biofilm (time point 24 h), FLC did not inhibit it. Clinical isolate 16755/1 with a strong biofilm did not respond to FLC at any time point, even at the concentration of 64 μg mL^{-1}.

All strains were susceptible to CAS when applied at time point 0 ($SMIC_{80}$ was 0.125 μg mL^{-1} to 0.5 μg mL^{-1}) and 1.5 h ($SMIC_{80}$ was 0.5 μg mL^{-1}), but not after that.

We also tested the susceptibility to FLC and CAS of non-adhered cells collected at the adherence stage (time 1.5 h) and of dispersal cells taken from 24-hour and 48-hour biofilm. Both non-adhered and dispersal

Table 2 *Susceptibility* of 48-hour mature biofilm of* C. albicans *treated with FLC and CAS at various time points*

C. albicans strains	$SMIC_{80}$ / μg mL^{-1}					
	for FLC			for CAS		
	T=0	T=1.5	T=24	T=0	T=1.5	T=24
SC 5314	1.0	1.0	≥64	0.125	0.5	≥16
16755/1	≥64	≥64	≥64	0.25	0.5	≥16
21922/1	1.0	1.0	≥64	0.25	0.5	≥16

** susceptibility was determined with a viability test using the XTT reduction assay after 48h;*
$SMIC_{80}$ - minimal inhibitory concentration that inhibits biofilm viability by 80 % compared to control biofilm formed - without antifungal drugs;
T=0 - drugs were added at the beginning of experiments;
T=1.5 - drugs were added after 1.5 h - adherence stage;
T=24 - drugs were added after 24 h - early-mature biofilm

cells (collected at either time point) remained susceptible to FLC (MIC_{80} was in the range of 1.0 μg mL^{-1} to 4.0 μg mL^{-1}), but susceptibility concentration of dispersal cells increased compared to the planktonic ones. The same was observed for CAS, with MIC_{80} range between 0.5 μg mL^{-1} and 1.0 μg mL^{-1}.

As the susceptibility of the dispersal cells was lower than of the planktonic cells, and these cells can spread in the human body through blood and can form a new biofilm, it was interesting to learn more about their adherence properties. To do that we compared the expression of *C. albicans* SC5314 *Als1* and *Als3* genes in dispersal cells and sessile biofilm with the expression of those genes in planktonic cells cultured overnight. The expression of the tested genes was normalised to the standard *Act1* gene under every set of testing conditions. Because of possible changes in *Act1* expression during the morphogenesis of *C. albicans* in biofilm, data were also normalised to the *Tef1* gene (data not shown). However, the results were very similar regardless of the housekeeping gene used. The qRT-PCR revealed up-regulation of both *Als1* and *Als3* genes in biofilm and dispersal cells in comparison with planktonic ones (Figure 1). However, the *Als1* gene was markedly up-regulated in dispersal cells, but not in sessile biofilm, while the *Als3* gene was significantly up-regulated in both (p<0.05).

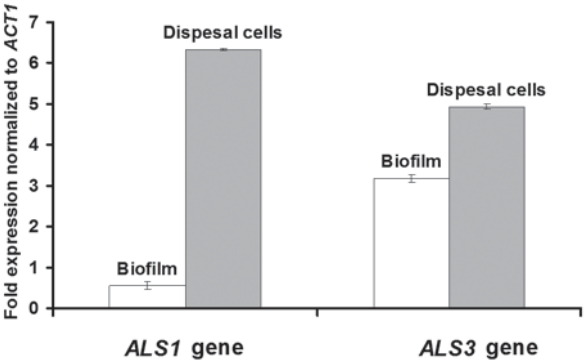

Figure 1 *Als1 and Als3 gene expression in sessile 48-hour-old biofilm and dispersal cells collected from 48-hour-old biofilm compared to planktonic cells (overnight liquid culture) of* C. albicans *SC 5314. Data are shown as the fold regulation calculated using the comparative Ct method. The tested genes were normalised to the Act1 gene level.*

DISCUSSION

Biofilm-associated infections are a general problem in immunocompromised or critically ill patients with foreign bodies (2, 26). The yeast *C. albicans* is the most common source of nosocomial bloodstream infections (4), mostly associated with the use of medical devices. FLC is often used to treat acute or chronic vaginal candidiasis and fungal infections in hospitalised patients or to prevent fungal infections in AIDS patients (27-29). However, the number of azole-resistant, mainly non-*C. albicans* isolates has increased in hospital care (30), limiting its application. Moreover, in critically ill patients receiving presumptive therapy, new azoles, amphotericin B, or echinocandins are used instead of FLC to avoid complications associated with fungal infections, biofilms in particular.

