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Research Article

In Vitro Anti-*Candida* Activity of Lidocaine and Nitroglycerin: Alone and Combined

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The aim of this work was to study the anti-*Candida* activity of lidocaine and nitroglycerin alone and in combination. Ten *Candida* strains were included, corresponding to 1 collection type strain (ATCC 10231) and 9 clinical isolates: 4 *C. albicans*, 2 *C. glabrata*, 1 *C. tropicalis*, 1 *C. krusei*, and 1 *C. parapsilosis*. The CLSI reference M27-A3 micromethod was used to determine the anti-*Candida* activity of the drugs alone; minimal inhibitory and lethal concentrations were determined. The classic checkboard technique was used to determine the activity of combined drugs. Lidocaine fungicidal effect was dosedependent. Nitroglycerin exhibited a higher effect. The drugs combination resulted in a reduction of the inhibitory concentration, corresponding to an additive effect. In conclusion, both drugs exhibited an interesting anti-*Candida* activity. The combination of lidocaine with nitroglycerin was shown to have an additive effect against *Candida* spp., predicting the interest to include, in the future, these drugs in a new delivery system for the treatment of mucocutaneous candidosis.

1. Introduction

Candida spp. are microorganisms frequently found in the human oral cavity, gastrointestinal tract, and vagina [1–4]. Among mucocutaneous infections, vulvovaginal candidosis (VVC) is the second most frequent vaginal infection, after vaginal bacteriosis and is one of the most common clinical diseases caused by *Candida* spp. It affects 70–75% of women at least once in their lifetime while 40–50% of them will experience a recurrence; 5–8% of adult women develop recurrent vulvovaginal candidosis (RVVC), defined as four or more episodes within a year [3–7]. The main goal of VVC treatment is the control and immediate relief of its signs and symptoms, related to vulvovaginal inflammation, as quickly as possible and the mycological cure to be confirmed some days later; recurrence prevention is also pursued [8]. VVC is usually treated very effectively with azoles, which are present in the most prescribed therapeutic regimens, unless a suspected or confirmed azole-resistant *Candida* strain is involved. On the other hand, most gynecologists believe that the control of RVVC requires both systemic and local therapy, also involving new antifungal drugs and strategies [3, 9, 10]. Despite RVVC being considered a *Candida* infection that is more dependent on the host characteristics, therapeutics approaches available and able to allow a remission of the symptoms between episodes are antifungal drugs used for a long period of time [8, 9, 11]. Some authors consider that new therapeutic strategies must be considered for RVVC control [3, 9, 10]. In addition to the limited number of available antifungal drugs, the restrictions to its use stress the need for the development and validation of new therapeutic strategies exhibiting distinct mechanisms of action and/or evasion of resistance [12–15].

Lidocaine is used as anesthetic, and its anti-*Candida* activity has been previously reported as a fungicidal drug exhibiting a dose-dependent effect and related with a

primary lesion of the cytoplasmic membrane [12]. Also its ability to inhibit Candida albicans germ tube formation was reported [16]. Nitroglycerin has been used to treat haemorrhoidal symptoms under an ointment pharmaceutical formulation, commercially available as Rectogesic [17]. An association of lidocaine with isosorbide-di-nitrate has also been proposed for the treatment of anorectal problems [18] on behalf of the expected benefits of combining their individual properties, namely, anaesthetic and blood supply promoter.

As in VVC, irritation leads to excoriations and fissuring [19]. We investigated the *in vitro* anti-*Candida* activity of lidocaine and nitroglycerin, both alone and in combination, in view of its possible future inclusion in a pharmaceutical formulation for the treatment of mucocutaneous candidosis.

2. Materials and Methods

2.1. Chemicals and Drugs. Pure and analytic grade compounds were used to prepare the tested solutions used in this work. A 6% lidocaine solution was prepared by solubilization of lidocaine cloridrate (Sigma-Aldrich, Portugal) in sterile water. Stock solution of 1% glycerol trinitrate (Merck, Germany) was used to prepare the work solution, meaning 0.5% nitroglycerin in RPMI 1640 culture medium (Biochrom AG, Berlin). Serial concentrations of the products were obtained by geometric dilution in RPMI.

2.2. Yeast Strains. A total of 10 *Candida* strains were used, including 5 *C. albicans*, 2 *C. glabrata*, 1 *C. tropicalis*, 1 C. *krusei,*and 1 *C. parapsilosis*. With exception of the type strains *C. albicans* ATCC 10231 from the American Type Culture Collection, all the other strains tested were isolates from patients with RVVC and showed variable degree of resistance to fluconazole (Table 1). Such isolates had been characterized to species level using API ID 32 (BioMérieux, Vercieux, France), and its susceptibility pattern to classic antifungals (fluconazole and amphotericin B) was determined according to the CLSI M27-A3 micromethod. The strains were kept frozen in Brain-Heart Broth (Difco Laboratories, Detroit, MI, USA) with 5% glycerol at −70°C until testing. After thawing, the strains were subcultured twice on Sabouraud agar (Difco) to assure optimal growth (37°C/24 h).

2.3. Anti-Candida Activity. The lidocaine and nitroglycerin anti-*Candida* activity was assessed according to the CLSI reference M27-A3 micromethod protocol [20]. Minimal inhibitory concentration (MIC) values were read visually after 48 h of incubation at 37°C. For each tested concentration, yeast growth was compared with the positive control (growth control). Only the total growth inhibition was considered as MIC. All determinations were performed in duplicate, and only concordant results from three independent experiments were considered.

The modified protocol proposed by Canton et al. [21] was used to determine minimal lethal concentrations (MLCS).

