Effect of a Selective Decontamination of the Digestive Tract Regimen Including Parenteral Cefepime on Establishment of Intestinal Colonization with Vancomycin-Resistant Enterococcus spp. and Klebsiella pneumoniae in Mice

David L. Paterson,1 Usha Stiefel,2,3 and Curtis J. Donskey2,3*

Division of Infectious Diseases, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania;1 Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio;2 Infectious Diseases Section, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio3

Received 16 January 2006/Returned for modification 15 March 2006/Accepted 12 April 2006

In mice, a selective decontamination of the digestive tract regimen consisting of orogastric tobramycin, polymyxin E, and amphotericin B in combination with subcutaneous cefepime inhibited gram-negative bacilli, including Klebsiella pneumoniae, and did not promote vancomycin-resistant Enterococcus spp. (VRE) colonization. However, concurrent administration of subcutaneous ampicillin-sulbactam resulted in promotion of VRE.

Selective decontamination of the digestive tract (SDD) is an infection prophylaxis strategy used in critically ill patients that is intended to inhibit colonization by aerobic gram-negative bacilli and fungi while preserving the anaerobic microflora (1, 4). SDD regimens typically include topical oropharyngeal and enteral nonabsorbed antimicrobials (usually tobramycin, polymyxin E, and amphotericin B) in combination with parenteral antibiotics (usually cefotaxime) for the first few days (1). In a recent prospective study conducted in The Netherlands, SDD prophylaxis was associated with significant reductions in mortality, length of stay, and overall antibiotic costs (1). Although these results are promising, there is concern that SDD regimens could promote colonization by gram-positive pathogens such as vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococcus aureus in intensive care units where these pathogens are endemic. In fact, the cefotaxime component of SDD regimens has been shown to promote overgrowth of indigenous enterococci in the intestinal tract (11, 14), and we have shown that this agent promotes overgrowth of VRE in mice (our unpublished data). In anticipation of an upcoming clinical trial of SDD in our organ transplant intensive care unit, we used a mouse model to examine the effect of SDD on the establishment of intestinal colonization by VRE and Klebsiella pneumoniae. Cefepime was substituted for cefotaxime as the systemic agent because it is minimally excreted into bile, causes minimal disruption of the anaerobic intestinal microflora of mice and humans, and does not promote VRE colonization in mice (4).

The isolates used were VRE strain C68, a clinical VanB isolate, and K. pneumoniae strain P62, a bloodstream isolate that produces an SHV extended-spectrum beta-lactamase (4, 7). The test strains have been used in several previous mouse studies and persistently colonize the intestinal tract when antibiotic selective pressure is maintained (2, 4). A VanB-type VRE isolate was selected for our prior mouse studies because these were the predominant VRE strains present in Cleveland area hospitals in the mid-1990s (2). The effects of antibiotics on the density and persistence of VRE colonization in mice have been shown to correlate well with findings in colonized patients (3). The broth dilution MICs for C68 and P62, respectively, were as follows: tobramycin, >500 and 32 μg/ml; colistin, >10,000 and 0.125 μg/ml; cefepime, >10,000 and 0.75 μg/ml; cefotaxime, >10,000 and 4 μg/ml; and ampicillin, 256 and >256 μg/ml.

The experimental protocol was approved by the Cleveland Veterans Affairs Medical Center’s Animal Care Committee. Female CF1 mice (Harlan Sprague-Dawley, Indianapoli, Ind.) weighing 25 to 30 g were housed individually. Initial experiments were performed to assess the effect of SDD on the indigenous intestinal microflora (n = 4 mice per group). The SDD regimen included tobramycin, polymyxin E, and amphotericin B given by orogastric gavage once daily for 5 days and cefepime given subcutaneously once daily for the first 2 days. Tobramycin and polymyxin E were given at three times the usual human dose (in milligrams per kilogram of body weight) and amphotericin and cefepime were given at the usual human dose (0.42, 0.51, 0.86, and 2 mg/day for each drug, respectively); Speekenbrink et al. (11) found that this dosage adjustment for tobramycin and polymyxin E was necessary to maintain inhibition of facultative gram-negative bacilli in mice. Fresh stool samples were diluted in saline and plated onto Enterococcus agar, MacConkey agar, brucella agar, and Bacteroides bile-esculin agar (Becton Dickinson) to measure concentrations of enterococci, total and facultative gram-negative bacilli, total anaerobes, and Bacteroides spp., respectively. Cultures of total anaerobes and Bacteroides spp. were performed inside an anaerobic chamber (Coy Laboratories). The concentrations of antibiotics in stool were determined by an agar diffusion assay with Escherichia coli as the indicator strain (10). The lower limits of detection were ~2.5 log_{10} CFU/g of stool. A second set of experiments was performed to examine the
The treatment groups included saline controls, SDD for 5 days, SDD for 5 days plus ampicillin-sulbactam subcutaneous daily for 2 days prior to beginning SDD, and ampicillin-sulbactam subcutaneous for 2 days. Ampicillin-sulbactam was included because this is the standard perioperative prophylaxis given in our liver transplant population. Mice received orogastric inoculation of 10,000 CFU of VRE C68 and *K. pneumoniae* P62 on day 2 of the SDD regimens. The organisms were suspended in 0.5 ml of phosphate-buffered saline and administered using a stainless steel feeding tube (Perfektum; Popper & Sons, New Hyde Park, N.Y.). The densities of VRE and ceftazidime-resistant gram-negative bacilli in stool were measured at baseline and on days 1, 3, and 6 after orogastric gavage as previously described (7). Two runs of experiments with four mice per group were performed (i.e., eight total mice/group).

