In Vitro Evaluation of Three Iodine-Containing Compounds as Antiplaque Agents

J. M. TANZER,* A. M. SLEE, B. KAMAY, AND E. R. SCHEER

University of Connecticut Health Center, Farmington, Connecticut 06032

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The antibacterial activity of three iodine-containing compounds was determined for strains of four dental plaque-forming microorganisms, using an in vitro preclinical plaque model system. Solutions of inorganic iodine, povidone-iodine, and Wescodyne were tested for antiplaque activity against preformed plaques of a number of strains of Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosus, and Actinomyces naeslundii. Solutions of inorganic iodine were more effective as antiplaque agents, with respect to minimal bactericidal concentration and time and frequency of treatments, than solutions of Wescodyne and povidone-iodine. Inorganic iodine appeared to be particularly effective against the most cariogenic (S. mutans) and periodontopathic (A. viscosus) organisms while allowing survival of the least orally pathogenic plaque-forming microorganism (S. sanguis). It is suggested that topical application of solutions of inorganic iodine may be useful in controlling dental caries and actinomyces-associated periodontal disease.

Dental plaque, the dense adhesive microbial mass that colonizes the teeth, is strongly implicated in both experimental animals and humans as the key etiological factor in dental caries (6, 10, 11, 19, 20, 28, 38) and periodontal disease (17, 25, 30, 41). Notable among those microorganisms associated with dental plaque accumulation are strains of Actinomyces viscosus, Actinomyces naeslundii (2, 12, 21, 22, 26, 40), Streptococcus mutans (13, 14, 42), and Streptococcus sanguis (4, 6). Infection of experimental animals by these microorganisms has been shown to cause periodontal disease and dental caries, with the possible exception of S. sanguis, where the evidence for pathogenicity with respect to such diseases is minimal (4, 6, 13).

The removal of dental plaque from oral surfaces by mechanical means, such as toothbrushing and dental flossing, results in a short-term remission of the signs of gingivitis/periodontitis and dental caries (17, 18, 41, 46). However, effective control of dental plaque by such techniques is dependent on patient compliance and is limited by the failure of mechanical devices (toothbrush, dental floss, etc.) to reach some of the critically plaque-infected areas. Thus, there exists great therapeutic potential for the development of antiplaque chemotherapeutic agents to control plaque-associated infections in humans (33). Because plaque is adherent to the surfaces of teeth and is not bathed in blood or interstitial fluid, topical antiseptic agents appear of greatest potential utility. However, to be effective, such agents must rapidly kill preformed plaques of target organisms upon transient application. In this context, compounds such as antibiotics, unless secreted in the saliva, would appear to be of relatively limited value.

This laboratory has been actively engaged in examining a number of compounds that may potentially have utility for topical use in the control of dental plaque and its associated diseases. Such agents may be administered either by patients themselves or professionally. The present report deals with in vitro evaluation of one class of such agents as it affects intact pure culture plaques.

Iodine is a widely used and effective antiseptic of long standing (37). However, its history of use intraorally is more limited. It is reported to be effective in preparation of oral mucosal sites before surgery. The prophylactic topical administration of iodine compounds has been shown to reduce the number of viable microorganisms in saliva (48) and in the gingival crevice (36). In fact, topical scrubs of S. mutans-infected tooth loci in children with rampant caries has succeeded in suppressing S. mutans at these tooth sites for several weeks (16). Furthermore, several investigators have shown that povidone-iodine, when used as a mouth rinse, effectively reduces the gingival surface microflora (1, 3, 35, 36). Iodine compounds may, therefore, also be useful in controlling dental plaque and asso-
associated diseases. To our knowledge, however, there have been no systematic studies directed toward identifying the most potent iodine preparation in this regard and estimating the optimal conditions for its use. Accordingly, this study examines the antibacterial activity of three iodine-containing compounds on strains of four dental plaque-forming microorganisms, three of which are well established as pathogenic with respect to dental caries and/or periodontal disease. A pregrown in vitro plaque model system for the evaluation of antiplaque agents was used in these studies (44, 45).

