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Identification of gentian violet concentration that does not stain oral mucosa, possesses anti-candidal activity and is well tolerated

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Abstract

Gentian violet (GV) is recommended for initial treatment of oral candidiasis in HIV-infected patients in resource-limited settings. Currently GV is not used because of its staining effects. In this study, we investigated the staining capacity of three different concentrations of GV to determine a concentration that does not cause staining. The selected concentration that did not cause staining was evaluated for its physical stability and antifungal activity. Fifteen healthy participants were randomized to rinse twice daily for 14 days with one of three GV concentrations: 0.1%, 0.0085%, or 0.00165%. Oral examination and intra-oral photographs were performed at baseline and at the end of therapy. Participants responded to a questionnaire to assess adverse events. Antifungal activity was evaluated using the Clinical and Laboratory Standard Institute methodology. GV at a concentration of 0.00165% did not stain the oral mucosa and was well tolerated. GV at a concentration of 0.00165% was stable and possessed antifungal activity when

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stored at certain temperatures for different time periods. Gentian violet solution at the concentration of 0.00165% does not stain the oral mucosa, is stable and possesses potent antifungal activity.

Introduction

Gentian violet (GV), a triphenylmethane dye, has gained increasing attention as a treatment for oral candidiasis in HIV-infected patients in resource-limited settings [1]. Its cost and ease of use make it a suitable alternative to other topical agents, e.g., nystatin, for the treatment of oral candidiasis. The cost of a two-week treatment of GV for oral candidiasis is estimated to be about \$0.50 compared to \$15 for nystatin [2]. In this regard, the World Health Organization recommended topical application of GV at a concentration of 1% for initial treatment of oral candidiasis in HIV-infected patients in resource-limited settings [3]. However, due to its staining properties and associated stigma GV is not used currently for treating this disease in these settings.

A pre-requisite to conducting a large clinical trial aimed at determining the safety and efficacy of GV solution in the treatment of oral candidiasis in HIV-infected adults in resource-limited settings is the identification of a concentration that does not stain the oral cavity. In this study, we conducted a pilot study and compared the staining potential, tolerability, and acceptability of three GV concentrations (0.00165%, 0.0082%, and 0.1%) in order to select the concentration that does not stain the oral mucosa and thereby is less stigmatizing. The GV concentration that did not cause staining was further assessed in vitro for its stability and antifungal activity.

Materials and methods

Preparation of GV solution

The primary objective of this study was to evaluate the staining potential and safety of three concentrations of GV (0.00165%, 0.0082% and 0.1% w/v). We used commercially available 1% GV solution (AmerisourceBergen, Valley Forge, PA) and sterile water for irrigation, USP (Baxter Healthcare Corp., Deerfield, IL), to prepare the GV solutions. Since it is well documented that light degrades GV [4], aliquots from each concentration prepared were placed in amber bottles to protect them from light.

Participant selection

Five participants were randomized in a blinded design fashion to receive one of the three GV solutions (total participants enrolled in the study was 15). The Institutional Review Board (IRB) at Case Western Reserve University/University Hospitals Case Medical Center approved the study protocol. Participants were healthy and ≥ 18 years of age. Participants were excluded from the study if they smoked or had any oral condition that might interfere with the study endpoints. Baseline demographic information was recorded (age, gender, and date of enrollment), an oral examination was administered by an oral medicine specialist and results were recorded. In addition, we obtained baseline intra-oral photographs of the left and right buccal mucosa, palate, and dorsum of the tongue. Participants were instructed to rinse with 5 mL of GV solutions twice daily for at least 2 minutes for 14 days. They also were instructed to refrain from eating for 30 min after rinsing with the GV solution. This procedure was designed to mimic the actual international clinical treatment protocol that will be undertaken in the near future to evaluate the safety and efficacy of the selected GV concentration in the treatment of oral candidiasis. The subjects were instructed to keep a medication diary to document the times of rinsing and any observations or comments related to taste, discoloration, mucosal changes, or ulceration. They returned to the clinic on day 14

to have the same intraoral photographs taken as well as to submit the medication diaries and to return the empty bottles. Pre-and post-therapy photos and medication diary information was compiled and analyzed for evidence of mucosal staining and adverse events. In addition, the residuum of GV solution in the bottles was measured as part of compliance assessment.

