In vitro MIC of Itraconazole Against Different Isolates of *Candida albicans*

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Abstract

In vitro antifungal susceptibility test of itraconazole was carried out against 38 isolates from nails, skin, oral cavity, vagina and wounds, This study was done in Ramadi Teaching Hospital in period from January to August 2010. According to the National Committee for Clinical Laboratory Standard (NCCLS) M 27- A by using the broth dilution method. Inoculum size was 1-5X10^3 CFU/ ml, while final concentrations of itraconazole ranged from 0.025 – 6.4 µg / ml by using RPMI – 1640 broth media and the fungus was incubated at 35 ^oC. No resistant stain was recorded. MIC ranged from 0.05 – 6.4 µg / ml and the Mean ± SEM was 0.89 ± 0.28. MIC for nail isolates was 0.05 – 6.4 µg/ ml, for skin isolates it was 0.05 – 6.4 µg / ml, for oral cavity isolates it was 0.05 – 0.4 µg/ ml, for vagina isolates it was 0.1 – 6.4 µg / ml and for wound isolates it was 0.1 – 1.6 µg/ ml. MIC_{50} was 0.2 µg / ml and MIC_{90} was 1.6µg / ml. Itraconazole acts as fungistatic more than fungicidal agent; in 25(65.8%) isolates it acts as fungistatic and as fungicidal in 13 (34.2 %) isolates.

Key words: Itraconazole, *C. albicans*.

الخلاصة

دواء الاتراكنازول من الادوية واسعة الطيف المضادة للفطريات. تم فحص حساسية ٣٨ عزلة من فطر المبيضات Candida albicans (٨ عزلات من الاظافر، ١٠ من الجلد ، ١٠ من التجويف الفموي ، ٥ من المهبل ، ٥ من الجروح) لهذا الدواء خارج الجسم بواسطة المثبط الادنى للنمو (MIC) وبطريقة التخفيف في الوسط السائل حسب (NCCLS). لم تظهر العزلات مقاومة لدواء الاتراكنازول. تراوح المثبط الادنى للنمو من ٠,٠٥–٦,٤ مايكروكرام/ مل، MIC_{50} يساوي ٠,٢ مايكوكرام/ مل، MIC_{90} يساوي ١,٦ مايكوكرام/مل وعمل الدواء كمثبط للنمو في ٢٥ عزلة وقاتل للفطر في ١٣ عزلة .

Introduction

Itraconazole is a broad spectrum azole antifungal agent. It is a synthetic triazole drug. [(1)] The antifungal activity of azole drugs results from reduction of ergosterol synthesis by inhibition of fungal cytochrome P450 enzymes. The selective toxicity of azole drugs results from their greater affinity for fungal than for human cytochrome P450 enzymes. [(2)] The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent that inhibits growth of a particular test organism.[(3)] Candida albicans (*C. albicans*) is a classic opportunistic pathogen.[(4)] It is a common inhabitant of the human gastrointestinal, genitourinary tract and skin and become pathogenic causing lesions in the skin, nail and mucous membrane. *C. albicans* is dimorphic; in addition to yeast and pseudohyphae,it can produce true hyphae. On agar media, candida grows as oval budding yeast cells (3-6 µm in size), soft white to cream – colored colonies at 37 ^oC for yeast form for 1- 3 days and 1- 2 weeks for hyphae form at 25- 30 ^oC [(5 ,6)].The aims of the present study were (1) to determine *in vitro* activity (MIC) of itraconazole against different clinical isolates of *C. albicans* (nails, skin, oral cavities, vagina, wounds) by using broth dilution method.(2) to determine if there is resistance to itraconazole from different clinical isolates.(3) to determine whether the itraconazole act as fungistatic and / or fungicidal.

Materials and Methods

38 isolates of *C. albicans* were taken from different infected sites like 8 isolates from the nails (5 females, 3 males) with the age ranged from 20 – 45 years. 10 isolates from the skin (7 females, 3 males) with the age ranged from 29 – 40 years. 10 isolates from the oral cavities (5 females, 5 males) with age ranged from 2 – 8 years. 5 isolates from vagina (5 females) with the age ranged from 23 – 38 years and 5 isolates from the wounds (2 females, 3 males) with the age ranged from 18 – 32 years. Excluded all isolates were taken from patients that received antifungal drugs before this study. *C. albicans* was identified by growth rate, morphology of the organism and germ tubes test by incubation in serum for about 90 minutes at 37 ^oC, yeast cell of C.albicans will begin to form true hyphae or germ tubes. [(7,8)] .

