EFFICACY OF TISSUE CONDITIONER ACTING AS EFFECTIVE FUNGICIDAL DRUG DELIVERY SYSYTEM - AN INVITRO STUDY

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Abstract

A variety of disorders like infections, inflammatory diseases, trauma, allergic manifestations,neoplasia etc. may affect the tissues and structures of the mouth, jaw bones and teeth. Treatment of candidal infection in denture wearers has always been a challenging task to the Prosthodontist. There are various treatment procedures for candidal infections. Three different drug delivery systems for the treatment of denture stomatitis were observed. The study proved that the tissue conditioner (Viscogel) can be used as a delivery system for the antifungal drugs like Nystatin, Clotrimazol and Ketoconazole.

Introduction

When the teeth are lost and replaced by a complete denture, it may stimulate the underlying tissues in the form of keratinisation and improving the health of these tissues. But as the duration of the denture use increases, without proper reconditioning of the prosthesis, it may irritate the tissue resulting in trauma and inflammation.

There are a wide variety of disorders like infections, inflammatory diseases, trauma, allergic manifestations, neoplasia etc. which may affect the tissues and structures of the mouth, jaw bones and teeth.

Among the infections that affect soft tissues of the oral cavity, candidiasis is the most common one. Among the precipitating factors of candidal infection, the role of ill fitting or unhygienic condition of the denture is well documented.

Treatment of candidal infection in denture wearers has always been a challenging task to the Prosthodontist. Topical application of the drugs can be used but the purpose is defeated by the copious flow of saliva. Systemic administration of drugs may not be that effective against candidal infection because the organism usually limits its activity to the oral mucosa. So in the treatment of denture stomatitis , it is always desirable to have the drug release at the site of infection constantly at therapeutic levels.

Here tissue conditioner was used for an effective drug delivery system. The efficacy of three antifungal drugs namely, Nystatin, Clotrimazole, and Ketoconazole were evaluated based on drug delivery system

Aims and Objectives

The present study was undertaken to find out:-

- Whether the tissue conditioner which is popularly used in dentistry for the management of abused denture supporting tissue could function as an effective drug releasing system.

- Whether this drug releasing system is having sufficient antifungal activity against the candidal organism.
- Whether this drug releasing system would release adequate quantity of the specified drugs for an optimum period at therapeutic levels to recoup the tissues to its normal health.

DRUG DELIVERY SYSTEM

There are various treatment procedures for candidal infections. There are two approaches which may be used. The first is designed to reduce denture trauma and the other to reduce the amount of candida in the mouth. The simplest way is to avoid the denture altogether. But leaving the denture during the daytime may be objectionable to some patients. So the other means is to reduce the concentration of candida, which may be achieved by improved denture hygiene and use of antifungals. The second is directed at the mucosa and include sucking of antifungal drug such as nystatin.

Barbara B Chamberlane et al comes to the conclusion that brushing the soft tissues with a soft brush when compared to denture brushing technique is more effective in the control of inflammation processes under the denture. Various denture cleansing agents such as alkaline peroxide , alkaline hypochloride, acid disinfectants, and enzymes are used.

Chlorhexidine gel can be applied to the fitting surface of the denture. But use of chlorhexidine has the drawbacks of staining the denture and reducing the materials strength. Other antiplaque agents like Pimafucin 2.58 suspension, octapinol, gentian violet, 28% potassium permanganate or soaking the denture in benzoic acid also can be tried.

Various antifungal drugs like nystatin, clotrimazole, ketoconazole, hamycin, and miconazole can be given to the patient orally or topically. But this may lead to fungal resistance (Martin, Dinsale in 1982). Topical application is sometimes ineffective as the drugs may be washed off due to copius flow of saliva and it may lead to development of fungal resistance and unpleasant taste. Systemic administration of the drug is not very effective because the organism usually limits its activity to the oral mucosa. So it is desirable to have the drug release at the site of infection constantly at therapeutic levels. If one considers the general nature of the therapeutic problem facing dentistry and the accessibility of the disease site in the oral cavity, there is no doubt that therapy by controlled drug delivery had a general applicability

DRUG RELEASING SYSTEM

Materials and Methods

The present study was done to find out:-

- Whether the tissue conditioner (viscogel) which is popularly used in dentistry could function as a effective drug releasing system.
- Whether this drug releasing system would release adequate quantity of the drug for an optimal period at therapeutic levels.
- Whether this drug releasing system is having sufficient antifungal activity against the candidal organisms.

