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Inhibition of plaque formation by a sustained release delivery system for cetylpyridinium chloride

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Summary

Cast films composed of ethyl cellulose and cetylpyridinium were prepared from an ethanol varnish and exhibited sustained inhibition of *Streptococcus mutans* growth in vitro. In a clinical study the varnish containing 2% of the drug was applied to removable appliances in 8 patients and the effect of sustained release of cetylpyridium on the total flora and on the plaque accumulation was measured. All oral hygiene procedures were withheld during the experiment. The results of the clinical study proved that a single application of cetylpyridinium varnish decreased plaque accumulation for a period of 3 days. No changes in the total salivary flora were observed during the study.

Introduction

Dental caries and periodontal disease, the two most important oral diseases, may be attributed to dental plaque (Axelsson and Lindhe, 1978). Plaque control is primarily concerned with plaque removal but, since complete mechanical plaque removal is difficult for the ordinary patient, control of the residual plaque by an antibacterial agent becomes important.

Among the chemical agents thus far clinically tested for their potential to inhibit the formation of plaque, chlorhexidine has shown the greatest promise (Loe, 1986). The high plaque-reducing property of chlorhexidine in vivo has been attributed to its high germicidal activity and its level of adsorption to enamel, tooth pellicle, oral mucosa and salivary proteins from which sites chlorhexidine is later released to provide prolonged inhibition of oral bacteria (Rolla, et al., 1970).

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound whose properties are similar to those of other surface-active cationic antiseptics (Reynolds, 1982), Gjermo et al., (1970) and Balanyk and Sandham (1985) showed that CPC in vitro had an inhibitory effect on oral streptococci and staphylococci which was equal to or better than that of chlorhexidine. However, when tested in vivo, the effectiveness of CPC is significantly lower than that of chlorhexidine (Llewelyn, 1980). Bonesvoll and Gjermo (1978) have shown that a

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moderate plaque inhibition was obtained when CPC was applied twice daily as a mouthwash. Only when the frequency was increased to 4 times daily did the plaque inhibitory effect approach that of chlorhexidine. The differences in clinical effect could be explained from the retention time of the drug: the quaternary ammonium compounds were cleared from the mouth more rapidly than was chlorhexidine (Bonesvoll and Gjermo, 1978).

The aim of this work was to develop a sustained-release delivery system for local application of CPC. This delivery system would supply the drug at an effective level for a long period of time, and thereby overcome the problem of the short retention time of CPC.

Materials and Methods

Preparation of ethyl cellulose films containing CPC

The technique of preparation of ethyl cellulose (EC) (Hercules, Wilmington, DE) films containing drugs has been presented in detail previously (Friedman and Golomb, 1982). Ethanolic solutions containing the requisite weights of CPC (Sigma, St. Louis, MO) and the ethyl cellulose polymer were poured at a concentration of 10% (w/v) of total solids. The films were cast on glass plates and the solvent was allowed to evaporate for 24 h. Films containing 0 (control) 10, 20 and 30% w/w of CPC in the dry film and a thickness of about 0.2 mm were prepared.

Dissolution rate determination

The procedure of the dissolution rate measurement is described in detail elsewhere (Friedman and Golomb, 1982). Briefly, films containing the drug were cut to a circular form of 12.5 cm^2 in area and accurately weighed. Two films, separated by Parafilm, were clamped between the two compartments of the dissembling dissolution apparatus. In such a system, each of the two cells served as a separate release experiment. 50 ml of dissolution media previously warmed to 37 ° C was added to the dissolution cells. At suitable time intervals the contents of the cells were withdrawn and replaced by fresh solution. The concentration of CPC released was measured by a UV spectrophotometer (Unicam Model SP1805) at 259 nm. In all of the dissolution experiments doubly distilled water served as the dissolution medium.

