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BISBIGUANIDE-INDUCED STAINING IN ORAL HYGIENE

Wu-huang Yang
Department of Industrial Pharmacy
Massachusetts College of Pharmacy
Boston, Massachusetts 02115

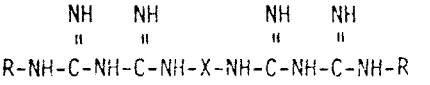
Gilbert S. Banker
Department of Industrial and Physical Pharmacy
Purdue University
West Lafayette, Indiana 47907

ABSTRACT

The use of a class of antimicrobial agents, the bisbiguanides, in oral hygiene and their involvement in the formation of teeth staining are reviewed. Particular attention is placed on a discussion of the mechanism of bisbiguanide-induced staining from a molecular level and the approaches taken to resolve this staining problem.

BISBIGUANIDE COMPOUNDS

The general chemical structure of the bisbiguanide compounds is:



Members of this series of compounds were first synthesized by the Imperial Chemical Industry Inc. in England in 1954 (1-3). The chemical synthesis of these bisbiguanide compounds is shown in Figure 1. Table 1 indicates that the selection of the group R and X from many possible chemical groups leads to a great number of bisbiguanide analogs.

These bisbiguanide compounds were found to possess antimicrobial activity and are effective against a wide range of vegetable bacteria, both Gram-positive and Gram-negative. Of these bisbiguanide compounds, chlorhexidine (R=p-chlorophenyl,

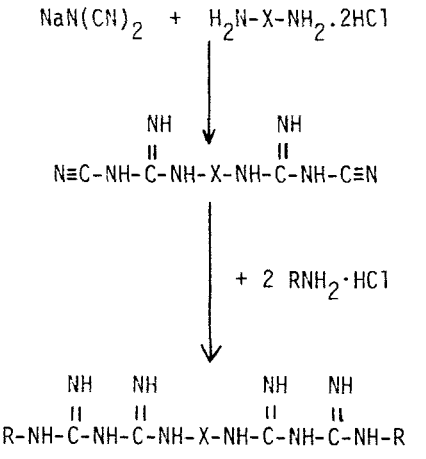


FIGURE 1

Chemical synthesis of bisbiguanide compounds

Table 1

Bisbiguanide Analogs

1. X = $(CH_2)_6$

R	Reference
C_6H_5	3
p-C_6H_4Me	3
p-C_6H_4OH	3
p-C_6H_4OMe	3
p-$C_6H_4CO_2H$	3
p-C_6H_4Cl (Chlorhexidine)	3
m-C_6H_4Cl	3
3,4-$C_6H_3Cl_2$	3
2,5-$C_6H_3Cl_2$	3
α-$C_{10}H_7$	3
β-$C_{10}H_7$	3
p-C_6H_4-SC_nH_{2n+1} (n=1 to 6)	4
3-Cl-4-SC_nH_{2n+1}-C_6H_3(n=1 to 3)	4
p-C_6H_4-$SO_2C_nH_{2n+1}$ (n=1 to 6)	4
C_nH_{2n+1} (n=4 to 9)	4

2. R = p-C_6H_4CL

X	Reference
$(CH_2)_n$ (n=2 to 10)	3
$(CH_2)_3O(CH_2)_3$	3
$(CH_2)_3O(CH_2)_2O(CH_2)_3$	3
$(CH_2)_3O$-C_6H_4(p)-$O(CH_2)_3$	3

X=hexamethylene) was found to have the greatest antimicrobial activity (3) and later alexidine (R=2-ethylhexyl, X=hexamethylene) was found to have antimicrobial activity comparable to chlorhexidine (4).

Many bisbiguanide salts are reported in the literature. These include the following forms for chlorhexidine: dihydrochloride (3), diacetate, digluconate, di-L-glutamate (5,6), distearate, dilaurate, dioleate, linoleate (7), diglucosaccharo-1,4-lactone salt (2:1 salt, monohydrate) (8), various bis(hydroxybenzoates) (9), N-(hydroxyethyl)ethylene diaminetriacetate, N,N-bis[2-[bis(carboxyethyl)]amino]etnyl glucine salt (3:2 salt), N,N-bis(carboxymethyl) glycine (1:1 salt), N,N-1,2-ethanediyl bis[N-(carboxymethyl)] glycine salt (1:1 and 1:2 salts), N,N-bis (2-hydroxyethyl) glycine salt (1:2 salt) (10), disuccinamate bis(iminodiacetate), bis(6-acetamidohexanoate) (6), diacetate, diglycoate (11), disarcosinate, and monoflurophosphate (12,13). A 20% aqueous solution of chlorhexidine digluconate is marketed under the trade name of HIBITANE.