Our previous *in vitro* study (11) confirmed the efficiency of anidulafungin and CAS against sessile biofilm when added at the early stage of formation. Our present study has confirmed that biofilm susceptibility changes in a time-dependent manner. Early-formed (24-hour) biofilm proved to be resistant to either FLC or CAS, regardless of the strain tested.

Moreover, in the strain 16755/1, forming a strong biofilm, FLC was not effective even at the early stage of administration. Similar results with *C. albicans* SC5314 biofilm, but under flow conditions, were published by Uppulari et al. (31). Unlike these authors, however, we did not observe a remarkable efficiency of CAS when the drug was added to early-mature biofilm at hour 24, probably because there is an eight-hour difference between their and our early biofilm (16-hour and 24-hour, respectively), which seems to be enough time for the resistance against CAS to develop, probably due to a production of extracellular matrix that reduced the penetration of the antifungal drug. In addition, Uppulari et al. (31) found increased MIC_{80} for FLC in dispersal cells in comparison with planktonic cells, but their MIC_{80s} remained far below the "breakpoint" (64 μg mL^{-1}). In contrast to Uppulari et al. (31), who found the same susceptibility profile to CAS for dispersal and for planktonic cells, our data showed differences in susceptibility; later CAS administration resulted in lower efficacy, but CAS concentrations were still within its therapeutic dose.

Dispersal cells have phenotypic properties that can significantly contribute to virulence (14). To date, only a few genes participating in dispersion have been profiled (15, 32). Our study has provided new data on the expression of two important genes encoding for adhesins Als1 and Als3, completing the earlier findings (11, 16, 19). In agreement with them the *Als1* gene was significantly up-regulated in the early biofilm stage, and the *Als3* gene in the mature one. However, up-regulation of the *Als1* gene in dispersal cells collected from 48-hour biofilm suggests that cell dispersion could be not only the last stage of biofilm development but also the stage where a new one begins. Moreover, the high expression of the *Als3* gene underlines high adherence capacity of dispersal cells and confirms that these cells differ from planktonic not only in phenotype, but also in the regulation of genes important for virulence.

In summary, the effects of both FLC and CAS on biofilm proved to depend on the application time; later administration resulted in an increase in $SMIC_{80}$ in biofilm and in MIC_{80} in dispersal cells. CAS proved to be more effective than FLC. In contrast to regulation in mature biofilm, both the *Als1* and *Als3* genes were significantly up-regulated in dispersal cells collected from the 48-hour mature biofilm. Our findings point to high virulence of dispersal cells and them as a high-risk source of infection.

Acknowledgements

This research was funded by grant 1/0966/12 from the Ministry of Education of the Slovak Republic. We wish to thank assoc. prof. Hana Drahovská, PhD for valuable advice on the evaluation of qReal-Time PCR.

REFERENCES

1. Elias S, Banin E. Multi-species biofilms: living with friendly neighbors. FEMS Microbiol Rev 2012;36:990-1004.
2. Kojic EM, Darouiche RO. *Candida* infections of medical devices. Clin Microbiol Rev 2004;17:255-67.
3. Djeribi R, Bouchloukh W, Jouenne T, Menaa B. Characterization of bacterial biofilms formed on urinary catheters. Am J Infect Control 2012;40:854-9.
4. Hajjeh RA, Sofair AN, Harrison LH, Lyon GM, Arthington-Skaggs BA, Mirza SA, Phelan M, Morgan J, Lee-Yang W, Ciblak MA, Benjamin LE, Sanza LT, Huie S, Yeo SF, Brandt ME, Warnock DW. Incidence of bloodstream infections due to *Candida* species and *in vitro* susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J Clin Microbiol 2004;42:1519-27.
5. Mukherjee PK, Chandra J. *Candida* biofilm resistance. Drug Resist Updat 2004;7:301-9.
6. Borecká-Melkusová S, Moran GP, Sullivan DJ, Kucharíková S, Chorvát D Jr, Bujdáková H. The expression of genes involved in the ergosterol biosynthesis pathway in *Candida albicans* and *Candida dubliniensis* biofilms exposed to fluconazole. Mycoses 2009;52:118-28.
7. Walker LA, Gow NA, Munro CA. Fungal echinocandin resistance. Fungal Genet Biol 2010;47:117-26.
8. Bal AM. The echinocandins: three useful choices or three too many? Int J Antimicrob Agents 2010;35:13-8.
9. Kucharíková S, Tournu H, Holtappels M, Van Dijck P, Lagrou K. *In vivo* efficacy of anidulafungin against mature *Candida albicans* biofilms in a novel rat model of catheter-associated candidiasis. Antimicrob Agents Chemother 2010;54:4474-5.
10. Fiori B, Posteraro B, Torelli R, Tumbarello M, Perlin DS, Fadda G, Sanguinetti M. *In vitro* activities of anidulafungin and other antifungal agents against biofilms formed by clinical isolates of different *Candida* and *Aspergillus* species. Antimicrob Agents Chemother 2011;55:3031-5.
11. Kucharíková S, Tournu H, Lagrou K, Van Dijck P, Bujdáková H. Detailed comparison of *Candida albicans* and *Candida glabrata* biofilms under different conditions and their susceptibility to caspofungin and anidulafungin. J Med Microbiol 2011;60:1261-9.
12. Ramage G, Vandewalle K, Wickes BL, López-Ribot JL. Characteristics of biofilm formation by *Candida albicans*. Rev Iberoam Micol 2001;18:163-70.
13. Finkel JS, Mitchell AP. Genetic control of *Candida albicans* biofilm development. Nat Rev Microbiol 2011;9:109-18.
14. Uppuluri P, Chaturvedi AK, Srinivasan A, Banerjee M, Ramasubramaniam AK, Köhler JR, Kadosh D, Lopez-Ribot JL. Dispersion as an important step in the *Candida albicans*

