Nat. Volatiles & Essent. Oils, 2021; 8(4): 1805-1815

Effect of Fluconazole on HWP1 and PLB Genes Expression and Antifungal Resistant Status in Candida albicans

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Abstract:

This study aims to consider the impacts of Fluconazole on HWP1 and PLB genes in Candida albicans. Six *Candida albicans* isolates were included In this study; three isolates were obtained from clinical cases of systemic Candidiasis, while the other three isolates were collected randomly from the mouth of healthy children aged 3-12 years old; all isolates were grown in two different conditions with and without Fluconazole for 24 hours (The sensibility dose for Fluconazole was detected by the MIC method). The Vitek 2 Compact system was used to recognize the antifungal sensitivity before and after exposure to the Fluconazole, and the qPCR technique was used to detect gene expression levels of HWP1 and PLB genes in these conditions.

Data showed that all sensitized *Candida albicans*isolates to Fluconazole exhibited no statistical differences from non-sensitized isolates and no difference between clinical and non-clinical isolates. Still, a significant increase in the MIC cut point was noticed for Fluconazole, Voriconazole, Caspofungin and Amphotericin-B but not Micafungin andFlucytosine. On the other hand, evident overexpression levels of the HWP1 genes (2.95 fold-up)and PLB genes (5.9 fold up) was noticed in the sensitized isolates to the Fluconazole.

This study indicates that exposure of *C. albicans* isolates to a low concentration of Fluconazole leads to an overexpression of HWP1 and PLB genes as two crucial virulence genes in *C. albicans.* At the same time, Fluconazole increased the MIC for Fluconazole, Voriconazole, Caspofungin and Amphotericin-B but not Micafungin and Flucytosine.

Keywords: HWP1 gene, PLB genes, Fluconazole, Antifungal agents.

Introduction:

Candida albicans are a common cause of fungal infections in humans, causing a 75% mortality rate, especially in immunocompromised peoples worldwide (Brown et al., 2012; Xiao et al., 2016). C. albicans colonization occurs due to adhesion to host epithelial cells through a complicated process involving many variables and receptor proteins(Jabra-Rizk et al., 2016).

HWP1 is one of the most critical genes responsible for the adhesion process, whose expression is induced by physical contact between the fungal cell and epithelial cells. It encodes hyphal wall protein 1 (Hwp1), a fungal cell wall mannoprotein specific for germ-tubes and hyphal forms, which on molecular mimicry, it forms a stable complex with transglutaminase active on the host epithelial surface (Wächtler et al., 2011). Hwp1 adhesion (the first known protein required in the process of biofilm formation) is regulated by Kex2 endoproteinase, which itself also regulates the activity of C. albicans proteinases, indicating its role for the fungus in providing virulence and drug resistance(Nobile et al., 2006;Modrzewska & Kurnatowski, 2015). Microbial analysis has indicated that azole antifungal drugs effectivelyC. albicans hyphae formation(Calabrese et al., 2013;Liu et al., 2017). Likewise, a role for HWP1 in C. albicans hyphal growth had been demonstrated by adhesins to host epithelial cell receptors(Moyes et al., 2015;da Silva Dantas et al., 2016;Desai, 2018).

Candida albicans produce a range of extracellular hydrolytic enzymes that are vital for establishingC. albicans infections as phospholipases(Khan et al., 2010). This enzyme can lead to impairment or even rupture of the host cell membrane, necessary for adherence and invasion (De Luca et al., 2012). It has been found that phospholipases activity is usually elevated during tissue damage because these enzymes are responsible for the hydrolysis of one or more ester linkages of glycophospholipids of the host cell membranes (Sardi et al., 2013). The family of the secreted phospholipases is classified into four different classes (PLA to PLD) based on the enzyme activity to cleave a specific ester bond in the phospholipid molecule (Barman et al., 2018). This action affects the stability of the host cell membranes and eventually causes cell lysis (Pereira et al., 2015). In C. albicans, Only five members from the B-class (PLB1-5) are secreted, andtheir expression is regulated by environmental signals, which have been described as having a virulence role in animal modelsCandidiasis (Mayer et al., 2013).

Excessive abuse of some antifungal agents (as Fluconazole) may lead to the development of resistance to the antifungal agents or may increase the virulence of C. albicans, which is considered as a part of human and animal microflora and may become a serious problem in our community.The present study aimed to evaluate the low concentration of Fluconazole on developing an antifungal-resistance status and HWP1 and PLB genes expression as vital virulence genes in C. albicans.

