CONCISE COMMUNICATION

Effect of Parenteral Antibiotic Administration on the Establishment of Colonization with Vancomycin-Resistant Enterococcus faecium in the Mouse Gastrointestinal Tract

Curtis J. Donskey, Jennifer A. Hanrahan, Rebecca A. Hutton, and Louis B. Rice

A mouse model of intestinal colonization with vancomycin-resistant enterococci (VRE) was used to study the effect of different β-lactam antibiotics on establishment of VRE colonization. A clinical VanB VRE isolate, Enterococcus faecium C68 (10⁶ or 10⁴ cfu), was inoculated by gastric gavage in conjunction with subcutaneous administration of antibiotics. The MIC of ceftriaxone and ticarcillin against VRE strain C68 is >10,000 µg/mL, and the MIC of piperacillin is 1250 µg/mL. Ceftriaxone and ticarcillin-clavulanate treatment groups developed persistently high levels of stool VRE compared with both the saline and the piperacillin-tazobactam (Pip-Taz) groups (P < .008). The level of stool VRE in the Pip-Taz group did not differ from that for the saline group. Thus, in this mouse model, β-lactam antibiotics with minimal anti-anaerobic activity promoted establishment of high-level VRE colonization, but Pip-Taz (a β-lactam antibiotic with more potent activity against VRE) did not.

We have used a mouse model to study the effect of antibiotics on intestinal colonization by vancomycin-resistant enterococci (VRE). After establishment of high-level VRE stool colonization in mice, antibiotics with potent anti-anaerobic activity (including piperacillin-tazobactam [Pip-Taz]) promoted persistent high-level colonization, while antibiotics with less potent activity against anaerobes (including cefepime and ceftriaxone [Ctri]) promoted VRE to a lesser degree or not at all [1]. These findings suggest that anti-anaerobic antibiotics are associated with VRE in clinical studies because they promote persistent high-density intestinal colonization.

Our findings in mice differ from 2 observations from clinical studies: (1) Pip-Taz and ampicillin-sulbactam have potent anti-anaerobic activity, but substitution of these antibiotics for third-generation cephalosporins has been associated with a decrease in the prevalence of VRE colonization [2], and (2) exposure to third-generation cephalosporins with minimal in vitro activity against anaerobes (e.g., ceftazidime) has been associated with VRE colonization or infection [3]. One possible explanation for these discrepancies is that antibiotics may inhibit or promote the establishment of colonization yet exert the opposite effect after high-level colonization has been established.

In humans, Pip achieves levels in bile (>1000 µg/mL [4]) that are above the MICs of many VRE strains [5]. In clinical settings, patients may intermittently ingest small inocula of VRE. We hypothesized that Pip-Taz is associated with a lower prevalence of VRE colonization because it inhibits small inocula of VRE in the upper intestinal tract, whereas broad-spectrum, β-lactam antibiotics with negligible activity against enterococci promote the establishment of VRE colonization in this setting. We examined the effect of β-lactam antibiotics with different levels of anti-enterococcal activity on the establishment of VRE stool colonization in mice.

Materials and Methods

The colonizing VRE strain. We used Enterococcus faecium C68, a previously described [1, 6] clinical VanB VRE isolate, to colonize study animals.

Quantification of stool organisms. Fresh stool specimens were processed as described elsewhere [1]. For days 3–16, when no VRE were detectable on initial screening, a larger aliquot (100 µL) was plated to increase detection of small numbers of VRE (lower limit of detection, ~2.0 log₁₀ cfu/g); if no VRE were detected from these samples, a number equal to the lower limit of detection was assigned.

Experimental model. Female CF1 mice (Harlan Sprague-Dawley, Indianapolis) weighing 25–30 g were housed individually. On
experiment day 0, gastric gavage of 0.5 mL of diluent from a frozen overnight culture was performed using a stainless steel feeding tube (Perfektum; Popper & Sons, New Hyde Park, NY). The goal was to inoculate $10^9$ or $10^6$ cfu of VRE. The actual inoculum was quantified. Stool colony counts of VRE and total enterococci were determined for a subset of mice prior to any intervention and on day 0 (after receiving subcutaneous antibiotic for 2 days prior to inoculation with oral VRE).

Two days before gastric gavage of VRE, subcutaneous injection (0.1 mL total volume) of saline, Ctri (2.4 mg/day), ticarcillin-clavulanate (Tic-Clv; 12 mg/day), or Pip-Taz (8 mg/day) was initiated at 12-h intervals. Ctri and Tic-Clv were studied because they have minimal activity against VRE strain C68 (MICs >10,000 μg/mL) compared with the activity of Pip-Taz (MIC 1250 μg/mL) [5]. Daily doses of antibiotics were equivalent to the daily dose (per kilogram) recommended for human adults.

