Evaluation of the antimicrobial effects of sodium benzoate and dichlorobenzyl alcohol against dental plaque microorganisms

An in vitro study

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Evaluation of antimicrobial agents is based on in vivo and in vitro studies. The minimum inhibitory concentrations (MICs) of sodium benzoate and dichlorobenzyl alcohol to 115 strains of plaque microorganisms were determined by a broth-dilution method. Sodium benzoate did not inhibit growth of any gram-positive cocci (MIC > 106,590 μM). MICs for *Porphyromonas gingivalis* and two strains of *Treponema socranskii* were 26,650 μM. The MIC of dichlorobenzyl alcohol to the reference strain of *Actinobacillus actinomycetemcomitans* was 723 μM and to *P. gingivalis*, two strains of *T. socranskii*, and *Candida albicans* 1,446 μM. MICs for other organisms were 2,892 to 5,784 μM. Saliva samples from 10 volunteers, collected at various times after toothbrushing with a dentifrice containing 10% sodium benzoate and 0.3% dichlorobenzyl alcohol, were analyzed gas-chromatographically. Immediately after toothbrushing mean levels of sodium benzoate and dichlorobenzyl alcohol were 372,626 μM and 7,529 μM, respectively. After 5 min mean levels were 38,700 μM and 734 μM. In conclusion, the concentrations of both antimicrobials dropped rapidly during the first 30 min, but for 5–10 min they were high enough to inhibit growth of potential periodontal pathogens.

☐ *Dentifrice; plaque microorganisms; saliva; toothbrushing*

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It has been well documented that plaque microorganisms are the primary etiologic agents of periodontal disease (1–3). Reduction and/or elimination of plaque is essential to reduce and prevent periodontal disease (4), and worldwide, the major feature of individual dental hygiene programs for populations is toothbrushing with a toothbrush and a dentifrice (5).

The benefit of anti-plaque agents added to dentifrices is recognized, and the plaque-reducing effect of various antimicrobial agents added to dentifrices is accepted (6). To obtain scientific evidence of the effect of new antimicrobial agents included in dentifrices, data from clinical investigations and in vitro studies of antimicrobial agents should be combined with data from studies of the pharmacokinetic modes of action of these agents in saliva and the oral cavity (7).

Various components, such as various metal ions, chlorhexidine, sanguinarine, triclosan, delmopinol, and related compounds, have been tested because of their plaque-reducing potential, and the results confirm that they possess in vitro activity against plaque microorganisms (8–12). Data on the oral retention of fluorides and some of the above-mentioned antimicrobial agents have also been published (13–15).

The aim of the present study was 1) to determine the susceptibility to sodium benzoate and dichlorobenzyl alcohol of reference strains and fresh oral isolates of

several microorganisms present in sub- and supra-gingival plaque, and 2) to examine the saliva concentration of sodium benzoate and dichlorobenzyl alcohol by gas chromatography after toothbrushing with a dentifrice containing the two agents.

Sodium benzoate is used as a food preservative and has a broad spectrum of in vitro activity at low pH values against microorganisms, including yeasts and denture-borne *Candida albicans* (16). It is recommended as an effective germicide for the disinfection of denture prostheses and removable orthodontic appliances (17) and is used empirically in dental practice to cure minor lesions of the oral mucosa.

Dichlorobenzyl alcohol has a broad spectrum as an antimicrobial agent. It is used as a fungicidal preservative in cosmetics and pharmaceuticals and as a bactericidally active agent in lozenges, skin antiseptics, and oral rinses (18).

Materials and methods

Subjects

For the minimum inhibitory concentration (MIC) determinations, plaque microorganisms were isolated from a population of 39 individuals clinically showing gingival inflammation and/or adult periodontitis, characterized by pocket depths of ≤6 mm.

Pregnant women and women using medical contraception were excluded from the study, as were subjects receiving medical treatment due to chronic diseases, subjects abusing alcohol or drugs, subjects who had received antibiotic treatment within the past 6 months, and subjects who had received surgical periodontal treatment within the past 6 months.

Subjects were instructed not to drink alcohol the night before the samples were collected, not to eat or drink anything but water, and not to perform any personal oral hygiene on the morning of the sampling.