Methods:

This study included six C. albicans isolates; threeisolates were obtained from clinical cases of systemic Candidiasis from a previous study in Iraq (Samaka et al., 2018), while the other three isolates were collected randomly from volunteered healthy peoples. The collected swabs were streaked directly on the Sabouraud dextrose agar plates (HiMedia Laboratories Pvt. Ltd., India) and CHROMagar-Candida plates (Rambach, France) incubated at 25°C for 48–72 hours. Vitek2 compact system was used for the final identification of study isolates.

Sensitization of the isolates to Fluconazole:

All study isolates were grown overnight in brain-heart broth at 25C^{o} and then grown in two different ways for 24hours: on brain-heart broth without any additives and on brain-heart broth with a sensitized dose of Fluconazole. The minimum inhibition concentration for the Fluconazole was measured to detect the Fluconazole sensitized dose using the HiComb MIC test Kit. Vitek2 compact system was used for checking the antifungal sensitivity of the study isolates to a set of antifungal agents before and after exposure to the Fluconazole.

RNA Extraction and qPCR technique:

RNA extraction and RNA to cDNA was done for all study isolates after grown in the two conditions (described above) using SV Total RNA Isolation System from (Promega, USA) and GoTaq® 1-Step RT-qPCR System (Promega, USA) for reverse transcription.

Primers and qPCR protocols from Samaranayake et al., 2013were followed to analyze HWP1 and PLB gene expression using EFB as a reference gene (Samaranayake et al., 2013); data were analyzed quantitative using the Livak method (Livak & Schmittgen, 2001).

Results:

All C. albicans isolates were susceptible to the tested antifungal agents after exposure to the Fluconazole. However, it isstill susceptible to the tested antifungal agents, but the MIC cut-point for Fluconazole, Voriconazole, Caspofungin and Amphotericin-B increased significantly ($p<0.05$), whileno change in the NIC cut-points for Micafungin and Flucytosine.(Tables 1)

Tables 1: The susceptibility of C. albicansisolates to a set of antifungals before and after sensitizing with Fluconazole

Sample	Fluconazle		Voriconazole		Caspofungin		Micafungin		Flucytosine		Amph-B	
	MIC	MIC+F	MIC	MIC+F	MIC	MIC+F	MIC	MIC+F	MIC	MIC+F	MIC	MIC+F
1	≤0.1	≤1	≤1	≤0.12	≤0.1	≤0.25	≤0.	≤0.6	≤1	≤1	1	≤0.2

	2				2		6					5
2	≤0.12	≤1	≤1	≤0.12	≤0.12	≤0.25	≤0.6	≤0.6	≤1	≤1	0.5	0.5
3	≤0.12	≤1	≤0.12	≤0.12	≤0.12	≤0.25	≤0.6	≤0.6	≤1	≤1	0.5	0.5
4	≤0.25	≤1	≤0.12	≤0.12	≤0.12	≤0.25	≤0.6	≤0.6	≤1	≤1	0.5	≤0.25
5	≤1	≤0.5	≤0.12	≤0.12	≤0.12	≤0.12	≤0.6	≤0.6	≤1	≤1	1	1
6	≤0.12	≤1	≤0.5	≤0.12	≤0.12	≤0.25	≤0.6	≤0.6	≤1	≤1	1	0.5
Mean ± Std.Error	0.12 ± 0.048	1± 0.543*	0.132 ± 0.048	0.743 ± 0.218*	0.22 ± 0.53	0.1533 ± 0.055*	----	----	----	----	0.75 ± 0.272	0.5± 0.273*

*p< 0.0

Expression of HWP1 and PLB genes:

The results indicate a higher rate of HWP1 gene expression (three-fold up) in Fluconazole-sensitized isolates with a significant difference ($p<0.05$) compared to non-sensitized isolates. (Figures 1). Likewise, the results indicate a higher rate of PLB gene expression (five-fold up) in sensitized isolates with a significant difference at the level of expression ($p<0.05$) compared to non-sensitized isolates. (Figures 1)

