Cellular apoptosis: an alternative mechanism of action of caspofungin in *Candida glabrata*

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Introduction: Although the mechanism of activity for echinocandins is known, the physiological mechanisms by which these antifungal agents cause cell death via the classical apoptotic pathways are not well-defined yet. Regarding this, the present study aimed to evaluate the mechanisms of caspofungin-induced *Candida glabrata* cell death.

Material and methods: For the purpose of the study, the minimum inhibitory concentration (MIC) of caspofungin against *C. glabrata* (ATCC 90030) was determined using the broth microdilution reference method (CLSI M27-A2 and M27-S4). The annexin V and propidium iodide staining was performed to determine the way through which caspofungin exerts activity against *C. glabrata* (i.e., through the induction of apoptosis and/or necrosis). Additionally, the possible effect of caspofungin on inducing the expression of two apoptotic genes, namely *MCA1* and *NUC*, was studied using the real-time polymerase chain reaction assay.

Results: According to the obtained MIC (0.5 µg/mL), *C. glabrata*, exposed to 0.25, 0.5, and 1 µg/mL of caspofungin, exhibited the features of late apoptosis/necrosis after 18 h of incubation. Furthermore, the use of 0.25, 0.5, and 1 µg/ml caspofungin induced apoptosis (early/late) in 14.67%, 17.04%, and 15.89% of the cells, respectively. There was a significant difference between the percentages of early-apoptotic cells at the three concentrations (P<0.05). The rate of necrosis was significantly greater in response to caspofungin. Accordingly, necrosis occurred in 71.26%, 71.26%, and 61.26% of the cells at

the caspofungin concentrations of 0.25, 0.5, and 1 µg/mL, respectively (P<0.05). The analysis of the data in REST software demonstrated that the expression of *MCA1* and *NUC1* genes had a significant increase (P<0.05).

Conclusion: As the findings of the present study demonstrated, caspofungin promoted both necrosis and apoptosis of *C. glabrata* cells at concentrations higher or equal to the MIC.

Table 1. Primers used for *MCA1*, *NUC1*, and *RDN5.8* genes expression

Name	Reference gene Accession No/ identifier	Primer's Sequence (5' → 3')	PCR product length
<i>MCA1</i> -F <i>MCA1</i> -R	KU739085.1	AGCTCGGTTACGAAAAGCA CGAACGTGTCGTCTGATGTT	121
<i>NUC1</i> -F <i>NUC1</i> -R	MF113057	GCGGGTTTTCAAGTATGGA TTTCAGGAATGGCTTCATCC	192
<i>RDN5.8</i> -F <i>RDN5.8</i> -R	AB032177.1	CTTGGTCTCGCATCGATGA GGCGCAATGTGCGTTCA	98

Table 2. Effects of caspofungin on early apoptosis, late apoptosis, and necrosis, as determined by annexin V and propidium iodide staining

Caspofungin Conc (µg/ml)	% of cells ^a		
	Annexin V+/PI- (early apoptosis)	Annexin V+/PI+ (late apoptosis/necrosis)	Annexin V-/PI+ (necrosis)
Control	0.13±0.006	0.4±0.03	17.61±0.4
0.25	0.46±0.05	14.21±0.7	61.26±0.5
0.5	0.56±0.2	16.48±0.4	71.26±0.1
1	0.86±0.04	15.3±0.6	71.26±0.6

Table 3. Expression pattern of apoptosis genes in standard isolate of *C. glabrata*

Gene	Type	Reaction Efficiency	Expression	Std. Error	95% C.I.	^t P(H1)	Result
RDN5.8	^b REF	1.0	1.000				
MCA1	^a TRG	1.0	4.547	4.067 - 5.111	3.829 - 5.409	0.000	UP
NUC1	TRG	1.0	12.338	10.820 - 14.211	9.944 - 15.350	0.000	UP

Ratio of the expression of two apoptotic major genes. *MCA1* and *NUC1* are representative for caspase-dependent and caspase-independent apoptosis induction in yeast, respectively.

Cell apoptosis determined by Annexin V-FITC staining.

Flow cytometer analysis of the apoptotic and necrotic cells (Q1: necrotic; Q2: late apoptotic; Q3: live; Q4: early apoptotic) after 24 hours of incubation with the 0.25 (A), 0.5 (B) and 1(C)µg/ml of caspofungin. Results are expressed as means ± standard deviation (n=3).

Chart 1. Effects of different concentration of caspofungin on early apoptosis, late apoptosis, and necrosis, as determined by annexin V and propidium iodide staining after 18 hr of incubation