Comparative antifungal efficacy of denture soft liners with Clotrimazole: An invitro study

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Introduction

Resilient liners are used to replace the fitting surface of denture when patients cannot tolerate the hard denture base. Soft liners helps in absorbing the impact energies of mastication and distributes it over a large area of tissues.¹ Liners are coated on dentures on a permanent or semi - permanent basis.² The most serious disadvantage is susceptibility to

microbial adhesion, specially candida albicans and their surface texture makes efficient mechanical cleaning difficult.² Denture stomatitis, is a common inflammatory condition that affects denture wearers, with a reported prevalence of 62%, 39% and 23% in edentulous patients by different researchers.³

Several studies have attempted to incorporate

ABSTRACT:

Background: Candidal colonization on denture fitting surfaces is the major causative factor in denture stomatitis. Though various treatment options are available, a method by combining antifungal agents into soft liners seems to be a promising approach.

Aim: To determine and compare antifungal activity of two soft liners combined with Clotrimazole against candida albicans.

Materials and method: Two soft liners Coe soft (Acrylic) and GC Reline soft (Silicone) combined with Clotrimazole pure powder form and microsphere form at different concentrations (0.5, 1 and 1.5% w/v) were tested against candida albicans by agar disc diffusion method. After 24 hours of incubation, inhibition diameters were noted. One way analysis of variance followed by Tukey's post hoc test was used to test the inter group difference. P value < 0.05 was considered statistically significant.

Results: Maximum inhibition was seen in the GC Reline soft with pure Clotrimazole followed by GC microsphere form followed by coe soft pure and microsphere forms at 1.5 and 1% W/V concentrations. There was no statistical significant inhibition between 1.5 and 1% W/V.

Conclusion: Denture soft liners, when mixed with antifungal agents showed satisfactory inhibition of candida albicans suggesting that incorporation of antifungal agents into soft liners can be recommended for clinical use.

Key words: Soft liners, Clotrimazole, microsphere, denture stomatitis.

antifungal agents into tissue conditioners for treatment of denture stomatitis. A method of treatment by combining tissue conditioner and antifungal agents was suggested by Douglas and Walker in 1973.⁴ This had the advantages that the action of the drug was prolonged, the cost was low and tissue recovery from trauma was encouraged.⁵ Invitro studies on ketoconazole, Miconazole, nystatin, Itraconazole combined with various tissue conditioners reported 100% efficacy in inhibiting *C. albicans*.²

This invitro study incorporates Clotrimazole pure powder form and microsphere form in two different soft liners and compares their efficacy in inhibiting *C. albicans*.

Materials and Method:

Clotrimazole microspheres were prepared by using cross linking method. Microbiological study was done in the Department of Microbiology, Kamineni Institute of Medical Sciences.

Inoculum preparation 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.24433. After inoculation the plate was incubated for 24 hrs at 35± 20C 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 centrifuged and turbidity was adjusted to 0.5 Mcfarland standard.

Susceptibility test procedure

45 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. Sterile cotton was used to streak the agar plates with the inoculum. In 4 plates, no further alteration was done and it served as the indicator of pure growth of *C. albicans*.

In another 30 plates, Coe soft and GC Reline soft liners were mixed in a sterile dappen dish following the recommended manufacturer's instructions. Clotrimazole pure form (CP) and microspheres (CM) were added to acrylic powder and

to silicone paste at 0.5%, 1%, 1.5% W/V (5 plates for each soft liner and for each concentration) concentrations (Table 1). 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. Four discs were equidistantly placed on each plate.

Table 1: Test materials used in the study

Product
Coe soft (Acrylic soft liner)
GC Reline soft (Silicone soft liner)
Clotrimazole pure powder

A total of 10 plates for soft liner controls (Coe soft and GC Reline soft) were prepared, 5 plates for each soft liner. Care was taken to use an aseptic technique in all the steps. All the plates were incubated at 37°C for 24 hours and inhibition diameters were noted using a metallic scale and divider.

Results

Table 2 shows the mean zone of inhibition of different test materials at different concentrations. Maximum inhibition was seen with GC Reline soft

Table 2 showing comparative mean inhibitory diameter different test and control disks

Group	Mean Inhibitory Zone diameter (mm)
Coe soft	0.00
GC Reline soft	0.00
CP 0.5% - Coe soft	6.01
CM 0.5% - Coe soft	3.12
CP 1% - Coe soft	8.32
CM 1% - Coe soft	6.72
CP 1.5% - Coe soft	12.2
CM 1.5% - Coe soft	11.6
CP 0.5% - GC	13.7
CM 0.5% - GC	7.2
CP 1% - GC	22.1
CM 1% - GC	20.3
CP 1.5% - GC	26.3
CM 1.5% - GC	25.3

CP - Clotrimazole pure form; CM - Clotrimazole microsphere form.

with pure Clotrimazole at 1.5% concentration followed by GC - CM. There is absolutely no inhibition of *C.albicans* observed in control group.

Discussion

Previous studies have used antifungal agents in the form of crushed tablets or suspensions.⁶⁻⁷ 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 such drug into liner material may result in varying concentration of the drug throughout the mix in the study. In our study, we used pure form of the drug instead of crushing the tablet, so that all the particles of the powder form should be uniform.

Microspheres from pure Clotrimazole are prepared, with uniform particle size. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μ m to 1000 μ m). Microspheres are sometimes referred to as microparticles.⁸⁻⁹ It is a well designed controlled drug delivery system. They are made up of polymeric substances, in which the drug is dispersed throughout the microsphere matrix.¹⁰ It has an advantage of maximum therapeutic efficiency delivered at the target site with minimal side effects.¹⁰

In this study there was absolutely no inhibition of *C albicans* seen in Coe soft and GC Reline soft. The results obtained are expected and are in accordance with previous studies suggesting that both the soft liners do not have any antifungal property of their own.^{2,5-7,11}

Test disks, Coe soft with CP at 0.5%, 1% and 1.5% and Coe soft with CM at 1% and 1.5% showed satisfactory inhibition diameters and were within the prescribed limits of inhibition for *C.albicans*, (National committee for clinical laboratory standards [NCCLS] document May 2004, HIMEDIA manual) suggesting the sensitivity of the strain to the used drugs.

GC Reline soft with CP at 0.5%, 1% and 1.5% and GC Reline soft with CM at 1% and 1.5% showed good inhibition diameters compared to Coe soft. There was no statistically significant difference between 1.5% and 1% W/V Clotrimazole in both pure and microsphere forms in both types of soft liner materials. Whereas 0.5% W/V Clotrimazole in Coe soft CM showed least inhibition comparatively.

Even though all test disks showed good antifungal efficacy GC CM and CP forms showed highest inhibition zones compared to all other disks at 1% concentration. As there is no significant difference of inhibition diameters between 1 and 1.5% W/V Clotrimazole, it is better to take lower concentration with almost similar efficacy. 1% W/V was considered as ideal for Clotrimazole incorporation, which was similar to a previous study who used same concentration Clotrimazole to silicone soft liner.¹²

Conclusion

In conclusion, soft liners can be incorporated with antifungal agents and can be used as one of the management approaches towards denture stomatitis.

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