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In Vitro Antiplaque Activity of Octenidine Dihydrochloride (WIN 41464-2) Against Preformed Plaques of Selected Oral Plaque-Forming Microorganisms

ANDREW M. SLEE* AND JOHN R. O'CONNOR

Department of Microbiology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144

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The antibacterial activity of octenidine dihydrochloride (WIN 41464-2) against intact preformed in vitro plaques of four indigenous oral plaque-forming microorganisms, *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Actinomyces naeslundii*, was studied. Both absolute (plaque bactericidal index) and relative (chlorhexidine coefficient) indices of antiplaque efficacy were established. Octenidine dihydrochloride compared favorably with chlorhexidine digluconate with respect to overall antiplaque potency in this in vitro plaque bactericidal model. These data indicate that prudent selection of treatment concentration and duration and frequency of exposure should provide an effective means to aid in controlling dental caries and *Actinomyces*-associated disease in vivo.

Dental plaque, the dense adhesive microbial mass that colonizes tooth surfaces, has been strongly implicated in both experimental animal models and in humans as the key etiological factor in dental caries (4, 7, 8, 13, 14, 21, 23) and various periodontal diseases (12, 18, 20, 25). Notable among the various microorganisms associated with dental plaque accumulations are strains of *Actinomyces viscosus*, *Actinomyces naeslundii* (2, 9, 15, 16, 19, 24), *Streptococcus mutans* (10, 11, 26), and *Streptococcus sanguis* (3, 4). Infection of experimental animal models by representative strains of these microorganisms has been shown to initiate the development of enamel lesions and cause periodontal tissue destruction, with the possible exception of *S. sanguis*, for which evidence of involvement in dental diseases is minimal (3, 4, 10).

Dental plaque removal from oral surfaces by various mechanical means has been shown to effect a short-term remission of the signs of gingivitis, periodontitis, and dental caries (12, 25, 32). The effective control of dental plaque and thereby of dental diseases of microbial etiology by such mechanical means is, however, dependent on patient compliance and appears to be limited by failure of the patient to reach certain plaque-infected sites. Hence, there currently exists great therapeutic potential for the development of antiplaque chemotherapeutic agents to assist in the control of dental plaque-associated infections in humans (1, 22). Because supragingival plaque adheres to tooth surfaces and is not bathed in either blood or interstitial fluids, topical antiseptic agents would appear to be of greatest utility. In addition, dental plaque is representative of a slow-growing microbial system (27, 31). Therefore, for an agent to be efficacious, it must be able to permeate the plaque matrix and rapidly kill at least the pathogenic members of the plaque microflora after only transient exposures.

In this context, the bispyridine octenidine dihydrochloride (WIN 41464-2) has been shown to have utility in controlling bacterial plaque formation. Preliminary studies have demonstrated that octenidine can inhibit the colonization of a substrate such as ceramic hydroxylapatite (durapatite) by *S. mutans* OMZ-61 at a concentration 10-fold less than that which promotes staining of such surfaces (J. O'Connor, D. A. Paris, and D. Bailey, J. Dent. Res. vol. 59, special issue A, abstract no. 743, 1980). In addition, in vivo studies have demonstrated that octenidine at a concentration of 0.5% (wt/vol) inhibited the implantation and subsequent ascendency of *S. mutans* in the oral ecology and concomitant development of enamel lesions in a rat caries model (R. Shern, E. Monell-Torrens, W. Bowen, and A. Kingman, J. Dent. Res. vol. 59, special issue A, abstr. no. 186, 1980). Octenidine at a concentration of 1.0% (wt/vol) also appears to be able to inhibit the growth of *Actinomyces* spp. in dental plaque in a primate model system (6).

Our studies have extended earlier assessments of the in vitro antiplaque activity of octenidine and have characterized the conditions required to kill preformed plaques of four indigenous dental plaque-forming microorga-

nisms. An in vitro antiplaque model was employed to determine the antiplaque activity of octenidine, the efficacy of which was compared with that of the extensively studied antiplaque agent chlorhexidine.

