

Denture stomatitis

Effects of chlorhexidine and amphotericin B on the mycotic flora

INGAR OLSEN

Department of Oral Surgery and Oral Medicine & Institute of Pharmacology, University of Oslo, Norway

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In 100 patients with denture stomatitis cultures and direct smears were used to evaluate 5 treatments, including sucking of chlorhexidine, amphotericin B or placebo tablets combined with denture soaking in 0.2 % chlorhexidine or water. After 14 days of treatment the amphotericin B lozenges had brought about a significant reduction in the quantity of fungus on the oral mucosa, whereas they barely affected the large amount present on the fitting surface of the upper denture. Denture immersion in chlorhexidine significantly reduced the number of organisms both on the mucous membrane and on the denture. It therefore seems at least as important to treat the denture as the patient in denture stomatitis. Fourteen days after withdrawal of the drugs the mycotic flora was largely re-established.

Key-words: Stomatitis; fungi; chlorhexidine; amphotericin B

Ingar Olsen, Institute of Pharmacology, University of Oslo, Blindern, Oslo 3, Norway

In a previous report (Olsen, 1974) it was demonstrated that yeasts are present in larger quantities on the fitting side of the upper denture than on the palatal mucosa in patients with denture stomatitis. A rational therapy should be directed against organisms in both sites. Denture disinfection with chlorhexidine has been reported efficacious in the treatment of denture stomatitis (Heyden *et al.*, 1971b, Budtz-Jørgensen & Løe, 1972). Sucking of amphotericin B tablets is frequently used in the management of candidal infection in the oral mucosa. The present study was performed to test the separate and the combined effects of these procedures on the fungal flora in denture stomatitis.

MATERIAL AND METHODS

Patients

One hundred patients, described previously (Olsen, 1974), were included. The participants fulfilled the following criteria: a) wearing full upper denture, b) having generalized simple or granular inflammation in the palate, and c) yielding positive cultures of fungi on agar models of the upper jaw and upper denture.

Efforts were made to match the patients in series of 5 for sex, age, intensity of inflammation and subjective symptoms, denture fit, and denture hygiene. The patients were then allocated randomly to one of 5 treatment groups.

Treatment

The combinations of tested drugs are outlined in Table 1. Each patient was supplied with either 56 lozenges of amphotericin B*, or their matching placebo tablets, or chlorhexidine chloride lozenges**. Written instructions for use were given. Four tablets, each taken at 4-hourly intervals during the day for a period of 14 days, were to be dissolved slowly in the mouth. During sucking, the denture(s) was (were) to be kept completely immersed in water or in 0.2% aqueous solution of chlorhexidine digluconate**. The soaking solution was delivered to the patients in plastic bottles of 1000 ml. The liquid in the vessel used for soaking was to be changed every day. Last sucking/soaking was performed in the evening before the examination day. Exclusion of dentifrices and soaps from the denture hygiene was stressed, but otherwise the patients were urged not to change their denture cleaning or denture wearing habits.

Table 1. *Treatments tested in 5 groups of 20 patients with denture stomatitis. A lozenge was dissolved in the mouth 4 times daily for a period of 14 days. During sucking the denture(s) was (were) kept in a soaking agent*

Lozenges	Soaking agent
Placebo	Water
Amphotericin B 10 mg	Water
Placebo	Chlorhexidine 0.2 %
Amphotericin B 10 mg	Chlorhexidine 0.2 %
Chlorhexidine 5 mg	Chlorhexidine 0.2 %

Appointments

The patients were examined by the same methods on the day before onset of the treatment (day 0), at the end of 14 days'

treatment (day 15), and at the relapse control 14 days after withdrawal of the drugs (day 29). Appointments for each patient were made at the same time of day to avoid diurnal variation in the intra-oral amounts of yeasts (*Williamson, 1972*). At each control it was ascertained that no significant changes had occurred in the patients' state of health.

