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Reduction of Oral Flora with Rifampin in Healthy Volunteers

PETER C. APPELBAUM,[1]* SHEILA K. SPANGLER,[1] CHARLES R. POTTER,[2]† AND FRED R. SATTLER[3]‡

Departments of Pathology (Clinical Microbiology),[1] Surgery (Otorhinolaryngology),[2] and Medicine (Infectious Diseases),[3] Hershey Medical Center, Hershey, Pennsylvania 17033

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The effect of a short course of rifampin on the oral microflora was evaluated in 17 healthy volunteers. Salivary specimens were collected before and after two 600-mg doses of rifampin administered 6 h apart. Salivary bacteria were identified to species, and total quantitative colony counts were determined for each isolate. For all 17 subjects, treatment with rifampin led to a reduction in total bacterial colony counts; the mean inhibitory activity was 85.8% (range, 48.3 to 99.8%). Both aerobic and anaerobic bacteria were inhibited in every case: 45.5 to 99.8% for aerobic bacteria, mean inhibitory activity of 85.8%; 40.9 to 100% inhibition for anaerobic bacteria, mean inhibitory activity of 87.6%. However, total counts were reduced by ≥2 logarithms in only 18% of individuals; for aerobes, in 29%; and for anaerobes, in 41%. All classes of bacteria were inhibited, with mean inhibitory activities ranging from 8.4 to 99.9%. However, only streptococci, *Haemophilus* spp., *Bacteroides* spp., and aerobic and anaerobic gram-positive nonsporeforming rods were reduced in counts close to 2 logarithms after treatment with rifampin. Clinical studies are needed to clarify the significance of these in vitro data and to delineate a possible role for rifampin in preoperative prophylaxis of patients undergoing head and neck cancer surgery.

Postoperative wound infections due to endogenous microflora (3, 7, 14) occur in a considerable proportion of patients undergoing head and neck cancer surgery and are a major cause of morbidity and mortality. Extension of these infections through fascial planes of the neck have resulted in carotid blowout and exsanguination. Experimental studies in animals and humans have shown that a minimal bacterial inoculum of approximately 10^5 organisms per ml had to be introduced into an incisional wound before infection was established (6). In head and neck surgery the wound is bathed with enormous concentrations of salivary microflora throughout the duration of the operation, which may last from 2 to 10 h. Thus, reduction of the inoculum of bacteria in saliva might diminish the incidence of wound infection.

Rifampin has bactericidal activity at low concentrations against virtually all aerobic and anaerobic bacteria present in normal oral flora, and salivary levels of the drug are approximately 90% of peak serum concentrations (5). We, therefore, sought to quantify the effect of rifampin on the salivary fluid microflora in 17 healthy adult volunteers. We selected two 600-mg doses to be given 6 h apart, since erratic or delayed absorption of a single dose might abrogate any potential benefit of the drug.

MATERIALS AND METHODS

Subjects, dosage, and procedures. The study was approved by the Institutional Clinical Investigational Committee of the Pennsylvania State University College of Medicine. Volunteers between the ages of 18 and 75 years were enrolled in the study after written informed consent was obtained. Criteria for exclusion were as follows: history of hypersensitivity to rifampin or of liver disease; pregnancy or lactation; treatment with antibiotics or any investigational drug within 2 weeks prior to the study; presence of signs or symptoms of inflammation of the oral cavity, pharynx, or larynx. At 2:00 p.m. on day 1, after 14 h of fasting, oral salivary samples were collected. Ten hours later, at 12 midnight (day 1), and again at 6:00 a.m. (day 2), subjects were given 600-mg doses of rifampin (Dow Chemical Co., Indianapolis, Ind.). At 2:00 p.m. on day 2, oral salivary fluid samples were again obtained after 10 h of fasting. Saliva specimens were collected without stimulants in volumes of approximately 0.5 ml; no statistical analysis of saliva amounts were performed. To assure adequacy of drug absorption from the gastrointestinal tract, blood was collected 1 and 3 h after the second dose of rifampin and tested for serum inhibitory activity.

