Acta Scientiarum. Health Sciences
ISSN: 1679-9291
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Universidade Estadual de Maringá
Brasil

dos Santos Oliveira Cunha, Maria da Conceição; de Almeida Filho, Tarcísio Paulo; Afrânio Cunha, Francisco; da Silva Farias, Ana Gesselena; Pereira de Souza, Ieda; Albuquerque Menezes, Everardo
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Acta Scientiarum. Health Sciences, vol. 39, núm. 2, julio-diciembre, 2017, pp. 129-131
Universidade Estadual de Maringá
Maringá, Brasil

Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9291
ISSN on-line: 1807-8648
Doi: 10.4025/actascihealthsci.v39i2.32413

In vitro synergistic activity of lidocaine and miconazole against *Candida albicans*

Maria da Conceição dos Santos Oliveira Cunha[1-2*], Tarcísio Paulo de Almeida Filho[1], Francisco Afrânio Cunha[1], Ana Gesselena da Silva Farias[2], Ieda Pereira de Souza[1] and Everardo Albuquerque Menezes[1]

*[1]Departamento de Análises Clínicas e Toxicológicas, Universidade Federal do Ceará, Rua Capitão Francisco Pedro, 1210, 60270-430, Rodolfo Teófilo, Fortaleza, Ceará, Brazil. [2]Universidade da Integração Internacional da Lusofonia Afro-Brasileira, Acarape, Ceará, Brazil. *Author for correspondence. E-mail: cecinhya@gmail.com*

ABSTRACT. *Candida albicans* is the main yeast isolated from vulvovaginal candidiasis(VVC) and a major antifungal used to treat VVC is miconazole (MZ), it shows local toxic effects, such as irritation and burns. The lidocaine (LD) is a local anesthetic. The aim of this study was to evaluate the synergistic activity of LD/MZ against 19 strains of *C. albicans* isolated from vaginal secretion. 78.9% of the strains were susceptible to the combination LD/MZ, demonstrating synergism of drugs. These drugs can be used to produce vaginal creams to treat VVC, especially drug resistant.

Keywords: vulvovaginal candidiasis, antifungal agents, local anesthetic.

Atividade sinérgica *in vitro* de lidocaína e miconazole contra *Candida albicans*

RESUMO. *Candida albicans* é a principal levedura isolada de candidíase vulvovaginal (CVV) e o principal antifúngico usado para tratar a VVC é miconazol (MZ), que apresenta efeitos tóxicos locais, tais como irritação e queimaduras. A lidocaína (LD) é um anestésico local. O objetivo deste estudo foi avaliar a atividade sinérgica de LD/MZ contra 19 cepas de *C. albicans* isoladas de secreção vaginal. Foram suscetíveis à combinação LD/MZ, 78.9% das cepas testadas, demonstrando sinergismo de drogas. Estes fármacos podem ser utilizados para a produção de cremes vaginais para tratar VVC, especialmente aquelas resistentes aos fármacos disponíveis.

Palavras-chave: candidíase vulvovaginal, agentes antifúngicos, anestésico local.

Introduction

Candida albicans is the leading yeast involved in fungal infections in the world, though other species start to detach, this species still remains isolated from samples of urine, blood, secretions, among other biological samples (Quindós, 2014).

The treatment of infections caused by *C. albicans* is performed using systemic and topical antifungal, mainly from the group of azole drugs, the main representatives are ketoconazole, miconazole, itraconazole, fluconazole, voriconazole and posaconazole, which have the same mechanism of action, inhibiting the synthesis of ergosterol. The foremost differences of this group of drugs are pharmacokinetic and toxicity (Guinea et al., 2014).

C. albicans is the most commonly isolated yeast vulvovaginal candidiasis (VVC). Treatment of VVC is usually accomplished by use of antifungal creams. The miconazole (MZ) is the active ingredient in vaginal cream used in the treatment of (VVC) and *Candida* spp. already show reduced sensitivity to this drug (Liu, Fan, Peng, & Zhang, 2014; Sobel, 2013).

Lidocaine (LD) is an anesthetic used in clinical medicine, which showed antifungal activity has already been described, alone or in synergy with antifungal agents like amphotericinB, itraconazole, voriconazole, and caspofungin, however it is important to emphasize that the use must be topical due to toxicity (Judd & Martin, 2009; Palmeira-de-Oliveira et al., 2012; Rodrigues, Araujo, & Pina-Vaz, 2006).

The aim of this study was to evaluate *in vitro* synergy between lidocaine(LD) and miconazole (MZ) against *C. albicans*, this combination has not been tested in previous works.

Material and methods

In this study, 19 strains of *Candida albicans* isolated from vaginal secretion cultures were selected, these strains are the most commonly isolated fungal infections in Ceará, Northeast Brazil. The identification was performed by micromorphology on rice agar Tween 80, germ tube

production, fermentation and assimilation of carbohydrates and molecular biology (Menezes, Cunha, & Cunha 2009).

