In Vitro Evaluation of Seven Cationic Detergents as Antiplaque Agents

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The bactericidal efficacy of seven cationic detergents was studied in vitro against intact preformed plaques of representative strains of four oral dental plaque-forming microorganisms: *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Actinomyces naeslundii*. Both absolute (plaque bactericidal index) and relative (chlorhexidine coefficient) indexes of antiplaque activity were established. Only cetyltrimethylammonium bromide was more efficacious than the reference agent, the bisbiguanide chlorhexidine, for the apparently more potent tooth-adherent cariogenic and periodontopathic elements, respectively, of the in vivo plaque microflora, *S. mutans* and *A. viscosus*.

Control of dental plaque, which is associated with gingivitis and dental caries, may be achieved by topical applications of antimicrobial agents (11, 18, 19). Topical rather than systemic administration of such agents to supragingival plaque is indicated because of its dense, tooth-adherent nature and, importantly, because plaque is not bathed in blood or interstitial fluids but is exposed to the environment of the oral cavity.

Cationic detergents have been known for a number of years to possess bactericidal and fungicidal properties (17). Cationic agents are also of interest because of potential reversible binding (substantivity) to anionic groups on oral mucous membranes (2, 21, 22, 25, 26), which could permit possible prolonged intraoral retention and sustained release of such cationic antiseptics. Recent studies have demonstrated modest in vivo and in vitro antiplaque activities of three quaternary ammonium detergents, namely, cetylpyridinium chloride, domiphen bromide, and benzylalkonium chloride (1, 6, 12, 27, 35). In those studies, however, the optimal conditions required for in vivo efficacy were not evaluated.

In the work reported herein an examination of seven cationic detergents was undertaken with a view to establishing the optimal conditions likely to achieve maximal efficacy in vivo. These agents were assessed for their antiplaque activities against strains of four oral plaque-forming microorganisms by using an in vitro preformed-plaque model system (30, 31), and their potencies were compared on both absolute

and relative bases to that of the potentially bactericidal bisbiguanide chlorhexidine.

MATERIALS AND METHODS

Cationic detergents. The structures of the agents employed in these studies are detailed in Fig. 1. Reagent-grade compounds were obtained from the following sources: Undecoylium chloride (UC), Chesebrough-Ponds Research Labs, Trumbull, Conn.; *p*-tert-octylphenoxyethoxyethyl dimethylbenzylammonium chloride (TOPEC), Pfaltz and Bauer, Stamford, Conn.; tetradecyldimethylbenzylammonium chloride (TDAC), Sterwin Chemical, New York; methylbenzethonium chloride (MC), benzethonium chloride (BC), cetylpyridinium chloride (CPC), and cetyltrimethylammonium bromide (CTAB), Sigma, St. Louis, Mo.

Microorganisms. The microorganisms used in these studies were *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Actinomyces naeslundii*. The strains and their sources and origins are given in Table 1. These microorganisms form plaques both in vitro and in vivo and, with the possible exception of *S. sanguis*, have a known association with the development of coronal caries and/or periodontal disease with attendant root surface carious lesions (3, 5, 9, 13, 23, 24). Identities were reconfirmed on the basis of biochemical and morphological data. Unless otherwise stipulated, the strains used in most of these studies were *S. mutans* 6715-13, *S. sanguis* ATCC 10558, *A. viscosus* M-100, and *A. naeslundii* ATCC 12104. All organisms were maintained either frozen or lyophilized except when used for experiments for which working cultures were maintained by monthly passage in fluid thioglycolate medium (Difco) containing 20% (vol/vol) meat extract and excess CaCO₃.

In vitro plaque formation. In vitro plaques were grown on no. 20 nichrome wires by using a slight modification of methods previously detailed (30, 31). Fluid thioglycolate cultures were used to inoculate a complex medium (14) supplemented with 5% (wt/vol)

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FIG. 1. Structures of the agents employed.

sucrose and 0.005% (wt/vol) sodium carbonate. The nichrome wires, fixed to culture tube closures (33), were transferred daily to fresh medium until the wire-adherent microorganisms (in vitro plaques) had grown to a rating of 3 to 4 as judged by the method of McCabe et al (20). *A. naeslundii* and *S. sanguis* cultures were grown in Morton closure-covered tubes in GasPak jars (BBL), *S. mutans* in rubber-stoppered tubes, and *A. viscosus* in Morton closure-covered tubes incubated aerobically. Such conditions were observed to maximize the plaque-forming ability of these microorganisms (32, 33), but not to influence resultant data. All cultures were incubated at 37°C.

