Effect of Vancomycin on Intestinal Flora of Patients Who Previously Received Antimicrobial Therapy

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To evaluate the ecological disturbances of peroral vancomycin administration following cephalosporin administration, 20 healthy volunteers received cefuroxime axetil tablets (250 mg) perorally twice a day for 1 week, and 10 of these volunteers subsequently received vancomycin capsules (125 mg) perorally four times daily for 7 days. The concentration of vancomycin in feces after 1 week of vancomycin administration was high (mean ± SD, 520 ± 197 mg/kg), which correlated with the ecological disturbances noted in the vancomycin recipients. Vancomycin administration resulted in a rapid decrease in the numbers of intestinal Enterococcus faecium, Enterococcus faecalis, and Enterococcus durans (P ≤ .05), while there was a significant emergence of motile enterococci with decreased susceptibility to vancomycin (Enterococcus gallinarum and Enterococcus casseliflavus; minimum inhibitory concentration, 4–16 mg/L) (P ≤ .01). Because of vancomycin administration, there was also a significant overgrowth of vancomycin-resistant Pediococcus species and lactobacilli as well as of Klebsiella species, Citrobacter species, and Enterobacter species (P ≤ .01). The numbers of bifidobacteria and Bacteroides species were significantly reduced during vancomycin administration. None of the enterococcal strains carried vanA or vanB. Twenty-two of the 27 motile enterococci carried the vanC-1 gene specific for E. gallinarum, whereas five strains carried the vanC-2(C-3) gene, thus implicating that they were E. casseliflavus or Enterococcus flavescens.

Enterococci have emerged as important human pathogens and are now common causes of nosocomial infections [1]. Most enterococcal infections are thought to arise from the patient’s own intestinal microflora, but resistant enterococci have also been shown to be easily spread among patients and medical staff. Several studies have shown a correlation between the administration of antimicrobial agents and the emergence of drug-resistant bacteria in the normal intestinal microflora. Since vancomycin is poorly absorbed, it is possible that its use may result in ecological disturbances in the intestinal microflora.

The aims of the present study were primarily to determine whether the use of oral vancomycin is associated with an increased frequency of vancomycin-resistant gram-positive cocci and secondarily to determine fecal concentrations of vancomycin and correlate the levels with quantitative and qualitative alterations of the intestinal microflora.

Materials and Methods

Subjects and drug administration. Twenty healthy volunteers, who had not been treated with any antimicrobial agents during the previous 3 months, were enrolled in the study. No concomitant treatment with other medication was allowed during the study period. The study was approved by the Local Ethics Committee of Huddinge University Hospital, Karolinska Institute, Stockholm. All subjects received cefuroxime axetil tablets (250 mg) perorally b.i.d. for 7 days. On the seventh day, the subjects were randomized to receive either no therapy (10 subjects, five women and five men [mean age, 37.4 years; range, 23–54 years]) or vancomycin capsules (125 mg) perorally q.i.d. for 7 days (10 subjects, five women and five men [mean age, 38.3 years; range, 22–52 years]).

Sampling procedures. Fecal samples from all subjects were collected in sterile plastic containers before cefuroxime axetil administration (day 0) and on days 7, 10, 14, 21, and 28. All samples were transported to the microbiological laboratory within 2 hours and stored at −70°C until assays were performed.

Assay of vancomycin. The fecal concentration of vancomycin was determined by the agar diffusion method. The test medium was dextrose sensitivity agar, and the indicator strain was Bacillus subtilis ATCC (American Type Culture Collection) 6633.

Microbiological analyses. After completion of the study, fecal samples were qualitatively and quantitatively analyzed as previously described [2].

Selective culture and biochemical identification of gram-positive cocci. The fecal samples were screened for vancomycin-resistant enterococci by plating all stool samples onto enterococcus agar supplemented with 5 mg of vancomycin/L. All catalase-negative gram-positive cocci were identified according to the method of Facklam and Sahm [3].
Antibiotic susceptibility tests. MICs of vancomycin were determined for all isolated strains by the agar dilution method with use of Antibiotic Sensitive Medium II (ABBIODISK, Stockholm). According to the National Committee for Clinical Laboratory Standards [4], susceptibility to vancomycin was defined as an MIC of $\leq 4$ mg/L, and resistance was defined as an MIC of $>32$ mg/L.

Detection of vanA, vanB, vanC-1, and vanC-2(C-3) genotypes by PCR analysis. All strains classified as enterococci that had decreased susceptibility to vancomycin (MIC, $\geq 2$ mg/L) were probed for the presence of the glycopeptide resistance genotypes vanA, vanB, vanC-1, and vanC-2(C-3) by PCR analysis as described by Dutka-Malen et al. [5].

Statistical analyses. The incidence of vancomycin resistance was compared between groups by using Fisher’s exact test. Analysis of variance with a repeated-measures design was used to estimate the significance of the two factors treatment and time. The results of quantitative stool cultures were further compared within treatment groups and between treatment groups by using the Wilcoxon signed-rank test and the Mann-Whitney U test, respectively.