MATERIALS AND METHODS

Antibacterial agents. Octenidine (WIN 41464-2; *N,N'*-(1,10-decanediyldi-1(4*H*)-pyridinyl-4-ylidene)-bis[1-octanamine] dihydrochloride), obtained from Sterling-Winthrop Research Institute, Rensselaer, N.Y., and chlorhexidine digluconate, obtained from Fermion, Finland, were used in these studies (Fig. 1). All agents were examined for purity before use and were shown to contain no detectable impurities with bactericidal properties.

Microorganisms. The microorganisms used in these studies were *S. mutans* NCTC 10449, *S. sanguis* ATCC 10558, *A. viscosus* M-100B, and *A. naeslundii* 631. These cultures were obtained from the collection of the Oral Microbiology Division, Department of Oral Diagnosis, School of Dental Medicine, University of Connecticut Health Center, Farmington, Conn., and their identities were confirmed with standard microbiological techniques before use. These bacteria form plaques both in vitro and in vivo and, with the possible exception of *S. sanguis*, have been associated with the development of coronal caries or periodontal disease with attendant root surface caries or both (2, 3, 9, 16, 23, 24). All microorganisms were stored either lyophilized or frozen (−70°C). For use in experiments, working culture stocks were maintained by bimonthly passage in fluid thioglycolate medium containing 20% (vol/vol) meat extract and excess $CaCO_3$.

In vitro plaque formation. In vitro plaques were grown on ceramic hydroxylapatite slabs (1.6 by 1 cm; Sterling-Winthrop Research Institute) which were individually suspended on no. 26-gauge nichrome wire by using a modification of methods previously detailed (29, 30). Fluid thioglycolate cultures were used to inoculate a complex medium (17) supplemented with 5% (wt/vol) sucrose and 0.005% (wt/vol) Na_2CO_3. The wire-attached ceramic hydroxylapatite slabs were transferred daily to fresh medium for 3 days. All cultures were incubated at 37°C under an anaerobic atmosphere (GasPak; BBL Microbiology Systems, Cockeysville, Md.). The in vitro plaques formed by this method were visually graded, and uniformly sized plaques of the target organisms were selected for assessment of agent efficacy (29).

Assessment of efficacy of octenidine. Efficacy was assessed by using previously detailed techniques (29, 30). Briefly, in vitro plaques were rinsed of growth medium by submersion in demineralized water for 2 min and then immersed in 10 ml of aqueous solutions of the test or control agents for various durations and frequencies. After treatment, the in vitro plaques were rinsed twice by immersion for 10 min in 15 ml of distilled water and then transferred to fresh broth growth medium having the same composition as that in which the plaques had grown but which now contained a pH indicator (bromocresol purple). Plaques were judged to be killed if culture acid production and turbidity increase ceased and if 24-h post-treatment plaque samples failed to grow when placed on appropriate agar media (29, 30).

RESULTS

Minimal bactericidal concentration. To determine the minimal concentration of octenidine and chlorhexidine that maximally inhibited preformed plaques of the four test microorganisms, plaques were immersed in various concentrations of these agents for 30 min at 37°C. Control plaques were similarly immersed in sterile demineralized water. Both octenidine and chlorhexidine were bactericidal as judged by the criteria described in Materials and Methods (Table 1). Octenidine at a concentration of 3.2 mM effectively killed all preformed plaques of a quadruplicate set for each bacterial strain examined. At a concentration of 1.6 mM aqueous octenidine, acid production was perturbed; however, upon extended incubation (36 h), the pH fell to the same level as that of the control plaques. At octenidine concentrations of less than 1.6 mM, there was little noticeable effect on acid production or plaque viability. Chlorhexidine was bactericidal for plaques of *S. sanguis*, *A. viscosus*, and *A. naeslundii* at 1.6 mM; however, 3.2 mM was required to kill all plaques in