The tissue conditioner (viscogel) was used as a releasing system for the antifungal drugs like nystatin, clotrimazole and ketoconazole in th treatment of candida induced denture stomatitis. The following drug systems were tested:-

1. Tissue conditioner (viscogel) – nystatin system

2. Tissue conditioner (viscogel) – clotrimazole system
3. Tissue conditioner (viscogel) – ketoconazole system

The drug releasing and the inhibition capacity of the drug delivery system as ascertained by treating colonies of candida albicans. The quantity of drug release and its therapeutic efficiency for a period of 1 4 days were evaluated through biological assay.

Details of the test are given below:-

Incorporation of the drug into tissue conditioner (viscogel):-

Incorporation of nystatin into tissue conditioner

5,00,000 IU of pure nystatin was powdered thoroughly and mixed with 8gms of viscogel powder in a mortar with pestle. Then 7ml of viscogel liquid was added to the powder and gel was made.

Incorporation of clotrimazole into tissue conditioner

200gms of pure _clotrimazole was powdered thoroughly and mixed with 8gms of viscogel powder in a mortar with pestle. Then 7ml of viscogel liquid was added to the powder and gel was made.

Incorporation of ketoconazole into tissue conditioner

200 mgs of pure ketoconazole was powdered thoroughly and mixed with 8gms of viscogel powder in a mortar with pestle. Then 7ml of viscogel liquid was added to the powder and gel was made.

Each of these materials was then poured into a sterile petridish to get a uniform thin layer. The petridish was covered with it covering plate. Six specimen were prepared and one control specimen was prepared without incorporating nystatin, clotrimazole or ketoconazole.

Test for antifungal activity of the drug delivery system:-

Incorporation of microbial growth was utilised for demonstrating the antifungal activity of the system.

Test procedure:

Apparatus: petridish, pipettes, forceps. All equipment used were cleaned and sterilised in a hot air oven.

Tests were carried out in sterile petridishes containing thin even layer of each fungicidal denture liner, on the top of this was poured 15 ml of sabouraund's agar media which formed a further layer of 3mm thick.

Medium contained the following:-

Peptone – 9.4gms
Yeast extracts – 4.7gms
Beef extracts -2.4gms
Dextrose – 10gms
Agar - 23.5gms
Sodium chloride – 10gms
Water upto 1000ml.

The final pH of the medium after sterilization was 6.2. The surface of agar medium was inoculated by 0.1 ml of a medium containing 3000 candida organisms/ml. Thus the candida organisms were not placed directly on to the denture liner. As a control a further 1ml of the candida suspension was placed on to the sabouraund's agar which was poured on the denture liner without incorporating drugs.

These cultures were incubated for 4 days at 37^0C andcolonies then counted. The number of colonies inhbited on the denture liner plate was expressed as a percentage of the total number of control agar. This quantity being designated as "fungicidal properties as a percentage". Following this the old agar

was stripped off and fresh sabouraund's medium applied and reinnoculated. Thus the fungicidal properties of the same denture liner was assessed for a further 4 days. The test were represented for 14 days.

By assessing fungicidal properties of the denture liner containing the drug that was needed to be incorporated with the tissue conditioner for destroying the organism for 14 days was standardized. The test showed not only the fungicidal properties of the system but also the ability of the matrix material to diffuse the drug slowly into the surrounding medium over a period of time. The test were carried out for all three drug delivery systems in test group as well as on the control specimens.

TEST TO MEASURE THE QUANTITY OF RELEASED DRUG AT ITS THERAPUETIC LEVELS

23ml of artifial saliva was poured on to each specimen contained in the petridish and the specimens are covered with the covering plates. Artificial saliva was prepared according to the formulae of S. Joyson- Beehal and EAM Kidd.

The specimens were kept in artificial saliva for 24hrs and then freshly prepared saliva was poured into each specimen. The procedure was repeated every 24 hrs for a period of 14 days. Artificial saliva was subjected to biological assay.

PROCEDURE FOR ASSAY

1.8 ml of sabourauds dextrose broth was taken in the first test tube, by using a calibrated pipette. In remaining tubes 1ml of sabourands dextrose was poured by using a pipette. In the first test tube 0.2ml of artificial saliva with drug was added and mixed to get a uniform solution. Then serial dilutions were employed to get a uniform solution. Then seriall dilution were employed by transferring 1ml of dilution from the first tube ; again from the second tube the same amount of dilution was added to the third tube. The sequential transfer of the dilution were carried out in the same way till 1ml from the seventh tube transferred to the eighth tube. These serial dilutions were carried out to achieve the dilutions of 1/20 , 1/40 , 1/80, 1/120 , 1/160 , 1/200 , 1/240 , 1/280 respectively. In the same way serial dilution procedures were repeated to get the dilutions of 1/30 , 1/60 , 1/100 , 1/140 , 1/180 , 1/220 , 1/260 respectively. Each tube was added with 1ml of suspension of candida albicans in sterile normal saline to obtain final concentrationof 1x10 organism or cells /ml in the tubes. The cells were counted in the Neubaure's chamber. Sabourauds dextrose broth with saliva containing drug were inoculated with artifial saliva containing drug were inoculated without organisms in a test tube which served as control. The test tubes were inoculated at 37^0 C and examined after 24 hrs . After overnight incubation, the minimum fungicidal concentrations were estimated by sub-culturing from the tubes on sabourauds dextrose agar plate. The concentration with highest dilution of the drug showing no visible growth was determined and noted. The drug concentrations were calculated by using the following formula.