MATERIALS AND METHODS

Microorganisms. The microorganisms used were strain of S. mutans, S. sanguis, A. viscosus, and A. naeslundii. The strains, their sources, and citations to their origins are given in Table 1. All strains are plaque forming both in vitro and in vivo, and all except those of S. sanguis have been implicated in coronal caries and/or periodontal disease with attendant root surface caries. Identification of these organisms was based upon biochemical and morphological data, and designations of actinomyces were further established with a serospecific fluorescent antibody by G. J. Hageage, Jr., St. Vincent Hospital, Toledo, Ohio. All strains were maintained in either frozen or lyophilized state except when used for experiments for which working stocks 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, using a slight modification of methods previously detailed (44, 45). Fluid thioglycolate cultures were used to inoculate a complex medium (24) supplemented with 5% (wt/vol) sucrose and 0.005% (wt/vol) sodium carbonate. The nichrome wires, fixed to culture tube closures, 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. (32). 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. All cultures were incubated at 37°C.

Agents. The following iodine-containing agents were used in these studies: inorganic iodine solutions of I₂ in KI at a ratio of 2:1 (wt/wt); povidone-iodine, polyvinyl-pyrrolidone-iodine (39), for which the commercial preparation Betadine Surgical Scrub (Purdue, Frederick Co., Norwalk, Conn.) was used; and Wescodyne (West Chemical Products, Inc., New York), which is a complex of polyethoxy-polypropoxypolyethoxethanol-iodine and nonyl-phenoxypoly(ethylenoxy)ethanol-iodine. The povidone-iodine and Wescodyne solutions contain 1.0 and 1.6% iodine, respectively, as determined by titration with sodium thiosulfate.

Assessment of efficacy of iodine agents. It has previously been demonstrated that serial tube dilution methods for inhibition of growing cultures and for inhibition of washed nongrowing cells are inappropriate for testing efficacy of agents against plaque microorganisms. Such methods require the presence of agent for a prolonged period, a situation unlikely to occur in the mouth, and they do not take into account the diffusion barriers apparently existing in intact dental plaque (43, 45). Thus, tube dilution techniques were demonstrated to overestimate the potency of agents by several orders of magnitude (45). Accordingly, agents were tested here by short-term immersion of preformed plaques in various agents using methods previously detailed (45). Control plaques were immersed in 10% potassium iodide, 10% alcohol, or water, as appropriate for the agent being examined. These placebos did not affect growth and fermentation and were not different in effect from one another. Therefore, water was routinely used for control plaques.

RESULTS

Minimal concentration. To determine the minimal concentrations of the three iodine-containing agents that maximally inhibit plaques of the four oral microorganisms, plaques were immersed in various concentrations of the agents for 30 min at 37°C. Inorganic iodine was generally more effective than Wescodyne or povidone-iodine (Table 2). Plaques of S. mutans, A. viscosus, and A. naeslundii treated with inorganic iodine were killed at a concentration of 0.04% as judged by the cessation of culture acid production and of turbidity increase and by failure of 24-h post-treatment plaque samples to grow when plated on appropriate agar media. Although growth and acid production by S. sanguis plaques were retarded at this concentration, this organism was not killed at less than 0.2% inorganic iodine.

Wescodyne was bactericidal at a concentration of 0.16% titratable iodine for all four test organisms. Povidone-iodine was bactericidal at a concentration of 1% titratable iodine for S. mutans and A. viscosus, but was bacteriostatic for S. sanguis and A. naeslundii. In all studies, control plaques treated with either sterile distilled water or a 10% solution of potassium iodine were not inhibited.

Minimum exposure time. To determine minimal duration of a single treatment producing maximal inhibition with inorganic iodine, plaques of the four test organisms were immersed in either 0.04% (for A. viscosus, A. naeslundii, and S. mutans) or 0.2% (for S. sanguis) of the agent for 1, 2, 5, 10, 20, or 30 min (Table 3). Both S. mutans and S. sanguis were killed when exposed for 5 min to 0.04 and 0.2% inorganic iodine, respectively. Single exposures for 1 and 2 min at those concentrations tran-
TABLE 1. Microorganisms studied