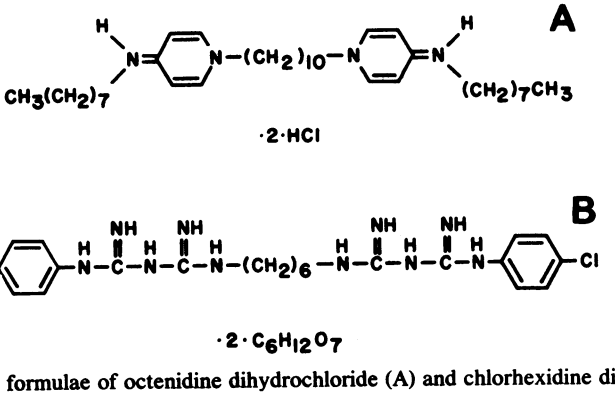

FIG. 1. Structural formulae of octenidine dihydrochloride (A) and chlorhexidine digluconate (B).

TABLE 1. Minimal concentration of agents required to be batericidal to plaques of the test microorganisms[a]

Agent	Concn (mM)[b] bactericidal for:			
	S. mutans	*S. sanguis*	*A. viscosus*	*A. naeslundii*
Octenidine	3.2	3.2	1.6	3.2
Chlorhexidine	3.2	1.6	1.6	1.6

[a] Plaques were run in quadruplicate. Results represent the uniform behavior of the quadruplicate set.
[b] Concentrations tested were 0.16, 0.8, 1.6, 3.2, 8.0, and 16.0 mM.

the quadruplicate set for *S. mutans*. These findings for chlorhexidine are similar to those previously reported by Tanzer et al. (29).

Minimal exposure time. The minimal duration of a single treatment which was bactericidal for these two agents was determined by immersion of preformed plaques at the previously established minimal bactericidal concentrations (Table 1) for periods of 1, 2, 5, 10, 15, 20, or 30 min. These studies demonstrated that octenidine at a concentration of 3.2 mM was bactericidal for *S. mutans* after exposure times of 15, 20, or 30 min. The same organism had to be exposed to 3.2 mM chlorhexidine for 30 min to achieve a similar bactericidal effect. The other three plaque-forming bacteria were susceptible to octenidine after only a 10-min exposure, in contrast to chlorhexidine, which at a concentration of 3.2 mM required an exposure time of at least 20 min to achieve a bactericidal effect (Table 2).

Frequency of exposure. Assuming that a mouth rinse is the delivery method of choice, the time that such an agent could normally be expected to be retained orally would not likely exceed 2 min. Studies were therefore conducted to evaluate whether shorter but more frequent exposures could achieve the desired bactericidal effects.

Two types of experiments were performed to assess this aspect of a potential treatment regimen. In the first, preformed in vitro plaques of the test microorganisms were treated for either 1 or 2 min once daily until plaque death occurred; such a protocol was viewed as analogous to a single daily mouth rinse. In the second protocol, in vitro plaques were treated for either 1 or 2 min, rinsed, incubated in growth medium for 6 h, and then retreated. The procedure was repeated daily until plaque death occurred, as judged by the criteria described above. This treatment protocol was viewed as analogous to the use of a mouth rinse twice daily.

Table 3 shows the concentration and frequency of use required for each agent to be efficacious in a once-daily regimen. *A. viscosus* and *A. naeslundii* plaques were killed after three successive once-daily, 2-min treatments with 3.2 mM octenidine, but they were not killed after five daily treatments if the exposure time was reduced to only 1 min (data not shown). It should be noted, however, that after the third daily 1-min treatment, acid production by these strains was greatly reduced in comparison with the water-treated controls. *S. mutans* was effectively killed by both agents after four successive once-daily, 2-min exposures, and acid production was also significantly reduced when the treatment time was reduced to 1 min in a similar period. *S. sanguis* had to be treated with octenidine for 5 days at the 2-min exposure time to be killed (Table 3); a once-daily, 1-min treatment for 7 days failed to kill the plaques (data not shown).

To compare the therapeutic utility of octenidine with that of chlorhexidine, a plaque bactericidal index (PBI) and a chlorhexidine coefficient (CC) were computed. The PBI is the integration of the effects of concentration (millimolar), duration (daily exposure time) and frequency of treatments (number of daily treatments necessary to kill plaques) required to achieve a plaque bactericidal effect. The CC is computed by dividing the PBI for chlorhexidine by that for octenidine.