Fungal cultures

Fungi residing on the palatal mucosa and on the fitting surface of the upper denture were cultivated on agar models and identified as described previously (*Olsen, 1974*). Quantitation of fungi was performed according to the following scale:

no colonies	= 0
1 — 9 colonies	= 1
10—24 colonies	= 2
25—100 colonies	= 3
> 100 colonies	= 4
confluent growth	= 5

Smears

These were taken from the palate, tongue, rhagades, and the fitting surface of the upper denture and prepared for semi-quantitative evaluation of yeast-like fungi (*Olsen, 1974*). The slides were coded and assessed blindly after the experiment, using the following scale:

no blastospores/hyphae	= 0
blastospores	= 1
single hyphae	= 3
scattered mycelium	= 4
large accumulations of mycelium	= 5

Statistical methods

Multinomial test series were derived from the material (*Sverdrup, 1967*), and the homogeneity test with ranked observers

*Fungilin®, Squibb AB, Lidingö, Sweden

**Hibitane®, I.C.I. Ltd., Macclesfield, England

(Feller, 1968) was used to indicate significance of differences at the 5 % probability level.

RESULTS

Fungal cultures

In the control group (placebo/water) both the quantitative (Fig. 1) and the qualitative (Table II) yield of fungi remained rather unaffected. Use of amphotericin B lozenges significantly reduced the number of colonies on the palatal models, whereas the growth on the denture models was only slightly affected. The chlorhexidine denture disinfectant brought about a significant growth reduction on both models, and, contrary to the amphotericin B lozenges, it also reduced the number of *Candida albicans* isolates. The highest reduction of growth was obtained on the palatal models when denture disinfection in chlorhexidine was combined with sucking of amphotericin B tablets. Essentially the same reduction of cultures was seen whether chlorhexidine or placebo tablets were combined with denture disinfection.

Fourteen days after treatment the

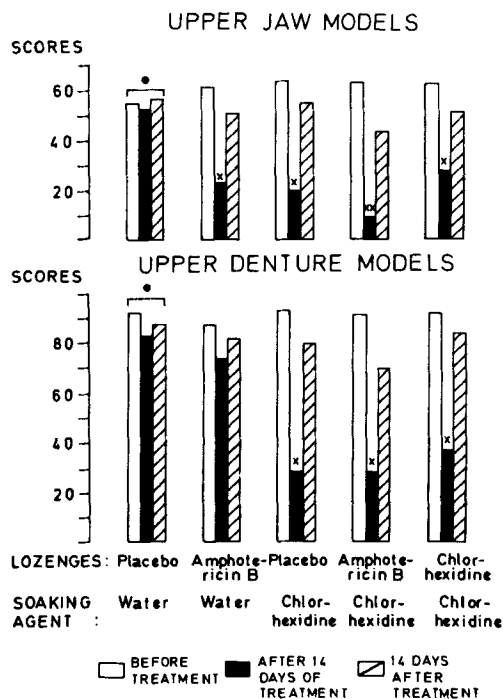


Fig. 1. Summarized fungal growth scores on agar models of the upper jaw and upper denture from 100 patients with denture stomatitis. (No colonies = 0, 1–9 colonies = 1, 10–24 colonies = 2, 25–100 colonies = 3, >100 colonies = 4, confluent growth = 5.)

× significantly reduced scores compared to the control •.

×× significantly reduced scores compared to ×. (5 % level of significance.)

Table II. Isolates of fungi obtained from 100 patients with denture stomatitis before treatment (day 0), at the end of treatment (day 15), and at the relapse control (day 29)

Lozenges/ Soaking agent	Placebo/ Water			Amphotericin B/ Water			Placebo/ Chlorhexidine			Amphotericin B/ Chlorhexidine			Chlorhexidine/ Chlorhexidine		
	0	15	29	0	15	29	0	15	29	0	15	29	0	15	29
<i>Candida albicans</i>	16	16	17	13	17	15	14	9	15	15	7	14	17	10	14
<i>C. krusei</i>	2	3	1	1											
<i>C. parapsilosis</i>	1	1			1				1	1		3	1		2
<i>C. pseudotropicalis</i>										1					
<i>C. tropicalis</i>	3	1	2	1	3	2	4			5	1	2	2		4
<i>Kluyveromyces fragilis</i>			1	1						1	1				
<i>Pichia membranaefaciens</i>									1						
<i>Saccharomyces fragilis</i>												1			
<i>Torulopsis glabrata</i>	6	5	1	8	3	4	9	4	5	5	3	3	5	6	6
<i>T. inconspicua</i>							2								
<i>Trichosporon capitatum</i>										1					