Staining assessment and adverse events

Staining was assessed by a blinded evaluator using a colorimetric scoring system (from 0 to 3). Staining was classified based on: no staining (0), mild (1), moderate (2), and severe staining (3). In addition, the study team assessed participants' adherence/acceptance and adverse events related to the different GV solutions used. Adherence was assessed using self-report aided by diaries and residual volumes of GV in the returned bottles. The acceptance of the three study solutions was assessed by a questionnaire concerning organoleptic perception (comfort, taste) completed by each participant and submitted to the investigator at the end of the treatment. Additionally, the participants were questioned and examined for any adverse sequelae (mucosal ulceration, gastrointestinal side effects, taste alteration or disturbance).

Physical stability testing

Once the GV solution that was not associated with mucosal staining was identified, it was subjected to further evaluation of its stability. GV solutions placed in amber bottles were stored at 4°C, 25°C, 37°C, 40°C, and 55°C. These samples were analyzed for stability by visual observation immediately after preparation and at 7, 14, 21, 28, 35, 42, 49, and 56 days and up to 12 months. Each stored solution was examined for any change in color, clarity or for the presence of particulate matter under standard laboratory lighting.

Antifungal activity testing

We determined the minimum inhibitory concentration (MIC) of the selected non-staining GV solution stored at various temperatures (4°C, 25°C, 37°C, 40°C and 55°C) and time periods (7, 14, 21, 28, 35, 42, 49 and 56 days) against four different *Candida* strains (*C. albicans*, fluconazole-susceptible and -resistant isolates, *C. glabrata* and *C. parapsilosis*) using the Clinical and Laboratory Standards Institute (CLSI) M-27A3 methodology [5] and as described in our publication [6]. An increase in the MIC of greater than three dilutions compared to baseline indicated that the solution had a reduced antifungal activity.

GV solutions also were evaluated for fungal and bacterial contamination by plating 100 µg of each stored GV solution on potato dextrose agar (Difco Laboratories, Detroit, Michigan) and brain heart infusion (Difco) plates. Petri dishes were incubated at 35°C for 24–48 hours and microbial growth was recorded.

Results

Staining potential of GV solutions

Fifteen participants were randomized to receive one of the three GV solutions. Because of severe mucosal staining five participants dropped out of the study on the first day, while ten participants stayed in the study until completion. Severe staining was observed in five participants, four of whom received GV at a concentration of 0.1% and one participant received the intermediate concentration (0.0085%) (Fig. 1). Staining was localized to the oral cavity in the six participants receiving GV 0.0085% and 0.1% solutions. Staining of the vermilion border of the lips and peri-oral tissues was not observed. In all cases, staining was transient and reversible, and no dental intervention was required to remove the stain. In contrast, no staining was seen in the four participants (4/5) who received the lowest

concentration of GV (0.00165%). Although, one participant in this group had a very mild staining of the tongue tip; this staining was noticed only by the evaluator, not by the participant himself.

Side effects

Aside from the oral mucosal staining, no serious side effects including mucosal burning, ulceration, and taste disturbance were reported in the participants receiving the low concentrations of GV. In contrast, several participants who received GV solutions at concentrations of 0.0085% and 0.1% reported a bitter taste (6/15) which resolved upon treatment discontinuation. One participant felt nauseated at GV concentration of 0.1%. No side effects were recorded or reported by participants who received GV at 0.00165% (Table 1).

Adherence

To assess adherence to the study medication, participants were instructed to record any issue that impacted on their taking the assigned GV concentration. Participants who withdrew early from the study noted that GV at 0.0085% and 0.1% stained the sink and their clothes. One participant who received GV at 0.1% was late to work because of a “purple” tongue and regarded this as stigmatizing. Participants who received GV at 0.00165% did not record any such comments. At the end of the study, participants returned the bottles with residual GV. The volume of residual solution was measured. The residual volumes and daily diaries were used to assess compliance (see Table 1). All participants who received GV at a concentration of 0.00165% were compliant and completed the 14-day course of GV rinse. None of those who were on the higher doses complied with the protocol.

Physical stability, antifungal activity and sterility

Having shown that the lowest GV concentration (0.00165%) did not cause significant staining, we evaluated its stability, antifungal activity and sterility.

There were no changes in physical appearance, color, or odor of GV 0.00165% solution stored at 4°C, 25°C, 37°C, and 40°C at 7 and 28 days of the study. However, GV solution stored at 55°C showed a color change and visible precipitation at day 7. GV solution was stable at 4°C, 25°C and 37°C when stored for 42 days. In contrast, solution stored at 40°C showed color change and precipitation at this time period. Long-term storage of GV 0.00165% solution (up to 1 year) showed that this solution is stable only when stored at 4°C.