(MICxVd)V=concentration of the drug released per day

MIC – minimum inhibitory concentration of the drug used.

Vd – highest dilution of the drug which inhibit the growth

V – total volume of the artificial saliva used.

The test were carried out for three various drug delivery system and the amount of drug released per day were estimated through the same assay.

Result

Tables II,III, IV show the fungicidal property as a percentage for nystatin-tissue conditioner system,clotrimazole-tissue conditioner system and ketoconazole-tissue conditioner system respectively. The test were performed in six specimen in test groups and one specimen served as the control. The result showed 100% inhibition of growth of organisms for 14 days. The result showed that 5lakh IU of nystatin,200mg of clotrimazole and 200mg of ketoconazole respectively when incorporated in tissue conditioner can effectively destroy the growth of organism for 14 days.in control specimen the growth was seen on the 2nd day of incubation. The above results revealed that all three drug delivery systems can effectively inhibit and destroy the candida organism for 14 days.

The drug release pattern of different systems were also studied. The graphic representation of the same is given in tables VIII to XI . The study shows the quantity of drug released each day by nystatin – tissue conditioner system for a period of 14 days. Initially 14.7 mg of drug was released . later it declined. A steady release of the drug was observed

The study shows the quantity of drug released each day by clotrimazole-tissue conditioner system for a period of 14 days. Initially 11mg of the drug was released . on the 7th day 36.8 mg of the drug was released and later it declined.

The study shows the quantity of drug released each day by ketoconazole denture liner for a period of 14 days. Initially 7.8 mg of the drug was released and it shows a steady increase to31.6 mg by the 7th day , then gradual decline to the 14th day. A comparative pattern of the release pattern of the drug are shown in graph XI. Blank specimen failed to give positive results.

The above observations revealed that all the three systems could be effective against the organism and each system was releasing the drug at therapeutic levels at their optimal level of concentration.

TABLE 1 -FUNGICIDAL PROPERTY AS PERCENTAGE FOR VARIOUS CONCENTRATION OF NYSTATIN: VISCOGEL SYSTEM

SPECIMEN	DRUG-DELIVERY SYSTEM	INHIBITION OF GROWTH BY PERCENTAGE			
		DAY 2	DAY6	DAY 10	DAY 14
1.	VISCOGEL –NYSTATIN (2,00,000IU)	60	35	24	00

2.	VISCOGEL –NYSTATIN (3,00,000IU)	73	60	48	35
3.	VISCOGEL –NYSTATIN (4,00,000IU)	89	71	62	45

TABLE 2: FUNGICIDAL PROPERTY AS A PERCENTAGE FOR NYSTATIN: 5,00,000IU TISSUE CONDITIONER

SPECIMEN	DRUG DELIVERY SYSTEM	INHIBITION OF GROWTH PERCENTAGE			
		DAY2	DAY6	DAY10	DAY14
1.	NYSTATIN-VISCOGEL	100	100	100	100
2.	NYSTATIN-VISCOGEL	100	100	100	100
3.	NYSTATIN-VISCOGEL	100	100	100	100
4.	NYSTATIN-VISCOGEL	100	100	100	100
5.	NYSTATIN-VISCOGEL	100	100	100	100
6.	NYSTATIN-VISCOGEL	100	100	100	100

TABLE III: FUNGICIDAL PROPERTY AS A PERCENTAGE FOR CLOTRIMAZOLE:200mg TISSUE CONDITIONER SYSTEM

SPECIMEN	DRUG – DELIVERY SYSTEM	INHIBITION OF GROWTH PERCENTAGE			
		DAY2	DAY6	DAY10	DAY14
1.	CLOTRIMAZOLE-VISCOGEL	100	100	100	100
2.	CLOTRIMAZOLE-VISCOGEL	100	100	100	100
3.	CLOTRIMAZOLE-VISCOGEL	100	100	100	100
4.	CLOTRIMAZOLE-VISCOGEL	100	100	100	100

