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In Vitro Evaluation of Actinobolin as an Antibiotic for the Treatment of Periodontal Disease[1]

P. J. ARMSTRONG, JR., AND D. E. HUNT

Department of Periodontics, Institute of Dental Research, Veterans Administration Hospital, and Department of Microbiology, School of Dentistry, University of Alabama in Birmingham, Birmingham, Alabama, 35233

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Actinobolin was evaluated in vitro by a paper disc-agar diffusion method for inhibitory activity against mixed microbial cultures obtained from patients with periodontal disease and against pure bacterial cultures tentatively identified as strains of *Bacteroides melaninogenicus*, *Fusobacterium fusiforme*, *Leptotrichia buccalis*, and *Veillonella parvula*. Every culture tested was inhibited to some degree by actinobolin. These observations suggest that actinobolin may be effective in the treatment of periodontal disease.

The antibiotic, actinobolin, has been reported to be inhibitory for cariogenic streptococci, for mixed bacterial cultures from human dental plaque (3), and to be highly effective in reducing the incidence of dental caries in rats (2). At present, actinobolin is not used in medical practice, is not appreciably absorbed when given orally, is essentially nontoxic (6), and is strongly inhibitory for bacteria under anaerobic conditions (3). These observations suggested that this antibiotic might be useful in the treatment of periodontal disease.

The purpose of this study was to evaluate the in vitro inhibitory activity of actinobolin against microbial cultures obtained from patients with deep periodontal pockets and against bacteria which are thought to be associated with periodontal disease.

MATERIALS AND METHODS

Clinical specimens were obtained from periodontal pockets (>6 mm in depth) of 15 patients, all of whom revealed symptoms of moderate to severe periodontitis. The clinical samples were evenly suspended in 2.5 ml of sterile saline (0.85% NaCl). After sterilizing the saline in an autoclave, it was kept in tightly covered screw-cap tubes until used, to maintain reduced conditions which many oral microorganisms require. Portions (0.15 ml) of the suspensions were rapidly transferred to plates of Brain Heart Infusion Agar (Difcδ; Detroit, Mich.). Sterile glass rods were used to distribute the inoculum over the surface of the plates.

Various concentrations of actinobolin sulfate (lot no. X8061, Parke, Davis and Co., Detroit, Mich.) were dissolved in sterile saline and used to charge filter-paper discs (no. 740-E; Schleicher and Schuell Co., Keene, N. H.). These discs were placed on the surface of the inoculated plates which were incubated anaerobically for 24 hr at 37 C in Torbal jars (model AJ-2; Torsion Balance Co., Clifton, N.J.) containing an atmosphere of 95% nitrogen and 5% carbon dioxide. The diameters of zones of growth inhibition surrounding the discs were used as a parameter to assess the relative antimicrobial activity of actinobolin. Pure bacterial cultures isolated from the clinical specimens and tentatively identified as strains of *Bacteroides melaninogenicus*, *Fusobacterium fusiforme*, *Leptotrichia buccalis*, and *Veillonella parvula* were also tested for sensitivity to actinobolin.

RESULTS AND DISCUSSION

Representative data (Table 1) show the inhibitory activity of actinobolin against mixed microbial cultures obtained from periodontal pockets of patients with clinically diagnosed periodontitis. Every culture tested was inhibited to some degree. We realize that testing mixed cultures for antibiotic sensitivity is contrary to routine clinical laboratory procedures, but it is technically very difficult to identify at the species level the myriad microorganisms involved in periodontal pathosis (7). Additionally, testing mixed cultures in this instance

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appeared to be justified because of the syntrophic relationship that exists in the periodontal disease state, e.g., the stimulation of the growth of *B. melaninogenicus* by other oral microorganisms which produce menadione (5). However, four bacterial species, reported to be associated with periodontal disease, were identified and tested as pure cultures for sensitivity to actinobolin. The data in Table 2 show that cultures of *B. melaninogenicus*, *F. fusiforme*, *L. buccalis*, and *V. parvula*, all of which may have a prominent role in periodontal disease (1), were strongly inhibited by actinobolin. The data in Tables 1 and 2 also reveal a dose-related response since higher concentrations of actinobolin generally produced larger zones of growth inhibition. The marked sensitivity of the strains of *Bacteroides* and *Leptotrichia* to actinobolin precluded an accurate measurement of the zones of growth inhibition. Although the concentrations of actinobolin tested were high, the low level of toxicity of actinobolin and the fact that this antibiotic is poorly absorbed when applied orally (6) indicate that these are therapeutically realistic concentrations readily obtainable in the oral cavity.

TABLE 1. *In vitro inhibitory activity of actinobolin against mixed microbial cultures obtained from periodontal pockets*

Microbial cultures obtained from periodontitis patients	Concn of actinobolin (μg/disc)			
	1,000	500	250	0
1 (CS)	1.7[a]	0	0	0
2 (DB)	2.6	2.0	1.7	0
3 (SW)	2.6	1.9	0	0
4 (TB)	2.7	2.0	0	0
5 (JB)	2.3	2.1	1.7	0
6 (PG)	2.3	1.8	1.6	0
7 (WM)	2.3	1.6	0	0
8 (WG)	2.2	2.0	0	0
9 (PG)	2.2	1.9	0	0
10 (RN)	2.6	NT[b]	NT	NT
11 (TV)	2.5	1.9	0	0
12 (GT)	2.0	0	0	0
13 (LP)	1.8	0	0	0
14 (RL)	2.5	2.1	0	0
15 (AF)	2.7	NT	NT	NT

[a] Numbers indicate diameters (centimeters) of zones of anaerobic growth inhibition surrounding paper discs. Diameter of discs, 1.27 cm.
[b] Not tested.

In a recent review (4), Keyes has listed a variety of antibiotics that have been reported to be of benefit in the control of dental caries or periodontal disease, or both. These antibiotics include bacitracin, erythromycin, lincomycin, penicillin, spiramycin, streptomycin, tetracycline, vancomycin, and virgimycin. Because of the present clinical utility of most of these antibiotics, especially those which are of therapeutic value in severe infections, their application in the control of dentobacterial infections has been questioned. Since actinobolin has a variety of properties which appear to make it highly appropriate for oral application (3), is not presently used in medical practice, and is strongly inhibitory for bacteria associated with periodontal disease, it seems justified to consider clinical trials with this antibiotic for the prevention, treatment, or as an adjunct in the treatment of periodontitis.

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TABLE 2. *In vitro inhibitory activity of actinobolin against bacteria frequently found to be associated with periodontal disease*

Bacteria	Concn of actinobolin (μg/disc)				
	1,000	500	250	100	0
Bacteroides melaninogenicus	>4.0[a]	>4.0	>4.0	NT[b]	0
Fusobacterium fusiforme	3.8	3.6	3.0	2.5	0
Leptotrichia buccalis	>4.0	>4.0	>4.9	>4.0	0
Veillonella parvula	3.7	3.4	3.1	2.5	0

[a] Numbers indicate diameters (centimeters) of zones of anaerobic growth inhibition surrounding paper discs. Diameter of discs, 1.27 cm.
[b] Not tested.

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