biofilm developmental cycle. PLoS Pathog 2010;26: e1000828.

15. Uppuluri P, Pierce CG, Thomas DP, Bubeck SS, Saville SP, Lopez-Ribot JL. The transcriptional regulator Nrg1p controls *Candida albicans* biofilm formation and dispersion. Eukaryot Cell 2010;9:1531-7.
16. Nailis H, Kucharíková S, Řičicová M, Van Dijck P, Deforce D, Nelis H, Coenye T. Real-time PCR expression profiling of genes encoding potential virulence factors in *Candida albicans* biofilms: identification of model-dependent and -independent gene expression. BMC Microbiol 2010;16:114.
17. Nett JE, Lepak AJ, Marchillo K, Andes, DR. Time course global gene expression analysis of an *in vivo Candida* biofilm. J Infect Dis 2009;200:307-13.
18. Řičicová M, Kucharíková S, Tournu H, Hendrix J, Bujdáková, H, Van Eldere J, Lagrou K, Van Dijck P. *Candida* albicans biofilm formation in a new *in vivo* rat model. Microbiology 2010;156:909-19.
19. Yeater KM, Chandra J, Cheng G, Mukherjee PK, Zhao X, Rodriguez-Zas SL, Kwast KE, Ghannoum MA, Hoyer LL. Temporal analysis of *Candida albicans* gene expression during biofilm development. Microbiology 2007;153:2373-85.
20. Nailis H, Vandenbroucke R, Tilleman K, Deforce D, Nelis H, Coenye T. Monitoring *ALS1* and *ALS3* gene expression during *in vitro Candida albicans* biofilm formation under continuous flow conditions. Mycopathologia 2009;167:9-17.
21. Gillum AM, Tsay EY, Kirsch DR. Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of *S. cerevisiae* ura3 and *E. coli* pyrF mutations. Mol Gen Genet 1984;198:179-82.
22. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeast. Approved standard M27-A3. Wayne (PA): National Committee for Clinical Laboratory Standards; 2008.
23. Li X, Yan Z, Xu J. Quantitative variation of biofilms among strains in natural populations of *Candida albicans*. Microbiolgy 2003;149:353-62.
24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001;25:402-8.
25. Green CB, Zhao X, Yeater KM, Hoyer LL. Construction and real-time RT-PCR validation of *Candida albicans* PALS-GFP reporter strains and their use in flow cytometry analysis of *ALS* gene expression in budding and filamenting cells. Microbiology 2003;151:1051-60.
26. Ramage G, Martínez JP, López-Ribot JL. *Candida* biofilms on implanted biomaterials: a clinically significant problem. FEMS Yeast Res 2006;6:979-86.
27. Clancy CJ, Yu VL, Morris AJ, Snydman DR, Nguyen MH. Fluconazole MIC and the fluconazole dose/MIC ratio correlate with therapeutic response among patients with candidemia. Antimicrob Agents Chemother 2005;49:3171-7.
28. Badiee P, Alborzi A, Davarpanah MA, Shakiba E. Distributions and antifungal susceptibility of *Candida* species from mucosal sites in HIV positive patients. Arch Iran Med 2010;13:282-7.
29. Martins HP, da Silva MC, Paiva LC, Svidzinski TI, Consolaro ME. Efficacy of fluconazole and nystatin in the treatment of vaginal *Candida* species. Acta Derm Venereol 2012;92:78-2.
30. Pfaller MA, Messer SA, Moet GJ, Jones RN, Castanheira M. *Candida* bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in Intensive Care Unit (ICU) and non-ICU settings in the SENTRY Antimicrobial Surveillance Program (2008-2009). Int J Antimicrob Agents 2011;38:65-9.
31. Uppuluri P, Srinivasan A, Ramasubramanian A, Lopez-Ribot JL. Effects of fluconazole, amphotericin B, and caspofungin on *Candida albicans* biofilms under conditions of flow and on biofilm dispersion. Antimicrob Agents Chemother 2011;55:3591-3.
32. Robbins N, Uppuluri P, Nett J, Rajendran R, Ramage G, Lopez-Ribot JL, Andes D, Cowen LE. Hsp90 governs dispersion and drug resistance of fungal biofilms. PLoS Pathog 2011;7:e1002257.