Assessment of efficacy of cationic detergents. It has previously been demonstrated that serial tube dilution methods are inappropriate for testing efficacy of agents against plaque microorganisms. Such methods require the presence of agent for a prolonged period at constant concentration, a situation unlikely to occur in the mouth, and do not take into account the diffusion barriers apparently existing in intact dental plaque (29, 31). Thus, tube dilution techniques were demonstrated to overestimate the potency of agents by several orders of magnitude (31). Accordingly, agents were tested here by short-term immersion of preformed plaques in various agents employing an in vitro preformed-plaque model system. The methods were previously detailed (30-33). Briefly, in vitro plaques were rinsed free of broth in demineralized water and then immersed in 10 ml of aqueous solution of the various test or control agents, at pH 6.8 to 7.0, for various durations and frequencies. The wire-adherent plaque samples were then rinsed twice in 15 ml of distilled water. Rinsing caused at least a 10⁶-fold dilution of any carried-over agent. Finally, the wire-adherent plaques were transferred to fresh broth containing a pH indicator. Plaques were judged to have been killed by the cessation of culture acid production and of turbidity increase and by failure of 24-h post-

TABLE 1. Microorganisms studied

Microorganism	Strain	Reference
<i>S. mutans</i> (Bratthall [4] serotype)		
a	E-49	8
b	FA-1	7
c	Ingbritt-1600	16
d	6715-13	28
e	LM-7	10
<i>S. sanguis</i>		
	ATCC 10558	30
	H7P	36
	A12R	36
<i>A. viscosus</i>		
	M-100	15
	UCHC HAS	33
	UCHC T04	33
	UCHC H-1	33
<i>A. naeslundii</i>		
	ATCC 12104	
	I-S-1000	24

treatment plaque samples to grow when plated on appropriate agar media.

RESULTS

Minimum bactericidal concentration. To determine the minimal concentrations of the agents that maximally inhibit plaques of the four oral microorganisms, preformed plaques were immersed in various concentrations of the agents for 30 min at 37°C. Control plaques were similarly immersed in sterile water. All of the cationic detergents examined were bactericidal as judged by the cessation of culture acid produc-

tion and of turbidity increase and by failure of 24-h posttreatment plaque samples to grow when plated on appropriate agar media (Table 2). CTAB was judged to be the most potent and CPC the least potent for all four plaque-forming microorganisms, and differential potency of agents for the various microorganisms was also apparent. UC was observed to be especially differentially efficacious, requiring between 10 and 100 times higher concentration to kill the *Actinomyces* species than the *Streptococcus* species examined.

Minimal exposure time. The minimal duration of a single treatment for the most potent of these agents was determined by immersion of preformed plaques in the previously established 30-min minimal bactericidal concentrations but for 1, 2, 5, 10, 20, or 30 min. Initial studies

indicated that in almost all cases the previously determined concentrations were only bactericidal when employing exposure times of 30 min. Shorter durations of exposure caused transient inhibition of culture acid production, which was reversed upon extended incubation (48 h). The concentrations of these agents had to be increased before bactericidal effects were apparent after exposure times of less than 30 min, and almost all agents required 2 min or more of exposure to be plaque bactericidal (Table 3).

Frequency of exposure. Assuming that a mouthrinse is the preferred method of delivery for such agents, the time such an agent would normally be retained orally would not likely exceed 2 min. Studies were, therefore, conducted to evaluate whether shorter but more frequent agent exposures could achieve bactericidal effect. Two types of experiments were performed with the previously identified most-potent agents. In the first, plaques of the test microorganisms were treated either once or once daily, analogous to a daily mouth rinse; in the second, plaques were treated for 2 min, rinsed, incubated in growth medium for 6 h, and retreated for 2 min, analogous to two daily mouth rinses. Table 4 shows the concentration and frequency of use of the five most potent cationic detergents examined. Generally, CTAB was the most potent of the agents against the various test microorganisms. The remaining agents killed plaques of *S. mutans*, *S. sanguis*, and *A. viscosus* after five or fewer daily treatments. Plaques of *A. naeslundii*, however, were not killed by those agents at the concentrations examined after multiple 2-min exposures.