For the saliva concentration determinations 10 individuals, 1 man and 9 women of the technical staff of the university department, volunteered after informed consent. The age of the participants was 19–54 years; mean age was 32 years.

None of the volunteers were pregnant, and none had been receiving antibiotic or other medical treatment for the previous 6 months. Furthermore, no volunteer showed lack of adequate dental treatment. Volunteers were instructed not to brush their teeth on the morning of the sampling, not to eat or drink anything and not to smoke from the start to the end of the sampling period of time, and to avoid swallowing.

Microorganisms

Reference strains were obtained from The American Type Culture Collection (Rockville, Md., USA) except for *Lactobacillus casei* 18, obtained from Dr. R. J. Fitzgerald, Veterans Administration Medical Center, Miami, Fla., USA, and one reference strain of *Treponema denticola* FDC T1 from Forsyth Dental Center, Boston, Mass., USA. Microbial strains were cultivated and maintained on media recommended in the ATCC Catalogue of Bacteria and Bacteriophages, 17th ed., 1989.

Table 1 specifies the reference strains and plaque isolates tested.

Antimicrobial agents and dentifrice

Sodium benzoate was in accordance with the European Pharmacopeia and obtained from Nomeco, Copenhagen, Denmark, and dichlorobenzyl alcohol was obtained from The Boots Company PLC, Chr. Krogh A/S, Hellerup, Denmark.

The test dentifrice contained 10.0% sodium benzoate and 0.3% dichlorobenzyl alcohol and was provided by Product Development Service ApS, Vanløse, Denmark. Other chemicals used were obtained from Merck and were of highest commercially available purity degree for gas chromatography.

Sampling

Supragingival microorganisms were isolated from plaque samples obtained with a sterile periodontal curette (Hu-Friedy

SM-13-14). Subgingival plaque microorganisms were obtained from periodontal pockets of 4–6 mm. Before sampling, supragingival deposits were removed, the area was dried, and three sterile paper points inserted to the bottom of the periodontal pocket, left for 10 sec, and transferred to 2 ml reduced transport medium (RTF) (19). The samples were immediately transported to the laboratory for processing.

Saliva samples were obtained by unstimulated expectoration into sterile 40-ml vials before and immediately after 2 min of toothbrushing and at the time intervals of 5 min, 15 min, 30 min, 1 h, and 2 h after toothbrushing with a new clean toothbrush (Tandex 40) carrying 2 g of the test dentifrice.

Isolation of microorganisms

Anaerobic procedures and incubations were carried out in an atmosphere of 70% of N_2, 20% of H_2, and 10% CO_2.

All samples were dispersed by vortex mixing and 10-fold serial dilution anaerobically in RTF. Aliquots of 0.1 ml of all samples were anaerobically inoculated on prereduced (48 h) tryptic soy agar (TSA, Difco) supplemented with horse blood (5%), hemin (50 μg/ml), menadione (0.5 μg/ml), and cysteine (0.02%). Furthermore, the supragingival plaque samples were inoculated on the following selective media: mitis-salivarius agar (MS, Difco) for isolation of viridans streptococci, Rogosa-SL agar (Merck) for *Lactobacillus* spp., and CNAC-20 agar (Difco) for *Actinomyces* spp.(20).

The selective media for subgingival plaque samples were Mandell & Socransky's medium for *Actinobacillus actinomycetemcomitans* (21), medium for *Campylobacter* spp. by Hammond & Mallonee (22), medium for *Capnocytophaga* spp. by Mashimo et al. (23), CVE medium by Walker et al. (24) for *Fusobacterium nucleatum*, and the modified medium of Singletary et al. (25) for *Prevotella* spp. MS plates were incubated anaerobically at 35°C for 2 days, followed by aerobic incubation at 37°C for 3 days; plates of selective media for *A. actinomycetemcomitans* and *Capnocytophaga* spp. were incubated at 35°C in atmospheric air and 7.5% CO_2 for 5 days, and the other plates were incubated anaerobically at 35°C for 5–7 days. At the end of incubation characteristic colonies were selected if possible and subcultivated on the same selective media until purity, based on colony morphology and Gram staining.