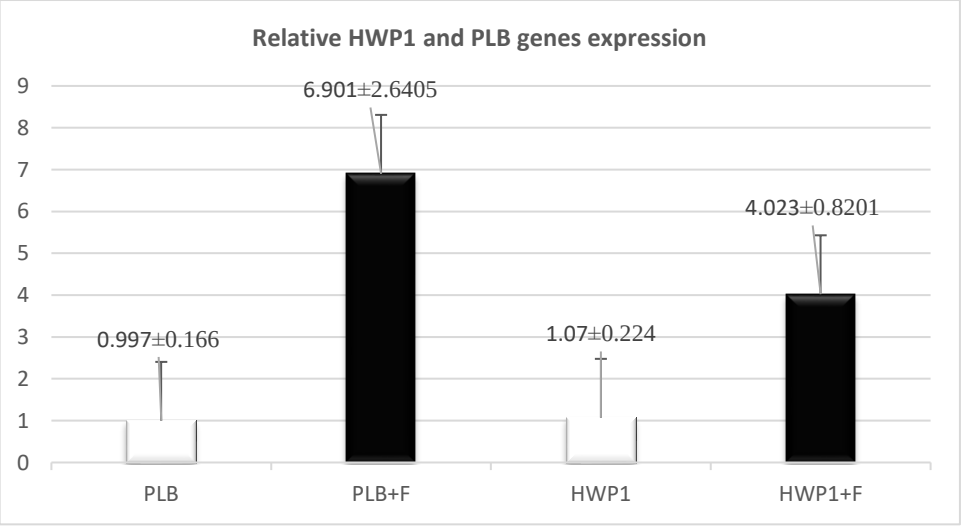

Figure 1: Histogram of the relative gene expression of HWP1 andPLB gene in experimental parameters relative to the sensitized isolation group.

Discussion

All study isolates from different sources exhibited susceptibility to the set of antifungals used in this study. Still, the sensitized isolates showed a significant increase in the MIC for Fluconazole, Voriconazole, Caspofungin and Amphotericin-B but not Micafungin and Flucytosine.These antifungals functionally converted to metabolites that inhibit fungal DNA and protein synthesis in different fungi (Vandeputte et al., 2012;Fang et al., 2017). While, Micafungin target a membrane-bound enzyme, squalene epoxidase, involved in converting squalene into squalene 2,3-epoxide(Campoy & Adrio, 2017). Thus, the action of both antifungals is far from the effect of the action of Fluconazole.

Fluconazole targeting 14 alpha-demethylase, an essential enzyme in the ergosterol biosynthetic. The azole-resistant mechanisms, including alteration of ERG11 gene expression(Jia et al., 2016), which encodes the 14α-demethylase enzyme, and an upregulated expression of this gene in biofilm C. albicans isolates may explain their resistance to azole(García-Sánchez et al., 2004;Jia et al., 2016). Intensive and long-term use of antifungals leads to a decline in the sensitivity and resistance development of Candida albicans isolates(Samaka et al., 2018). Jia et al. (2016) explain a synergistic relationship between the Erg11 gene and a group of genes like ALS3, CDR1, MDR, ERG11 and HWP1genes, while PLB was not mentioned. However, it is associated with one of the genes mentioned by the scientist as they work together to facilitate penetration of the fungus to cause tissue damage, iron acquisition, and dissemination within the host. In addition, the secreted hydrolytic enzymes help the fungal pathogen overcome and render the host immune system ineffective (Tsai et al., 2013).

The sensitized isolates showing a clear up-expression in HWP1 and PLB gene then non-sensitized isolates. The reason for the lack of sensitivity to Fluconazole may be due to its use for long periods or in low concentrations, as it affects the gene expression of Erg11, which have a synergistic effects with some virulence genes(Jia et al., 2016), including the genes of our study, and this may explain the increase in expression of virulence genes in our study and someother studies support this finding(Shahriari et al., 2020). While other study disagreement with our studywhich indicate a high efficacy of Fluconazole to inhibit biofilm formation which was linked to downregulated HWP1 gene and PLB gene expression in C. albicans.(Khodavandi et al., 2011;Li et al., 2016)

Conclusion:

The Fluconazole led to an increase in the HWP1 and PLB genes expression and increased the MIC cut-point of C albicanssignificantly and may interfere with the virulence of this microorganism.

Acknowledgement

This study was carried out in the laboratories of the Faculty of Veterinary Medicine at the University of Kufa, Iraq

Authors' Contributions

S. H.M. and S. A.A. contributed together to the design and implementation of the research, analysis of the results, and preparation of the manuscript.

Conflict of Interest

The authors declare that there were no conflicts of interest in the authorship or publication of this research.

Financial Disclosure

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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