The effect of some cationic antiseptics on the acidogenicity of dental plaque *in vivo*

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Two series of experiments were performed in order to compare the ability of different cationic antiseptics to inhibit the acid production in plaque. In addition an attempt was made to evaluate the influence of oral retention on the acid-inhibiting properties of these agents. In one series of experiments acid production, following sucrose applications on plaque, was measured *in situ* prior to and at given time intervals after rinsing with the individual agents. In a second series the effect of eluting the antiseptics retained in the oral cavity by means of 5 consecutive acetic acid (6 mM) rinses was evaluated. The results showed that chlorhexidine (0.5 mM) was more effective than benzalkonium chloride (1mM) and piperazine (1mM). Cetylpyridinium chloride (1mM) was the least effective. Acidic elution markedly reduced the inhibitory effect of single rinses of chlorhexidine (0.5 mM), benzalkonium chloride (1mM) and cetylpyridinium chloride (1mM). This effect was less pronounced with a higher concentration (2.2mM) of chlorhexidine. The results gave support to the view that retention of an agent in the mouth and in plaque is of significance for its ability to inhibit acid production of dental plaque.

Key-words: Acid production; inhibition; preventive dentistry

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It has been shown previously that the cationic chlorhexidine has a marked and prolonged effect on the acidogenicity of dental plaque (11). Uncharged or anionic antiseptics with comparable antimicrobial activity against plaque bacteria did not display similar effects (12). It was suggested in these studies that the long-lasting effect of chlorhexidine on the acid production in dental plaque might be related to the oral retention of this agent. It would, therefore, be of interest to investigate whether other cationic agents known to be retained in the mouth, such as some quaternary ammonium bases (3), would exhibit a similar inhibitory effect.

The aim of the present study was to compare the inhibiting effect of chlorhexidine on the acid production in dental plaque with that of some other antiseptics having common chemical properties with chlorhexidine. An attempt was also made to estimate the significance of the retention of the agents in the oral cavity for this effect.

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MATERIAL AND METHODS

Test panel

Nine subjects aged averagely 23 years, with full dentitions and healthy gingival conditions participated in the experiments. Clinical and radiographic examinations showed no carious lesions among the participants.

Test agents

Chlorhexidine solutions were prepared from a 20% aqueous solution of chlorhexidine di-gluconate (HIBITANE®, ICI, MacClesfield, England). Cetylpyridinium chloride (PYRISEPT®) was obtained from WEIFA Pharmaceutical Industries, Oslo, Norway. Benzalkonium chloride was purchased from Denochemo A/S (Copenhagen, Denmark) and piperazine [1,4-bis-(hexylcarbamylguanidinopronyl) piperazine dimethanesulfonic acid] was obtained from Cooper Laboratories, Inc. (New Jersey, USA). The latter is referred to in this paper by its generic name piperazine.

pH measurements

Plaque pH measurements were performed *in situ* with the aid of an Ingold flat surface glass electrode. Two contralateral teeth in each jaw were selected for measurements. Changes in plaque pH, following an application of 50 µl og 15% w/v sucrose solution on the tooth surface, were recorded every 15 seconds for a period of 5 minutes. The method has been described more extensively elsewhere (11).

General experimental design

Each experiment lasted for 4 days divided in a pre-experimental period of 3 days and an experimental period of 24 hours. At the start of the pre-experimental period the participants had their teeth scaled and polished to remove supragingival deposits. All mechanical oral hygiene was then suspended until the end of the experiment. Sucrose (15% w/v) rinses were carried out every other hour from 8:00 a.m. to 8:00 p.m. during the three first days in order to enhance plaque formation (7). The participants discontinued the sucrose rinses and avoided further sucrose intake 12 hours prior to and throughout the experimental period. Plaque pH readings were performed at least 0.5h after any food intake to minimize the influence of meals on the measurements.

Experimental series

The effect of the test agents on acid formation in plaque was compared in a series of experiments with three subjects participating. The experimental period started with the application of sucrose and recording of acid production by pH measurements. These results were considered the individual's base line data. The participants then rinsed for 1 minute with 10 ml of one of the following substances: Chlorhexidine (0.5mM), cetylpyridinium chloride (1mM), benzalkonium chloride (1mM) or piperazine (1mM). Plaque pH measurements, following new sucrose applications, were then performed 0.5, 2, 4, 6, and 24 hours after the rinsing. All participants were tested with all agents.