To establish that the conditions for the five most efficacious cationic detergents were generally appropriate, a number of strains of the test microorganisms were studied. Table 5 shows the results for the minimum bactericidal concentration determinations for various strains of the

TABLE 2. Minimum bactericidal concentrations of cationic detergents for preformed plaques after exposure for 30 min at 37°C^a

Agent	Minimum bactericidal concn (mM)			
	<i>S. mutans</i>	<i>S. sanguis</i>	<i>A. viscosus</i>	<i>A. naeslundii</i>
CTAB	0.27	0.27	1.35	1.35
CPC	29.4	2.94	29.4	29.4
TDAC	0.25	2.52	2.52	2.52
BC	2.23	2.23	2.23	2.23
MC	2.16	2.16	0.22	2.16
UC	2.59	2.59	25.9	259.0
TOPEC	2.31	2.31	2.31	2.31

^a Tests were run in quadruplicate. Results indicate the uniform behavior of the quadruplicate set. The agents were initially tested at the following concentrations (mM): CTAB—27.0, 13.5, 2.70, 1.35, 0.270, 0.135, 0.027, 0.0135; CPC—294.0, 29.4, 2.94, 0.294, 0.0294, 0.00294; TDAC—252.0, 25.2, 2.52, 0.252, 0.0252, 0.00252; BC—223.0, 22.3, 2.23, 0.223, 0.0223, 0.00223; MC—216.0, 21.6, 2.16, 0.216, 0.0216, 0.00216; UC—259.0, 25.9, 2.59, 0.259, 0.0259, 0.00259; TOPEC—23.1, 2.31, 0.231, 0.0231, 0.00231.

TABLE 3. Minimum bactericidal concentration and minimum killing time of cationic agents^a

Microorganisms	Agent									
	CTAB		TDAC		BC		MC		TOPEC	
	MBC (mM)	MKT (min)	MBC (mM)	MKT (min)	MBC (mM)	MKT (min)	MBC (mM)	MKT (min)	MBC (mM)	MKT (min)
<i>S. mutans</i>	2.70	5	2.52	20	4.46	5	4.32	20	4.62	10
<i>S. sanguis</i>	2.70	2	5.04	2	4.46	1	4.32	5	4.62	2
<i>A. viscosus</i>	2.70	2	5.04	2	4.46	2	2.16	30	4.62	2
<i>A. naeslundii</i>	2.70	10	5.04	20	4.46	10	4.32	20	4.62	5

^a MBC, minimum bactericidal concentration; MKT, minimum killing time. Plaques were run in quadruplicate. Results indicate the uniform behavior of the quadruplicate set. The agents were tested at the following concentrations (mM): CTAB—5.40, 2.70, 1.35, 0.675; TDAC—5.04, 2.52, 1.26; BC—8.92, 4.46, 2.23; MC—8.64, 4.32, 2.16; TOPEC—9.24, 4.62, 2.31.

four microorganisms after a single 30-min treatment. The results described above (Table 2) for four other plaque-forming species appeared generally applicable.

PBI and CC. To employ a potential therapeutic utility index that could reflect the integration of the effects of concentration, duration, and frequency of treatment required to achieve a bactericidal effect, the product of these parameters was computed for each of the five most potent agents and for each of the test microorganisms and was expressed as a plaque bactericidal index (PBI) (32). In addition, to allow for a ready comparison of bactericidal indexes of these compounds with the extensively studied bisbiguanide chlorhexidine, a chlorhexidine coefficient (CC) was derived by dividing the PBI for chlorhexidine (see ref. 32 for derivation) by that of the individual cationic agents for each of the test microorganisms. The CC for chlorhexidine is thus 1.00 (32). The CC thus allows ready comparison of the antiplaque efficacy for each of the five most potent cationic agents against the different species of plaque-forming microorganisms, in reference to chlorhexidine. A CC value of >1.00 indicates a greater bactericidal potency than chlorhexidine. It should be noted that the use of a PBI predicts that if, for example, CTAB at a concentration of 2.7 mM used once daily for 2 min on 2 successive days (PBI = 10.8) will kill an *S. mutans* plaque, then 5.4 mM should achieve the same result after one 2-min treatment. Furthermore, 1.35 mM used for 2 min for three successive daily treatments should also kill an *S. mutans* plaque. These predictions were tested and shown to be valid. Similarly, because TOPEC, at a concentration of 4.62 mM, killed an *A. viscosus* plaque after two successive daily 2-min treatments, then concentrations of 9.24 mM, 2.31 mM, and 1.15 mM should kill *A. viscosus* plaques after one, four, and eight successive daily 2-min treatments, respectively. These predictions were also shown to be valid.

PBI and CC computations are shown in Table 6. It may readily be seen that for the apparently most potent cariogenic microorganism, *S. mutans*, three agents compared favorably with the bisbiguanide chlorhexidine, namely, CTAB (CC = 1.77), TDAC (CC = 0.95), and TOPEC (CC = 1.04). For the apparently more potent periodontopathic microorganism, *A. viscosus*, only CTAB compared favorably with chlorhexidine and was, in fact, more potent (CC = 1.70).