Because higher doses of tobramycin have been shown to disrupt the anaerobic microflora (9), a third set of experiments was performed to assess the effect of higher doses of SDD antibiotics on establishment of VRE colonization (*n* = 4 mice per group). The treatment groups included saline controls, orogastric tobramycin plus polymyxin E daily with both agents at 12 times the usual human dose (1.68 and 2.04 mg/day, respectively), and subcutaneous ceftazidime at 12 times the usual human dose (24 mg/day). The adjustment to 12 times the usual human dose is based on human equivalent doses calculated by the technique of Freireich et al. (5); human equivalent doses are administered to mice when similar serum levels of drugs are desired. The treatments were continued for 5 days, and mice received orogastric inoculation of 10,000 CFU of VRE C68 on day 2 of treatment.

Because cefotaxime has been used in most previous SDD studies, we performed a final set of experiments to confirm our previous unpublished observation that this agent may promote VRE colonization to a greater extent than ceftazidime. Mice (*n* = 6 per group) received saline, ceftazidime (2 or 24 mg/day), and cefotaxime (3 or 36 mg/day) for 5 days and received orogastric inoculation of 10,000 CFU of VRE C68 on day 2 of treatment.

Data analyses were performed with the use of Stata software (version 6.0; Stata, College Station, Tex.). A one-way analysis of variance was performed to compare the groups with *P* values adjusted for multiple comparisons using the Scheffe correction. For the assessment of the effect of SDD on the intestinal microflora, Student’s *t* test was used to compare mean densities during treatment with baseline densities.

Figure 1 shows the effect of the SDD regimen on the stool microflora. Densities of total anaerobes and *Bacteroides* spp., facultative gram-negative bacilli, and *Enterococcus* spp. in the stool of mice. Stool samples were collected and plated onto selective media to determine bacterial densities. If organisms were not detected in stool, the lower limit of detection (−2.5 log_{10} CFU/g) was assigned. Error bars represent standard errors.
levels 2 days after discontinuation of treatment (i.e., day 6 in Fig. 1). The level of enterococci was significantly reduced on day 3 in comparison to pretreatment levels (P = 0.005). The mean concentration of tobramycin in stool was 125 µg/g, whereas cefepime was not detectable (limit of detection, 1 µg/g). Polymyxin E could not be measured using the bioassay because it did not diffuse into agar (i.e., no zones were apparent in the control wells containing known concentrations of antibiotics).

Figure 2 shows the effect of the treatment regimens on the establishment of colonization with VRE (Fig. 2A) and K. pneumoniae (Fig. 2B). At baseline, the mice did not have detectable VRE or ceftazidime-resistant gram-negative bacilli in stool. Ampicillin-sulbactam promoted overgrowth of VRE and K. pneumoniae (P of <0.001 in comparison to saline controls); the addition of the SDD regimen inhibited ampicillin-sulbactam-associated overgrowth of K. pneumoniae (P of 0.999 in comparison to saline controls). The SDD regimen and the cefepime component of the regimen did not promote overgrowth of either pathogen (P of >0.94 in comparison to saline controls).

Figure 3 shows the effect of the higher doses of SDD antibiotics on the establishment of colonization with VRE. High-dose tobramycin plus polymyxin E promoted overgrowth of VRE (P of 0.046 in comparison to saline controls), whereas high-dose cefepime did not (P = 0.99).

Figure 4 shows the effect of cefotaxime versus cefepime on the establishment of colonization with VRE. Cefotaxime promoted overgrowth of VRE in comparison to saline controls (P of 0.043 for the lower dose and P of <0.001 for the higher dose), whereas cefepime at either dose did not (P > 0.88).

In summary, we found that the SDD regimen including parenteral cefepime inhibited gram-negative bacilli but did not suppress total anaerobes or Bacteroides spp. and did not promote VRE colonization. In contrast to cefotaxime, cefepime monotherapy did not promote the establishment of VRE colonization even when administered at 12 times the usual human dose. The absence of promotion of VRE by cefepime is likely to be in part related to the fact that minimal amounts of this antibiotic are excreted into the bile of mice or humans in comparison to amounts associated with other extended-spectrum cephalosporins (i.e., 0.16% of subcutaneously administered cefepime was estimated to be excreted into the intestinal tracts of mice versus 3% of ceftazidime and 11.1% of ceftriaxone) (4, 13). Our findings suggest that substitution of cefepime for cefotaxime in SDD regimens could offer the benefit of causing less alteration of colonization resistance. However, ampicillin-sulbactam treatment for 2 days (i.e., the standard perioperative prophylaxis used in our liver transplant patients) promoted overgrowth of VRE with or without concurrent SDD. Others have similarly observed that the benefit of SDD regimens may be reduced if antibiotics that disrupt the anaerobic microflora are given concurrently (6, 8).

Our findings have important implications for future clinical studies of SDD in our institution and in other U.S. hospitals with high rates of VRE. One strategy to prevent the overgrowth of gram-positive pathogens in our transplant patients would be to alter the regimen used for perioperative prophylaxis. Alternatively, an oral nonabsorbed agent with activity against gram-positive pathogens could be added to the SDD regimen. However, this strategy could result in nonsuppressive decontamination because agents such as ramoplanin and bacitracin may disturb the anaerobic microflora and promote overgrowth of gram-negative bacilli (12; also unpublished data). Finally, some caution should be indicated for clinical trials of SDD in the United States, because the higher doses of tobramycin plus polymyxin E did promote VRE colonization in mice.

This work was supported by a grant from Elan Pharmaceutical and an Advanced Research Career Development Award from the Department of Veterans Affairs to C.J.D.

REFERENCES