In order to test the effect of acidic elution of the antiseptics a second series of experiments was carried out with cetylpyridinium chloride (1mM), benzalkonium chloride (1mM) and chlorhexidine (0.5 mM and 2.2 mM). Two groups, with three participants each, constituted the test panel. One group tested the quaternary ammonium compounds and the other group chlorhexidine. After the base line readings the participants rinsed for 1 minute with 10 ml of the test agents followed by 5 consecutive rinses with acetic acid pH 3.0 (6 mM) according to a method described by Gjermo et al (8). Acid formation upon new sucrose appli-

Table 1. *Mean plaque pH and Δ pH 5 min after a sucrose (15% w/v) application before (base line) and at different time intervals after (experimental) rinsing with the four test agents*

Agent	Base line values		Experimental values											
			0.5 h		2 h		4 h		6 h		24 h		sp**	
	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH
Benzalkonium chloride 1 mM	4.73	2.14	5.93	0.78*	5.68*	1.42*	5.09*	1.60*	4.84	2.13	4.81	2.16	0.20	0.12
Cetylpyridinium chloride 1 mM	4.69	2.10	5.55*	1.20*	5.28*	1.55*	4.83	1.86	4.87	2.01	4.83	1.99	0.42	0.16
Chlorhexidine 0.5 mM	4.70	2.05	6.01*	0.80*	5.85*	1.03*	5.73*	1.14*	5.43*	1.50*	5.07	1.82	0.21	0.28
Piperazine 1 mM	4.83	2.04	5.81*	1.11*	5.57*	1.39*	5.30*	1.56*	5.04	1.83	4.98	2.10	0.25	0.21
sp	0.23	0.19	0.37	0.26	0.31	0.20	0.17	0.18	0.31	0.22	0.31	0.31		

' – Δ pH is the difference between plaque pH values before and 5 min. after the sucrose application
* –indicates significant differences from the base line ($p < 0.05$)
| –indicates significant differences between agents ($p < .005$)
** –(sp) pooled standard deviation

cations was then monitored after the time schedule of the first series up to the time when the acid production corresponded to the base line data. At this time an extra rinse with the original concentrations of the antiseptics was performed as a final control. The acid production in the presence of sucrose was then measured after 0.5 and 2 hours. The experimental design is illustrated in Fig. 1.

Statistical analyses

The mean pH drop (Δ pH) was expressed as the difference between the initial pH values and the pH values observed 5 min after the sucrose challenge (11). The statistical significance of the results was tested by an analysis of variance employing a pooled standard deviation (4).

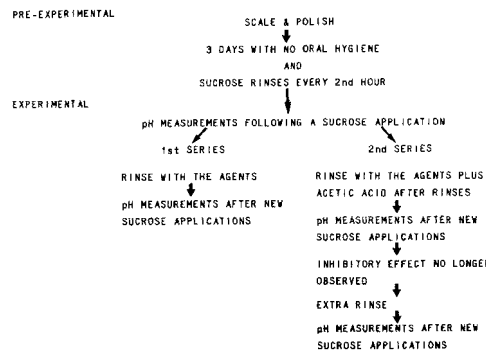

Fig. 1. The experimental design.

RESULTS

Table 1 shows the results of the first series of experiments where the inhibitory effect of the different test agents was compared. Chlorhexidine (0.5 mM) was the most effective agent, showing statistically significant differences from the control values 6h after rinsing. Benzalkonium chloride (1mM) and piperazine (1mM) had a comparable effect up to 4h after treatment. Cetylpyridinium chloride (1mM) was the least effective, yielding statistically significant reduction 2h after rinsing. The same agents prevented pH from dropping below 5.50 for 4h, 2h, 2h and 0.5h respectively.

Acetic acid elution markedly reduced the inhibitory effect of chlorhexidine (0.5 mM), cetylpyridinium chloride (1mM) and benzalkonium chloride (1mM) (Table 2). The pH drop observed at 0.5h after treatment approached the base line values. No differences could be observed between the pH values obtained 2h after treatment and those of the base line. The inhibitory effect observed following extra rinses with the test agents was similar to that observed in the first series of experiments. Acetic acid after rinses did not markedly reduce the effect of a 2.2 mM chlorhexidine rinse up to 6h, but the inhibitory effect was lost after 24 hours (Table 2). A control experiment with acetic acid rinses alone did not influence the acid production as measured following a sucrose application 0.5 h after the rinsing.

DISCUSSION

The choice of the test agents used in the present study was based on data from different sources concerning their respective plaque inhibiting effect, oral retention and antimicrobial activity (1, 3, 6, 7, 18, 19). Piperazine was included because it is claimed to have plaque-inhibiting activity comparable with chlorhexidine, and because it is cationic (15). In the original work where chlorhexidine was eluted from the mouth by means of acidic rinses no other agents were tested (8). Since the oral retention of chlorhexidine has been ascribed to its cationic nature (2, 5, 14), it was assumed that other cationic antiseptics, such as the quaternary ammonium bases, would also be similarly released by acid.