The experiments using the lowest inoculum of VRE C68 ($10^2$ cfu) were done twice, using a total of 48 mice. The first set of experiments consisted of 32 mice (6 received saline, 10 Pip-Taz, 8 Ctri, and 8 Tic-Clv). The second set of experiments consisted of 16 mice (8 received Pip-Taz, 4 Ctri, and 4 Tic-Clv). Stool was collected on days 3, 6, 9, 13, and 16 after gastric gavage of VRE. Starting on day 16 of the first set of experiments, 2 saline-treated mice and 8 Pip-Taz–treated mice were given oral vancomycin in their water (250 μg/mL) to assess whether low levels of VRE were present that could be promoted. To confirm that isolates recovered during the course of the experiment did not differ from VRE C68, agar-dilution MICs were determined on selected stool isolates.

The experiment using the $10^3$ inoculum of VRE was done once and utilized 17 mice (3 received saline, 8 Pip-Taz, 3 Ctri, and 3 Tic-Clv). Stool was collected on days 3, 6, and 9 after gastric gavage of VRE. The saline- and Pip-Taz–treated mice received continued injections and were reinoculated by gavage with $10^3$ VRE on day 9 and $10^4$ VRE on day 13. Stool was collected on days 13, 16, and 19. This was done to determine the inoculum of VRE required to establish high-level colonization in Pip-Taz–treated mice.

Statistical analysis. Mixed-effect linear modeling for repeated measures was performed using SAS software (SAS Institute, Cary, NC).

Results

Experiments using the $10^2$ inoculum of VRE. Prior to any intervention, 18 of 18 mice tested were colonized with vancomycin-susceptible enterococci (range, 4.5–7.0 log$_{10}$ cfu/g), and none had detectable VRE. On day 0, all 48 of the experimental mice had no detectable VRE (lower limit of detection, <2.7 log$_{10}$ cfu/g).

A 118-cfu inoculum of VRE was administered to the first group of 32 mice, and a 70-cfu inoculum was administered to the second group of 16 mice. The effect of subcutaneous saline or antibiotics on establishment of VRE colonization is shown in figure 1. Four (67%) of 6 saline-treated mice had low levels of stool VRE for at least one time point (range, 2.4–4.6 log$_{10}$ cfu/g), but none had detectable VRE on day 16. For mice receiving Pip-Taz, the level of stool VRE did not differ from that for the saline controls at any time point. All mice receiving Ctri or Tic-Clv (12 in each treatment group) developed high-level VRE colonization (>7 log$_{10}$ cfu/g) by day 3, and moderate to high levels of VRE (>4 log$_{10}$ cfu/g) persisted in most of these mice. VRE levels for the Ctri and Tic-Clv groups were higher than those for the saline and Pip-Taz groups at all time points ($P<.008$). Variation in VRE counts within all treatment groups was <2 log$_{10}$ cfu/g, with the exception of the Ctri and Tic-Clv groups on day 16 ($±3D$, 3.0 and 3.2 log$_{10}$ cfu/g, respectively). Antibiotic susceptibilities of 12 VRE isolates recovered from stool during antimicrobial administration were not significantly different from those for VRE C68 (data not shown).

After day 16, administration of oral vancomycin to 2 mice from the saline group did not result in detectable levels of VRE in stools. Administration of oral vancomycin to mice from the Pip-Taz group resulted in high levels of VRE (>8 log$_{10}$ cfu/g) in 8 of 8 mice tested. Only one of these mice had detectable VRE (2.6 log$_{10}$ cfu/g) before receiving vancomycin.

Experiment using the $10^3$ inoculum of VRE. Prior to any intervention and at day 0, 17 of 17 mice tested had no detectable VRE (lower limit of detection, <2.8 log$_{10}$ cfu/g). A 9500-cfu inoculum of VRE was administered. The effect of subcutaneous saline or antibiotics on establishment of VRE colonization is shown in figure 2. All 3 of the mice that received saline had low levels of stool VRE on days 3, 6, and 9. For mice treated with Pip-Taz, the level of VRE did not differ from that of the
saline group at any time point. All mice treated with Ctri or Tic-Clv developed high-level VRE colonization (>7 log_{10} cfu/g) by day 3, and moderate to high levels of VRE (>4 log_{10} cfu/g) persisted in all mice through day 9. VRE levels for the Ctri and Tic-Clv groups were higher than those for the saline and Pip-Taz groups at all time points (P < .001). Variation within all treatment groups was <2 log_{10} cfu/g at all time points.

