In Vitro Comparison of Actinobolin and Other Antibiotics Used in the Treatment of Periodontal Disease and Dental Caries[1] (36689)

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Actinobolin, an antibiotic isolated from cultures of *Streptomyces griseoviridis* var. *atrofaciens* (1), is a potentially useful cariostatic agent (2). When added to the diets of weanling rats at a concentration of 37.6 ppm, this antibiotic exerted a cariostatic effect equal to or greater than 25 ppm of fluoride (3). Considerable interest in this antibiotic has developed because it appears to have a multifaceted cariostatic action that may be attributed to its antistreptococcal activity (2), its subcellular antibacterial mechanism of action (4), and its ability to increase the hardness of human enamel (T. I. Koulourides, personal communication). A recent *in vitro* study (5) has shown also that actinobolin, at concentrations of 100–250 μg/disk, strongly inhibited bacteria that are associated with periodontal disease. Additionally, this antibiotic has a variety of chemical, pharmacological, and biochemical properties that make it ideally suited for topical application in the control of dental caries and periodontal disease (2). To assess further the potential of this antibiotic for the treatment of these dental and gingival infections, a study was initiated to compare the antibacterial activity of actinobolin, against microorganisms associated with periodontal disease, with a variety of clinically useful antibiotics that have been reported to be of value in the control of dental caries and periodontal disease.

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Materials and Methods. Actinobolin sulfate lot X8061 was obtained from Parke, Davis and Co. For comparison, ten clinically useful antibiotics (See Table I for the specific antibiotics) were tested in the form of sensitivity disks (Difco, Detroit). The antibiotic concentrations in the commercially prepared sensitivity disks reflect therapeutic levels that can be used *in vivo.* Consequently, blank disks of the same size and quality (Schleicher and Schuell No. 740-E, 0.64 cm in diameter) were charged with aqueous solutions of actinobolin that reflect therapeutic concentrations which could be readily applied topically in the oral cavity. The *in vitro* inhibitory activity of the antibiotics tested was determined by placing the antibiotic-containing disks on the surface of brain–heart-infusion agar (Difco) plates that had been uniformly inoculated with clinical specimens from periodontal pockets greater than 6 mm in depth. Since the microorganisms in periodontal pockets are facultative or strict anaerobes, the inoculated plates were incubated at 37° for 24 hr in an atmosphere of 95% nitrogen and 5% carbon dioxide. The diameters of zones of growth inhibition surrounding the paper disks were used to assess the relative antibacterial activity of the antibiotics.

In additional studies directed toward determining patterns of antibiotic cross-resistance between actinobolin and three representative clinically useful antibiotics: Ampicillin, demethylchlortetracycline (Dmt), and erythromycin (purchased from the University Hospital Pharmacy, Birmingham) were used. These antibiotics were selected for cross-resistance studies because they each represent a large family of clinically useful antibiotics.

E I. *In Vitro* Comparison of the Antibacterial Activity of Actinobolin, Ampicillin, Bacitracin, Cephalothin, Erythromycin, Lincor
cillin, Tetracycline, and Vancomycin Against Mixed Clinical Isolates from Periodontitis Patients.[a]

	Actinobolin 100[b]	Ampicillin 2[b]	Bacitracin 2[c]	Cephalothin 30[b]	Erythromycin 2[b]	Lincomycin 2[b]	Penicillin 2[b]	Tetracycline 5[b]
al cultures obtained riodontitis patients	Zone size (cm)	Zone size (cm)	Zone size (cm)	Zone size (cm)	Zone size (cm)	Zone size (cm)	Zone size (cm)	Zone size (cm)
1. (M.P.)	2.4[d]	3.4	2.2	4.0	3.0	2.7	2.8	2.3
2. (F.J.)	2.2	2.4	2.2	4.4	3.1	2.3	3.6	2.3
3. (C.S.)	2.7	2.5	1.9	4.2	2.9	2.0	3.6	1.1
4. (J.W.)	1.4	2.7	2.2	5.0	1.7	2.4	2.9	1.3
5. (T.J.)	1.8	2.3	2.1	3.8	2.1	2.4	3.4	—[e]
6. (L.S.)	1.9	2.7	1.3	3.7	0	1.6	2.4	0
7. (L.B.)	1.6	2.6	1.6	3.4	3.0	0	1.8	0
8. (W.W.)	1.7	2.4	1.4	3.3	—[e]	0	1.8	0
9. (J.W.)	2.0	3.0	2.2	3.6	2.3	2.3	2.6	1.9
10. (W.J.)	2.2	3.4	0	4.1	0	1.8	2.8	2.2
11. (C.W.)	2.0	3.2	0	4.0	0	1.6	2.4	2.7
12. (D.M.)	2.0	3.1	1.9	3.5	2.8	1.6	2.6	1.9