Sustained inhibition of bacteria growth measurements

Discs of 5 mm diameter were cut from the films and used in the release rate measurements. The discs were placed on agar plates containing *Streptococcus mutans* and were kept at 37 ° C in an incubator for 24 h. *Streptococcus mutans* 6715 was obtained from the stock of the Department of Oral Biology, Jerusalem. At the end of each incubation period the diameter of the zone of inhibition was measured and the film sample transferred to a new plate for continuation of the experiment. Each experiment was performed in triplicate; the mean calculated inhibition diameters were reproducible to within 10% of the means.

Clinical experiment

Eight patients between 6 and 12 years of age, each treated by an upper removable orthodontic appliance, were chosen for this study from the students' orthodontic clinic at the Hebrew University-Hadassah School of Dental Medicine. Prior to the study, the parents of the children were informed of the benefits and risks associated with the study and their consent was obtained. At the start of the experiments, the participants were brought to plaque index Pl.I. = 0 by means of scaling and brushing with a brush and abrasive paste. The orthodontic appliances were coated with an ethanolic varnish containing 8% w/v EC and 2% w/v CPC, using a soft brush. The acrylic portion of each plate was coated on the palatal and oral surfaces with a total of 15 mg $\pm$10% of the drug. The solvent was allowed to evaporate for 12 h. Salivary samples, 1 ml in volume, were taken before the plates were delivered to the participants and again during and at the end of the study, in order to evaluate the effect of the sustained release of CPC on the oral microflora.

The saliva samples were vortexed for 60 s and then serially diluted in isotonic saline. Duplicates of each dilution (1 : 100; 1 : 1000) were plated onto blood agar plates with an automatic plater (Spiral

Systems Inc., Cincinnati, OH) (Jarvis et al., 1977) and the plates were incubated aerobically at 37 °C for 48 h. At the end of the incubation period, the total numbers of aerobic colony-forming units (CFU's) were counted and the mean value of the counts was calculated for each sample. The given counts represent the total number of aerobic bacteria in 1 ml of saliva sample. The recording of the Pl.I scores was made daily for 4 days using the method of Turesky et al. (1970).

In the control experiment the orthodontic appliances were coated by ethanolic varnish containing the ethyl cellulose polymer only. The participants were asked to refrain from oral hygiene during the experiment. The initial preparation of the patients, the measurements of the plaque indices and the microbial counts of the saliva were performed by the same methods as described previously. A two week break period was given to the participants between the experiment and the control study. The results of the experimental and control periods were compared using the paired *t*-test.

Results

Release of CPC from ethyl cellulose films and bacteria growth inhibition

Fig. 1 shows the release rate profiles of CPC from EC film as a function of time. In each film studied, CPC was released at a rate that decreased with time. The increase in the initial drug concentration in the film resulted in an increase in the release rate. The diameter of inhibition obtained from discs containing 0, 10, 20 and 30% CPC as a function of time is presented in Table 1. Non-significant differences in the inhibition diameters were observed in films containing 10 and 20% of CPC. However, the increase in the initial CPC concentration to 30% produced a corresponding increase in the inhibition diameter. No antibacterial activity was exhibited by discs of ethylcellulose without CPC.

TABLE 2

The effect of sustained-release application of CPC on the plaque indices (mean ± S.E.M.) (n = 8)

Days	Control	CPC	*P*
1	1.40 ± 0.09	0.23 ± 0.06	< 0.01
2	1.73 ± 0.16	0.61 ± 0.08	< 0.01
3	2.34 ± 0.21	0.75 ± 0.05	< 0.01
4	2.40 ± 0.29	1.65 ± 0.12	n.s.

