An Innovative Method of Incorporating Antifungal Agents into Tissue Conditioners: An In Vitro Study

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This in vitro study aimed to test the efficacy of ketoconazole and itraconazole, combined with two tissue conditioners (Viscogel [Dentsply] and GC Soft [GC India]), in inhibiting the growth of Candida albicans. Four control groups tested were Viscogel (VGC), GC Soft (GCC), ketoconazole (KTZ) and itraconazole (ITZ). Four combination groups tested were ketoconazole with GC Soft (KGC), ketoconazole with Viscogel (KV), itraconazole with Viscogel (IV) and itraconazole with GC Soft (IG). Various tissue conditioners were mixed and antifungal susceptibility test discs were embedded completely in the mix. 24 hour old culture of C albicans strain (American type culture collection [ATCC] no.10231) was used for inoculation. After 24 hours of incubation, inhibition diameters of various groups were noted. The data was analyzed using a Students t test. There was absolutely no inhibition of C albicans observed in VGC and GCC. For KTZ and ITZ, all the inhibition diameter values were within the standard prescribed limits. There was absolutely no inhibition in IG and IV. For ketoconazole combinations, KGC was found to be significantly more inhibiting than KV (p value < .001). The inhibition of drug effect by embedding the drug into tissue conditioners was significant in all the groups (p value < .001). VGC and GCC do not have any antifungal property of their own. IG and IV were found to be completely ineffective. KGC was found to be significantly more inhibiting than KV.

Introduction

Placement of a removable prosthesis in the oral cavity produces profound changes in the oral environment that may have an adverse effect on the integrity of oral tissues. Denture associated stomatitis is one of the most common clinical presentations of oral candidosis, affecting 24–60% of otherwise well denture wearers. The inflammation is causally associated with Candida albicans. However, the condition is not a specific disease entity because other causal factors exist such as bacterial factors, mechanical irritation and allergy.

A method of treatment by combining tissue conditioner and antifungal agents was suggested by Douglas and Walker in 1973. These authors have investigated the combined effect of nystatin with tissue conditioners and found them to be fungicidal in varying degrees. In a study to clinically evaluate nystatin in the treatment of denture related candidiasis, it was found that re-infection with the organism occurred after 10 days of cessation of treatment. This necessitates closer look on other antifungal agents.

The various combinations tried in the past used drug in the powdered form or suspensions. In an in vitro study, it was observed that itraconazole dissolved Viscogel within three minutes of hand mixing and it is not possible to incorporate more than 5% wt/wt itraconazole into tissue conditioners. Hence, there arises a need to search for different methods for local drug delivery.

This in vitro study tests the efficacy of antifungal susceptibility test discs of itraconazole and ketoconazole (Himedia, Mumbai) incorporated into tissue conditioners (Viscogel and GC Soft) in inhibiting the growth of C albicans.

Material and Methods

Microbiological study was done in the Department of Microbiology, K.S. Hegde Medical Academy, Nitte University, Mangalore.

Preparation of the inoculum for susceptibility test procedure

A Sabourauds dextrose agar medium was prepared and poured into a sterile plate. After drying the plate was inoculated with C albicans using a standard American type culture collection (ATCC) strain no.10231. After inoculation the plate was incubated for 24 hrs at 35+/-2°C and the one day old culture was thus obtained.
Five distinct colonies of approximately 1 mm were picked up from this culture using a sterile inoculation loop. Colonies are suspended in 5ml of sterile saline (0.9%). The resulting suspension was vortexed using the Spinix centrifuge and turbidity was adjusted to 0.5 Mcfarland standard.

Susceptibility test procedure

100 Plates were prepared with Muller Hilton agar and 2% glucose with 0.5mg/ml Methylene blue dye i.e. GMB medium. The prepared medium was poured into the plates to an approximated depth of 4 mm. The plates were allowed to dry for 15 minutes in the incubator.

A wooden non toxic sterile cotton swab on the wooden applicator was dipped into the standardized inoculum. The soaked swab was rotated firmly against the upper side wall of the tube to express excess fluid. Then the entire agar surface of the agar plate was streaked three times turning the plate at 60° angle between each streaking. The inoculum was allowed to dry for 15 min with the lid in place.

In 4 plates, no further alteration was done and it served as the indicator of pure growth of C albicans. In another 8 plates antifungal test discs i.e. itraconazole (ITZ) and ketoconazole (KTZ) were placed (4 plates each) using sterile tweezers. Four discs were equidistantly placed on each plate.

Tissue conditioners were mixed in a sterile dappen dish following the recommended water powder ratios by the manufacturers and the sterile discs were completely embedded in the mix. The discs were carefully lifted up from the mix with the help of a sterile tweezer and gently placed over the agar plate such that the disc is completely embedded in the mix from all the sides. Care was taken to not disturb the shape of the disc while handling. A total of 8 plates i.e 4 each for a tissue conditioner (Viscogel [VGC] and GC Soft [GCC]) were prepared in this manner. Four discs were equidistantly placed on each plate.