Identification of microorganisms

Supragingival isolates. For *Actinomyces* spp. and *Lactobacillus* spp. the gram-positive rods were differentiated on the basis of growth on CNAC-20 agar and Rogosa SL agar, catalase and oxidase tests, nitrate reduction, branching and filamentous forms of the rods by microscopy of microcolonies on brain–heart infusion agar, and production of organic acids by fermentation in oxygen-free peptone–yeast extract–glucose broth (PYG) identified by gas liquid chromatography (26). *Actinomyces* spp. were further characterized using the following criteria: oxygen tolerance, urease test, acetoin, H_2S and indole production, methyl red reaction, and hydrolysis of esculin, starch, and gelatin. All tests were performed as described by Gerencser & Slack (27).

The lactobacilli were separated into thermobacteria, streptobacteria, and betabacteria, using the criteria of Edwardsson (28): gas production from glucose and gluconate, acid from ribose, deamination of arginine, hydrolysis of esculin and hippurate, and growth at 15°C and 45°C. The tests were performed in accordance with Cowan & Steel (29).

Viridans streptococci were identified in accordance with Kilian et al. (30). Gram-positive, catalase-negative cocci were submitted to the following tests: hydrolysis of arginine and esculin, H_2O_2 production, production of acid from the following carbohydrates: mannitol, sorbitol, salicin, amygdalin, and inulin, and production of acetoin (Voges–Proskauer test) and of extracellular polysaccharide dextran from sucrose. Detection of the enzyme activities β-glucosaminidase, β-mannosidase, α-L-fucosidase, and alkaline phosphatase was by use of the Api Zym test kit (30).

Table 1. Minimum inhibitory concentrations (MICs) of sodium benzoate (μM)

Organism	Reference strains	No. of strains	No. of supragingival isolates	No. of subgingival isolates	MICs of reference strains	No. of isolates with MIC = 26,650	No. of isolates with MIC = 35,530	No. of isolates with MIC = 53,290	No. of isolates with MIC = 71,057	No. of isolates with MIC = 106,590	No. of isolates with MIC > 106,590
Actinobacillus actinomycetemcomitans	ATCC 43718	3	0	2	53,290		1	1			
Actinomyces naeslundii	ATCC 12104	4	3	0	53,290			1		1	1
Actinomyces viscosus	ATCC 19246	5	4	0	53,290			2	2		
Campylobacter spp.		4	0	4							4
Candida albicans	ATCC 44503	4	3*	0	35,530		2		1		
Capnocytophaga spp.	ATCC 33612	4	0	3	106,590					1	2
Fusobacterium nucleatum	ATCC 25586	6	0	3	35,530		1	1		1	
Fusobacterium polymorphum	ATCC 10953				53,290						
Fusobacterium vincentii	ATCC 49256				53,290						
Lactobacillus betabacterium	ATCC 9338	7	6	0	106,590			1		2	3
Lactobacillus streptobacterium	L 18	8	7	0	106,590				1	1	5
Lactobacillus thermobacterium	ATCC 4356	2	1	0	71,057					1	
Peptostreptococcus spp.	ATCC 27337	4	1	2	>106,590					1	2
Porphyromonas gingivalis	ATCC 33277	2	0	1	26,650	1					
Prevotella intermedia	ATCC 25611	4	1	2	35,530		1	2			
Prevotella melaninogenica	ATCC 25845	4	1	2	35,530			1	1	1	
Streptococcus anginosus	ATCC 33397	5	0	2							4
Streptococcus constellatus	ATCC 27823				>106,590						
Streptococcus intermedius	ATCC 27335										
Streptococcus gordonii	ATCC 10556	6	5	0	>106,590						5
Streptococcus mitis	ATCC 33399	7	6	0	>106,590						6
Streptococcus mutans	ATCC 25175	8	7	0	>106,590						7
Streptococcus oralis	ATCC 35037	8	7	0	>106,590						7
Streptococcus salivarius	ATCC 7073	5	4	0	>106,590						4
Streptococcus sanguis	ATCC 19558	5	4	0	>106,590						4
Treponema denticola	ATCC 33520 FDC T1	5	0	3†	106,590					3	
Treponema socranskii ss. buccale	ATCC 35534	5	0	3†	26,650	1				2	
Treponema socranskii ss. socranskii	ATCC 35536				53,290						

* Isolates from the mucosa of the cheek, tongue, and palate, kindly provided by Dr. J. L. Rindum, School of Dentistry, University of Copenhagen, Copenhagen, Denmark.
† These treponemes are described as 2:4:2 and 1:2:1 spirochetes, respectively, and were kindly provided by Dr. N.-E. Fiehn, School of Dentistry, University of Copenhagen, Copenhagen, Denmark.

