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Inhibition of Experimental Dental Caries by Antibiotics

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A variety of antibiotic and chemotherapeutic agents were tested for their ability to inhibit the development of dental caries in Sprague-Dawley rats receiving the drugs in a coarse-particle sucrose-containing diet. Drugs which inhibit gram-positive microorganisms were effective inhibitors of caries, whereas agents which are active solely against gram-negative bacteria did not inhibit caries development. In vivo efficacy of the agents tested generally, but not invariably, paralleled in vitro inhibition of the growth of *Streptococcus mutans* strain FA-1, an organism which was isolated from carious Sprague-Dawley rats and which is known to induce caries in gnotobiotic Sprague-Dawley rats. Caries was significantly inhibited when 1-ephenamine penicillin (20 units/mg) was administered intermittently in the diet, 1 day per week or 1 week of every 4 weeks, but protection against caries was greatest when the same amount of the drug was fed continuously.

The original observation by McClure and Hewitt (19) that experimentally induced dental caries in the rat could be inhibited by the administration of penicillin in the diet and drinking water has been repeatedly confirmed and extended to other antibiotics and other test animals [see Shaw (24) and Bibby (1) for reviews of earlier literature]. Comparisons of the anticaries effects with the antimicrobial spectra of individual antibiotics indicated that caries development was most probably associated with the activities of one or more members of the gram-positive segment of the oral microflora (5, 25).

This concept received convincing support from studies with gnotobiotic rats in which monoinfection with an enterococcus resulted in the induction of typical carious lesions (21). Subsequent investigators showed the cariogenic potential of *Streptococcus mutans* (2, 4, 11) in gnotobiotic rats or conventional hamsters which did not normally harbor a caries-conducive microbiota (7, 8). Other investigators have since confirmed these findings and added additional species to the list of strains which can induce caries in rats or hamsters. At present, all known cariogenic strains are gram-positive. With the exception of certain filamentous organisms which cause root surface lesions, but not coronal caries (13, 15), all are either streptococci or lactobacilli (6). The significance of these observations has been enhanced by the finding that strains of streptococci closely related to some of the animal types have been isolated from human tooth surfaces and have also induced caries in animal test models (28).

These discoveries have rekindled interest in the role of specific microorganisms as a major factor in caries and have evoked the suggestion that effective control measures for this disease must ultimately include a means of controlling the microbial vectors (9, 23).

In the studies to be described, a number of antibiotic agents were tested for their inhibitory effects on the development of dental caries in a rat model system.

MATERIALS AND METHODS

Animal test system. Female Sprague-Dawley rats from the National Institutes of Health Animal Production Unit were put on test immediately after weaning at 21 days of age. Animals were randomly distributed into units of three per cage, and were housed in stainless-steel cages with 1.27-cm wire-mesh floors. Animal weights were recorded at the beginning of the test and at biweekly intervals thereafter. At the commencement of these studies, the Sprague-Dawley rats normally harbored a caries-conducive microflora as evidenced by consistently high caries activity over a period of several years when challenged with appropriate diets.

Diet. The coarse-particle Diet 585 of Stephan et al. (25) was selected as the test diet on the basis of prior experience which indicated that it was highly caries-conducive in the Sprague-Dawley rats. The lesions which developed were located in the molar sulci, most frequently in the first and second mandibular molars. Diet 585 is composed of 30% whole milk powder (Land O'Lakes), 42% yellow hominy

grits (Quaker Oats Co.), 25% granulated sucrose (Domino), 2% whole liver powder (Wilson Laboratories), and 1% salt mixture (25).

Test drugs. The sources of the agents tested are listed in Table 1. Each week the desired amounts of drugs were triturated into 100 g of Diet 585 by use of a mortar and pestle. The resulting mixture was blended with successively larger portions of the diet in a mixing machine to give an amount sufficient for *ad libitum* consumption for 1 week. The diet was stored at 4 C and provided to the animals every 2 days in spill-proof cups. Any diet unconsumed after 2 days was discarded. Food consumption was determined over a 2-day period about the thirtieth day and again about the sixtieth day of the test by measuring the weight loss of the food cups, corrected for any spillage collected on paper sheets placed beneath the cages. It was recorded as grams consumed per animal per day. Distilled water was provided fresh daily in glass jars fitted with stainless-steel drinking tubes.