The remaining 80 plates were divided into 4 equal groups i.e Viscogel with ketoconazole (KV), Viscogel plus itraconazole (IV), GC Soft plus ketoconazole (KGC) and GC Soft plus itraconazole (IG). Using the same technique the antifungal susceptibility test discs were completely embedded in the tissue conditioner mix and gently placed on the agar plates such that the disc is completely covered by the mix from all the sides. Care was taken to use an aseptic technique in all the steps. Four discs were placed on each plate.

All the plates were placed back into the incubator within 15 min of placing the discs. The plates were incubated at 35±2°C for 24 hours. Inhibition diameters were noted using a metallic scale and a divider. Students t test was employed to analyze the data.

Results

There is absolutely no inhibition of C albicans observed in VGC and GCC. For ketoconazole and itraconazole controls, the inhibition diameter values obtained ranged from 23–32mm and 16–20mm with the mean inhibition diameter being 29.45mm and 17.95mm respectively (Table 1).

For ketoconazole combinations, KGC found to be significantly more inhibiting than KV (p< .001) as shown in fig1. The inhibition diameters of KV and KGC at 24 hours are shown in fig 2 and fig 3 respectively. There was absolutely no inhibition in itraconazole combined with any of the tissue conditioners (fig4, 5). The inhibition of drug effect by embedding the drug into tissue conditioners was significant in all the groups (p < .001).

![Fig 1: Comparison of mean inhibition diameter(mm) ketoconazole Control and combinations.](image1)

![Fig 2: Ketoconazole–Viscogel combination](image2)

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| 100 | 29.45 | 17.95 | 11.34 | 29.11 |
| 200 | 2.36  | 1.36  | 3.03  | 3.15  |
| 300 | 23.20 | 16.00 | 5.06  | 17.10 |
| 400 | 32.00 | 20.00 | 19.00 | 29.00 |
Discussion

Previous studies have used antifungal agents in the form of crushed tablets or suspensions. This method, however, found to be efficacious could have possible disadvantages as crushing the antifungal tablet may result in non-uniform particle sizes of the drug and mixing of the powdered drug with the tissue conditioner may result in varying concentration of the drug throughout the mix. In a study, it was observed that itraconazole completely dissolved Viscogel within 3 minutes of hand mixing and the authors quoted that this combination is not recommended for clinical use.

Anti-fungal sensitivity test discs were used in this in vitro study instead of crushed tablets to know the efficacy of this method of local drug delivery. The possible advantages of using these discs over powdered drug are by placing the disc in a particular area, the effect can be possibly localized and by placing the disc equidistantly a near uniform concentration can be achieved. In an in vitro study when 1 µg/ml of miconazole in 33% collagen solution was plated on resin discs and dried to yield a thin membrane, the growth of *C. albicans* on the resin discs was nearly completely inhibited. The present study utilizes pure form of the drug in the concentration of 10 µgm incorporated in paper disc. The diameter of the paper disc used was 6mm and the thickness was 0.4mm.

In this study, there was absolutely no inhibition of *C. albicans* seen in VGC and GCC. The results obtained are expected and are in accordance with previous studies suggesting that both the tissue conditioners i.e. Viscogel and GC Soft do not have any antifungal property of their own.

For KTZ and ITZ, all the inhibition diameters obtained in this study fall within the prescribed limits of inhibition for *Candida albicans*, (National committee for clinical laboratory standards [NCCLS] document May 2004, HIMEDIA manual) suggesting the sensitivity of the strain to the used drugs.

In this in vitro study, itraconazole combinations with any of the tissue conditioners, i.e. IV and IG were found to be completely ineffective. The results obtained can be possibly explained by an interaction between the tissue conditioners and the drug rendering the drug completely ineffective. The results obtained are similar to the observations made by Chow *et al* who found that itraconazole completely dissolved Viscogel within 3 minutes of hand mixing. Also the present study utilizes the antifungal drug in the form of discs instead of the powdered form hence creating an interphase between the drug and the tissue conditioner. However further studies are needed in this regard.

For ketoconazole combinations, KGC was found to be significantly more inhibiting than KV. This might be explained by the difference in chemical constituents of the two tissue conditioners. This study suggests that for ketoconazole combinations, GC Soft can be more effective than combinations with Viscogel. However further studies are needed to quantify this.

This study utilizes the drug dosage of as less as 10 micrograms, while using the same concentration or even higher concentration of the inoculum of *Candida albicans*. The inhibition found at this less concentration could be possibly explained by a phenomenon known as post antifungal effect as observed by Ellepola and

Fig 3: Ketoconazole–GC Soft combination (lateral view)

Fig 4: Itraconazole–GC Soft combination

Fig 5: Itraconazole–Viscogel combination
Samaranayake. This effect implies that antifungals, even at sub therapeutic concentrations, may suppress the virulent attributes of yeast, especially intra orally where tropical drug levels fluctuate dramatically during dosing intervals. Also, this study utilizes pure form of the drug and is in contrast with those studies which used crushed tablets or suspensions.

This study can be of clinical significance, as incorporation of antifungal drug ketoconazole sensitivity disc into GC Soft shows good inhibition and can be recommended for clinical use. However further studies are required to quantify this.

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References