Subgingival isolates. A. actinomycetemcomitans was identified by catalase activity, reduction of nitrate, and fermentation of mannitol, lactose, glucose, sucrose, and xylose (31). *Campylobacter* spp. were identified by lack of growth in atmospheric air, motility, lack of oxidase activity, and inability to ferment glucose (32). *Capnocytophaga* spp. were identified by growth in atmospheric air, anaerobically and in atmospheric air and CO_2, and by catalase and oxidase activities, indole and H_2S production, sensitivity to actinomycin D, and by acid end products of gas chromatographic analysis (33). *F. nucleatum* was identified by growth in atmospheric air and atmospheric air plus 7.5% CO_2, catalase and oxidase activities, indole production, sensitivity to actinomycin D, and glucose fermentation to acid end products (26, 34, 35). *Peptostreptococcus anaerobius* was identified as gram-positive, catalase-negative anaerobe streptococci in accordance with Sutter et al. (36). Black-pigmented, anaerobe gram-negative coccoid rods were separated into *Porphyromonas gingivalis, Pr. intermedia*, and *Pr. melaninogenica* by indole production, fluorescence in long-wave ultraviolet light (37) and by the Api Zym test kit (38).

Susceptibility testing

For microbial susceptibility testing sodium benzoate was dissolved in sterile distilled water, and dichlorobenzyl alcohol was dissolved in 70% w/v of propylene glycol. Both were added to the first medium (see below) by filter sterilization, as a twofold dilution.

All strains were anaerobically cultivated in fluid brain–heart infusion (BHI, Difco). Spirochetes were cultivated in BHI supplemented by 15% inactivated rabbit serum, 0.07% Noble agar, and 5 μg/ml of cocarboxylase (39). Spirochetes were incubated at 37°C for 4 days (40); all other strains were incubated at 37°C for 2 days.

The susceptibility of strains to sodium benzoate, dichlorobenzyl alcohol, and the agents in combination was determined by the broth dilution method of Ericsson & Sherris (41) and tested under strictly anaerobic conditions. For all strains fluid cultures in the late logarithmic to early stationary growth phase with about 10^7 cells/ml medium were used, determined by repeated countings in a Petroff–Hauserss counting chamber. One milliliter of the adjusted culture was mixed with 1 ml broth incorporating the antimicrobial agent in a test tube. Concentrations in test tubes ranged from 4.44 to 106,590 μM for sodium benzoate and from 0.723 to 11,570 μM for dichlorobenzyl alcohol. Control tubes containing broth without antimicrobial agent were included for every strain tested. The susceptibility of the strains to propylene glycol was also tested as a control. The testing of every microbial strain was repeated six to eight times.

The MIC was defined as the lowest concentration of the agent resulting in no visible growth of microorganisms, determined by the clear eye and compared with a control.

Saliva preparation and analysis

Saliva samples were prepared for analysis by modifying the method of Gilbert et al. (15) for preparation of saliva samples for analysis of triclosan. To the weighed saliva samples were added 5 ml of an acid, saturated solution of sodium chloride (pH 1), 1 ml of 20% (w/v) ethanol, and 3 ml of a solution of 3 ml of dichlorophenol, 0.675 mg/ml (the selected internal standard for the analysis) in 12 ml of dichloromethane (99.5%) to a concentration of 0.125 mg/ml, and hydrochloric acid (32%) was added to acidify the solution. Samples were stored on ice for 15 min and centrifuged at 3,500 *g* for 10 min. The lower layer of the supernatant was extracted, 5 ml of dichloromethane was added twice, each time followed by centrifugation at 3,500 *g* for 10 min and extraction of the lower layer of the supernatant (dichloromethane). The three extractions collected were then centrifuged at 3,500 *g* for 10 min. Samples of 1 ml were then derivatized by mixing with 0.1 ml of *N*-methyl-*N*-(trimethylsilyl)-2,2,2-trifluoroacetamide (MSTFA) and were placed on a waterbath at 60°C for 15 min. For analysis, 1 μl of the prepared samples was injected in the gas chromatograph.

