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Comparison of Antifungal Activities of Gentian Violet and Povidone-Iodine Against Clinical Isolates of *Candida* Species and Other Yeasts: A Framework to Establish Topical Disinfectant Activities

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Abstract We evaluated antifungal activity as assessed by the contact time in topical use of gentian violet (GV) and povidone-iodine (PI) against *Candida* strains. A total of 102 yeast isolates were used in this study. A markedly lower minimal inhibitory concentration $(MIC)_{90}$ of GV than of PI was detected for all yeast isolates. No remarkable difference in the MICs was observed among the identical strains isolated from different clinical sites for both GV and PI. Although the minimal fungicidal activities (MFCs) of PI were identical for all tested time points, the fungicidal activity of GV decreased during the time course of incubation. These results indicate that, whereas GV is more effective than PI, the topical disinfectant efficacy of GV should be estimated using the $MFC_{5\ min}$ and not the MIC or the $MFC_{24\ h}$ for overall prevention of catheter-related bloodstream infections and oral infections.

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Introduction

Candida species belong to the normal flora of the skin and the gastro-intestinal tract but become opportunistic pathogens of the skin and mucosal when the balance between the host and the commensal fungus is broken in immunocompromised patients [1, 2]. *Candida* species also cause catheter-related bloodstream infections (CR-BSI) and are ranked as the forth most frequent nosocomial bloodstream pathogens [3]. Recently, the prevalence of non-albicans *Candida* species that are resistant to various antifungal drugs has increased [4]. Gentian violet (hexamethyl pararosaniline chloride; GV), a triphenylmethane dye, is effective against Gram-positive cocci as well as numerous types of pathogenic yeast such as various *Candida* species [5, 6]. GV has been used in aqueous solutions at concentrations of 1–10% without significant contact dermatitis [7]. The World Health Organization (WHO) recommends GV as an efficient, cost-effective, and safe topical disinfectant at concentrations of 0.5–1.0% [8]. Whereas several clinical trials of GV in HIV candidiasis demonstrated GV's efficacy in treating oral or urinary infections [9–12], the knowledge about the minimal inhibitory

concentration (MIC) or the minimal fungicidal concentration (MFC) of GV is still limited. In particular, the correlation between the MFC and contact time has not been elucidated.

In this study, we evaluated the antifungal activity with respect to the contact time for the topical use of GV and the commonly used alternative povidone-iodine (PI) against *Candida* strains.

Materials and Methods

Clinical Isolates

A total of 102 clinical isolates of yeast, obtained from the culture collection at the Juntendo University Hospital, were used in this study: *C. albicans* ($n = 33$); *C. parapsilosis* ($n = 30$); *C. glabrata* ($n = 11$); *C. tropicalis* ($n = 7$); *C. lusitaniae* ($n = 3$); *C. guilliermondii* ($n = 2$); *C. krusei* ($n = 1$); other *Candida* spp. ($n = 8$); and other yeast isolates ($n = 7$). These isolates were obtained from various anatomic sites and clinical specimens including the blood ($n = 15$), intravenous indwelling catheters ($n = 28$), the respiratory tract ($n = 17$), the ear ($n = 9$), and the vagina ($n = 9$). Isolates were identified using CHROMagar Candida (Kanto Kagaku, Tokyo, Japan) and an API ID32C system (bioMérieux, Durham, NC). Tested strains were subcultured from frozen stocks (−80°C) onto Sabouraud dextrose agar and incubated at 35°C for 24–48 h. For the susceptibility assay, *C. albicans* ATCC 24433, *C. parapsilosis* ATCC 20019 and 90018, and *C. krusei* ATCC 6258 were used as control strains.

Antifungal Agents and Culture Media

GV was purchased from Sigma-Aldrich (Tokyo, Japan), and povidone-iodine (PI) 10% was obtained from Meiji seika (Tokyo, Japan). All compounds were prepared in RPMI 1640 medium (with L-glutamine, without sodium bicarbonate, and with phenol red as a pH indicator) from GIBCO/Invitrogen Corp. (Grand Island, NY, USA), which was sterilized using commercial filter membrane units. Drug dilutions were prepared according to the Clinical and Laboratory Standards Institute (CLSI) document (M27-A3). Serial twofold dilutions of GV (0.03–32 μg/ml, equivalent percent 0.000003–0.0032%), and PI (0.0003–0.5%) were prepared.