Experiments using 10^6 and 10^9 inocula of VRE. The saline and Pip-Taz groups from the 10^4-inoculum experiment were gavaged with 1.0 × 10^4 cfu of VRE on day 9 and with 6.5 × 10^4 cfu on day 13. Two of 3 saline-treated mice had detectable levels of VRE in stools on days 16 and 19 (mean, 4.2 log_{10} cfu/g). On day 13, 5 of 8 Pip-Taz–treated mice had VRE levels >4 log_{10} cfu/g. All 8 Pip-Taz–treated mice had high levels of stool VRE on days 16 and 19 (>8 log_{10} cfu/g for all mice), and the levels of VRE were higher than those for the saline controls (P < .001).

Discussion

VRE colonization and infection have been associated with exposure to vancomycin [7, 8], third-generation cephalosporins [3, 8–10], and antibiotics with potent activity against anaerobes [11–12]. Pip-Taz and ampicillin-sulbactam have potent anti-anaerobic activity and promote persistent high-density VRE colonization in mice [1], but substitution of these antibiotics for third-generation cephalosporins has been associated with a decrease in the prevalence of stool VRE colonization [2]. In this study, we found that Tic-Clv and Ctri promoted the establishment of high-level stool colonization in mice after gastric inoculation of small numbers of VRE, while Pip-Taz (a β-lactam antibiotic with greater anti-enterococcal activity) did not. The protective effect of Pip-Taz could be overcome by administering a large inoculum of VRE. These findings suggest that for patients ingesting small numbers of VRE while receiving antibiotics, the likelihood of developing high-level intestinal colonization varies depending on the relative anti-enterococcal activity of the antibiotic regimen.

Pip-Taz has significantly greater activity against many enterococcal strains (VRE C68 MIC, 1250 μg/mL) than Tic-Clv or Ctri (VRE C68 MICs, >10,000 μg/mL) [5, 13], but these antibiotics are similar in other ways. All 3 antibiotics/antibiotic combinations are secreted in significant concentrations in the bile of humans [1, 4, 14], and all 3 antibiotics have similar potent activity against Enterobacteriaceae. Both Pip-Taz and Tic-Clv have potent activity against anaerobes, while Ctri has moderate in vitro activity against intestinal anaerobes.

Our data, in combination with our previous mouse experiments [1], suggest that the effect of Pip-Taz on VRE colonization in mice may represent a balance between inhibition due to anti-enterococcal activity and promotion of VRE due to anti-anaerobic activity. All of the mice that were challenged with oral vancomycin after completion of the course of Pip-Taz developed high-level VRE colonization; therefore, Pip-Taz did not eliminate the gastric inocula of VRE, but continued treatment inhibited the development of high-level colonization. When larger inocula of VRE (10^6 and 10^9 cfu) were administered, the anti-enterococcal activity of Pip-Taz was not sufficient to prevent establishment of high-level colonization.

In our previous mouse model, Ctri promoted persistent high-density VRE colonization less than did antibiotics with more potent in vitro activity against intestinal anaerobes (including Pip-Taz and Tic-Clv). In this study, Ctri promoted the initial establishment of high-level colonization to the same degree as Tic-Clv. Studies with other cephalosporins, such as ceftazidime or cefepime, would be useful to further test our hypothesis that cephalosporins that lack potent anti-anaerobic activity may be associated with VRE because they promote the initial establishment of colonization. Third-generation cephalosporins vary widely in their levels of biliary excretion and anti-anaerobic activity, and therefore it is likely that they promote VRE to different degrees in humans [1].

The applicability of this mouse model to human VRE colonization is limited by several factors, including differences between mice and humans in terms of intestinal flora and the pharmacokinetics of antibiotics. This model also assessed the effect of antibiotics on only 1 VRE strain. The effect of antibiotics on VRE colonization may vary for strains with different
levels of antibiotic resistance. Furthermore, in clinical settings, many factors in addition to antibiotics may influence VRE colonization.

Despite the potential limitations of this model, our results, in combination with our previous mouse experiments [1], provide a theoretical model for the effect of antibiotic treatment on intestinal VRE colonization in humans. We hypothesize that antibiotics with significant anti-enterococcal activity (e.g., Pip-Taz) inhibit the establishment of high-level intestinal VRE colonization in humans, whereas broad-spectrum antibiotics with minimal anti-enterococcal activity promote establishment of high-level colonization. We hypothesize that, after high-level intestinal VRE colonization has been established, anti-anaerobic antibiotics promote continued high-density colonization, while antibiotics lacking anti-anaerobic activity do not [1]. This model of VRE intestinal colonization is consistent with findings from clinical studies [2, 11–12] and is microbiologically plausible. If proven applicable to humans, these findings could be applied to the selection of antibiotics in clinical settings.

References