In autoclaving chlorhexidine digluconate aqueous solutions, it was discovered that a very small amount of p-chloraniline was formed due to the hydrolysis of chlorhexidine (14-17). The decomposition is the least at pH 5-6 and increases as the pH is raised or lowered (15-17). The decomposition was found to increase sharply at temperatures higher than 110° (16,17). It was concluded, however, that the low level of p-chloroaniline thus

formed could not induce any toxic effects and the loss of the antimicrobial activity of chlorhexidine so treated was negligible (15).

The dissociation constants (pK_a values) of chlorhexidine are given as 10.3 and 2.2, corresponding to the formation of a mono- and di-cation, without any specific detail on the method of the determination (18). The corresponding pK_a values for alexidine were determined to be 11.49 and 2.47 using a spectrophotometric method (99). For purpose of comparison, the dissociation constants of a number of diprotonated biguanides were compiled by Ray (19), and the pK_a values ranged from 10.2 to 10.8 for one and 2.0 to 3.0 for the other depending on the substituents attached.

Like biguanides, bisbiguanides can be complexed with metal ions. Silver(III) ethylene bisbiguanide salts have been synthesized from ethylene-bisbiguanide sulfate and a silver salt in the presence of potassium or sodium persulfate (20).

The uses and drug delivery systems which were found in the literature for various bisbiguanides are shown in Table 2.

SORPTION OF BISBIGUANIDES

As noted previously the bisbiguanide compounds are primarily used in salt forms. The most widely used salt forms are diacetate, dihydrochloride, and digluconate for chlorhexidine and dihydrochloride for alexidine. Thus bisbiguanides exist in aqueous solution

Table 2

Uses and Drug Delivery Systems of Bisbiguanides

Formulation	Use	References
Aqueous Solution	Mouth rinse for plaque inhibition	21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39
	Topical application for plaque inhibition	31, 40
	Ophthalmic	41, 42
	Bactericide for paper towels and diapers	43, 44, 45, 46, 47
	Instrument sterilization	48, 49, 50
	Bladder irrigation	51
	Disinfection of floor	52, 53
	Disinfection of telephone mouth- and ear-piece	54
	Disinfection of skin	55
	Hand washing for disinfection cleaning	53, 56, 57, 58
	Mouthwash for tartar treatment	34, 59
	Acne treatment	60, 61, 62
	Preservation for soft contact lens	63, 64, 65
	Burn treatment	66

"Table 2 Cont."

Formulation	Use	References
	Preservation of histological slides	67
Alcoholic Solution	Suture preservation	68
Dentifrice	Teeth cleaning	12, 13, 22, 23, 24, 33, 36, 38, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78
Mold	Plaque inhibition	79
Aerosol	i. Bactericidic	80, 81
	ii. Agricultural and Horticultural nematocide	82
Tablet	i. Teeth cleaning	36, 38, 83
	ii. Agricultural and Horticultural nematocide	82
Cream	Disinfection of skin	51
Ointment	Antiseptic for surface wounds on animals	84
Lozenge	i. Plaque inhibition	85
	ii. Treatment of throat infection	66
	iii. Treatment of aesophagitis	66
Gum	Tartar treatment	34

as the mono-positively charged species at pH conditions typically found in pharmaceutical preparations, and the cations are capable of being bound by anionic substances with the electrostatic attraction as the driving force. Chlorhexidine has been shown to be bound by hydroxyapatite (86-90), tooth surface (88,90), anionic materials such as CP-11 cellulose (phosphate), CM-sephadex (carboxyl), and SP-sephadex (sulfate) (91,92). Chlorhexidine showed significant ionic association with the stearate acid film (92). The protein in saliva and serum were precipiated by chlorhexidine (90,93). A histochemical staining method for chlorhexidine with azocarmine B revealed that the areas of the bacterial cell where chlorhexidine accumulated were those areas known to contain negatively charged chemical groups (94). It was concluded that a chlorhexidine-resistant bacteria might have acquired its resistance to chlorhexidine, at least in part, by a decrease in surface phosphate groups (95,96). The effect of pH on sorption of chlorhexidine by bacteria was consistent with the electrostatic attraction hypothesis (18).