DISCUSSION

The seven cationic detergents (quaternary ammonium compounds) examined have a range

TABLE 4. Minimum concentration and frequency of 2-min treatments required for bactericidal effect upon preformed plaque^a

Microorganism	Agent					
	CTAB		TDAC		BC	
	mM ^b	Frequency ^c	mM	Frequency	mM	Frequency
<i>S. mutans</i>	2.70	2 × 2 min, 1 day	2.52	1 × 2 min, 4 days	4.46	2 × 2 min, 1 day
		1 × 2 min, 2 days				1 × 2 min, 3 days
<i>S. sanguis</i>	2.70	2 × 2 min, 1 day	2.52	2 × 2 min, 1 day	4.46	2 × 2 min, 1 day
		1 × 2 min, 2 days		1 × 2 min, 5 days		1 × 2 min, 1 day
<i>A. viscosus</i>	2.70	2 × 2 min, 1 day	2.52	1 × 2 min, 2 days	4.46	2 × 2 min, 1 day
		1 × 2 min, 1 day				1 × 2 min, 2 days
<i>A. naeslundii</i>	2.70	1 × 2 min, 5 days	5.04	Ineffective ^d	4.46	Ineffective
					4.32	1 × 2 min, 4 days
					4.32	2 × 2 min, 1 day
					4.32	1 × 2 min, 3 days
					4.32	1 × 2 min, 3 days
					4.32	2 × 2 min, 1 day
					4.32	2 × 2 min, 2 days
					4.32	Ineffective
					4.62	2 × 2 min, 1 day
					4.62	1 × 2 min, 2 days
					4.62	2 × 2 min, 1 day
					4.62	2 × 2 min, 3 days
					4.62	2 × 2 min, 1 day
					4.62	1 × 2 min, 2 days
					4.62	Ineffective

^a Plaques were run in quadruplicate. Results indicate the uniform behavior of the quadruplicate set. The agents were tested at the following concentrations (mM): CTAB—5.40, 2.70, 1.35, 0.675; TDAC—5.04, 2.52, 1.26; BC—8.92, 4.46, 2.23; MC—8.64, 4.32, 2.16; TOPEC—9.24, 4.62, 2.31.

^b Minimum agent concentration.

^c Number of treatments × time of each treatment for number of days.

^d Ineffective. Treatments for greater than 5 days at the concentrations indicated failed to kill all four plaques in a quadruplicate set.

TABLE 5. Minimum bactericidal concentrations of cationic detergents for preformed plaques of various strains of the test microorganisms after exposure for 30 min at 37°C^a

Microorganism	Strain	Minimum bactericidal concn of agent (mM)				
		CTAB	TDAC	BC	MC	TOPEC
<i>S. mutans</i>	Ingbritt-1600	0.540	0.252	1.125	2.160	2.310
	FA-1	0.270	0.252	1.125	1.080	1.155
	E-49	0.270	0.252	2.230	1.080	2.310
	LM-7	0.135	0.252	2.230	2.160	2.310
<i>S. sanguis</i>	H7P	0.270	2.520	2.230	2.160	2.310
	A12R	0.270	2.520	2.230	2.160	2.310
<i>A. viscosus</i>	UCHC HAS	0.675	1.260	2.230	0.216	2.310
	UCHC TO4	1.350	2.520	2.230	0.216	2.310
	UCHC H-1	0.675	1.260	2.230	0.432	1.155
<i>A. naeslundii</i>	I-S-1000	0.675	2.520	2.230	2.160	2.310

^a Tests were run in quadruplicate. Results indicate the uniform behavior of the quadruplicate set. The agents were tested at the following concentrations (mM): CTAB—5.40, 2.70, 1.35, 0.675, 0.540, 0.270, 0.135, 0.068, 0.027; TDAC—5.04, 2.52, 1.26, 0.504, 0.252, 0.126; BC—4.46, 2.23, 1.125, 0.446, 0.223; MC—8.64, 4.32, 2.16, 1.08, 0.432, 0.216, 0.108; TOPEC—4.62, 2.31, 1.155, 0.462, 0.231, 0.116.

TABLE 6. PBI and CC^a

Agent	Strain							
	<i>S. mutans</i>		<i>S. sanguis</i>		<i>A. viscosus</i>		<i>A. naeslundii</i>	
	PBI	CC	PBI	CC	PBI	CC	PBI	CC
CTAB	10.80	1.77	10.80	0.85	5.40	1.70	27.00	0.47
TDAC	20.16	0.95	25.20	0.36	10.08	0.91	— ^b	—
BC	26.76	0.72	8.92	1.03	17.84	0.52	—	—
MC	34.56	0.56	25.92	0.35	25.92	0.35	—	—
TOPEC	18.48	1.04	27.72	0.33	18.48	0.49	—	—
Chlorhexidine	19.20	1.00	9.20	1.00	9.20	1.00	12.90	1.00

^a PBI values were computed for once-per-day agent exposure conditions using values presented in Table 4.