These data indicate that, for the most potent cariogenic and periodontopathic components of in vivo plaque examined here (*S. mutans* and *A. viscosus*), the antiplaque activity of octenidine compares very favorably with that of bisbiguanide chlorhexidine (Table 3). In addition, it is noted that higher concentrations or more frequent exposures to octenidine are required to kill the relatively innocuous plaque former *S. sanguis*, as compared with the conditions required for chlorhexidine to kill this species.

Table 4 shows the concentration, duration,

TABLE 2. Minimal exposure time required of agent at minimal bactericidal concentration to kill plaques[a]

Microorganism	Minimum killing time (min) for agents at minimal bactericidal concn of 3.2 mM	
	Octenidine	Chlorhexidine
S. mutans	15	30
S. sanguis	10	20
A. viscosus	10	20
A. naeslundii	10	20

[a] Plaques run in quadruplicate. Results represent the uniform behavior of the quadruplicate set.

TABLE 3. Comparison of the therapeutic utility of chlorhexidine with that of octenidine, one treatment daily[a]

Microorganism	Chlorhexidine		Octenidine		CC (chlorhexidine PBI/octenidine PBI)
	Minimal concn (mM) × duration (min) × frequency (no. of days) required for bactericidal effect	PBI	Minimal concn (mM) × duration (min) × frequency (no. of days) required for bactericidal effect	PBI	
S. mutans	3.2 × 2 × 4	25.6	3.2 × 2 × 4	25.6	1.00
S. sanguis	3.2 × 2 × 3	19.2	3.2 × 2 × 5	32.0	0.60
A. viscosus	3.2 × 2 × 4	25.6	3.2 × 2 × 3	19.2	1.33
A. naeslundii	3.2 × 2 × 3	19.2	3.2 × 2 × 3	19.2	1.00

[a] Plaques run in quadruplicate. Results represent the uniform behavior of the quadruplicate set.

and frequency of treatment required to achieve a bactericidal effect when preformed plaques were treated twice a day. Octenidine was shown to compare favorably with chlorhexidine with respect to antiplaque efficacy for all test organisms, with the possible exception of *S. sanguis*. If used for 2 min twice a day, octenidine at a concentration of 1.6 mM had a bactericidal effect for all plaque formers after four to five twice-daily treatments, with the exception of *S. sanguis*. If, however, the exposure time was reduced to only 1 min twice a day, a sufficient number of organisms survived in the plaque to grow and produce acid (Table 4).

DISCUSSION

A novel bispyridine, octenidine dihydrochloride (WIN 41464-2), was assessed for bactericidal activity in vitro against preformed plaques of representative strains of four indigenous oral plaque-forming bacteria. This agent was shown to be efficacious and to compare favorably with chlorhexidine with respect to overall antiplaque activity.

These studies were designed to evaluate the conditions required for octenidine to kill preformed plaques in vitro and also to estimate the conditions that are likely to be required for the agent to be effective in vivo (29, 30). The dense adhesive character of in vivo dental plaque appears to constitute a diffusion barrier to many antiseptics (29, 30), and this probably accounts for the inability of a number of otherwise bactericidal agents to be effective in controlling dental plaque development. Because freely suspended cells of plaque-forming microorganisms are or-

TABLE 4. Comparison of the therapeutic utility of chlorhexidine with that of octenidine, two treatments daily[a]

