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In Vitro Antiplaque Properties of a Series of Alkyl Bis(biguanides)

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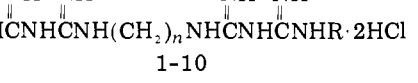
A series of eight alkyl bis(biguanide) analogues of alexidine, *N,N''''*-1,6-hexanediyl bis[*N'*-(2-ethylhexyl)imidodicarbonimidic diamide] (1), was prepared. Five of these analogues constituted a series isolipophilic with 1 but with varying bridge length between biguanide moieties. The compounds were evaluated in vitro for antibacterial and antiplaque properties against *Streptococcus mutans*, *Actinomyces viscosus*, and *Actinomyces naesludii*. One analogue, *N,N''''*-1,6-hexanediyl bis[*N'*-(*n*-octyl)imidodicarbonimidic diamide], appeared to be more effective than either 1 or chlorhexidine against this spectrum of dental plaque forming microorganisms.

In the search for chemotherapeutic agents for the control of dental diseases, the bis(biguanides) **1–10**, especially



1-10

chlorhexidine (**10**, R = *p*-chlorophenyl, $n = 6$), have received much attention.[1-8] The effectiveness of chlorhexidine in controlling the formation of dental plaque in humans has been ascribed to its antibacterial properties and substantivity in the oral cavity.[3,5,9]

Although studies by Davies[10] indicated that optimal antibacterial properties were associated with bis(biguanides) with hexamethylene bridges, Cutler[11] has shown that lipophilicity was the principal property correlating with this activity. Gjermo[6] evaluated a series of 1,6-bis(biguanidohexanes) for both in vitro antibacterial and in vivo antiplaque properties and found an alkyl derivative (R = cyclohexylmethyl) superior to chlorhexidine. Variations of bridge length, $n = 2$–12, of bis(biguanides) with *p*-chlorophenyl terminal groups were examined by Warner[7] who found comparable antibacterial activity in vitro among members in this series, while $n = 2$ or 10 resulted in lower antiplaque activity against *Streptococcus mutans* in vitro. Recently, a series of eight hexamethylene bridged alkyl bis(biguanides) was evaluated for in vitro antiplaque properties employing a bioassay involving preformed plaques on nichrome wires with drug solution contact for 30 min.[12]

In this study an isolipophilic series of alkyl terminal group bis(biguanides) of various bridge lengths was prepared and evaluated, as their dihydrochloride salts, for in vitro antibacterial and antiplaque activity against *Actinomyces viscosus*, *A. naesludii*, and *S. mutans*. The lipophilicity of members in this series (compounds **2–6**) was chosen to be nearly equal to that of alexidine (**1**, R = 2′-ethylhexyl, $n = 6$) which has been reported to be clinically effective in decreasing plaque scores.[13-15] Three other alkyl bis(biguanides) (**2–9**) with slightly different lipophilicity were also tested. Chlorhexidine (**10**) was included for purposes of comparison.

In these previous studies involving the variation of bridge length with constant terminal groups, both the lipophilicity and separation of cationic centers varied simultaneously, thereby potentially obscuring the relationship of antiplaque properties upon antibacterial properties vs. binding abilities. The rationale for this study was the expectation that while only small differences in antibacterial activities among compounds of comparable lipophilicity may be observed, larger variations in antiplaque properties may reflect differences in binding ability which may be produced by changes in spatial separation of cationic centers.[9]

The synthesis of the analogues listed in Table I followed the method of Rose and Swain[16] wherein an α,ω-di-

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Table I. Alkyl Bis(biguanides) with in Vitro Antiplaque Properties

compd[a]	R	n	mp, °C	plaque MIC[b]		
				S. mutans[c]	*A. naesludii*[d]	*A. viscosus*[e]
1[f]	2'-ethylhexyl	6	219-221	>100	5.0	0.5
2	2'ethylheptyl	4	221-222	100	0.5	10
3	*n*-nonyl	4	187-189	70	1.0	10
4[g]	*n*-octyl	6	175-176	8	0.5	5
5	*n*-heptyl	8	174-176	>10	0.5	>10
6	*n*-hexyl	10	173-175	10	0.5	>10
7[g]	*n*-heptyl	6	169-171	50	0.5	>10
8	*n*-hexyl	8	192-194	3	1.0	>10
9[g]	*n*-hexyl	6	184-186	100	0.8	0.8
10[h]	*p*-chlorophenyl	6		10	>100	>10