Evaluation of results. After 90 days on test, final weights were recorded and the animals were chloroformed. The heads were defleshed in a colony of dermestid beetles (22). Caries was scored on the unsectioned jaws according to the system of Cox et al. (3) with the use of 15 times magnification. A preliminary comparison with the more exacting method of Keyes (14), which involved hemisectioning of the molars, indicated that both methods permitted comparable assessments of the relative efficacy of a series of antibiotics with Diet 585. The simpler scoring method of Cox et al. (3) was routinely employed because it was primarily oriented toward diagnosis of caries in the molar pits and fissures, whereas Keyes' procedure includes smooth (buccal and lingual) surface lesions which seldom are found with Diet 585. An untreated group of control animals was included in each test, and scores were compared with those of the drug-treated groups. For the group sizes employed, 18 to 24 animals, a difference in caries score of 25% was significant at the probability level of 0.02 to 0.01 by Student's t test. In many of the trials, a drug that had been previously tested was included as a further control measure.

In vitro tests. The ability of various drugs to inhibit growth of *S. mutans* FA-1 was tested by the serial broth-dilution technique. Drugs were diluted in twofold steps in the medium described by Jordan et al. (12), in which the glucose concentration was 0.4%. Screw-capped culture tubes containing 10 ml of this medium and dilutions of the test drugs were inoculated with a 24-hr culture of strain FA-1 diluted so that the inoculum contained approximately 10^4 colony-forming units. After 48 hr of incubation at 37 C in an atmosphere of 95% nitrogen and 5% carbon dioxide, the concentration of drug at which growth was inhibited by 50% or more was considered the minimal inhibitory concentration (MIC).

The FA-1 strain of *S. mutans* was originally isolated (7) from a Sprague-Dawley rat, and serologically identical strains have been repeatedly isolated from these animals since that time.

RESULTS AND DISCUSSION

Diet 585 does not support the development of smooth surface lesions on the rat molars, probably because of its abrasive physical characteristics (18). Lesions are confined primarily to the molar fissures, and the carious process is apparently intensified in these areas both because the coarse hominy grits promote impaction of diet deep into the sulci and because they tend to enlarge carious lesions by mechanically debriding softened tooth structure. This test system is considered to represent a more severe test of an anticaries agent than that posed by fine-particle diets, on which smooth surface lesions also develop, because there is some evidence that caries of all types are much more susceptible to inhibition by antibiotics when fine-particle diets are used than when coarse-particle diets are used (5).

Table 1 summarizes the results of a number of individual experiments in which various antibiotics were contained in Diet 585. The average number of carious teeth per animal, the average number of carious surfaces per animal, and the average caries score (3) are shown. Although Cox et al. (3) cautioned that their caries score is not linearly derived and therefore not strictly amenable to statistical analysis, in our experience with Diet 585 the caries score gives a fairly close approximation of the amount of tooth structure destroyed. We have utilized these scores only to make statistical comparisons between the untreated control group and individual drug-treated groups.

In several cases, e.g., subtilin and nalidixic acid, increased caries over control scores was noted. In these cases, since growth rates and food consumption were not affected and there were no other overt signs of toxicity, the drug may have inhibited some members of the oral microbiota which normally compete with the cariogenic organisms within the oral or intestinal ecosystem. Subtilin affects gram-positive microorganisms almost exclusively, but it was not inhibitory to the growth of *S. mutans* in vitro at levels up to 40 μg/ml (Table 3). It is therefore possible that this antibiotic inhibited other gram-positive organisms which might have competed with *S. mutans*. Nalidixic acid, which inhibits gram-negative microorganisms, may have produced a more favorable environment for the proliferation of the gram-positive cariogenic microflora.