Table 2. *Mean plaque pH and Δ pH 5 min following a sucrose application, before (base line) and after rinsing with cetylpyridinium chloride (CPC), benzalkonium chloride (BC) or chlorhexidine (CH) plus 5 acetic acid after rinses. Extra rinses with the antiseptics alone were performed when, within the 24-hour experimental period, the inhibitory effect of the agents was no longer observed*

Agent	Base line		Time period after rinses								Time period after extra rinses				sp	
			0.5 h		2 h		6 h		24 h		0.5 h		2 h			
	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH
CPC 1 mM	4.86	1.95	5.10	1.62	4.93	1.88	nd	nd	nd	nd	5.65	1.16	4.96	1.83	0.18	0.20
BC 1 mM	4.93	2.00	5.28	1.33	4.98	1.76	nd	nd	nd	nd	5.95	0.88	5.61	1.18	0.11	0.15
CH 0.5 mM	4.48	2.33	5.05	1.85	4.67	2.34	nd	nd	nd	nd	5.85	1.15	5.86	1.14	0.22	0.19
CH 2.2 mM	4.55	2.17	6.46	0.60	6.14	0.85	5.67	1.21	5.10	1.76	nd	nd	nd	nd	0.21	0.18

- Δ pH is the difference between plaque pH values before and 5 min after the sucrose application
- sp = pooled standard deviation
- underlined figures are significantly different from the respective base line ($p < .005$)
  nd = not determined

The results of the present study showed that the test agents inhibited acid production in dental plaque at significant levels when compared with their respective base line. The test agents, following sucrose applications, kept the pH values for different time periods after treatment above 5.50. This effect may be of clinical interest, since this pH value is within the pH range where enamal demineralization does not occur (9). Previous studies have shown that anionic or neutral antiseptics failed to inhibit acid production in plaque to the same extent (12, 17). The present observations showed that the inhibitory effect, previously observed for chlorhexidine (11), is shared with other cationic agents with some common chemical properties with chlorhexidine. Among them the well recognized antibacterial activity of chlorhexidine and the quaternary ammonium compounds (for review see 13) cannot be disregarded. The comparable *in vitro* activity against oral bacteria displayed by these agents can, however, hardly explain the differences in the duration of the inhibitory effect observed in the present study. It should, however, be recognized that *in vitro* tests have obvious limitations in predicting events in the oral cavity. Pharmacodynamic differences in the oral retention and release of chlorhexidine and some quaternary ammonium bases have been reported, the latter being released more rapidly than chlorhexidine (3). A correlation thus appears to exist between the oral retention and release of the individual cationic agents and their ability to exert a long lasting influence on plaque metabolism. Similar observations have previously been made concerning the capacity of these agents to inhibit plaque formation (3).

The results obtained in the second series of experiments confirmed that the oral retention of the antiseptics is of importance for their inhibitory effect. Acetic acid after rinses markedly reduced the duration of the inhibitory effect of chlorhexidine (0.5 mM) and of the two quaternary ammonium bases (1mM) on the acidogenicity of dental plaque. Thus, the inhibitory effect of these agents cannot be due only to an irreversible change of the plaque bacteria. On the other hand, acetic acid after rinses did not induce a similar reduction on the inhibitory effect of a 2.2 mM chlorhexidine rinse up to 6h after treatment. It appears that a bactericidal effect may prevail at this concentration. However, a certain effect of the acidic elution was observed also in this case because the pH drop after 24 hours (following a sucrose application) was more marked than when acidic after rinses were not performed (11).

The mechanisms by which these agents inhibit the acid production in dental plaque are yet unclear. The long-term bacteriostatic milieu provided by the oral retention of these agents (10, 14) is of importance. Antibacterial agents, once retained in plaque, may interfere with bacterial metabolism in different ways. One interesting possibility may be a direct inhibition of the carbohydrate transport into the bacterial cell. Some evidence for this has been provided by the observation that chlorhexidine and benzalkonium chloride inhibit the glucose phosphotransferase system of *Streptococcus mutans* (16).

In conculusion the results of the present study support the view that the oral retention and release of an antibacterial agent is of significance for its potential to influence the metabolic activity in dental plaque for a prolonged period of time. These properties should thus be considered of importance for the observed inhibiting effect and not only the immediate antibacterial activity of the agents *per se*.

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