[a] Compounds 2-9 were analyzed, as their dihydrochloride salts, for C, H, N, and Cl; the values found were within ±0.4% of theoretical values. [b] Lowest molar concentration (M × 10^4) used to treat tooth slabs which produced >90% inhibition of growth of adherent cells; uniform results obtained from triplicate sets. [c] Strain 6715-13WT. [d] Strain ATCC 12104. [e] Strain M-100-2000. [f] Alexidine (dihydrochloride salt), lit.[18] mp 220-223 °C. [g] Reference 18. [h] Chlorhexidine (digluconate salt, Imperial Chemical Industries, Ltd.).

aminoalkane was treated with sodium dicyanamide to give α,ω-bis(N^3-cyano-N^1-guanidino)alkanes. These were converted to the desired bis(biguanides) by treatment with the appropriate amine.

Biological Activity. Each analogue was evaluated for antiplaque activity in an in vitro assay similar to that employed by Turesky et al.,[8] but using uniformly sized, saliva-coated bovine tooth slabs rather than whole teeth. The culture media, methods of incubation, and description of this assay have been previously reported.[17] Each compound was also evaluated for antibacterial activity in the standard tube dilution assay modified by dispersion of adherent cells before estimation of cell densities spectrophotometrically (540 nm). Almost all of the test compounds exhibited the same minimum inhibitory concentration (10^{-5} M) against the three microorganisms in the tube dilution assay. A few were more active against *A. viscosus* (1, 5×10^{-7} M; 7–9, 10^{-6} M), while only one, 3, was less active (10^{-4} M) against *S. mutans*.

A greater variation in plaque-inhibiting activity was observed (Table I). Although alexidine (1) was most effective against *A. viscosus*, several compounds in the isolipophilic series, 2–6, and the less lipophilic compound, 8, were more effective against both *S. mutans* and *A. naesludii*. In the series 2–6, optimal activity against both *S. mutans* and *A. viscosus* was observed in the hexamethylene bridged compound 4. Chlorhexidine was effective against *S. mutans* but less effective than several alkyl bis(biguanides) (1–4 and 9) against *A. naesludii* and *A. viscosus*.

Although the log *P* values of compounds in the isolipophilic series were not experimentally determined, the terminal group size varied inversely with the bridge length resulting in isomeric ($C_{26}H_{56}N_{10}$·2HCl) structures in which only minor variations in lipophilicity, arising from branching differences, would be expected. In this in vitro study, 4 appears to be more effective than either alexidine or chlorhexidine against this spectrum of plaque-forming organisms. Although these results may be considered to strengthen the requirement for the hexamethylene bridge, one cannot neglect the influence of the contribution to hydrophobic binding by the terminal group. Since the latter varied with bridge length in this study, the critical factors influencing binding have not been determined. Compound 8 was the most active of those tested against *S. mutans*. The effectiveness of compounds 8 and 9, which were not members of the isolipophilic series, suggests that the optimal lipophilicity for activity against the test microorganisms may be less than that chosen for the isolipophilic series.

Experimental Section

Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR and NMR spectra were used to verify the assigned structures. IR spectra were recorded on a Perkin-Elmer 237 spectrophotometer. NMR spectra were recorded on a Varian T-60 spectrometer.

***N,N''''*-α,ω-Alkanediyl Bis[*N'*-(alkyl)imidodicarbonimidic diamides]** (1–9). An intimate mixture of α,ω-di(N^3-cyano-N^1-guanidino)alkane[7] (12 mmol) and the appropriate alkylamine hydrochloride salt (24 mmol) was heated in an oil bath at 155 °C for 2 h. Upon cooling the solidified melt was recrystallized from EtOH–EtOAc.

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