| Microorganism       | Strain               | Reference                |
|---------------------|----------------------|--------------------------|
| *Streptococcus mutans* Perch (34) serotype: |                       |                          |
| a                   | E-49                 | 11                       |
|                     | AHT                  | 47                       |
|                     | OMZ-61               | 20                       |
|                     | FA-1                 | 10                       |
|                     | BHT                  | 47                       |
| b                   | NCTC 10449           | 29                       |
|                     | Ingbrt-1600          | 28                       |
|                     | GS-5                 | 15                       |
|                     | UCHC-EHL             | J. M. Tanzer, clinical isolate |
|                     | 6715-13              | 42                       |
|                     | OMZ-176              | 19                       |
|                     | B-15                 | 8                        |
|                     | LM-7                 | 15                       |
|                     | B-2                  | 8                        |
|                     | 82-SA                | I. L. Shklair, clinical isolate |
|                     | OMZ-175              | 34                       |
|                     | SL-1                 | 9                        |
| d                   |                      |                          |
|                     |                      |                          |
| e                   |                      |                          |
|                     |                      |                          |
| f                   |                      |                          |
| SL                  |                      |                          |
| *Streptococcus sanguis* |                  |                          |
|                     | ATCC 10558           | 44                       |
|                     | H7P                  | 23                       |
|                     | A12R                 | 23                       |
|                     | UCHC-JTS             | J. M. Tanzer, clinical isolate |
| *Actinomyces viscosus* |                  |                          |
|                     | M-100                | 26                       |
|                     | UCHC-HAs             | J. M. Tanzer, clinical isolate |
|                     | UCHC-TO4             |                          |
|                     | UCHC-BIB             |                          |
|                     | UCHC-R3A             |                          |
|                     | UCHC-H1              |                          |
|                     | UCHC-BE1             |                          |
| *Actinomyces naeslundii* |                  |                          |
|                     | ATCC 12104           | 40                       |
|                     | I-S-1000             |                          |

TABLE 2. Minimum bactericidal concentrations of iodine-containing compounds for preformed plaques after exposure for 30 min at 37°C

| Agent       | Minimum bactericidal concn (%) for: |
|-------------|------------------------------------|
|             | *S. mutans*                          |
|             | ATCC 10558                          |
|             | *S. sanguis*                         |
|             | ATCC 10558                          |
|             | *A. viscosus*                        |
|             | M-100                               |
|             | *A. naeslundii*                      |
|             | ATCC 12104                          |
| Inorganic iodine | 0.04                   |
| Povidone-iodine | 1.00                   |
| Wescodyne | 0.16                   |

* The following concentrations of agents, as titratable iodine, were studied: inorganic iodine, 5.0 to 0.008%; povidone-iodine, 10 to 0.001%; Wescodyne, 1.6 to 0.0016%. Plaques were run in quadruplicate. The results indicate the uniform behavior of the quadruplicate set.

* Concentration expressed as titratable iodine. Thus, a 0.16% Wescodyne solution as titratable iodine is a 10-fold dilution of proprietary stock Wescodyne solution; a 1.0% povidone-iodine solution as titratable iodine is a 10-fold dilution of proprietary stock Betadine solution.

* Bacteriostatic.

siently retarded culture acid production. *A. viscosus* and *A. naeslundii* were killed when exposed to 0.04% inorganic iodine for 20 and 30 min, respectively. Exposure of these organisms for shorter periods caused a transient retardation of culture acid production.

To determine whether more frequent but brief treatment with the agent was effective, two types of experiments were performed with *A. viscosus* and *S. sanguis* as test organisms. In the first type of experiment, plaques were treated either once or once daily at the mini-
mum inhibitory concentration of inorganic iodine (0.04 and 0.2%, respectively) for 2 min. In the second plaques, were treated for 2 min, rinsed, incubated for 6 h in broth, and then retreated with inorganic iodine for an additional 2 min, rinsed, and reincubated in broth. The results of these experiments are shown in Table 3. A. viscosus plaques were killed after a single 2-min treatment for 2 successive days. For S. sanguis, however, the plaques had to be treated with single 2-min treatments daily for 5 successive days before they were killed. Two 2-min treatments at 0.2% inorganic iodine for 1 day succeeded in killing S. sanguis plaques.

To establish that these conditions for inorganic iodine are generally appropriate, a number of strains of A. viscosus, A. naeslundii, S. mutans, and S. sanguis were studied. Table 4 shows the results for the minimum concentration and minimum treatment time for various strains of the four organisms, using a single treatment for 1 day. The results described above for the four plaque-forming species appeared generally applicable. The Actinomyces strains were killed at a concentration of 0.04% for 30 min. Attempts to shorten the duration of treatment showed that A. naeslundii and A. viscosus strains, treated at twice this concentration, still required treatment for 30 and 20 min, respectively, in order to achieve a bactericidal effect.