Clinical study

The effects of local application of a sustained-release delivery system of CPC on plaque accumulation and on total bacterial counts are summarized in Tables 2 and 3. Table 2 clearly indicates that the plaque accumulation was significantly inhibited during the first 3 days after the application when compared with the plaque indices of the control study. There was no signifi-

TABLE 3

The bacterial count in saliva (CFU/ml, mean ± S.E.M.). Before and during treatment with sustained release dosage form of CPC

	Before treatment	During treatment (days)	
		2	4
Control	$(1.2 \pm 0.9) \times 10^7$	$(0.85 \pm 0.75) \times 10^7$	$(1.41 \pm 1.2) 10^7$
CPC	$(1.37 \pm 1.1) \times 10^7$	$(2.01 \pm 1.92) \times 10^7$	$(1.81 \pm 1.6) 10^7$

TABLE 1

The inhibition diameters of streptococcus mutans as a function of CPC concentration in ethyl cellulose films

Percentage of CPC in film (w/w)	Diameter of inhibition (mm) in time (days)									
	1	2	3	4	5	6	7	10	12	17
0	0									
10	8.1	7.0	7.2	6.8	6.6	6.2	6.4	6.0	6.1	6.1
20	8.6	9.0	8.5	8.2	8.0	7.8	7.6	7.2	6.9	6.2
30	11.1	11.4	11.0	10.9	10.6	10.1	10.4	9.8	9.4	9.1

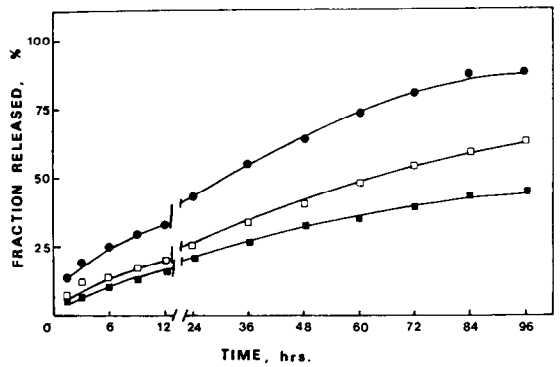

Fig. 1. Release profiles of CPC from films as a function of time. Key (concentration of the drug in film, % w/w): ■, 10; □, 20; ●, 30.

cant difference in plaque indices between the control and the CPC study after 3 days. From Table 3 it can be seen that there was no significant difference in the total number of bacteria in the salivas during the control and experimental periods.

Discussion

The mechanical removal of plaque by toothbrushing procedures still appears to be the most effective and widely employed oral hygiene method. However, since complete plaque removal is very difficult for the ordinary patient, control of the residual plaque by antiseptic agents may become an important ancillary method. Antiseptic drugs are commonly marketed today for topical application in the form of dentifrices, solutions, gels and mouthrinses. None of these dosage forms is able to release the antiseptic drug to the oral cavity for a prolonged period. Sustained-release dosage forms of antiseptic agents which can supply the drug at a therapeutic level for a long period of time increase significantly the efficacy and compliance, and decrease the side effects of the drug.

The relatively low efficacy of CPC in clinical studies could be related to the short retention time of the drug in the oral cavity. The current study has proved that by release of embedded CPC from ethyl cellulose polymer, prolonged antibacterial activity could be achieved both in vitro and in vivo.

The release rate data obtained in this study conform to our previously published data on the kinetics of drug release from ethyl cellulose polymer (Friedman, 1980, 1981; Friedman and Golomb, 1982).

The most important conclusion of the results of microbiological studies is that embedding CPC in ethyl cellulose polymer did not inhibit the biological activity of the drug.

Our previous clinical studies (Friedman et al., 1984, 1985) demonstrated that a single application of chlorhexidine prevented plaque formation for a period of 3 days when applied in a sustained-release dosage form. The current study shows that similar results could be achieved by sustained-release application of CPC. The results of the current study showed that the sustained-release delivery dosage form of CPC effectively reduced plaque formation, but did not reduce the total salivary flora. Thus plaque reduction by CPC in the sustained-release dosage form may be due to the high concentration of the drug on the tooth surface achieving a local antiseptic effect. Although the number of patients participating in this study was small, the results were unequivocal and leave no doubt as to the effectiveness of the treatment.

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