The antifungal activity of the stored GV 0.00165% solution against *Candida* strains was determined using the CLSI M27A3 methodology [5]. There were no changes in the MIC values of the tested solutions stored at 4°C, 25°C, 37°C, and 40°C at 28 days of the study. In contrast, GV solution stored at 55°C and 42°C showed a marked increase in the MICs (more than three dilutions) against the tested isolates at 7 and 42 days, respectively. GV solution was active in vitro at 4°C, 25°C and 37°C when stored for 42 days. Long-term storage of GV (up to 1 year) showed that this solution retained its antifungal activity only when stored at 4°C.

Having shown that GV 0.00165% solution is stable and possesses antifungal activity, we examined whether this solution is sterile. Our data showed that this solution was sterile since no fungal or bacterial growth was noted when aliquots were cultured indicating that the solution remained sterile under the storage time and temperatures tested.

Discussion

Our data showed that GV 0.00165% solution does not cause oral staining and is well-tolerated. In comparison, GV 0.1% and 0.0085% solutions were associated with staining of the oral mucosa, tongue, and gingiva. The 0.1% solution was also associated with poor patient compliance. In this group the majority of subjects complained of bitter taste and mucosal discoloration. A major concern of our study was the possibility of irreversible staining of esthetic anterior dental restorations. Therefore, we excluded these subjects from the study, as a precautionary measure. Moreover, these solutions did not stain the linea alba in patients where this normal oral structure was pronounced.

In previous studies, GV showed clinical efficacy in the treatment of oral candidiasis in AIDS patients. In this regard, Nyst et al. [2] conducted a randomized open-label study and compared the efficacy of GV 0.5% solution with nystatin mouthwashes and oral ketoconazole in the treatment of oral candidiasis in AIDS patients in the Democratic Republic of Congo. At day 14, the clinical cure rate, defined by lesion disappearance, was 42% (11/26 patients) in the GV arm compared to 43% and 9% in the ketoconazole and nystatin arms, respectively. Both GV and ketoconazole were clinically superior to nystatin in the treatment of oral candidiasis. Side effects of GV therapy reported were local irritation and ulceration, which were infrequent and reversible. In addition, a recent double-blind placebo controlled study conducted in Malawi (T. A. Hodgson, personal communication) on HIV-infected children with oral candidiasis resulted in a 58% cure rate (as measured by resolution of mucosal lesions and of symptoms) following treatment with a 0.00165% solution of GV compared to 52.6% with topical nystatin. The study of Hodgson and colleagues showed that a low concentration of GV (0.00165%) was as effective as a 1% solution and with fewer side effects, and no staining. In the present study, we demonstrated that GV 0.00165% solution is well-tolerated and does not stain the oral mucosa which makes this concentration appealing as a clinical alternative in the treatment of OC.

This study shows that GV 0.00165% concentration is stable when stored in an amber bottle at 4°C, 25°C and 37°C for 6 weeks, maintained its antifungal activity and is sterile. Based on these findings and considering the environment and resources available (e.g. refrigeration) in resource-limited settings, we propose that prepared GV 0.00165% solution be stored at room temperature for 6 weeks.

In this pilot study we demonstrated that GV 0.00165% solution does not stain the oral cavity, is stable, well tolerated, and possesses potent anti-*Candida* activity. Therefore, this solution has the potential to be an inexpensive highly effective treatment for oral candidiasis in HIV-infected patients in resource-limited settings. This data provides the bases for initiating an international multicenter phase III clinical trial to assess the safety and efficacy of gentian violet compared to nystatin oral suspension in the treatment of oral candidiasis. This interventional trial is part of the Oral HIV/AIDS Research Alliance (OHARA) program in collaboration with the AIDS Clinical Trial Group.

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Fig. 1.
Staining potential of three different dilutes of gentian violet

Table 1

Gentian violet (GV) staining of oral mucosa and associated adverse events

GV concentration	No stain(score 0)	Mild stain(score 1)	Moderate stain(score 2)	Severe stain(score 3)	Location of stain		Taste disturbance		Number of compliant subjects
					Dorsum of tongue	Generalized	No	Yes	
0.00165%	4/5	1/5	0/5	0/5	1/5	0/5	5/5	0/5	5
0.0085%	2/5	2/5	0/5	1/5	1/5	2/5	4/5	1/5	4
0.1%	1/5	1/5	0/5	4/5	0/5	4/5	0/5	5/5	0
Total	7/15; 46%	3/15; 20%	0/15; 0%	5/15; 33%	2/15; 13%	6/15; 40%	9/15; 60%	6/15; 40%	9/15; 60%