S. sanguis strains were killed under conditions similar to those previously used for S. sanguis ATCC 10558. Similarly, S. mutans strains were killed under similar conditions as the reference 6715-13 strain. Thus, plaques of the S. mutans strains were the most sensitive to killing by inorganic iodine.

Various serotype representatives of S. mutans were also examined with respect to the minimum concentration required for bactericidal effect. All of these serotype representatives appeared to require a similar concentration to that found to kill S. mutans 6715-13

### Table 3. Minimum exposure time and frequency of treatment with inorganic iodine required for bactericidal effect upon preformed plaques

| Microorganism       | Concen of inorganic iodine tested (%) | Minimum killing time (min) | Frequency of treatment |
|---------------------|--------------------------------------|---------------------------|------------------------|
| S. mutans 6715-13   | 0.04                                 | 5                         | ND                     |
| S. sanguis ATCC 10558 | 0.20                                 | 5                         | 2 × 2 min for 1 day    |
| A. viscosus M-100   | 0.04                                 | 20                        | 1 × 2 min for 5 days   |
| A. naeslundii ATCC 12104 | 0.04                              | 30                        | ND                     |

* Plaques were tested in quadruplicate. The results indicate the uniform behavior of the quadruplicate set.

* Concentration expressed as percent molecular iodine (wt/vol).

* ND, Not determined.

### Table 4. Minimum bactericidal concentrations and the minimum bactericidal exposure time for inorganic iodine for preformed plaques of various strains of the test microorganisms

| Microorganism       | Strain   | Minimum concen (%) | Minimum killing time (min) at concen (%) |
|---------------------|----------|--------------------|-----------------------------------------|
| S. mutans 6715-13   |          | 0.04               | 5 at 0.04                               |
|                     | FA-1     | 0.04               | 5 at 0.08                               |
|                     | SL-1     | 0.04               | 5 at 0.08                               |
|                     | LM-7     | 0.04               | 5 at 0.08                               |
|                     | E-49     | 0.04               | 5 at 0.08                               |
| S. sanguis ATCC 10558 |         | 0.20               | 5 at 0.20                               |
|                     | H-P      | 0.20               | 5 at 0.40                               |
|                     | A9R      | 0.20               | 5 at 0.40                               |
|                     | UCHC-JTS | 0.20               | 5 at 0.40                               |
| A. viscosus M-100   |          | 0.04               | 20 at 0.08                              |
|                     | UCHC-HAS | 0.04               | 20 at 0.08                              |
|                     | UCHC-TO4 | 0.04               | 20 at 0.08                              |
|                     | UCHC-BIB | 0.04               | 20 at 0.08                              |
|                     | UCHC-RaA | 0.04               | 20 at 0.08                              |
|                     | UCHC-H1  | 0.04               | 20 at 0.08                              |
|                     | UCHC-BE1 | 0.04               | 20 at 0.08                              |
| A. naeslundii ATCC 12104 |        | 0.04               | 30 at 0.08                              |
|                     | I-S-1000 | 0.04               | 30 at 0.08                              |

* Plaques were tested in quadruplicate. The results indicate the uniform behavior of the quadruplicate set.

* Concentration expressed as percent (wt/vol) molecular iodine.

(Table 5). There appeared to be no uniform differences between the Perch serotype representatives.

### DISCUSSION

These in vitro studies indicate that inorganic iodine solutions are likely to be more potent antiplaque agents than either Wescodyne or povidone-iodine. Brief exposure to 0.04 to 0.08% inorganic iodine is bactericidal to S. mutans plaques and also bactericidal, albeit requiring longer and/or more frequent exposures, to A. viscosus plaques. A. naeslundii plaques are less sensitive to this agent, and the relatively non-odontopathic S. sanguis plaques are the least sensitive. These data suggest that it is
the plaque pathogens appear achievable clinically by topical application and appear to have promise for suppressing the plaque-forming pathogens investigated here. However, because iodine is toxic upon ingestion, professionally administered topical application with ordinary and due care to avoid the patient’s swallowing the agent is indicated.