ical specimens were obtained from periodontitis patients and were used to inoculate plates of brain–heart–infusion agar. Anti
lisces were placed on the surface of the inoculated plates, and after incubation, the zones of growth inhibition surrounding the ar
disks were used as a parameter to assess the relative inhibitory activity of the antibiotics tested.
disk.
ts/disk.
nbers refer to diameters (cm) of zones of anaerobic growth inhibition surrounding paper disks.
tested.
: Concentrations of 30 μg/disk of kanamycin (not shown in Table) were found to be noninhibitory for the cultures tested.

Bacteria used in the cross-resistance studies were: *Escherichia coli* ATCC 9637, *Staphylococcus aureus* ATCC 6538, and *Streptococcus faecalis* ATCC 11420. Although these bacteria have no known role in the etiology of dental caries or periodontal disease, they were chosen for cross-resistance studies because they represent genera or species frequently encountered in serious systemic infections. Therefore it seemed important to determine if actinobolin-resistant strains of *S. faecalis, S. aureus* or *E. coli* might be refractory to representative clinically useful antibiotics that are presently used to treat systemic infections caused by these or related bacteria. Conversely, it seemed useful to determine if bacterial resistance to ampicillin, erythromycin, or Dmt might also confer resistence against actinobolin and thereby reduce the effectiveness of this antibiotic for dental and gingival infections. Laboratory-derived antibiotic-resistant strains of *E. coli, S. aureus,* and *S. faecalis* were designated as follows: Name of parental strain–abbreviated name of antibiotics-r. These resistant strains were obtained by an agar-gradient plate method (6), and all were greater than fivefold more resistant than the parental strains from which they were derived. Antibiotic cross-resistance was determined by placing paper disks containing various concentrations of the respective antibiotics on the surface of brain–heart-infusion agar plates which had been previously inoculated with the antibiotic sensitive or resistant bacterial strains. After incubation for 24 hr at 37°, the diameters of zones of growth inhibition surrounding the disks were used to assess simultaneously the relative sensitivity of the parental and resistant strains to the various antibiotics.

Results and discussion. The data presented in Table I show that actinobolin, at a concentration of $\geqq$ 100 μg-disk, strongly inhibited mixed cultures obtained from patients with periodontitis. It should be noted that although testing mixed cultures for antibiotic sensitivity is contrary to routine clinical laboratory procedures, mixed cultures were used in this part of the study because of the syntrophic microbial relationship that appears to be responsible for periodontal disease (7). Compared to the concentrations of the clinically useful antibiotics in Table I, the levels of actinobolin tested were high; however, concentrations of 100–250 μg of actinobolin/disk reflect levels that could be readily used in the mouth since this antibiotic has a very low order of toxicity when applied orally (8).

Bacterial colonies, which appeared with varying frequency within the zones of growth inhibition of all the clinically useful antibiotics tested, were subcultured and tested for sensitivity to actinobolin. All of these strains were sensitive to inhibition by $\geqq$ 250 μg actinobolin/disk (See Table II). Additional data shown in Table III revealed that laboratory-derived strains of *S. faecalis, S. aureus,* or *E. coli* resistant to actinobolin were not resistant to ampicillin, Dmt, or erythromycin. Laboratory-derived strains of these three species resistant to ampicillin, Dmt, or erythromycin were not resistant to actinobolin.

TABLE II. *In Vitro* Inhibitory Activity of Actinobolin Against Bacterial Isolates Showing *In Vitro* Resistance to Various Antibiotics That Have Been Reported to be Potentially Useful in the Control of Dental Caries and Periodontal Disease.[a]

Bacterial colonies resistant to:	Actinobolin (μg/disk)	
	100	250
	Zone size (cm)	
Ampicillin (10 μg/disk)	2.8[b]	3.4
Bacitracin (10 units/disk)	2.1	2.6
Cephalothin (30 μg/disk)	0	0.7
Erythromycin (15 μg/disk)	0	2.1
Lincomycin (2 μg/disk)	3.3	3.8
Penicillin G (10 units/disk)	1.9	2.6
Tetracycline (30 μg/disk)	0	2.1
Vancomycin (30 μg/disk)	2.9	3.6

[a] Colonies were selected which appeared within the zones of growth inhibition surrounding the antibiotic sensitivity disks indicated in the above left column. These colonies, consisting mostly of strains of *Bacteroides melaninogenicus, Fusobacterium fusiforme, Leptotrichia buccalis,* and *Veillonella parvula,* were subcultured and tested for sensitivity to actinobolin.