^b —, Not possible to compute.

of bactericidal activities for the four oral plaque-forming microorganisms tested. The two pyridinium compounds, CPC and UC, were the least efficacious of this series of agents for preformed in vitro plaques, particularly those of the *Actinomyces* species. CTAB was the most efficacious of the agents examined (PBI = 10.8 and CC = 1.77 for *S. mutans*). In addition, the data suggest that it is conceivable to suppress in vivo the apparently more cariogenic and periodontopathic elements of plaque microflora (*S. mutans* and *A. viscosus*, respectively) while permitting the survival of the less orally pathogenic microorganisms (*S. sanguis* and *A. naeslundii*) by using TOPEC. CTAB similarly could possibly be used to select for the survival of *A. naeslundii* while fostering the suppression of the other plaque-forming species. Such may be more desired effects than the total elimination of all elements of the plaque microbiota.

Studies on the antiplaque activities of cationic detergents have been inconclusive with respect to their effectiveness against in vivo dental plaques (1, 6, 12, 27, 35). The studies of Sturzenberger and associates indicated that CPC (1.23 mM) or domiphen bromide (0.6 mM) used as adjuncts to tooth brushing effectively reduced amounts of bacterially undefined in vivo dental plaques as compared to a placebo tooth-brushing control (27). However, these same workers demonstrated that the use of CPC alone at a final concentration of 0.685 mM did not significantly reduce in vivo dental plaque. A similar study of Barnes et al. (1) using CPC plus domiphen bromide as well as CPC-containing commercial mouthwashes demonstrated an in vivo reduction of bacterially undefined plaque when such mouthwashes were used as adjuncts to toothbrushing. In contrast, Gjermo et al. (12) indicated that CPC at a concentration of 2.74

mM used as a mouthrinse for 30 s twice daily was ineffective in reducing *in vivo* plaque. Such a finding is predicted by those of the current report. In the context of these earlier studies the findings reported here suggest that the conditions (concentration, duration, and frequency) previously employed for CPC were perhaps inadequate. In fact, *S. sanguis* is so much more susceptible to CPC than either *S. mutans* or *A. viscosus* as to suggest that, whereas this agent might certainly reduce total *in vivo* plaque scores, it might serve to suppress the most orally innocuous species of the plaque flora and to select for the more orally pathogenic ones, *S. mutans* and *A. viscosus*.

It is presently not possible to account for differential agent susceptibility of the microorganisms examined. However, the effect of an agent against an intact plaque must be a function of the inherent susceptibility of the species in point and the barrier to permeation of an agent to the plaque depth that is constituted by the organisms and their adhesive and cohesive extracellular matrixes. With the present *in vitro* plaque/agent assessment model, because plaques were monitored for up to 120 h post-treatment, survival of even a few cells in the depth of the plaque became clear because of an increase in culture turbidity, change in color of the growth medium pH indicator, and growth of cells following plating of plaque samples on appropriate agar growth media.

The activities of these agents, as they reflect both bactericidal and plaque permeation properties, are not simply predictable from the structure of the agent molecules alone. However, the data suggest that the substitution by a pyridinium moiety for a trimethylammonium group in the alkylmethyl ammonium molecule, as occurs with the agents CPC and UC, significantly reduces those molecules' antiplaque efficacy. Similarly, the substitution of a phenoxy or a cresoyl moiety with the alkyl side chain as occurs in the agents BC, TOPEC, and MC (Fig. 1) also reduces the antiplaque activities of these compounds as compared to CTAB.

The PBI computations (Table 6) allow ready evaluation of the sum (integral) of treatment conditions (concentrations, duration, and frequency) for bactericidal effect on intact plaques of the four test microorganisms. It is not surprising that PBI validly expresses bactericidal properties as a function of these parameters, because intact plaques are very slowly growing (29, 34) and, therefore, represent virtually a stable mass of cells available for killing. Thus, effects of antiseptic treatments appear to be cumulative. The PBI, therefore, is an index of potency of an agent independent of the treat-

ment concentration, duration, or frequency. The CC merely relates the relative potency of an agent to that of chlorhexidine, probably the best studied of potential topical antiplaque agents. PBI and CC calculations indicate that CTAB is more efficacious than chlorhexidine for the apparently most potent cariogenic and periodontopathic elements of the plaque microflora studied, *S. mutans* and *A. viscosus*, respectively.

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