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Antifungal susceptibility test was done by broth dilution method according to the (NCCLS) M 27- A, a reference procedure for testing yeast form. The quality control (QC) in this study was *C. albicans*. MIC of QC was repeated 7 times (0.2 µg / ml). According to the ATCC90028, the MIC of itraconazole for *C. albicans* was 0.25 – 2 µg / ml. Itraconazole (Janssen pharmaceuticals Beers, Belgium) 3mg was dissolved in 1ml of dimethylsulfoxide (3000 µg /ml), then 150 µg/ml was prepared by adding 1ml of stock (3000 µg/ml) to 19 ml of RPMI- 1640 media. Then 51.2 µg / ml (1ml of 150 µg /ml to 1.93 ml of RPMI– 1640), 25.6µg /ml (1ml of 150 µg /ml to 4.86 ml of media) were prepared. From 12.8 µg /ml (1ml of 150 µg /ml to 10.7 ml of media), we can prepare (0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3. 2, 6.4 µg/ ml) by serial dilution method. Inoculum size of fungus was 1- 5 X 10^3 cell forming unit (CFU), broth media was RpMI -1640 (Sigms, St. Louis, Mo.), which was prepared by picking 5 colonies from pure culture, at least 1mm in diameter, then 5 ml of sterile normal saline (Nacl 0.9 %) was added, mixed and standardized to 0.5 Mcfarland scale (1- 5 X10^6 CFU/ ml) by adding normal saline to the suspension until the visually match between suspension and Macfarland tube occur. After that, add 1ml of suspension to 9 ml of RPMI-1460 broth media to obtain (1-5X 10^5 CFU /ml), by 1: 10 dilution obtained (1- 5 X10^4 CFU/ml) and finaly (1- 5 X10^3 CFU/ml) for the quality control and test organism.[9,10,11] .Three quality controls were used, (control: 1) consisted of only RPMI – 1640 broth media, (control: 2) consisted of 1ml of RPMI – 1640 media and 50 µl of final inocula, (control:3) consisted of 50 µl of inocula into tubes containing solvent (dimethylsulfoxide) in RPMI– 1640 media by the same method of drug preparation.The same procedure was used for preparing inoculum size, drug concentrations to QC and test organisms. [12]. Take 10 test tubes and add 1 ml of 12.8 µg /ml of drug to the tube No.10, and by serial dilution prepare 9test tubes with drug concentrations (0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 µg /ml) then add 50 µl of inoculum to all MIC tubes, incubate at 35^{o}C for 24 hours or 48 hours. At the end point, MIC was read visually to detect whether the fungus was susceptible or resistant to itraconazole. Take 0.01 ml from MIC tube directly after reading and culturing on the Sabourauds dextrose agar and incubate at 35C^o for 24 hours or 48 hours to detect whether the drug acts as fungistatic (MIC) or fungicidal (MFC) according to the number of colonies. [13,14,15]

Results

38 isolates from nails, skin, oral cavity, vagina and wounds were susceptible to itraconazole and no resistant to the drug. MIC ranged from 0.05– 6.4 µg /ml, MIC_{50} was 0.2 µg /ml and MIC_{90} was 1.6 µg /ml with Mean ± SEM o.89 ± o.28 (table 1). MIC of nail isolates were 0.05 –6.4 µg /ml, skin isolates were 0.05 – 6.4 µg /ml, oral cavity isolates were 0.05 – 0.4 µg /ml, vagina isolates were 0.1 – 6.4 µg /ml and wound isolates were 0.1 – 1.6 µg /ml (table 2). This drug acted as fungistatic (MIC) in 25(65.8 %)isolates and fungicidal (MFC) in 5 (13.2 %) isolates as complete fungicidal and incomplete fungicidal in 8 (21%) isolates. Itraconazole acts as fungistatic agent in 7, 6, 7, 2, 3 isolates from nails, skin, oral cavity, vagina and wounds, respectively. Also itraconazole acts as fungicidal agent (MFC) in 1, 4, 3, 3, 2 isolates from nails, skin, oral cavity, vagina and wounds, respectively (table 2).