Microbiological techniques. All specimens were processed and plated in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) within 5 min of collection. After dispersal by vortexing for 30 s (Vortex Genie Mixer; Scientific Products, McGaw Park, Ill.), serial 10-fold dilutions of salivary specimens were prepared in prereduced Ringer solution (16). All anaerobic plates were prepared aerobically and prereduced in the chamber 2 to 4 h prior to plating. Media were as follows: enriched brucella blood agar (16) (prepared in-house; incubated anaerobically for 2 to 4 days); kanamycin-vancomycin laked blood agar (prepared in-house; incubated anaerobically for 2 to 4 days); FM agar (Nissui Seiyaku Co., Tokyo, Japan; incubated anaerobically for 2 days); cadmium sulfate agar (Remel Laboratories, Lenexa, Kan.; incubated anaerobically for 4 days); veillonella agar (Remel; incubated anaerobically for 2 days); lactobacillus selective agar (Remel; incubated anaerobically for 2 days); brucella blood agar (prepared in-house; incubated in air plus 10% CO_2 for 2 days); mitis salivarius agar (Remel; incubated in air plus 10% CO_2 for 2 days); chocolate bacitracin agar (Remel; incubated in air plus 10% CO_2 for 2 days). Specimens were incubated at 37°C. All organisms were identified to species according to standard procedures (10, 13, 16). Quantitative colony counts were determined by standard plate count techniques, using 0.1-ml samples of specimen dilutions spread on plates. Total counts were performed on nonselective media, and specific counts were done on selective media (16). We defined inhibitory activity as pretreatment CFU − post-treatment CFU/pretreatment

* Corresponding author.
† Present address: Springfield Clinic, Springfield, IL 62708.
‡ Present address: Southern California Permanente Medical Group, Los Angeles, CA 90027.

TABLE 1. Bacterial inhibition (I) by rifampin (R) in specific subjects

Subject	All organisms			Aerobes only			Anaerobes only		
	CFU/ml		I (%)	CFU/ml		I (%)	CFU/ml		I (%)
	Pre-R	Post-R		Pre-R	Post-R		Pre-R	Post-R	
1	5.3×10^8	1.1×10^8	79.3	1.3×10^8	2.1×10^7	83.9	4.0×10^8	9.3×10^7	76.8
2	1.4×10^8	1.0×10^7	92.9	8.1×10^7	6.2×10^6	92.4	5.6×10^7	4.1×10^6	92.7
3	2.6×10^8	6.1×10^7	76.5	5.5×10^7	3.3×10^6	94.0	2.0×10^8	5.8×10^7	71.0
4	8.5×10^6	2.1×10^6	75.3	5.8×10^6	2.0×10^6	65.5	2.7×10^6	8.2×10^4	97.0
5	2.5×10^{10}	1.2×10^9	95.2	2.5×10^{10}	1.0×10^9	96.0	3.5×10^8	1.5×10^8	57.2
6	1.8×10^9	7.3×10^7	95.9	1.0×10^9	1.1×10^7	98.9	7.5×10^8	6.2×10^7	91.7
7	1.0×10^9	1.6×10^8	84.0	5.8×10^8	1.6×10^8	72.4	4.6×10^8	1.0×10^5	99.9
8	4.1×10^8	2.0×10^6	99.5	3.9×10^8	6.7×10^5	99.8	1.5×10^7	1.3×10^6	91.3
9	9.4×10^8	9.5×10^7	89.9	6.7×10^8	9.5×10^7	85.8	2.7×10^8	5.2×10^3	100.0
10	1.2×10^9	6.2×10^8	48.3	1.1×10^9	6.0×10^8	45.5	1.0×10^8	2.5×10^7	75.0
11	1.8×10^9	6.8×10^8	62.2	1.7×10^9	6.1×10^8	64.1	1.1×10^8	6.5×10^7	40.9
12	1.1×10^9	3.1×10^8	71.8	1.1×10^9	3.1×10^8	71.8	4.5×10^7	2.2×10^6	95.1
13	1.2×10^{10}	9.4×10^8	92.2	1.2×10^{10}	9.4×10^8	92.2	7.6×10^8	0	100.0
14	5.9×10^{11}	5.1×10^9	99.1	5.9×10^{11}	5.1×10^9	99.1	8.4×10^8	1.8×10^5	99.9
15	7.2×10^{11}	3.4×10^9	99.5	5.7×10^{11}	3.3×10^9	99.4	1.5×10^{11}	8.0×10^7	99.9
16	6.4×10^{10}	1.3×10^8	99.8	5.3×10^{10}	1.2×10^8	99.8	1.1×10^{10}	5.0×10^6	99.9
17	8.1×10^{11}	2.1×10^{10}	97.4	8.0×10^{11}	2.1×10^{10}	97.4	8.0×10^9	4.0×10^3	99.9

CFU $\times 10^2$. Gram stains were prepared from the initial specimens. Serum inhibitory activity was measured by microtiter technique against *Micrococcus luteus* ATCC 4698 (American Type Culture Collection, Rockville, Md.) according to standard techniques (1).