Sažetak

PROFIL OSJETLJIVOSTI NA KASPOFUNGIN I FLUKONAZOL I EKSPRESIJA GENA ALS1 I ALS3 U STANICAMA BIOFILMA TE PLANKTONSKIM STANICAMA VRSTE *CANDIDA ALBICANS*

Tvorba biofilma u vrste *Candida albicans* smatra se uzročnikom infekcija koje se prenose krvlju. Rasprostranjenju biofilma pridonose oslobođene, tzv. planktonske stanice, koje nastaju u posljednjoj fazi oblikovanja biofilma. U ovome istraživanju usporedili smo osjetljivost stanica biofilma i planktonskih stanica (prekonoćna bujonska kultura) *C. albicans* na kaspofungin (CAS) i flukonazol (FLU) u uvjetima kada su lijekovi dodavani: i) na početku pokusa; ii) nakon 1,5 h (faza priljepljivanja, adherencije); iii) nakon 24 h (rana zrelost biofilma). Nakon 48 h u fazi zrelog biofilma provedeno je mjerenje primjenom testa redukcije XTT-a. Dobiveni rezultati potvrđuju da kasnija primjena lijeka povisuje sesilnu minimalnu inhibicijsku koncentraciju, $SMIC_{80}$ (od engl. *sessile minimal inhibitory concentration*) i FLU i CAS (u rasponu od 1 μg mL^{-1} do ≥64 μg mL^{-1} te u rasponu od 0,125 μg mL^{-1} do ≥16 μg mL^{-1}). Nadalje, uočili smo smanjenu osjetljivost planktonskih stanica na lijekove. U drugom dijelu pokusa usredotočili smo se na ekspresiju *Als1* i *Als3* gena u *C. albicans* SC5314, kako u sesilnim stanicama iz biofilma starog 48 h tako i u stanicama koje su se izdvojile iz njega te smo ih usporedili s kulturom planktonskih stanica. Ekspresiju smo u svakome od ispitanih uvjeta testiranja normalizirali prema genu *Act1* kao standardu. Primjenom kvantitativnog PCR-a u realnom vremenu (engl. *Quantitative Real Time PCR*) dokazali smo snažno pojačanu ekspresiju gena *Als1* u zrelim stanicama koje se oslobađaju iz biofilma, ali ne i u sesilnom biofilmu, kao i visoku ekspresiju gena *Als3* i u biofilmu i u stanicama koje su se izdvojile iz njega. Takvi rezultati upućuju na to da se osjetljivost stanica udruženih u biofilm te stanica oslobođenih iz njega na FLC i CAS smanjuje s kasnijim vremenom primjene lijeka, a, dodatno, visoka ekspresija gena *Als1* i *Als3* govori u prilog hipotezi da su stanice oslobođene iz biofilma visoki čimbenik rizika i izvor infekcije.

KEY WORDS: Act1*, infekcije putem krvi, MIC, PCR, test redukcije XTT-a*

CORRESPONDING AUTHOR:

Helena Bujdáková
Comenius University in Bratislava
Faculty of Natural Sciences,
Department of Microbiology and Virology
Mlynská dolina B-2
842 15 Bratislava, Slovak Republic
E-mail: *bujdakova@fns.uniba.sk*