The test sensitivity and synergism of LD and MZ was performed according to the protocol M27-A3 by method the microdilution in broth RPMI (pH 7.0), and by checkerboard method, the range of LD used was 2,500 to 15.0 μg mL^{-1} and MZ 32.0 to 0.015 μg mL^{-1}. The MIC was the lowest concentration that inhibited 50% of fungal growth. The tests were performed with drugs alone (MZ and LD) and also combined at fixed concentrations. The synergistic effect of MZ and LD was calculated based FICI (fractional inhibitory concentration index), (FICI = $[MZC].[MZA]^{-1} + [LDC].[LDA]^{-1}$), where [MZC] and [LDC] are the concentrations of miconazole and lidocaine that showed action when combined. [MZA] and [LDA] are the concentrations of the same drugs acting alone. The interpretation of results was performed according to the value of FICI < 0.5, synergism (SYN), 0.5 < FICI < 4.0 - Indifferent (IND) and FICI > 4.0 – antagonism (ANT) (Menezes et al., 2012; Menezes, Vasconcelos Júnior, Ângelo, Cunha, & Cunha, 2013).

Results

The Table 1 shows the results of the synergy between MZ and LD, 78.9% of the strains have sensitivity to combination of drugs and effect synergic was found.

Table 1. Synergistic Effect of miconazole (MZ) and lidocaine (LD) against *Candida albicans.*

Strain	MZ[a] MIC_{50} (μg mL^{-1})	LD[a] MIC_{50} (μg mL^{-1})	MIC MZ+LD (μg mL^{-1})	FICI MZ + LD	Int
C. albicans	1.0	2,500	025/15	0.26	SYN
C. albicans	2.0	2,500	0.50/500	0.45	SYN
C. albicans	0.50	2,500	0.25/15	0.50	SYN
C. albicans	1.0	2,500	0.25/250	0.35	SYN
C. albicans	0.25	2,500	0.12/125	0.55	IND
C. albicans	0.50	2,500	0.03/500	0.26	SYN
C. albicans	0.50	2,500	0.06/250	0.22	SYN
C. albicans	0.50	2,500	0.25/15	0.53	IND
C. albicans	1.0	2,500	0.25/15	0.26	SYN
C. albicans	1.0	2,500	0.25/30	0.26	SYN
C. albicans	2.0	2,500	0.50/30	0.26	SYN
C. albicans	1.0	2,500	0.25/250	0.35	SYN
C. albicans	0.50	600	0.25/15	0.53	IND
C. albicans	0.50	600	0.25/15	0.53	IND
C. albicans	0.50	2,500	0.015/15	0.04	SYN
C. albicans	0.06	600	0.015/30	0.30	SYN
C. albicans	0.50	600	0.03/250	0.48	SYN
C. albicans	0.25	300	0.06/15	0.29	SYN
C. albicans	0.25	2,500	0.015/125	0.11	SYN

[a]MZ–Miconazole, LD–Lidocaine. Int- interpretation.

The LD alone has antifungal action only at high doses, but when combined with MZ concentrations fall too, which shows the potential of this combination. The antifungal properties of anesthetics had already been presented in previous works (Judd & Martin, 2009; Wright, Durieux, & Groves, 2008).

Discussion

Fungal infections are increasing worldwide, affects all age groups, but especially seriously ill patients, HIV patients, transplant recipients and those using corticosteroids and they are associated with increased morbidity and mortality. Another important factor is fungal resistance a phenomenon has been detected in various parts of the world and worries, because the amount of antifungal agents available is very limited (Delaloye & Calandra, 2014; Dimopoulos, Antonopoulou, Armaganidis, & Vincent, 2013; Paramythiotou, Frantzeskaki, Flevari, Armaganidis, & Dimopoulos, 2014).

C. albicans is yeast that has several virulence factors and is the main agent isolated from VVC, with about 70-75% of the isolates. Antifungal drugs available for the treatment of VVC are generally for topical use and may cause local toxic effects, such as burns and irritation, in addition, the sensitivity of the strains to these drugs is becoming less (Liu et al., 2014). In our study, we used 19 strains of *C. albicans* isolated from vaginal secretion cultures; samples were identified and used in the tests.

Drugs without antifungal effects known have been associated with traditional antifungal agents with the goal of increasing their activity and diminish the toxic effects. The various associations tested the most common are those where fluconazole is used as a prototype (Liu et al., 2014; Stylianou et al., 2014). In our work we used a widely used antifungal miconazole and rarely described when evaluating synergy.

LD is a drug used as a local anesthetic and has no antifungal indication and its association with miconazole shows that this drug can be potentiated in the presence of an antifungal agent (Table 1). Due to some mechanism not yet fully elucidated synergy occurs between LD and MZ (Palmeira-de-Oliveira et al., 2012).

In a study using 10 strains of *Candida* spp. was evaluated and observed the antifungal effect of lidocaine; however the synergistic antifungal effect was not assessed (Palmeira-de-Oliveira et al., 2012). We observed an antifungal effect of LD alone and combined with the MZ (Table 1).

Conclusion

In conclusion, the synergistic activity of the combination of LD and MZ was observed *in vitro*, new studies with a larger number of strains should

be used to confirm the results, and this combination could be an alternative in the production of vaginal cream for the treatment of VVC.

Acknowledgements

The financial support received from the CNPq, Capes and Funcap (Brazilian agencies).

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Received on June 26, 2016.
Accepted on November 17, 2016.