The literature supports this prediction, although no systematic studies of dosage conditions have appeared. Thus, Kligerman and Bissada (27) reported that an aqueous solution (0.02%) iodine mouthwash used twice a day for 3 weeks had no effect against bacterially undefined dental plaque. However, a 2% iodine in 2.4% sodium iodide solution applied directly to the teeth controlled both dental plaque and gingivitis approximately as well as an enforced mechanical plaque removal regimen. Duany et al. (7) indicated that there were no significant differences in the caries increment during a 9-month study among groups treated with either 0.5% povidone-iodine or placebos. The data obtained from our studies suggest that the concentrations of iodine used as a mouthwash in the studies of Kligerman and Bissada (27) and of Duany et al. (7) were too low to have pronounced antiplaque activity. Gibbons et al. (16), by contrast, demonstrated effective suppression of S. mutans at infected tooth sites for several weeks’ duration by topical scrubs of 0.2% iodine in 2% KI, a concentration which our data suggest may be unnecessarily high.

The results of the preclinical antiplaque agent screening procedure used here were previously demonstrated to be remarkably consistent with those established empirically in assessing the minimal conditions for efficacy of the bisbiguanide chlorhexidine in human clinical trials testing its antiplaque, antimicrobial, and antacaries effects (5, 31, 45). It is felt that such preclinical screening of agents against target plaque-forming microorganisms might avoid costly, time-consuming, and potentially hazardous empirical approaches to human clinical trials.

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| Perch serotype | Strain   | Minimum bactericidal concn (%) |
|---------------|---------|-------------------------------|
| a             | E-49    | 0.04                          |
|               | AHT     | 0.04                          |
|               | OMZ-61  | 0.04                          |
| b             | FA-1    | 0.08                          |
|               | BHT     | 0.04                          |
| c             | Ingbrit-1600 | 0.04                      |
|               | GS-5    | 0.04                          |
|               | NCTC-10449 | 0.08                      |
|               | UCHC-EHL | 0.04                          |
| d             | 6715-13 | 0.04                          |
|               | OMZ-176 | 0.04                          |
|               | B-15    | 0.08                          |
| e             | LM-7    | 0.08                          |
|               | B-2     | 0.04                          |
|               | 82-SA   | 0.04                          |
| f             | OMZ-175 | 0.04                          |
| SL            | SL-1    | 0.04                          |

* Plaques were tested in quadruplicate. The results indicate the uniform behavior of the quadruplicate set.

* Concentration expressed as percent (wt/vol) molecular iodine.

conceivable to suppress in vivo the apparently more cariogenic and periodontopathic elements of plaque flora (S. mutans and A. viscosus, respectively) and to select for the survival of the less orally pathogenic of the plaque-forming microorganisms (S. sanguis) studied. These results were established to be generally applicable to several strains of the test organisms.

It is not presently possible to account for the differences in iodine sensitivity of the various species. However, the effect of an agent against an intact plaque is surely a function of both the inherent sensitivity of the species in point and the inherent barrier to permeation of an agent to the plaque depth which is constituted by the cells and their adhesive and cohesive extracellular matrix. With this model, because plaques are monitored for up to 120 h post-treatment, survival of even a few cells in the depth of a plaque becomes clear because of increase in culture turbidity, change of color of the broth pH indicator, and growth after plating of plaque on appropriate agar. Furthermore, it is not easy to explain the decided differences in potency between the inorganic iodine solutions and those of the iodophors. Apparently, activity, as it reflects both bactericidal properties and plaque permeation properties, is not predictable merely by the amount of titratable iodine.

The conditions indicated as appropriate for bactericidal effect of inorganic iodine against

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TABLE 5. Minimum bactericidal concentrations of inorganic iodine for preformed plaques of various S. mutans serotypes treated for 30 min*

| Perch serotype | Strain       | Minimum bactericidal concn (%) |
|---------------|--------------|-------------------------------|
| a             | E-49         | 0.04                          |
|               | AHT          | 0.04                          |
|               | OMZ-61       | 0.04                          |
| b             | FA-1         | 0.08                          |
|               | BHT          | 0.04                          |
| c             | Ingbrit-1600 | 0.04                          |
|               | GS-5         | 0.04                          |
|               | NCTC-10449   | 0.08                          |
|               | UCHC-EHL     | 0.04                          |
| d             | 6715-13      | 0.04                          |
|               | OMZ-176      | 0.04                          |
|               | B-15         | 0.08                          |
| e             | LM-7         | 0.08                          |
|               | B-2          | 0.04                          |
|               | 82-SA        | 0.04                          |
| f             | OMZ-175      | 0.04                          |
| SL            | SL-1         | 0.04                          |

* Plaques were tested in quadruplicate. The results indicate the uniform behavior of the quadruplicate set.

* Concentration expressed as percent (wt/vol) molecular iodine.

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