2.4. Anti-Candida Activity of Lidocaine Plus Nitroglycerin. The classical checkerboard methodology, as described by Vitale et al. [22], was used to determine the MIC resulting from the products association. One *C. albicans* ATCC 10231 was included. Briefly, a two-dimensional microplate with 50 μl of each product was prepared; microplates were incubated during 24 h at 37 °C.

MIC for products association was calculated from three independent experiences with concordant results. To evaluate the compound interactions, the fractional inhibitory concentration index (FICI) was calculated as follows: (MIC of Drug A in combination/MIC of Drug A alone) + (MIC of Drug B in combination/MIC of Drug B alone). The interpretation of the FICI corresponds to a synergic effect for values ≤0.5: additive effect when >0.5 but <4.0 and antagonism when ≥4.0 [22].

3. Results and Discussion

Lidocaine and nitroglycerin exhibited antifungal activity upon *Candida* spp. MIC varied from 10 mg/mL to 30 mg/mL for lidocaine and from 0.15 mg/mL to 0.30 mg/mL for nitroglycerin (Table 2). The antifungal susceptibility pattern of the selected strains to classical antifungals, namely, fluconazole and amphotericin B, was unrelated to the tested compounds activity, predicting distinct mechanisms of action.

Lidocaine fungicidal effect was confirmed for concentrations corresponding to at least double MIC, varying from 15 mg/mL to 30 mg/ml, showing a dose-dependent effect. *C. krusei* and *C. albicans* ATCC10231 were the most susceptible strains to lidocaine (MIC 10 mg/mL). Other *C. albicans* exhibited an increased MIC, 15 mg/mL, similar to *C. parapsilosis*. On the other hand, *C. glabrata* and *C. tropicalis* were the less susceptible, having their growth inhibited by lidocaine at 20 mg/mL. Our results are in accordance with other authors that confirm the higher *C. albicans* susceptibility to this drug [12]. However, some MIC and MLC differences were noticed (onefold dilution). This is probably related to the fact that those authors choose the macromethod from the same protocol that we used for the micromethod.

Regarding nitroglycerin, the effect was fungicidal at concentrations able to inhibit *Candida* growth, being MIC and MLC coincident. *C. albicans* was the most susceptible species tested (0.15 mg/mL). Other species exhibited higher MIC values (0.30 mg/mL), coincident with MLC for *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* AP 426: lower to MLC for *C. glabrata* AP 425 (0.6 mg/mL) and *C. krusei* (1.25 mg/ml).

The possible antifungal advantage of the two products association was studied by the *checkerboard* procedure upon *C. albicans*. A reduction effect was evident for both MIC products (lidocaine alone—7,5 mg/mL, lidocaine in combination—5 mg/mL; nitroglycerin alone—0,15 mg/mL, nitroglycerin in combination—0,075 mg/mL) expressed by the resulting FICI value (1.17) that corresponds to an additive effect (>0.5 and <4.0).

Table 1: *Candida* spp. origin and susceptible profile to classic antifungals, namely, fluconazole and amphotericin B. Minimal inhibitory concentrations (MICS) are presented in μg/mL.

Species	Origin	Fluconazol MIC (μg/mL)	Amphotericin B MIC (μg/mL)
C. albicans ATCC 10231	Collection	1	0.5
C. albicans AP440	Vaginal	0.25	0.25
C. albicans AP416	Vaginal	<0.125	1
C. albicans AP437	Vaginal	2	0.5
C. albicans AP439	Vaginal	0.5	1
C. glabrata AP426	Vaginal	32	0.25
C. glabrata AP425	Vaginal	32	0.25
C. krusei AP3	Vaginal	>64	0.5
C. tropicalis AP407	Vaginal	2	1
C. parapsilosis AP11	Vaginal	1	0.5

Table 2: *Candida* spp. susceptibility to nitroglycerin and lidocaine. Minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) are presented in mg/mL.

Species	Nitroglycerin		Lidocaine	
	MIC	MLC	MIC	MLC
C. albicans ATCC 10231	0.15	0.15	10.0	15.0
C. albicans MC440	0.15	0.15	15.0	20.0
C. albicans MC416	0.15	0.15	15.0	20.0
C. albicans MC437	0.15	0.15	15.0	20.0
C. albicans MC439	0.15	0.15	15.0	20.0
C. glabrata MC426	0.30	0.30	20.0	30.0
C. glabrata MC425	0.30	0.625	20.0	30.0
C. krusei OL103	0.30	1.25	10.0	20.0
C. tropicalis MC407	0.30	0.30	20.0	30.0
C. parapsilosis AP11	0.30	0.30	15.0	25.0

The possible pH variation with the addition of both solutions to culture medium was limited by the tampon effect of the RPMI medium. Additionally, the pH value at the drugs most concentrated conditions was measured and confirmed to be between 6.9 and 7.1, guarantying no pH influence on the results.

4. Conclusions

In this study we tested the anti-*Candida* activity of lidocaine and nitroglycerin alone and in combination in view of their use as topical treatment of acute VVC. Nitroglycerin's vasodilator effect may promote healing while at the same time the anesthetic effect of lidocaine may relief pain, burning, and pruritus. Our results show that adding to the well-known therapeutic effects of these two individual drugs an anti-*Candida* activity may also be relevant. The combination of lidocaine with nitroglycerin showed to have an additive effect against *Candida* spp.

In the future, the development of a new delivery system including both lidocaine and nitroglycerin at concentrations higher than MIC values here reported and that allow compounds to produce local effect limiting possible systemic absorption, stands up as an interesting approach for VVC topical treatment. These *in vitro* studies showing their significant antifungal activity must now be followed by *in vivo* studies in animal models, to evidence the efficacy of this cheap and believed to be safe new strategy.

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