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Comparative Effect of Tetracycline and Doxycycline on the Occurrence of Resistant *Escherichia coli* in the Fecal Flora

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Antibiotic-induced changes in the fecal microflora after oral administration of tetracycline hydrochloride and doxycycline for 8 to 10 days were compared. A significant difference was noted in the concentrations of *Escherichia coli* resistant to tetracyclines. With tetracycline hydrochloride, there was a mean increase of approximately 10^4 resistant strains per g compared to only 10^1/g for doxycycline. This difference is ascribed to reduced intestinal concentrations of bioactive drug with recommended oral dosage for doxycycline.

Tetracyclines have enjoyed extensive use as broad spectrum antimicrobials for oral treatment of many common infections. Two important side effects are diarrhea and suprainfection. The activity of these agents against the major microbial components of intestinal tract has tempted speculation that changes in the flora are responsible for diarrhea. A similar mechanism may contribute to suprainfection since the bowel serves as a potential source of resistant organisms.

Doxycycline is a relatively new entry in the tetracycline class. Excellent oral absorption and protracted half-life permit significantly lower dosage to achieve blood levels comparable to those obtained with the parent compound. This raises the possibility that doxycycline may induce fewer changes in the intestinal flora. The purpose of this study was to compare the effects of oral doxycycline and tetracycline on the fecal microflora.

MATERIALS AND METHODS

Subjects. Thirty healthy adult volunteers were randomly divided to receive either doxycycline or tetracycline. No subject had been treated with antimicrobials for 1 month previously. Other antibacterial agents were not administered concomitantly during the study. The 15 volunteers given doxycycline received 200 mg of this agent the first day, followed by 100 mg in a single daily dose. The second group of 15 subjects received tetracycline hydrochloride, 1 g per day in four divided doses. Each subject submitted a fresh stool sample prior to antimicrobial administration. A second specimen was submitted after 8 to 10 days of treatment. The test agent was discontinued after the second stool was submitted.

Bacteriological studies. Fresh stools were obtained in sterile cartons and transferred immediately after collection into the anaerobic chamber for processing. Each specimen (100 mg) was suspended in 9.9 ml of thioglycolate broth. Serial 100-fold dilutions were prepared to give final concentrations of 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8}. One-tenth milliliter of each dilution was dispersed with a glass spreader on the following agar plates: 5% sheep blood, mannitol-salt, mycosel, sheep blood with neomycin (10 μg/ml), MacConkey agar, MacConkey agar containing doxycycline (10 μg/ml), and MacConkey agar containing tetracycline (10 μg/ml). The blood agar plates were incubated in 5% CO_2, and the other plates were incubated in air. In addition to these aerobic media, prereduced brucella-base menadione blood agar was plated and incubated in the anaerobic chamber containing 85% nitrogen, 10% CO_2, and 5% H_2.

Aerobic isolates in concentrations $>10^3$/g were enumerated, isolated, and identified according to conventional classification schemes. No attempt was made to enumerate isolates on plates showing confluent growth. Total anaerobic counts were determined by the difference in total bacterial populations on anaerobic plates (brucella-base menadione blood agar) and aerobic plates (sheep blood agar). Anaerobic isolates were not identified. However, the predominant bacteria on anaerobic media were tested for aerotolerance and Gram stained to confirm that they were obligate anaerobes. *Escherichia coli* resistant to doxycycline and tetracycline were enumerated on the MacConkey plates incorporating these agents.

RESULTS

Overall results showed that neither tetracycline hydrochloride nor doxycycline had a major impact on the total populations of aerobic or anaerobic bacteria when measured after 8 to 10 days of administration (Table 1). Nine subjects acquired new aerobic strains during therapy. In the group receiving tetracycline hydrochloride, these were *Candida albicans* (three subjects),

TABLE 1. *Effect of oral tetracycline and doxycyline on fecal flora*

Determinants[a]	Pretreatment specimens	Post-treatment specimens
Tetracycline treatment		
Total anaerobic counts	11.00 ± 0.14[b]	10.88 ± 0.10
Total aerobic counts	8.28 ± 0.42	8.02 ± 0.76
E. coli	6.95 ± 0.52	6.43 ± 1.62
E. coli-resistant tetracycline[c]	3.01 ± 1.34	6.35 ± 1.58
E. coli-resistant doxycyline[c]	2.25 ± 1.30	6.27 ± 1.56
Doxycycline treatment		
Total anaerobic counts	10.95 ± 0.10	10.81 ± 0.10
Total aerobic counts	8.50 ± 0.60	7.48 ± 0.80
E. coli	7.43 ± 0.82	5.48 ± 1.34
E. coli-resistant tetracycline[c]	4.30 ± 1.92	4.97 ± 1.58
E. coli-resistant doxycycline[c]	3.83 ± 1.80	4.86 ± 1.58

[a] Fifteen subjects used in each treatment.
[b] Mean $\log_{10}$ per gram ± 2 standard error of the mean.
[c] Six subjects had no detectable resistant *E. coli* in the pretreatment specimen (three in each treatment group).

enterococci (two), and *Citrobacter freundii* (one). With doxycycline, newly acquired strains were *C. albicans* (three), enterococci (one), and *Staphylococcus aureus* (one). The mean population levels of *C. albicans* among the six subjects who became colonized during treatment was $10^{3.9}$/g. Levels of *Candida* did not change significantly among three subjects in whom this organism was detected in the pretreatment stool.