One drug, anisomycin, resulted in an apparent inhibition of dental caries, although it has no antibacterial activity, being effective against a number of protozoa and fungi (26). In this case,

TABLE 1. *Effects of antibacterial agents on caries development in rats*

Expt	Drug[a]	No. of carious teeth[b]	No. of carious surfaces	Avg caries score[c] (SE)
A	Potassium penicillin G[11], 20 units	4.9	5.7	6.7 (±0.7)
	1-Ephenamine penicillin G[4], 20 units	3.1	3.2	3.5 (±0.4)
	Ristocetin[1], 20 μg	6.2	8.8	17.3 (±1.9)
	Kanamycin[3], 20 μg	6.1	8.9	17.6 (±2.2)
	Anisomycin[9], 200 μg	6.3	7.9	12.2 (±1.7)
	Sulfadiazine[6], 1,000 μg	6.3	8.5	13.0 (±1.1)
	Sulfamerazine[6], 1,000 μg	8.4	12.2	18.2 (±1.3)
	Sulfaguanidine[6], 1,000 μg	9.3	14.3	23.5 (±1.9)
	Phthalylsulfathiazole[8], 1,000 μg	7.4	10.4	18.3 (±1.6)
	Controls	7.5	11.4	22.6 (±1.5)
B	Erythromycin[1], 20 μg	3.7	3.7	4.4 (±0.6)
	Carbomycin[9], 20 μg	5.0	5.5	7.5 (±0.5)
	Chlortetracycline[6], 50 μg	2.2	2.3	3.7 (±0.6)
	Chloramphenicol[8], 50 μg	4.1	5.5	10.6 (±0.6)
	Controls	4.8	8.8	21.7 (±1.7)
C	Zn bacitracin[4], 20 units	4.2	4.2	5.0 (±0.5)
	Tetracycline[6], 20 μg	3.1	3.9	7.1 (±1.5)
	Oxytetracycline[9], 20 μg	3.8	4.4	6.2 (±1.2)
	Subtilin[14], 70 μg	5.1	9.2	22.8 (±2.4)
	Antibiotic 14[14], 20 μg	4.3	6.4	13.1 (±1.9)
	Antibiotic 14[14], 100 μg	4.8	7.3	15.3 (±2.2)
	Antibiotic 14[14], 500 μg	4.7	8.9	20.8 (±2.4)
	Antibiotic 281DY[14], 10 mg	3.8	6.3	12.9 (±2.1)
	Antibiotic 1968D[14], 10 mg	4.7	7.6	17.1 (±2.0)
	Controls	4.8	7.5	16.4 (±1.9)
D	1-Ephenamine penicillin G[4], 10 units	0.8	0.8	0.8 (±0.3)
	Erythromycin[1], 10 μg	0.6	0.9	1.9 (±0.8)
	Zn bacitracin[4], 10 units	2.4	3.0	6.2 (±1.3)
	Carbomycin[9], 10 μg	3.4	4.6	10.0 (±1.2)
	Novobiocin[12], 20 μg	4.5	6.2	10.2 (±1.1)
	Cycloserine[4], 100 μg	4.0	5.2	11.5 (±1.5)
	Celesticetin[12], 20 μg	5.3	6.5	11.8 (±1.2)
	Neomycin[15], 10 μg	3.6	5.4	13.8 (±1.5)
	Antibiotic 115-1D (124)[14], 10 mg	4.9	7.2	13.9 (±1.2)
	Antibiotic 829C-DD[14], 100 μg	5.4	7.6	14.3 (±1.7)
	Antibiotic 204-1D (111)[14], 10 mg	5.6	10.4	22.9 (±1.7)
	Controls	5.7	8.3	16.2 (±1.8)
E	Bacitracin[4], 25 units	2.6	2.7	3.3 (±0.2)
	Vancomycin[7], 20 μg	5.4	6.8	8.2 (±0.8)
	Anisomycin[9], 100 μg	6.4	7.4	7.7 (±0.9)
	Controls	7.2	8.7	13.9 (±1.1)