Since locally high concentrations were observed on the tooth surface after using chlorhexidine, it was suggested that chlorhexidine molecules may be involved in the development of staining products (97). One observation which is consistent with this thought is the fact that upon discontinuation of the use of chlorhexidine the staining disappears (98). An extensive study on the adsorption and desorption of chlorhexidine and alexidine by

hydroxyapatite and extracted human teeth showed that the sorption obeys Langmuir theory (99). The fact that chlorhexidine can be slowly desorbed from the tooth surface following its fast adsorption has been thought to be a prophylactic principle in the prevention of periodontal diseases (100,101).

MODE OF ACTION OF BISBIGUANIDES AS ANTIMICROBIAL AGENTS

The mode of action of bisbiguanides as antimicrobial agents can be summarized as follows (100): (a) Adsorption of bisbiguanides to the surface of the bacterial cell. The driving force for this adsorption was previously described as an electrostatic attraction between the positively charged bisbiguanide species and the negatively charged bacterial surface. (b) The damage of the cell membrane to alter its premeability and thus facilitate the entry of bisbiguanides (102-105). (c) The precipitation of the cytoplasm and the prevention of the repair of the cell membrane (106-109).

THE USES OF BISBIGUANIDES IN ORAL HYGIENE

As can be seen in Table 2, chlorhexidine has been extensively investigated for its use in oral hygiene, mainly in the form of a mouthwash. Various short term studies on human subjects revealed activity in inhibiting dental plaque formation (110-112). A two year study of the oral use of chlorhexidine in 120 human subjects has been conducted in Denmark (98, 113-117). The re-

sults showed that when compared to a placebo solution, chlorhexidine treatment substantially reduced the formation of plaque and the incidence of gingivitis. There were no local side effects or alterations of the structure of the oral mucosa, tongue, salivary glands and pharyngeal complex following the prolonged use of chlorhexidine (98,115). The chlorhexidine treatment resulted in a 30 to 50 percent reduction in the number of the bacteria in saliva without producing a detectable shift in the bacterial flora (113). One study, however, showed that chlorhexidine treatment produced a slight change in the distribution toward those organisms which were less sensitive to chlorhexidine (114).

BISBIGUANIDE-INDUCED TEETH STAINING IN ORAL HYGIENE

One consistent side effect exists when chlorhexidine is used in the oral hygiene area. This is the staining of the teeth surface (98, 115, 118-121) and sometimes the staining of the tongue (119, 120). The staining was also observed when alexidine was used in the oral hygiene. The staining is an esthetic rather than a health problem. However the high incidence of the staining reaction would be objectionable to some users. Inquiries into the possible relationship between the discoloration and smoking or the intake of certain food stuffs have been inconclusive (101). The staining on the teeth is reportedly relatively easily removed by brushing, polishing or other mechanical procedures (101).

A number of suggestions and approaches have been directed at the resolution of the staining problem with chlorhexidine. The

stained material has been scrapped off the teeth and analyzed; "but it has an amino acid combination of pellicle material and nothing else. It's just the protein you find on the teeth" (66). It was suggested that the brown staining might be due to the formation of mucin salts or their degradation products (90). The staining has also been related by some to the denaturation of protein (90, 120). In an in vitro test, yellow to brown colors were observed when chlorhexidine was incubated with certain aldehydes and ketones, such as acetaldehyde, benzaldehyde, formaldehyde, D-glyceraldehyde, acetone, acetoacetic acid, pyruvic acid, α-keto-glutaric acid, glucose, fructose, galatose, sucrose, and lactose (122). It was proposed that a Schiff base formation between chlorhexidine and carbonyl compounds, which are intermediates normally found in both mammalian and microbial metabolism was responsible for the color formation. This color formation was also observed for alexidine in a similar in vitro test (99). Brown colored compounds were isolated from the incubation mixtures of alexidine and chlorhexidine with acetaldehyde respectively, using preparative thin layer chromatography. These colored compounds have been identified to be Schiff base type compounds using NMR and IR spectroscopic data (99).