Fungal Susceptibility Tests and Fungicidal Activity Tests

The MICs of GV and PI were determined according to CLSI M27-A2. The MIC was determined as the lowest concentration that inhibited fungal growth compared with growth control. The minimum concentrations of the drug that inhibited 90 and 50% of the isolates were defined as the MIC_{90} and MIC_{50}, respectively. Susceptibility plates were prepared 1 day before testing and kept at −4°C. Fungicidal activity was determined for contact time lengths of 1, 5, 15, 30, and 60 min and 24 h at room temperature. At the end of each contact time point, 10 μL of each test mixture ($1–5 \times 10^3$ cfu.) was added into RPMI 1640 medium that was then incubated at 35°C. After a 48 h incubation, the MFC was determined as the lowest concentration showing no visible growth, which means >99% killing. All experiments were performed in duplicate. The MFC of the drug with a contact time of 5 min was defined as the $MFC_{5\ min}$.

Results

MICs of GV and PI for Yeast Isolates

As shown in Table 1, the ranges of MICs of GV and PI for all tested yeast isolates were 0.12–8 μg/ml (0.000012–0.0008%) and 600–5,000 μg/ml (0.06–0.5%), respectively. Whereas *C. parapsilosis* and *Trichosporon* spp. showed relatively high MIC_{90} values for GV (4 and 8 μg/ml), the MIC_{90} of GV for other yeast isolates was 0.5 μg/ml (0.00005%). We detected markedly high MIC_{90} values for PI for all yeast isolates (5,000 μg/ml, equivalent to 0.5%). No remarkable difference in the MICs of GV and PI was observed among the identical strains isolated from different anatomic sites (details not shown).

MFCs of GV and PI for Yeast Isolates

As shown in Table 2, GV demonstrated fungicidal activity, with an $MFC_{5\ min}$ of 50 μg/ml (0.005%) for *C. albicans*, *C. tropicalis* and *C. krusei*. and an $MFC_{5\ min}$ of 500 μg/ml (0.05%) for the other yeast isolates. Notably, the MFCs decreased during the time course of the incubation, and the $MFC_{24\ h}$ was 0.5–50 μg/ml (0.00005–0.005%). The MFCs of PI

Table 1 Susceptibility testing of gentian violet and povidone-iodine for *Candida* and other yeast isolates

	No. of isolates	GV MIC (μg/ml)			PI MIC (%)		
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Species							
C. albicans	33	0.25–1	0.5	0.5	0.06–0.5	0.5	0.5
C. parapsilosis	30	0.12–8	2	4	0.06–0.5	0.5	0.5
C. glabrata	11	0.25–2	1	2	0.12–0.5	0.5	0.5
C. tropicalis	7	0.25–0.5	0.25	0.5	0.5	0.5	0.5
C. lusitaniae	3	2	2	2	0.12–0.5	0.25	0.5
C. guilliermondii	2	0.5	0.5	0.5	0.5	0.5	0.5
C. krusei	1	1	1	1	0.5	0.5	0.5
C. ciferrii	1	1	1	1	0.12–0.5	0.5	0.5
C. famata	1	0.5	0.5	0.5	0.5	0.5	0.5
C. intermedia	1	2	2	2	0.5	0.5	0.5
C. melibiosica	1	2	2	2	0.5	0.5	0.5
C. norvegensis	1	0.25	0.25	0.25	0.5	0.5	0.5
C. pelliculosa	1	0.5	0.5	0.5	0.25	0.25	0.25
C. sake	1	1	1	1	0.5	0.5	0.5
C. utilis	1	0.25	0.25	0.25	0.5	0.5	0.5
Pichia ohmeri	1	0.5	0.5	0.5	0.5	0.5	0.5
Rhodotorula sp.	1	0.5	0.5	0.5	0.25	0.25	0.25
S. cerevisiae	1	2	2	2	0.12	0.12	0.12
Cryptococcus sp.	1	1	1	1	0.25	0.25	0.25
Trichosporon spp.	3	1–8	4	4	0.5	0.5	0.5
All yeasts	102	0.12–8	0.5	4	0.06–0.5	0.5	0.5
Control strains							
C. albicans		0.5	0.5	0.5	0.12–0.25	0.12	0.25
C. krusei		2	2	2	0.12–0.25	0.12	0.25
C. parapsilosis		0.5–2	0.5	2	0.06–0.25	0.12	0.25