[a] The amounts shown for the drugs represent the amount contained in 1 g of Diet 585. The superscript numbers indicate the sources of the drugs, as follows: (1) Abbott Laboratories, North Chicago, Ill.; (2) Boots Pure Drug Co. Ltd, Nottingham, England; (3) Bristol Laboratories, Syracuse, N.Y.; (4) Commercial Solvents Corp., Terre Haute, Ind.; (5) Hoffman La Roche, Inc., Nutley, N.J.; (6) Lederle Laboratories, Pearl River, N.Y.; (7) Eli Lilly & Co., Indianapolis, Ind.; (8) Parke, Davis & Co., Detroit, Mich.; (9) Chas. Pfizer & Co., Brooklyn, N.Y.; (10) R.I.T., Genval, Belgium; (11) E. R. Squibb & Sons, New Brunswick, N.J.; (12) The Upjohn Co., Kalamazoo, Mich.; (13) Winthrop Laboratories, New York, N.Y.; (14) Western Utilization Research Branch, U.S. Department of Agriculture, Albany, Calif.

[b] Average number per animal.

[c] The caries score is the sum of the number of various surfaces multiplied by a severity factor of 1, 2, or 3, to denote enamel caries, penetration of the dentin, or deep dentinal penetration, respectively.

the anticaries effect was attributed to the fact that the rats ate very little of the anisomycin-containing diet. This was reflected both in the food consumption determinations and in the weight gain of the animals. Animals receiving 200 μg of anisomycin per gram of diet consumed an average of 5.0 g of diet per day throughout the 90-day test period. In contrast, control animals averaged 7.1 g of food at 7 days, 9.8 g at 35 days, and 10.5 g at 60 days. Both groups of animals weighed 45 g at the start of the test; 6 weeks later, the controls weighed 144 g and the anisomycin group weighed 56 g. The drug was discontinued for 1 week, and the anisomycin group gained 28 g (weight, 84 g) while the controls gained 14 g (weight, 158 g). After 90 days, the controls weighed 194 g and the anisomycin group still weighed 84 g. Thus, the reduction in caries was most probably due to decreased intake of cariogenic diet, resulting from aversion of the rats to the drug which it contained.

The experience with anisomycin illustrated the importance of including in the experimental design ancillary procedures to aid in the recognition of side effects of the test agents which may complicate the interpretation of the effects of the drug on caries development. In this connection, periodic measurement of weight gains and food intake is especially informative, particularly during the early postweaning stage when the teeth are most susceptible to caries (10, 17) and when changes in the amount of cariogenic diet consumed or frequency of eating would have disproportionately large effects on subsequent caries development. It is also important that weights be measured regularly during the course of the test, because deviations from normal values are often the first toxic manifestation of an orally administered drug. Furthermore, the fact that every animal must be handled individually provides an excellent opportunity to make gross observations for other untoward symptoms such as diarrhea, unthriftiness, neuromuscular disorders, and the like.

The drug tests extended over a period of several years and encompassed a period in which efforts were being made in the breeding colony to obtain healthier breeding stock free from common laboratory animal infections. Concomitantly with these efforts, there was a gradual decline in the apparent caries activity of the colony, as can be seen in Table 1 by the decreasing caries scores in control animals of successive tests. To determine whether this was due to some systemic change in the animals resulting in increased resistance to caries or to a change in the cariogenic potential of the oral microbiota, an experiment was performed in which all of the animals received an oral inoculation of a 10% aqueous suspension of pooled feces from Osborne-Mendel rats maintained on the high-sucrose Diet 2000 of Keyes and Jordan (15). The high caries score in the control animals (Table 2) suggests that the declining caries activity in the Sprague-Dawley rat colony was most probably due to alterations in the cariogenic

TABLE 2. *Effects of antibacterial agents on caries development in rats superinfected with a cariogenic microflora*

Drug[a]	No. of carious teeth[b]	No. of carious surfaces[b]	Avg caries score[c] (SE)
Potassium penicillin G[11], 20 units	3.6	3.9	4.6 (±0.7)
Potassium penicillin G[11], 50 units	3.6	3.7	4.3 (±0.7)
Staphylomycin[10] (ST[d]), 50 μg	3.9	4.1	4.4 (±0.5)
Staphylomycin[10], 50 μg	4.7	5.4	6.8 (±1.5)
Ampicillin[3], 50 units	5.2	5.7	7.7 (±1.7)
Staphcillin[3], 100 μg	5.1	5.8	7.7 (±0.9)
Lincomycin[12], 50 μg	6.9	8.3	12.3 (±1.5)
Quinacillin[2], 100 μg	6.6	8.7	15.5 (±2.2)
Humatin sulfate[8], 50 μg	6.3	12.3	27.4 (±2.6)
Nalidixic acid[13], 50 μg..β	6.1	13.9	33.0 (±3.1)
Dodecyl lysine·HCl[5], 50 μg	5.1	9.3	24.8 (±3.4)
Palmitoyl lysyl lysine·HCl[6], 50 μg	7.3	13.7	29.6 (±2.7)
Controls	7.4	13.2	26.4 (±2.6)