Microorganism	Octenidine		Chlorhexidine	
	Minimal concn (mM) × duration (min) × frequency (no. of days) required for bactericidal effect	PBI	Minimal concn (mM) × duration (min) × frequency (no. of days) required for bactericidal effect	PBI
S. mutans	3.2 × (2 + 2) × 2	25.6	3.2 × (2 + 2) × 2	25.6
	3.2 × (1 + 1) × 4	25.6	3.2 × (1 + 1) × 3	19.2
	1.6 × (2 + 2) × 5	32.0		
	1.6 × (1 + 1) × >5	NC[b]		
S. sanguis	3.2 × (2 + 2) × 3	38.4	3.2 × (2 + 2) × 2	25.6
	3.2 × (1 + 1) × 5	32.0	3.2 × (1 + 1) × 3	19.2
	1.6 × (2 + 2) × >5	NC		
	1.6 × (1 + 1) × >5	NC		
A. viscosus	3.2 × (2 + 2) × 2	25.6	3.2 × (2 + 2) × 3	38.4
	3.2 × (1 + 1) × 3	19.2	3.2 × (1 + 1) × 4	25.6
	1.6 × (2 + 2) × 4	25.6		
	1.6 × (1 + 1) × >5	NC		
A. naeslundii	3.2 × (2 + 2) × 2	25.6	3.2 × (2 + 2) × 2	25.6
	3.2 × (1 + 1) × 3	19.2	3.2 × (1 + 1) × 3	19.2
	1.6 × (2 + 2) × 4	25.6		
	1.6 × (1 + 1) × >5	NC		

[a] Plaques were run in duplicate. Results represent the uniform behavior of the duplicate set.
[b] NC, Could not be computed.

ders of magnitude more susceptible to agents than are intact plaques (22, 27, 28), efficacy was evaluated against intact bacterial plaques in this study. Such a model is more representative of the conditions and constraints an agent can be expected to encounter in vivo (22, 28, 30).

Octenidine was found to possess bactericidal activities similar to those of the well-characterized bisbiguanide chlorhexidine. This may be readily seen by comparing the PBIs for the two agents against the various test microorganisms. The PBI computation allows for a ready evaluation of the sum of treatment conditions (concentration, duration, and frequency) required to achieve a bactericidal effect on intact in vitro plaques. The derivation and validity of this index has been previously detailed (29). Briefly, it should be noted that the index permits comparison of efficacy because dental plaque grows slowly and, therefore, plaque as such represents virtually a stable-sized mass of cells available for killing. The effects of antiseptic treatment, therefore, appear to be cumulative. Thus, the PBI is essentially an index of agent potency against a specific plaque mass and is independent of concentration, duration, or frequency of treatment. The CC relates the relative potency of an agent to that of chlorhexidine, which still represents the most extensively studied antiplaque agent (5).

The PBI and CC computations predict that octenidine should compare favorably with chlorhexidine (when both are used in a final concentration of 3.2 mM) in controlling plaque development by *S. mutans* and *A. viscosus*, the most cariogenic and periodontopathic elements of the dental plaque microflora examined here. Indeed, recent studies in a rat and a monkey dental plaque model have indicated that this may certainly be so; octenidine has demonstrated efficacy equal to or greater than that of chlorhexidine in controlling the ascendency of these two pathogenic plaque-formers in their respective oral ecologies (6; Shern et al., J. Dent. Res. vol. 59, special issue A, abstr. no. 186, 1980; C. Emilson, W. Bowen, S. Robrish, and C. Kemp, J. Dent. Res. vol. 59, special issue A, abstr. no. 489, 1980).

The oral cavity represents a dynamic ecosystem and, it may be reasoned, it would not be totally advantageous to eliminate all elements of the oral microflora in an effort to control dental plaque-associated infections. Rather, it may be more ideal to remove only the most cariogenic and periodontopathic elements of the dental plaque microflora while permitting the more innocuous elements to remain. The PBI computations from this in vitro study suggest that prudent selection of the octenidine concentration would permit the inhibition of *S. mutans* and *A. viscosus* while allowing for the survival of the more innocuous *S. sanguis*. Thus, the bispyridine octenidine appears to have utility in controlling dental plaque-related infections. In addition, selection of the correct dosage may allow manipulation of the plaque ecology to permit survival of the relatively nonpathogenic organisms and thereby provide a mechanism for sustained inhibition of recolonization and growth of the more pathogenic members of the dental plaque microflora. Furthermore, the spectrum of antimicrobial activity displayed by this agent suggests that, at the indicated dose levels, organisms such as yeasts and molds would not be given a selective advantage in the oral cavity, a problem of obvious concern for agents used frequently. Clinical evaluations of this agent for in vivo efficacy in controlling plaque-associated diseases in humans are currently under way.

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