The method of The National Food Agency

Table 2. Minimum inhibitory concentrations (MICs) of dichlorobenzyl alcohol (μM)

Organism	MIC of reference strains	No. of isolates with MIC = 1446	No. of isolates with MIC = 2892	No. of isolates with MIC = 5784
Actinobacillus actinomycetemcomitans	723	2		
Actinomyces naeslundii	2,892		2	1
Actinomyces viscosus	2,892		4	
Campylobacter spp.			4	
Candida albicans	1,446	3		
Capnocytophaga spp.	2,892		2	1
Fusobacterium spp.	2,892		3	
Lactobacillus betabacterium	2,892		4	2
Lactobacillus streptobacterium	2,892		5	2
Lactobacillus thermobacterium	2,892			1
Peptostreptococcus spp.	2,892		2	1
Porphyromonas gingivalis	1,446	1		
Prevotella intermedia	2,892		3	
Prevotella melaninogenica	2,892		2	1
Streptococcus milleri	2,892		2	2
Streptococcus gordonii	2,892		1	4
Streptococcus mitis	2,892		2	4
Streptococcus mutans	5,784		1	6
Streptococcus oralis	2,892		2	5
Streptococcus salivarius	2,892		2	2
Streptococcus sanguis	2,892		2	2
Treponema denticola	2,892 5,784			3
Treponema socranskii	1,446 5,784	1	1	1

of Denmark for analysis of benzoic acid and sorbic acid in food was used in the study (42).

The concentration of sodium benzoate and dichlorobenzyl alcohol in saliva samples was determined by gas chromatography (Chrompack, CP 9000) with an FI detector and a C-R3A recorder. Every sample was analyzed twice. Carrier gas was nitrogen. The flow was 10.8 ml/min, the oven temperature was 165°C, the detector temperature was 250°C, and the injector temperature was 250°C. The column (Chrompack) was a 3-m stainless steel column; the outside diameter was 0.32 mm, and the inside diameter 2 mm. The concentration was 6%, the liquid phase was CP-SIL-8, the adsorbent was Chromsorb WHP, and mesh size was 80–100. Linear standard curves for the agents and the internal standard dissolved in saliva in the concentration interval of current interest were obtained before analysis.

Gas chromatographic quantitative analysis of saliva samples with an FI detector and an internal standard were reproducible and accurate to the level of 1 μg/ml, and there was a straight linearity between the concentration of the two agents and the peak areas.

Results

Tables 1 and 2 show the MIC values of sodium benzoate and dichlorobenzyl alcohol. Generally, all microorganisms were about 20 to 30 times more susceptible to dichlorobenzyl alcohol than to sodium benzoate.

The MICs of sodium benzoate to gram-positive cocci, approximately 50% of *Lactobacillus* spp., and one strain of *Actinomyces naeslundii*, were higher than 106,590 μM. For other supragingival bacterial isolates and reference strains MICs were 53,290–106,590 μM. The MIC of the most susceptible species, *Por. gingivalis*, one oral isolate, and one reference strain of *Tr. socranskii*, was 26,650 μM. The MICs of *Cam-*

pylobacter spp. and 50% of strains of *Capnocytophaga* spp. were >106,590 μM. MICs of other subgingival isolates and reference strains were 35,530–106,590 μM. Three strains of *Candida albicans* showed MICs of 35,530 μM and one strain 71,057 μM.

All organisms were approximately equally susceptible to dichlorobenzyl alcohol. The most susceptible strain was the reference strain of *A. actinomycetemcomitans*, with an MIC of 723 μM. Oral isolates of *A. actinomycetemcomitans*, *C. albicans*, *Por. gingivalis*, and two strains of *Tr. socranskii* had MIC values of 1,446 μM. MICs for all other strains were 2,892–5,784 μM.

The effect of the two components combined was equal to the MIC of the most active component, dichlorobenzyl alcohol.