5.	CLOTRIMAZOLE-VISCOGEL	100	100	100	100
6.	CLOTRIMAZOLE-VISCOGEL	100	100	100	100

TABLE 4 :FUNGICIDAL PROPERTY AS PRCENTAGE FRO KETOCONAZOLE : 200MG TISSUE CONDITIONER SYSTEM

SPECIMEN	DRUG – DELIVERY SYSTEM	DAY 2	DAY6	DAY10	DAY 14
1	Ketoconazole-viscogel	100	100	100	100
2	Ketoconazole-viscogel	100	100	100	100
3	Ketoconazole-viscogel	100	100	100	100
4	Ketoconazole-viscogel	100	100	100	100
5	Ketoconazole-viscogel	100	100	100	100

Table VIII
Graphic representation of the drug releasing pattern for nystatin: Viscogel system

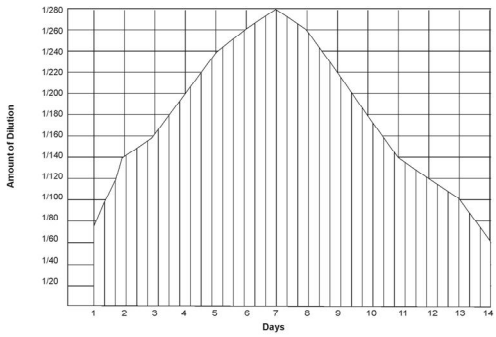


Table IX

Graphic representation of drug release pattern for clotrimazole: Tissue conditioner system

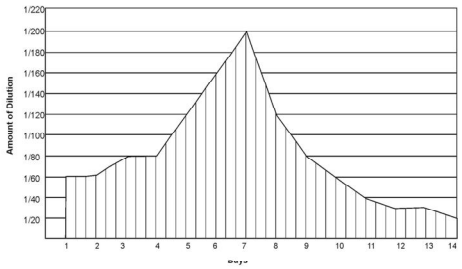


Table X

Graphic representation of the drug release pattern for ketaconazole: Tissue conditioner system

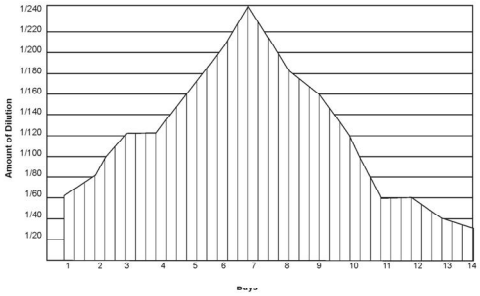
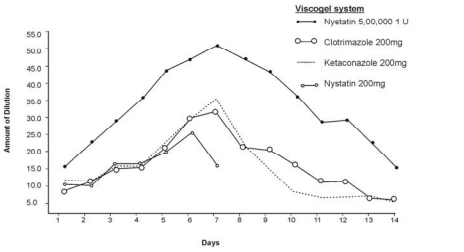


Table XI

Graphic representation of different concentration of Nystatin with clotrimazole and Ketaconazole :



Discussion

After the tests were performed and results analysed it was found that clotrimazole-tissue conditioner system was also very effective and there were no harmful effect for clotrimazole as concentration of the drug was also very minimal compared to its systemic dosages.

Ketoconazole-tissue conditioner system was also very effective against the organism. The concentration of the drug used was also very minimal. But the risk of liver damage is present.

While evaluating and comparing the efficacy of 3 antifungal drugs ie. Nystatin, clotrimazole and ketoconazole the concentration of nystatin used in tissue conditioner system was higher than that of clotrimazole and ketoconazole, so clotrimazole and ketoconazole were more therapeutically efficient at lower concentrations than nystatin.

The present study showed that the drugs can be dispersed through the acrylic matrix in a regular fashion and it was therapeutically effective against candidal organism. A clinical application of the in vitro study on three patients were conducted for all delivery system for a period of 14 days. All the cases were selected on the basis of Newtons classification II for denture stomatitis (Generalised simple inflammation diffused erythema). All the drug delivery systems were effective against the organism and initial symptoms subsided within 8th day with Nystatin, 7th day with Ketoconazole and 5th day with Clotrimazole.

Summary

Three different drug delivery systems for the treatment of denture stomatitis were observed. Nystatin (5,00,000 IU), Clotrimazole (200 mg) and ketoconazole (200 mg) were incorporated into the tissue conditioner. Their antifungal activities were tested by treating cultures of candida and the drug releasing pattern of these systems at its therapeutic levels were studied by biological assay for a period of 14 days.

The study proved that the tissue conditioner (Viscogel) can be used as a delivery system for the antifungal drugs like Nystatin, Clotrimazol and Ketoconazole. It also showed the efficiency of the acrylic matrix to disperse the drug into the surrounding medium at constant therapeutic levels. The drug Clotrimazole had therapeutic advantage and it did not have adverse side effects when compared with Nystatin and Ketoconazole. The clinical application of the in vitro study indicated that candida infections can be effectively treated by all three drug releasing systems.

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