RESULTS

In all 17 cases, rifampin led to a reduction in total bacterial colony counts, with a mean inhibitory activity of 85.8% (range, 48.3 to 99.8%); both aerobic and anaerobic bacteria were inhibited in every case (aerobes: 45.5 to 99.8% inhibition, mean inhibitory activity of 85.8%; anaerobes: 40.9 to 100%, mean inhibitory activity of 87.6%). However, total bacterial counts were reduced by ≥2 logarithms in only 3 of 17 individuals (18%); for aerobes, in 5 of 17 (29%); and for anaerobes, in 7 of 17 (41%) (Table 1). All classes of organisms were inhibited, with mean inhibitory activities as follows: staphylococci, 65.5%; streptococci, 82.9%; *Neisseria* spp., 68.9%; *Haemophilus* spp., 99.5%; aerobic gram-positive nonsporeforming rods, 99.9%; *Enterobacteriaceae* and oxidase-positive fermenters, 54.8%; *Bacteroides*, *Capnocytophaga*, and *Leptotrichia* spp., 94.5%; *Fusobacterium* spp., 78.6%; anaerobic cocci, 76.4%; clostridia, 8.4%; anaerobic gram-positive nonsporeforming rods, 84.7%. However, only streptococci, *Haemophilus* spp., *Bacteroides* spp., and aerobic and anaerobic gram-positive nonsporeforming rods were reduced in counts close to 2 logarithms following rifampin administration (Table 2).

Commonly encountered organisms, and numbers of subjects positive (in parentheses), were as follows: *Staphylococcus aureus* (9), *Staphylococcus epidermidis* (15), micrococci (12), *Streptococcus mitis* (10), *Streptococcus sanguis* II (10), *Streptococcus salivarius* (15), *Neisseria flavescens* (10), *Neisseria meningitidis* (8), *Neisseria sicca* (6), *Neisseria lactamica* (7), *Haemophilus influenzae* (5), *Haemophilus parainfluenzae* (11), *Corynebacterium* spp. (12), *Bacteroides ureolyticus* (11), *Bacteroides melaninogenicus/intermedius* (10), *Bacteroides oralis* (5), *Bacteroides fragilis* (4), *Capnocytophaga ochracea* (4), *Fusobacterium naviforme* (8), *Fusobacterium nucleatum* (4), *Peptostreptococcus magnus* (10), *Peptostreptococcus anaerobius* (4), *Veillonella parvula* (5), *Streptococcus intermedius* (6).

Blood from all volunteers 1 and 3 h after the second dose of rifampin produced serum inhibitory activity (titers, 1:512 to 1:2,048), which indicated that gastrointestinal absorption was adequate in each subject. There were no clinical adverse drug effects.

DISCUSSION

Results of several studies have indicated that prophylaxis with systemic antibiotics decreased the incidence of postoperative infections in major head and neck cancer surgery when the mucosa of the upper aerodigestive tract was entered through the neck (2, 4, 7–9, 11, 12, 15). However, infection rates in even well-controlled studies have approached 38% in groups given prophylaxis with cephalosporin antibiotics (2). In an attempt to lower this unacceptably high rate, Johnson and co-workers (11) demonstrated that prophylaxis with clindamycin plus gentamicin was superior to cefazolin alone (11 versus 53%; $P < 0.05$). The need to use

TABLE 2. Effect of rifampin on specific organism groups

Organism group	Range of inhibition (%)	Mean inhibitory activity (%)
Staphylococci	0–99.9	65.5
Streptococci	28.0–99.9	82.9
Neisseria spp.	0–100	68.9
Haemophilus spp.	98.0–100	99.5
Aerobic gram-positive nonsporeforming rods	99.9–100	99.9
Enterobacteriaceae + oxidase + fermenters	0–100	54.8
Bacteroides spp.[a]	49.0–100	94.5
Fusobacteria	0–100	78.6
Anaerobic cocci	0–100	76.4
Clostridia	0–16.7	8.4
Anaerobic gram-positive nonsporeforming rods	0–100	84.7

[a] Includes *Capnocytophaga* and *Leptotrichia* species.

potentially toxic antibiotics is worrisome, and a search for more effective and safer prophylactic regimens is, therefore, needed.

In the current study treatment with rifampin led to a demonstrable decrease in total aerobic and anaerobic colony counts in salivary specimens, though counts were reduced by ≥ 2 logarithms in $<50\%$ of cases. In many volunteers $\geq 10^8$ CFU of various bacteria per ml persisted after treatment with rifampin. In experimental models, these concentrations would be more than sufficient to cause incisional infection (6). However, it is unknown whether all or most classes of bacteria in the normal oral flora must be suppressed below "threshold" concentrations to prevent wound infection in clinical circumstances. Contrariwise, there may be even higher concentrations of bacteria in gingival crevices or on the buccal mucosa (not investigated in the current study) to amplify the risk for infection. If this were the case more prolonged treatment, namely, repetitive doses of rifampin for 24 to 48 h, would likely provide greater bacterial suppression.

Clinical studies will be necessary to determine whether a two-dose regimen of rifampin, or more prolonged treatment with this antibiotic, could supplant prophylaxis with potentially toxic parenteral antibiotics or at least augment the effectiveness of prophylaxis with cephalosporin drugs.

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