Effect of Erythromycin and Clindamycin on the Indigenous Human Anaerobic Flora and New Colonization of the Gastrointestinal Tract

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Erythromycin and clindamycin were given orally to ten subjects in recommended doses for seven days in order to study the effects of these antibiotics on human flora. Saliva and faecal specimens were collected for up to 29 days after administration of the antibiotics. Erythromycin caused only minor changes in the saliva flora while the aerobic and anaerobic colon flora were considerably disturbed. Clindamycin depressed both the anaerobic saliva and colon flora. Both erythromycin and clindamycin induced new colonization of the oral cavity and colon. The levels of free volatile fatty acids sank in saliva and faeces when erythromycin and clindamycin were given. The ecological disturbances caused by antibiotics require further investigation and should be taken into consideration in therapy.

The normal flora of the digestive tract seems to act as a factor in resistance to colonization with new bacteria. In animal studies it has been shown that suppression of the anaerobic gut flora facilitates colonization and infection with pathogenic aerobic bacteria (1). Van der Waaij et al. (2) have suggested the term “colonization resistance” for the ability of the anaerobic flora to withstand new colonization of the digestive tract. Apparently, the administration of antibiotics which disturb the normal anaerobic flora may lower the colonization resistance. Erythromycin is a macrolide antibiotic that has proved effective in the therapy of upper respiratory tract infections (3), Campylobacter enteritis, Legionnaires disease (4) and pulmonary infections caused by Mycoplasma pneumoniae (5). It is active against most strains of group A streptococci, pneumococci, staphylococci, Haemophilus influenzae and anaerobic bacteria (6), but is generally not active against enterobacteria. Clindamycin is sometimes used as a penicillin substitute, and is widely used in the treatment of anaerobic infections, especially when Bacteroides fragilis is suspected as infecting organism (7). Clindamycin is also used in the treatment of staphylococcal osteomyelitis (8) and orofacial infections with penicillin-resistant anaerobes (9). Clindamycin has low activity against enterobacteria in vitro. When given orally, both erythromycin and clindamycin are only partially absorbed and high concentrations are found in faeces (10). Since erythromycin and clindamycin are also freely available in saliva (11, 12), they possess the potential to influence sensitive microorganisms of the indigenous flora in the oral cavity and colon. The purpose of the present investigation was to study the effect of orally administered erythromycin and clindamycin on the normal human oral and colon microflora, and the subsequent colonization of the gastrointestinal tract with new bacteria.

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Materials and Methods

Subjects. Twenty healthy volunteers (12 males and 8 females) between 20 and 55 years of age participated in the study. None of the volunteers had a history of gastrointestinal, hepatic or renal disease, or had taken antibiotics during the six months preceding the investigation period. Ten subjects were given 500 mg erythromycin stearate tablets (Abbott, S.p.a., Campoverde L.T., Italy) every 12 h for seven days, and the other ten subjects were given 150 mg clindamycin capsules (Upjohn, Kalamazoo, USA) every 6 h for seven days. The antibiotics were taken orally before the morning meal with 50 ml of water.

Sampling Procedures for Assay of Erythromycin and Clindamycin. Mixed saliva samples were obtained in sterile glass cups. Samples were collected prior to the first antibiotic dose and then every day 90 min (erythromycin group) and 60 min (clindamycin group) after the morning dose. Samples were also collected two days after the drugs had been withdrawn. Stool specimens from the erythromycin group were taken before antibiotic administration daily during administration, two, four and nine days after discontinuing administration. In the clindamycin group stool specimens were taken before antibiotic administration, daily during administration and two, nine and 33 days after discontinuing administration. The specimens were collected in sterile plastic containers, placed in ice chests and usually processed in the laboratory within one hour of collection.

Assay of Erythromycin and Clindamycin. The saliva and faecal concentrations of erythromycin and clindamycin were determined by the agar diffusion method of Safling et al. (13). The test medium was Bacto Penassay Seed Agar (Difco), and the indicator strain Sarcina lutea ATCC 9341. Erythromycin (Astra) and clindamycin (Upjohn Co.) were dissolved in 0.15 M phosphate buffer, pH 7.2, to give concentrations of 256 μg/ml active drug. From these stock solutions, standard solutions were prepared with mixed saliva and faeces samples respectively. The range of the standard series was 0.125–16.0 μg/ml for saliva samples and 1–256 μg/g for faecal samples.

Collection and Processing of Specimens for Microbiological Study. Saliva samples were collected by subjects spitting into sterile glass tubes immediately before taking the initial antibiotic dose and then daily for seven days 90 min (erythromycin group) or 60 min (clindamycin group) after swallowing the drug and again two, four (erythromycin group), nine and 22 days (clindamycin group) after discontinuing the antibiotics. Stool specimens were collected as described above. Saliva samples were suspended in pre-reduced peptone-yeast extract medium, diluted, inoculated on selective media, and treated as described elsewhere (14). The aerobic agar plates were incubated for 24 h at 37 °C and anaerobic plates for 48 h at 37 °C in anaerobic jars (GasPak, BBL, Cockeysville, USA), then examined under a stereo-microscope. A 1 g sample of faeces was homogenized in 9 ml peptone-yeast extract medium. Tenfold serial dilutions were made up to 10⁻⁸. The samples were inoculated and treated as described elsewhere (14). After incubation, total colony counts were made from the anaerobic blood agar plates. Different colony types were enumerated, isolated in pure culture and identified, as were the different colonies appearing on the selective media.

Identification of Microorganisms. Aerobic and facultative anaerobic bacteria were identified biochemically as described elsewhere (14). Anaerobic bacteria were identified by biochemical tests and gas-liquid chromatography (15).

Antibiotic Susceptibility Tests. The minimum inhibitory concentrations (MIC) of erythromycin and clindamycin for all colonizing bacterial strains isolated were determined by the agar dilution method (16, 17).

Gas Chromatographic Analysis of Volatile Fatty Acids in Saliva and Faeces. Mixed saliva samples and faeces samples were prepared for gas chromatographic analysis by the following procedure. The samples were homogenized in glass mortars, centrifuged at 3,000 g for 15 min, and the supernatants sucked off for gas chromatographic analysis. Four μl of the saliva samples and 2 μl of the faecal samples respectively were injected. A Varian gas chromatograph 1400 (Palo Alto, California, USA) with a hydrogen flame ionization detector was used. The operation conditions were: injector port temperature 195 °C, column temperature 135 °C, detector temperature 195 °C and nitrogen/oxygen carrier gas 40 mg/min. The glass column (3 feet long and 1/8 inch in diameter) contained Carbowax 20M, 0.5 % H₃PO₄ on 60/80 Carbopack B (Supelco, Bellefonte, Pennsylvania, USA).

Cytotoxicity Assays. Cytotoxicity assays of tissue cultures were performed according to the method of Bartlett et al. (18).

Results

Concentrations of Erythromycin and Clindamycin in Saliva and Faeces

The concentrations of erythromycin and clindamycin in mixed saliva varied from 0–1.8 μg/ml and 0–1.7 μg/ml respectively. In faeces high concentrations of erythromycin and clindamycin were detected in the interval between first day of administration until two days after the antibiotic had been withdrawn (Table 1).