Table 2 MFCs of GV for *Candida* and other yeast isolates

	MFC (μg/ml)					
	1 min	5 min	10 min	30 min	60 min	24 h
Strains						
C. albicans	50	50	50	50	5	0.5
C. parapsilosis	1,000	500	500	500	50	1
C. glabrata	500	500	500	500	500	50
C. tropicalis	50	50	50	50	50	1
C. krusei	50	50	50	50	50	1
Trichosporon spp.	1,000	500	500	500	500	1
Control strains						
C. albicans	50	50	50	50	50	0.5
C. parapsilosis	500	500	500	500	50	0.5
C. krusei	50	50	50	50	50	0.5

MFCs were tested for contact time length of 1, 5, 15, 30, 60 min and 24 h

Table 3 MFCs of PI for *Candida* and other yeast isolates

	MFC (%)					
	1 min	5 min	10 min	30 min	60 min	24 h
Strains						
C. albicans	0.5	0.5	0.5	0.5	0.5	0.5
C. parapsilosis	0.5	0.5	0.5	0.5	0.5	0.5
C. glabrata	0.5	0.5	0.5	0.5	0.5	0.5
C. tropicalis	0.5	0.5	0.5	0.5	0.5	0.5
C. krusei	0.5	0.5	0.5	0.5	0.5	0.5
Trichosporon spp.	0.5	0.5	0.5	0.5	0.5	0.5
Control strains						
C. albicans	0.5	0.5	0.5	0.5	0.5	0.5
C. parapsilosis	0.5	0.5	0.5	0.5	0.5	0.5
C. krusei	0.5	0.5	0.5	0.5	0.5	0.5

MFCs were tested for contact time length of 1, 5, 15, 30, 60 min and 24 h

were identical in all tested species and at all tested time points (5,000 μg/ml, 0.5%) (Table 3). The MFC/MIC ratio is known to be useful to predict drug tolerance [12]. GV showed an $MFC_{5\ min}/MIC_{90}$ ratio of 50–250, which suggests that caution is needed when using drug tolerance for predictions.

Discussion

The antifungal susceptibility results indicate that the clinical yeast isolates are highly susceptible to GV, with lower MIC and MFC values for GV than for PI. Our findings about the GV efficacy were almost concordant with those of a previous study that reported the GV antifungal activity in HIV patients [10]. The $MFC_{5\ min}$ of GV (50 μg/ml, 0.005%) found in the present study was 100 times higher than the $MFC_{24\ h}$ value which was similar to the MIC (0.5–1 μg/ml). Tolerance of *Candida* for amphotericin B has been well studied, and the antifungal activity of amphotericin B is estimated by the value of a 6-h killing rate, which is correlated with MFC [13–15]. The previous study demonstrated that $MFC_{1\ min}$ or $MFC_{5\ min}$ is the best index for estimating the efficacy of these disinfectants [16]. Previously reported MFCs of GV for yeast isolates were lower than those obtained in this study [10], possibly because earlier studies focused on $MFC_{24\ h}$.

Our results indicate that the routine microbial testing results of MIC or even $MFC_{24\ h}$ are not sufficiently reliable to estimate the antifungal activity of topically applied GV. Of note, however, the $MFC_{5\ min}$ of GV was still markedly lower than the concentrations of GV usually used in clinical practice (0.5–2%).

We also demonstrated no remarkable difference in the susceptibility against GV dependent on the isolated sites for all of our tested strains. However, because GV is a water-soluble dye, it may remain on the skin for a relatively long time but not on the mucosa. Whether the disinfectant activity of GV for MRSA and Flu virus has been already established [17–19], further clinical investigation is required to elucidate the antifungal efficacy and therapeutic concentrations for topical use of GV on the different treatment sites.

Contrary to findings for GV, the MFCs of PI were consistently independent of the tested timing, and the MIC_{90} of PI (5,000 μg/ml, 0.5%) was equal to the MFC. These results suggested that 0.5% PI might have the stable antifungal activity needed for effective topical use. For oral candidiasis, however, a standard PI solution for mouth wash (approximately 0.23–0.46% of PI) may not be active enough.

In conclusion, whereas GV is more effective than PI to deter candidiasis with a concentration less than 0.1%, the efficacy of the topical disinfection of GV should be estimated using the $MFC_{5\ min}$ value but not the MIC or $MFC_{24\ h}$ values for overall prevention of CR-BSI as well as oral infections.

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