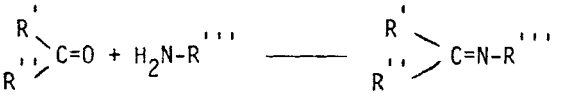
MECHANISM OF STAIN FORMATION AND ITS PREVENTION

It was proposed (99) that the mechanism of the stain formation, when either chlorhexidine or alexidine is used in oral hygiene, involves the following steps: (a) The cationic chlorhexidine or

alexidine species is adsorbed onto the negatively charged tooth surface due to the electrostatic attraction. (b) The biguanido group at the other end of the molecule then reacts with a carbonyl compound normal to the flora of the mouth to form a colored Schiff base type compound. This mechanism is illustrated in Figure 2.

According to this model, prevention of staining should be directed to a search for antistain agents capable of inhibiting the Schiff base formation, while at the same time retaining the antimicrobial activity of the bisbiguanide. The possible ways to achieve this goal are:

(a) Modification of the Bisbiguanide Molecule: Schiff base is formed when a carbonyl group reacts with a primary amino group, thereby converting a C=O double bond to a C=N double bond:



Theorectically it would be possible to modify the chemical structure of the bisbiguanide so that the contribution of a primary amino group is decreased. It should be borne in mind, however, that any modification of the chemical structure should not decrease the antimicrobial activity. It was found that the major factor influencing the antimicrobial activity of the bisbiguanide was the hydrophilic-lipophilic balance (HLB) (4). It is thus quite possible to resolve this staining problem by a structure modification process, provided a proper HLB in the chemical structure is maintained.

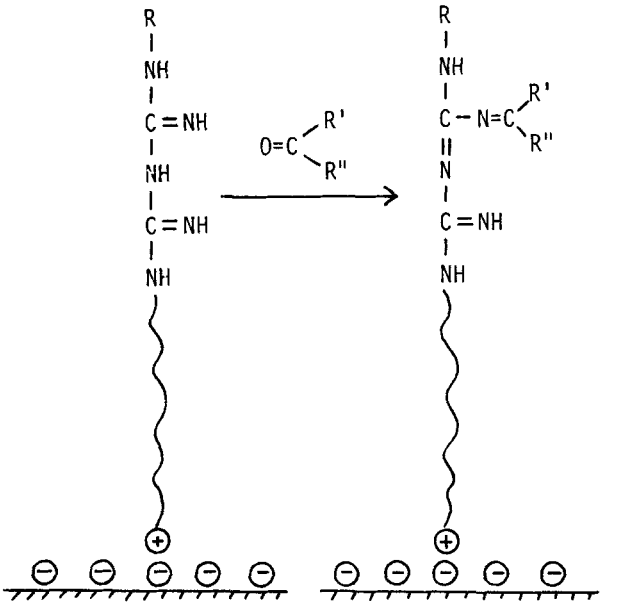
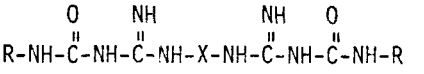


FIGURE 2

A model which shows the adsorption of a bisbiguanide onto a solid surface and the formation of a Schiff base with a carbonyl compound

A modification of the biguanido group to a carbonylguanido group was reported to be effective in reducing the stain formation tendency while retaining the antimicrobial activity (123). The general chemical structure of this type of compound is:



(b) Protection of the Reacting Sites in Bisbiguanides: The other possible way to block the staining reaction based on the hypothesis proposed above could be to use a large counter ion to

block the approach of the carbonyl compound to the reactive sites in the adsorbed bisbiguanide. It was found that the incorporation of poly(methyl vinyl ether/maleic anhydride) and poly(ethylene/maleic anhydride) in a mouthwash formulation was effective in reducing the stain formation tendency. A formulation containing the former polymer has been patented (124).

Other methods were reported to reduce the stain formation tendency. Chelating agents such as kojic acid which can react completel with the bisbiguanide was used in one proposed formulation (125). Others have used reducing agents such as gallic acid or dihydrocoumarin (20), ascorbic acid (21), insoluble salt forms (12, 13, 126), or amino carboxylate compounds such as nitrilo triacetic acid (22,23). The incorporation of urea into the formulation was claimed to reduce the staining tendency (24).

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