Results

Concentration of vancomycin in feces. The mean fecal concentrations of vancomycin in feces $\pm$ SD on days 10 and 14 were 523 $\pm$ 157 mg/kg and 520 $\pm$ 197 mg/kg, respectively. One subject still had detectable levels (135 mg/kg) 1 week after discontinuation of drug administration.

Biochemical identification of gram-positive cocci. Sixty-five of 103 different catalase-negative gram-positive cocci that were isolated from the screening agar plates were identified as Enterococcus species. According to the ability of the strains to form acid from the carbohydrates, 32 were identified as Enterococcus faecium (MIC, $<0.25$–2 mg/L); 4, as Enterococcus faecalis (MIC, 1–2 mg/L); and 2, as Enterococcus durans (MIC, 0.5–2 mg/L); of 27 strains for which the motility test was positive, 22 were identified as Enterococcus gallinarum, and five pigmented strains were identified as Enterococcus casseliflavus (MIC$_{50/90}$ for the motile enterococci, 8/8 mg/L; range, 4–16 mg/L). Thirty-four strains were identified as Pediococcus species (MIC$_{50/90}$, $>256$ mg/L; range, 4 to $>256$ mg/L).

Impact of cefuroxime axetil on the intestinal microflora. Administration of cefuroxime axetil significantly increased the numbers of $E$. faecium, $E$. faecalis, and $E$. durans on day 7 as compared with the numbers before treatment ($P \leq .01$); only two subjects became colonized by $E$. gallinarum and Pediococcus species after the administration period. The numbers of Escherichia coli were moderately depressed by cefuroxime axetil administration, and five subjects were colonized by Klebsiella species, Citrobacter species, or Enterobacter species on at least one sampling occasion. The numbers of lactobacilli and bifidobacteria in the anaerobic microflora were significantly reduced on day 7 ($P \leq .01$).

Impact of vancomycin on the intestinal microflora. Administration of vancomycin following cephalosporin administration resulted in a rapid decrease in the numbers of $E$. faecium, $E$. faecalis, and $E$. durans ($P < .05$). Vancomycin administration strongly promoted emergence of motile enterococci with decreased susceptibility to vancomycin; eight of 10 subjects were colonized with high numbers of $E$. gallinarum or $E$. casseliflavus on day 21 ($P < .01$). During and after vancomycin administration, seven of 10 subjects became colonized with high numbers of vancomycin-resistant Pediococcus species. The numbers of $E$. coli were not affected by vancomycin administration, whereas there was a significant overgrowth of Klebsiella species, Citrobacter species, and Enterobacter species ($P < .01$) (figure 1). A significant overgrowth of lactobacilli in the anaerobic intestinal microflora was noticed in the week following vancomycin administration ($P < .01$). Bacteroides strains were strongly suppressed or eliminated during vancomycin administration ($P < .01$), but the numbers of these organisms increased to pretreatment levels in eight of 10 volunteers on day 28 (figure 2).

Presence of vancomycin resistance genotypes in enterococci with decreased susceptibility to vancomycin. Twenty-seven motile enterococcal strains (MIC, 4–16 mg/L) and two $E$. faecium strains (MIC, 2 mg/L) were analyzed for the presence of vanA, vanB, vanC-1, and vanC-2(C-3) genes by PCR analysis. None of the strains carried vanA or vanB. Twenty-two of the 27 motile enterococci carried the vanC-1 gene specific for $E$. gallinarum, whereas five strains (which were also pigmented) carried the vanC-2(C-3) gene, thus implicating that they were $E$. casseliflavus or Enterococcus flavescens.

Discussion

The microbiological findings of the present study are in accordance with a recent study by Van der Auwera et al. [6] who investigated the influence of peroral vancomycin on the fecal flora of six volunteers. In this study, an $E$. faecium strain highly resistant to vancomycin was recovered from one subject. The occurrence of infections caused by vancomycin-resistant enterococci thus far in Sweden is uncommon compared with that in many other European countries and the United States [7]. Several studies have reported that besides length of hospital stay, treatment with antimicrobial agents—especially cephalosporins and glycopeptides—predisposes patients for infections with vancomycin-resistant enterococci [8].

The normal microflora of the gastrointestinal tract constitutes a large reservoir of genes that code for resistance potentially transferable to pathogenic microorganisms. The vanC gene (which expresses low levels of vancomycin resistance) will probably not be transferable to other microorganisms since the vanC type of resistance is an intrinsic property of motile enterococci, unlike the vanA and vanB types of resistance (which are carried by transposons and easily disseminated to other bacteria) [9].
In conclusion, the results of the present study show that oral administration of vancomycin to healthy volunteers leads to high intestinal concentrations of vancomycin and to significant emergence of intrinsically vancomycin-resistant gram-positive microorganisms (such as *E. gallinarum*, *E. casseliiflavus*, *Pediococcus* species, and lactobacilli) in the normal intestinal microflora.

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References