When the saliva samples were analyzed gas-chromatographically, sodium benzoate and dichlorobenzyl alcohol were well separated on the chromatograms. The retention time of sodium benzoate was 6.5 min, and that of dichlorobenzyl alcohol was 14.5 min. Sodium benzoate and/or dichlorobenzyl alcohol was not detected in any of the samples collected before toothbrushing. Figs. 1 and 2 show the concentrations of sodium benzoate and dichlorobenzyl alcohol in saliva after toothbrushing versus time, respectively. Initial mean concentrations of sodium benzoate and dichlorobenzyl alcohol after toothbrushing were 372,626 μM and 7,529 μM, respectively. After 5 min mean concentrations had fallen to 38,700 μM and 734 μM. Sodium benzoate was detected in all samples 15 min after toothbrushing and in 30% of samples 30 min after toothbrushing (mean concentration, 105 μM). One hour after toothbrushing no sodium benzoate was detected in any of the samples. Dichlorobenzyl alcohol was found in all samples but one (90%) 10 min after toothbrushing. After 15 min it was found in 50% of the samples (mean, 90 μM) and after 30 min and 1 h in none of the samples.

Discussion

The selection of antimicrobial agents suitable for incorporation in oral hygiene products is based on clinical studies, on the susceptibility of the relevant oral microorganisms to the agent, and on the pharmacokinetic modes of action of the agents in the oral cavity, with regard to toxicity and adverse side effects (43). A general scheme for delivery of anti-plaque agents in the oral cavity has been presented, and it is stated that antimicrobial agents could have an effect in subinhibitory concentrations, because of various pharmacokinetic and chemical

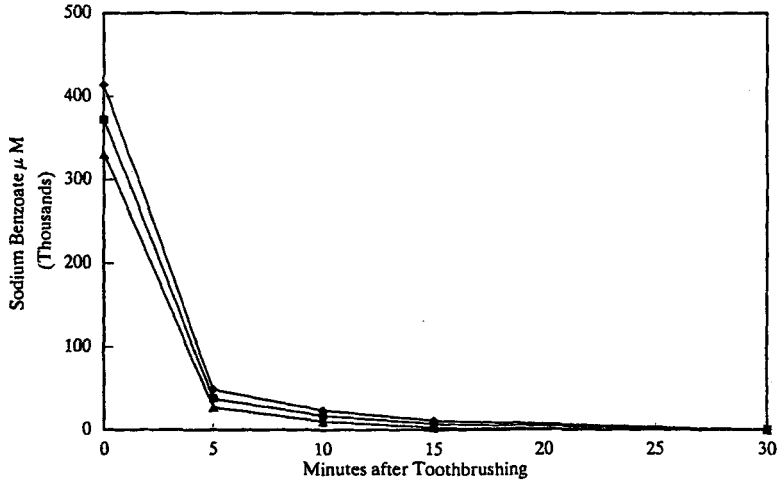

Fig. 1. Mean saliva concentration (μM) of sodium benzoate versus time (■), the mean + SD (◆), and the mean − SD (▲) ($n = 10$).

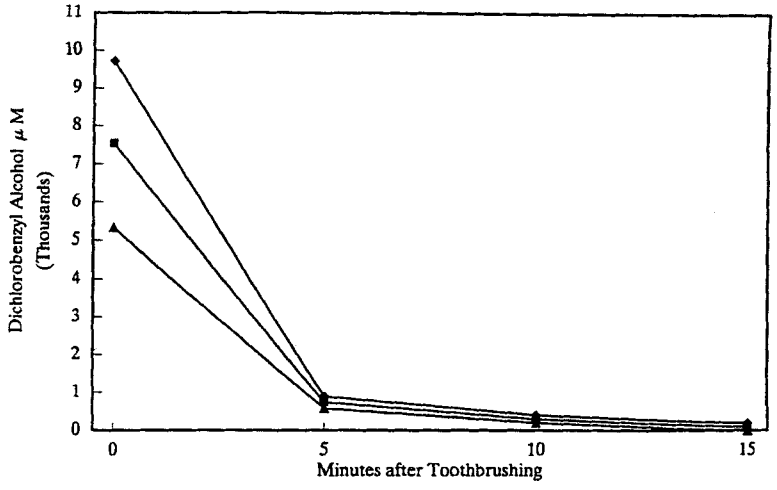

Fig. 2. Mean saliva concentration (μM) of dichlorobenzyl alcohol versus time (■), the mean + SD (◆), and the mean − SD (▲) (n = 10).

mechanisms and interactions (44), but it is also stated that in vitro data on the properties of antimicrobial components cannot necessarily be extrapolated to effects in vivo (45).