• Control

quantitative yield of fungi was nearly the same as before treatment. The growth scores were not statistically different from pretreatment values in any of the groups. Furthermore, the pretreatment number of isolates from the main species had almost been reached in all groups given active drugs.

Smears

In the control the palatal and denture smear scores were not consistently changed by the treatment (Fig. 2). As seen with the growth scores from upper jaw models, there was a significant reduction in the palatal smear scores in all groups treated with active drugs. Further, the denture scrapings from amphotericin B-treated patients demonstrated only a slight reduction in the amounts of yeast-like fungi. Smears from chlorhexidine-disinfected dentures revealed that the concentration of fungi had been reduced, but the yeast or the mycelial form, or both still persisted. Besides, positive smears were obtained from dentures yielding negative cultures. Therefore, in contrast to the growth

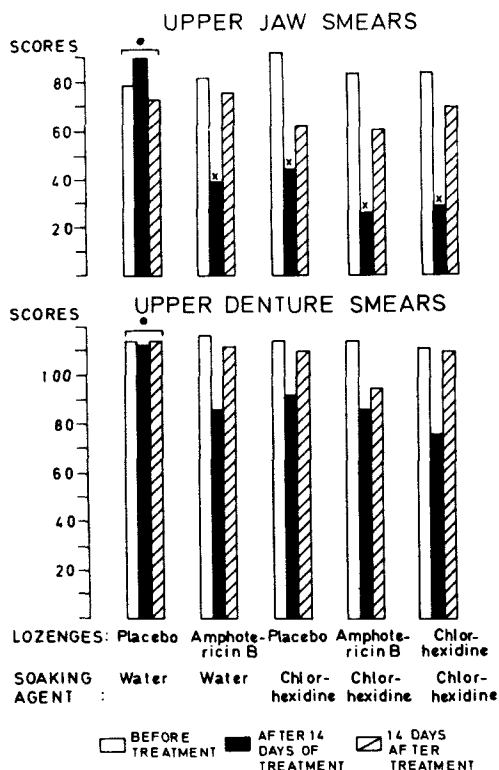


Fig. 2. Summarized scores on yeast-like fungi in smears from the upper jaw and upper denture in 100 patients with denture stomatitis. (No blastospores/hyphae = 0, blastospores = 1, single hyphae = 3, scattered mycelium = 4, large accumulations of mycelium = 5.)
* significantly reduced scores compared to the control. (5 % level of significance.)