It is noteworthy that there were no instances in which *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Proteus*, or *Serratia* were acquired during treatment. Further, there were no instances in which the counts of these organisms increased more than 2 logs/g among subjects initially colonized. Thus, the principal change in terms of ingrowth of new aerobic species was the acquisition of *C. albicans*, and this effect occurred with equal frequency in the tetracycline and doxycycline groups. It should be noted, however, that the techniques used in this study did not permit detection of species changes nor emergence of resistant strains for anaerobic bacteria.

The major finding in this study was the emergence of resistant *E. coli* strains (Fig. 1). There was a significant increase in resistant *E. coli* recovered in 10 subjects receiving tetracycline compared to a significant increase in only three subjects receiving doxycycline. Among these 13 individuals, six had no detectable resistant *E. coli* in the pretreatment specimen whereas seven had a 2-log or greater increase over pretreatment counts. The data were further analyzed by comparing the difference in resistant *E. coli* between pre- and post-treatment stools for all subjects receiving either tetracycline or doxycycline. For the 15 subjects receiving tetracycline, the mean increase in doxycycline-resistant *E. coli* was $10^{4.021}$/g and for tetracycline-resistant strains it was $10^{3.34}$/g. By contrast, the increase in resistant strains during treatment with doxycycline was only

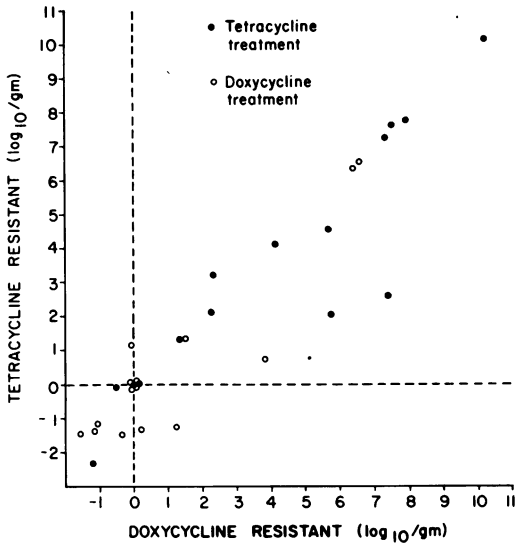

FIG. 1. *Comparative effect of orally administered tetracycline and doxycycline on the occurrence of resistant E. coli in the fecal flora. Results are expressed as the increase in E. coli strains ($\log_{10}$ per gram of feces) resistant to doxycycline (abscissa) and tetracycline (ordinate). Resistance to the two antimicrobials is parallel and shows no interaction (coefficient of correlation is 0.927). It is noted that the major changes in resistant strains are associated with tetracycline treatment.*

$10^{1.03}$/g and $10^{0.67}$/g for doxycycline and tetracycline, respectively.

According to an analysis of variance (bacterial counts times treatment times media), the increase of resistant *E. coli* was significantly greater with tetracycline treatment compared to that for doxycycline ($F = 6.49$; degrees of freedom = 1,28; $P < 0.05$). This conclusion can be further examined by dichotomous testing. Fisher's exact test was used to compare the frequency of growth above (or below) the median. Tetracycline produced 11 above median growths and doxycycline produced four. Again, this analysis confirmed that the difference between the two agents is significant ($P < 0.02$).

DISCUSSION

Tetracycline-induced alterations in bowel flora have been studied extensively with variable results. Dearing and co-workers noted a significant reduction in anaerobes, coliforms, and streptococci after chlortetracycline or oxytetracycline (2, 3). In concert with these changes, there was an ingrowth of resistant organisms not generally found in stool, principally *Candida*, *S. aureus*, and *Pseudomonas*. A similar observation was reported with tetracycline hydrochloride by Hinton (6). Other investigators, however, have noted minimal alterations of the normal flora with this class of antimicrobials (1, 5). The results of our studies are most consistent with the latter reports. There were no significant changes in total populations of anaerobic and aerobic bacteria with either tetracycline or doxycycline. Ingrowth of *C. albicans* was noted in six subjects, but other changes in the aerobic constituents were seldom encountered.

The major finding in this study concerned the susceptibility of intestinal *E. coli* to the agents tested. *E. coli* were recovered from all pre- and post-treatment specimens and their mean concentrations did not change significantly with either antimicrobial. The relative proportion of resistant strains, however, increased with tetracycline administration and this effect was significantly greater than with doxycycline. Similar changes after oral tetracycline have been previously reported by Hirsh et al. (7). These investigators noted that seven of eight subjects shed tetracycline-resistant *E. coli* during ingestion of this antimicrobial, and that five subjects continued to harbor large populations of resistant strains at least 28 days after therapy was discontinued.

The failure of doxycycline to produce a comparable effect is unknown. Perhaps the best explanation concerns the relative doses and pharmacokinetics of the two agents. Previous studies indicate that 100 mg of doxycycline is therapeutically equivalent to a daily dose of 1 g of tetracycline hydrochloride (4). Therefore, the recommended oral dosage of doxycycline is only one-tenth that of tetracycline. Gastrointestinal absorption rates with oral administration are 50 to 70% for tetracycline compared to 90 to 95% for doxycycline (4, 8). Thus, in terms of unabsorbed antimicrobial, it is anticipated that the levels of tetracycline in the intestinal tract would greatly exceed those with doxycycline. Concerning excretion, isotope dilution studies have shown that intestinal secretion is a major disposition route for doxycycline (9). In contrast, tetracycline is primarily excreted via the urinary tract (8). But the intestinal form of excreted doxycycline is principally a bound compound which is biologically inactive (9). It is postulated that the net effect of these observations is reduced antimicrobial pressure to induce resistance in the fecal flora.

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