[b] Numbers in table refer to diameter (cm) of zones of growth inhibition surrounding paper disks. Diameter of disks, 0.64 cm.

TABLE III. Patterns of Reciprocal Antibiotic Cross-Resistance between Bacterial Strains Resistant to Actinobolin (Acb-r), Ampicillin (Amp-r), Erythromycin (Ery-r), of Demethylchlortetracycline (Dmt-r).

Bacteria		Zone size in cm with the following antibiotics											
		ACB			AMP			ERY			DMT		
		500[a]	50	10	30	3	1	5	0.5	0.1	3	1	0.5
S. faecalis	ATCC 11420[c]	1.9[b]	1.0	1.1	2.4	1.6	1.2	1.8	1.1	0	1.6	1.3	1.0
	Acb-r	0	0	0	2.5	1.9	1.3	2.1	1.5	1.1	1.6	1.3	1.1
	Ery-r	2.4	1.1	0	2.7	2.1	1.4	0	0	0	1.9	1.5	1.3
	Amp-r	2.6	1.3	1.1	1.6	0	0	2.3	1.2	1.1	1.7	1.4	1.2
	Dmt-r[d]	3.8	2.4	0.5	2.6	2.0	1.4	2.3	1.7	1.1	0	0	0
S. aureus	ATCC 6538	2.2	1.1	0	2.5	1.9	1.1	1.8	1.3	1.0	1.5	1.2	1.0
	Acb-r	0	0	0	2.7	2.2	1.6	2.3	1.7	1.3	1.7	1.3	1.2
	Ery-r[d]	3.1	1.8	1.4	3.4	3.2	2.9	1.2	0	0	2.0	1.7	1.5
	Dmt-r	2.8	1.5	0	2.8	2.3	1.8	2.3	1.7	1.3	1.1	0	0
E. coli	ATCC 9637	2.4	1.6	0	2.4	2.0	1.5	0	0	0	1.5	1.0	0
	Acb-r	0	0	0	2.4	2.0	1.5	0	0	0	1.5	1.0	0
	Amp-r	2.7	1.5	1.1	1.4	0	0	0	0	0	1.0	0	0
	Dmt-r	2.6	1.6	0	2.4	2.0	1.5	0	0	0	0	0	0

[a] μg antibiotic/disk.

[b] Numbers in table refer to diameter (cm) of zones of growth inhibitions surrounding paper disks. Diameter of disk, 0.64 cm.

[c] Resistant strains are designated as: Name assigned by American Type Culture Collection–antibiotic-r to which it is resistant. All resistant strains are $\geq$ fivefold more resistant than the parental strains from which they were derived.

[d] Representative antibiotic resistant strains which showed an increased sensitivity to inhibition by actinobolin.

In certain instances, bacterial strains resistant to one of the clinically useful antibiotics showed an increased collateral sensitivity to actinobolin. For example, *S. aureus* Ery-r, *S. faecalis* Dmt-r, and *E. coli* Amp-r were more sensitive to inhibition by actinobolin than the parental strains. Similar observations have been reported by other investigators (9).

The significance of this report is that actinobolin, compared with a variety of clinically useful antibiotics, strongly inhibited bacteria associated with periodontitis. In addition, bacterial resistance to actinobolin or to a variety of medically useful antibiotics should pose no reciprocal problems in a clinical situation in which actinobolin is applied orally for the management of dental and gingival infections. These observations, in addition to the fact that actinobolin is not currently used in medical practice, appear to circumvent some of the impediments that have prevented the clinical use of currently available antibiotics for the control of dental caries and periodontal disease.

Summary. When compared *in vitro* with a variety of clinically useful antibiotics at concentrations that reflect therapeutic levels that can be used *in vivo*, actinobolin (100–250 μg/disk) strongly inhibited bacterial cultures obtained from patients with periodontitis. *In vitro* bacterial resistance to ampicillin, demethylchlortetracycline, or erythromycin did not confer resistance to actinobolin, and resistance to actinobolin did not confer resistance to the above indicated clinically useful antibiotics.

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