The aim of this study was to determine the oral retention and the antimicrobial properties of the two compounds sodium benzoate and dichlorobenzyl alcohol, with the purpose of assessing whether any preparation of a dentifrice or mouthrinse formulation, including one or both compounds, could be expected to benefit oral health, mediated through the antibacterial effects.

The study has first of all provided oral microbiologic data to confirm formerly proved antibacterial activity of sodium benzoate and dichlorobenzyl alcohol (18, 46, 47).

With regard to the susceptibility testing, the growth of microbial species dominating the supragingival plaque of individuals with healthy periodontal tissues was not inhibited by sodium benzoate, but sodium benzoate did inhibit growth of some gram-negative species. Dichlorobenzyl alcohol inhibited growth of all microorganisms tested in the study but showed highest activity against *A. actinomycetemcomitans* and *Por. gingivalis*, organisms related to juvenile and destructive forms of periodontitis (48, 49). *C. albicans* was inhibited by the same concentrations as the above-mentioned microorganisms.

The essential difference between the two tested antimicrobial components was the inability of sodium benzoate to inhibit most of the gram-positive plaque flora. Under healthy conditions such an inhibition would not be desirable, because instability of the plaque community and oral ecologic balance could disturb the host defense (50).

The curves of saliva concentrations of sodium benzoate and dichlorobenzyl alcohol versus time were comparable to similar curves for other anti-plaque agents. With regard to the oral retention, sodium benzoate was present in saliva for a longer time than dichlorobenzyl alcohol. Five to 10 min after toothbrushing the concentrations of both antimicrobials were high enough to kill potential periodontal pathogens.

In Denmark the highest permitted concentration of the compounds when used as antiseptics in the cosmetics industry is 0.5% for sodium benzoate and 0.15% for dichlorobenzyl alcohol. Higher concentrations of both compounds are allowed as long as concentration and purpose of the use are declared on the product (51). In the oral cavity a dentifrice is continuously diluted and washed out by the flow of saliva and the gingival fluid. Therefore, decreased concentrations of the antibacterial compounds can be expected to reach the plaque matrix and target bacteria. Sodium benzoate is

highly soluble in saliva and could be added to dentifrice or mouthrinse formulations in high concentrations (52). The solubility of dichlorobenzyl alcohol is poor in water but high in etheric oils (18). The present MIC values were all concentrations that could be added to oral hygiene products.

The effect of benzoic acid has an optimum in the pH range of 2.5–4.0 (53). Acidified benzoate is approximately 100 times more effective than a neutral solution, because the undissociated acid and not the benzoate ion is the active form (54). The pH of test tubes was neutral. The average pH for unstimulated saliva is 6.75 (55), but under certain conditions the pH in saliva and outer layers of plaque decreases. These conditions often exist after the intake of sweet food components. This may result in an increased inhibiting activity of sodium benzoate to potential periodontal pathogens in the oral cavity.

The importance of the influence of supragingival plaque control on the subgingival microflora in periodontal pockets has been stated (56, 57). Deposits of periodontal pathogenic microorganisms occur on the oral mucous membranes, the tongue, and tonsils, and the intraoral spread of these bacteria can take place via saliva (58, 59). The use of antimicrobials for supragingival plaque control might affect these deposits and decrease the risk and rate of recolonization.

So far, the antimicrobial and anti-plaque properties of chlorhexidine (9, 14) have been superior to the properties of all other known anti-plaque agents. The present study suggests the possibility of improving dentifrices or mouthrinse formulations by adding sodium benzoate or dichlorobenzyl alcohol to increase individual self-performed plaque control, mediated through the antimicrobial effect. However, it is essential to carry out several other studies to determine adverse side effects and, further, in vitro properties and in vivo activities of the two compounds in oral hygiene products or used as subgingival irrigators in local delivery systems, to obtain confirming scientific evidence of the benefit of sodium benzoate and dichlorobenzyl alcohol as therapeutic agents in oral hygiene programs.

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