Table 1: MIC of Itraconazole against yeast form of 38 isolates of *C. albicans*

No.of isolates	Inoculum size Cfu/ml	Range of drug concentration µg/ml	MIC rang	Mean ± SEM	MIC_{50}	MIC_{90}
Nails: (8) (1 – 8) Skin: (10) (9- 18) Oral cavity: (10) (19-28) Vagina: (5) (29-33) Wounds: (5) (34-38)	1-5x 10^3 1-5x 10^3 1-5x 10^3 1-5x 10^3 1-5x 10^3	0.025-6.4	0.05-6.4	0.89±0.28	0.2	1.6

Table 2: MIC of Itraconazole for 38 isolates of *C. albicans*.

Isolate number	MIC µg/ml	No. of Colonies*	Fungistatic (M IC)*	fungicidal (M F C)*	
				complete	Incomplete
1	0.05	7	0.05		
2	0.2	9	0.2		
3	6.4	2			6.4
4	0.2	6	0.2		
5	0.2	7	0.2		
6	0.1	10	0.1		
7	0.05	13	0.05		
8	0.2	8	0.2		
9	0.4	7	0.4		
10	6.4	0		6.4	
11	0.8	6	0.8		
12	3.2	6	3.2		
13	6.4	2			6.4
14	0.8	3			0.8
15	0.2	6	0.2		
16	1.6	0		1.6	
17	0.2	6	0.2		
18	0.05	9	0.05		
19	0.05	10	0.05		
20	0.05	9	0.05		
21	0.2	6	0.2		
22	0.2	6	0.2		
23	0.2	2			0.2
24	0.2	2			0.2
25	0.4	0		0.4	
26	0.05	10	0.05		
27	0.05	12	0.05		
28	0.05	8	0.05		
29	0.1	2			0.1
30	6.4	0		6.4	
31	0.2	3			0.2
32	0.1	8	0.1		
33	0.8	7	0.8		
34	0.8	3			0.8
35	0.8	9	0.8		
36	1.6	0		1.6	
37	0.2	7	0.2		
38	0.1	8	0.1		
			25 65 . 8 %	5 13 . 2 %	8 21 %

* M IC = minimum inhibitory concentration
* M F C = minimum fungicidal concentration
* No. of Colonies = 0 (complete fungicidal) ,5 or Less (incomplete fungicidal), More than 5 (Fungistatitic)

Discussion

MIC of itraconazole against *C. albicans* was 0.05 – 6.4 µg / ml. MIC_{50} was 0.2 µg / ml which means that the MIC value of the strains that appeared in the 50 % of isolates, MIC_{90} was. 1.6 µg /ml which means the MIC value of the strains that appeared in the 90 % of isolates.[16,17,18]. Itraconazole acts as fungistatic (MIC) in 25 isolates which means that the minimum concentration of drug inhibits growth of fungus while it acts as fungicidal (MFC) in 13 isolates which means that the minimum concentration of drug can kill the fungus.[19,20] . The present study recorded different values of MIC of itraconazole against different isolates of *C. albicans* because *C.albicans* is part of normal human flora, thus candidiasis represents opportunistic infection and many antigens have been characterized, including secreted proteases, an immunodominant enolase and heat shock proteins. Also predisposing factors for candidiasis are preceding surgery, immunosuppression, intravenous catheters, prolonged administration of antimicrobial agents and burns.[8,21,22] . Many studies reported different values for the MIC of itraconazole against *C. albicans* (0.002-32 µg/ml ,0.03-16 µg/ml and 0.25-0.5 µg/ml)[12,23,24] .Moreover differences in the site for obtaining the isolate lead to differences in their response to the effect of Itraconazol , i.e. different MIC values[25-27] . In conclusion, the results of the present *in vitro* study suggest that itraconazole has good activity against *C. albicans* by using the procedures recommended by NCCLS. Further assessment of the correlation of these MIC endpoints and the efficacy of this drug *in vivo* should be accomplished.

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