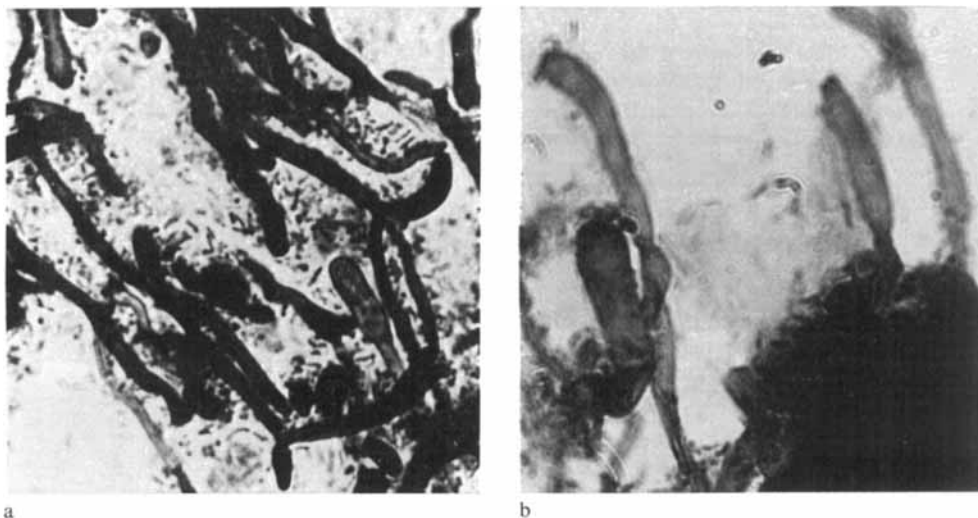


Fig. 3. Denture smears. PAS-hematoxylin. $\times 1180$.

a) Blastospores and hyphae before treatment.

b) Persisting hyphae showing swelling and reduced staining after denture soaking in 0.2 % aqueous chlorhexidine digluconate 4 times daily for 14 days.

scores, the smear scores from chlorhexidine-treated dentures were not significantly diminished. Changes such as reduced staining, swelling or washing out of cellular contours were observed in many of the yeast-like cells in smears from dentures yielding no growth (Fig. 3b). The tongue smears essentially confirmed the drug effects indicated by the palatal smears. Scrapings from treated rhagades demonstrated that in this site the chlorhexidine disinfectant was less efficacious than the other active medications.

In smears taken 14 days after treatment the yeast-like cells were nearly as numerous as before treatment. Again the largest concentrations were found on the denture scrapings. The smear scores were not statistically different from pretreatment values in any of the groups.

DISCUSSION

Denture disinfection revealed itself as an essential procedure. This agrees with the fact that the denture harbours the bulk of the micro-organisms involved. Denture soaking in chlorhexidine also reduced the amounts of yeast-like fungi on the palate and the tongue, and in the mouth angles. This may be ascribed to the antifungal effect of chlorhexidine brought into the oral cavity on the denture. A binding of chlorhexidine to acrylic denture base materials has been demonstrated by Heyden *et al.* (1971a). However, it may also reflect the reduction of the denture microflora.

Sucking of antifungal tablets proved to be the least rational procedure. Although it significantly reduced the amount of fungus on oral tissue, that on the denture was rather unaffected. This explains why amphotericin B lozenges did not reduce the number of *C. albicans* isolates. It

should be noted that 4 amphotericin B lozenges a day, as recommended by the manufacturer, are a relatively low dosage, being equivalent to about 0.8 mega units nystatin. In the treatment of denture stomatitis 1.5 mega units nystatin are currently used.

The combination of amphotericin B lozenges and chlorhexidine denture disinfection appeared as the most effective antifungal regimen, probably because it allowed a more effective contact between drugs and involved organisms.

As observed by Davenport (1970), negative cultures occurred at the end of treatment, while denture smears were still positive. A defect in the sampling technique may have been responsible, but this seems unlikely as the agar was kept in contact with the remaining fungi on the denture fitting side for 1 hour. A more reasonable explanation is that the changes observed in yeast-like cells were expressions of reduced viability induced by the treatment.

In the response rating scale used for smears score 2 was omitted to stress the difference between blastospores and hyphae, as the former are widely held to be the nonpathogenic and the latter the pathogenic form of *Candida* (Kozinn & Taschdjian, 1962; Cawson, 1965; Jepsen & Winther, 1965; Budtz-Jørgensen & Bertram 1970).

Both cultures and smears demonstrated that the mycotic flora was not completely eradicated by the presently used procedures. Since yeasts may be present as saprophytes, negative cultures or smears are not necessarily criteria of treatment success. Actually, a clinical cure may be obtained in the persistence of yeasts (Ewing, 1967). Nevertheless, it is desirable that at least the denture yeast flora is eliminated as it seems to be a key factor

in the development of denture stomatitis. This flora was re-established after a rather short period. As the denture thereby may promote re-infection, it is recommended to include disinfection in the regular denture hygiene, particularly in susceptible individuals.

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