[a] The amounts shown for the drugs represent the amount contained in 1 g of Diet 585. The superscript numbers indicate the sources of the drugs as given in footnote *a* of Table 1.

[b] Average number per animal.

[c] See footnote *c* of Table 1.

[d] Stabilized.

microflora rather than to any inherent increase in caries resistance of these animals. These observations indicate the value of the practice suggested by Larson (16) of maintaining a "donor" colony of caries-active animals on an appropriate cariogenic diet as a uniform source of inoculum for test animals which may be suspected of variability in caries susceptibility for one reason or another.

In general, antibacterial agents which have been reported to be effective in vitro against gram-positive microorganisms exhibited anticaries activity in the present study. However, it was not always possible to predict the in vivo efficacy against caries from the in vitro activity when *S. mutans* FA-1, derived from rats and known to be capable of inducing caries in gnotobiotic rats (7), was used as the in vitro test organism. Table 3 lists the in vitro minimal effective concentrations of various drugs based on 50% or more inhibition of growth of *S. mutans* FA-1 after 48 hr. Similar results were obtained with hamster strain HS-6 (8) and human strain K-1R. For comparative purposes, results of the anticaries tests in rats receiving Diet 585 are included. The anticaries activity is given in arbitrary units ranging from ++++, which represents greater than 75% caries reduction as compared to the untreated controls, to −, which represents no significant inhibition. Ristocetin and vancomycin were less effective in vivo than might be expected from their activity in vitro, and the converse was true for carbomycin and quinacillin. With consideration of the many factors which could account for disparities between in vivo and in vitro efficacy of drugs and the fact that the differences observed in the present study were quantitative rather than qualitative, the *S. mutans* test system appears to be adequate for use as a preliminary screen for detecting potential anticaries drugs. It remains to be determined just how well the results of in vitro test systems or even the in vivo animal tests will correlate with effectiveness in human caries. However, in view of the expense and compexity involved in conducting human trials, practical considerations would dictate that only those agents which display the highest activity both in vitro and in vivo should be considered for tests in humans. Scherp (23) recently reviewed many of the considerations relating to the appli-

TABLE 3. *Comparison of in vitro effects of antibiotics against Streptococcus mutans and in vivo effects on sulcal caries in rats*

Drug	Antibacterial MIC[a]	Anticaries tests	
		Amt of drug/g of Diet 585	Relative anticaries activity
Penicillin G	0.16 units	20 units	++++
Erythromycin	0.16 μg	10 μg	+++
Staphylomycin	0.6 μg	50 μg	+++
Tetracycline	1.0 μg	20 μg	++
Bacitracin	1.2 units	25 units	+++
Ristocetin	1.5 μg	20 μg	±
Vancomycin	1.5 μg	20 μg	+
Chloramphenicol	2.5 μg	50 μg	++
Oxytetracycline	2.5 μg	20 μg	++
Celesticetin	2.5 μg	20 μg	+
Staphcillin	3.0 μg	100 μg	+++
Chlortetracycline	5.0 μg	50 μg	++++
Carbomycin	5.0 μg	20 μg	+++
Novobiocin	5.0 μg	20 μg	+
Lincomycin	10.0 μg	50 μg	++
Kanamycin	12.0 μg	20 μg	+
Neomycin	50.0 μg	10 μg	±
Subtilin	>40 μg	70 μg	−[b]
Cycloserine	>40 μg	100 μg	+
Quinacillin	>100 μg	100 μg	++
Anisomycin	>100 μg	100 μg	+[c]
Nalidixic acid	>100 μg	50 μg	−[b]

[a] The minimal inhibitory concentration, in micrograms or units per milliliter, which inhibited growth by 50% or more after 48 hr at 37 C in Jordan's medium (12) in a 5% CO_2-95% N_2 atmosphere.

[b] Increased caries.

[c] Toxic, see text.

cation of chemotherapeutic procedures in the prevention and control of human caries.

The initiation and progression of dental caries require the colonization of potentially cariogenic microorganisms directly on the tooth surfaces in masses called dental plaque deposits (6). It thus appears plausible that caries could be brought under some measure of control simply by taking advantage of the fact that, once the established population of caries-conducive microorganisms on the teeth or in the oral cavity is drastically reduced, some interval of time would be required before they could become reestablished in sufficient numbers to reinitiate the disease process. A comparable analogy is the use of penicillin or sulfonamides prophylactically for the control of rheumatic fever associated with group A streptococci.

Table 4 summarizes the results of an experiment in which 1-ephenamine penicillin was administered to rats in Diet 585 on three dosage schedules: continuously, 1 day of each week, or 1 week of every 4 weeks. Significant, although not maximal, caries reductions were achieved with the intermittent schedules of administration of the antibiotic, even though the total amounts of drug available were only one-seventh and one-fourth, respectively, of that received under the continuous administration schedule. Since the rate of caries progression in humans is ordinarily much slower than the acute type of disease induced in animal test systems, intermittent treatment with suitable agents might prove an advantageous alternative to continuous prophylaxis, in terms of treatment time, cost of treatment, and patient cooperation.

Control of caries in humans through the application of antibacterial agents is an especially attractive concept, because it is an approach which is aimed directly at the eradication of the causative microorganisms, in contrast to measures like fluoridation or dietary control which serve to ameliorate the disease process by indirect mechanisms.

A certain risk of undesirable effects accompanies the use of any therapeutic agent for whatever purpose. However, the likelihood that administration of anticaries drugs might eventually be widespread throughout population groups, and most probably for extended periods of time, would undoubtedly tend to increase the chance for untoward effects to materialize. Therefore, the ideal anticaries agent should possess the following properties: (i) a relatively narrow antimicrobial spectrum, preferably confined to cariogenic microorganisms; (ii) a high therapeutic index and a very low order of toxicity; (iii) a very low potential for the induction of resistant microorganisms; (iv) a very low potential for allergenicity; (v) low susceptibility to inactivation by substances within the oral cavity (but susceptibility to destruction in the intestinal tract would be desirable); (vi) a rapidly bactericidal mode of action, preferably combined with a selective affinity for teeth or microbial dental plaque deposits; (vii) high stability under conditions of storage and compatability with components of the vehicle in which it is formulated; (viii) no unpleasant organoleptic properties; (ix) relatively inexpensive to manufacture in a pure state and to compound in a therapeutic vehicle which is convenient to apply; (x) long lasting efficacy with relatively few applications; and (xi) not in general use for other therapeutic applications.

It is not very likely that a single antimicrobial drug will be found that possesses all of these qualities, but it is reasonable to expect that some agents possessing enough of these properties to be worth testing in humans could eventually be developed. At present, vancomycin (20), actinobolin (D. E. Hunt, J. M. Navia, and H. Lopez, Proc. 48th General Meeting, Int. Ass. Dent. Res., p. 164, 1970), and a macrolide antibiotic (27) have already been proposed as control agents for human dental health purposes.

TABLE 4. *Anticaries effects of continuous versus intermittent administration of 1-ephenamine penicillin G in Diet 585*

Drug concn (units/g of Diet 585)	Dosage schedule	No. of carious teeth[a]	No. of carious surfaces[a]	Avg caries score[b] (SE)
20	Continuous	3.7	3.9	3.9 (±0.2)
20	1 day per week	4.2	4.3	5.4 (±0.5)
20	1 week of every 4 weeks	4.9	5.1	6.7 (±0.7)
50	Continuous	2.6	2.7	3.3 (±0.2)
—	Controls	7.2	8.7	13.9 (±1.1)

[a] Average number per animal.
[b] See footnote *c* of Table 1.

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