ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2004, p. 4427–4429 Vol. 48, No. 11
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.11.4427–4429.2004

Evidence for Biliary Excretion of Vancomycin into Stool during Intravenous Therapy: Potential Implications for Rectal Colonization with Vancomycin-Resistant Enterococci

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Received 24 March 2004/Returned for modification 28 April 2004/Accepted 11 July 2004

Sixty-three stool samples and five bile samples were prospectively collected from 33 patients receiving intravenous vancomycin therapy and were quantitatively analyzed for vancomycin by a competitive immunoassay. Vancomycin was excreted via bile into the stools of almost all patients at concentrations of 3.3 to 94.8 μg/ml after ≥5 days of a therapy of 1 g every 12 h.

Vancomycin-resistant enterococci (VRE) have emerged as significant nosocomial pathogens worldwide and are one of the most common etiologies of hospital-acquired infection in the United States (3, 15). In spite of the rapid emergence and dramatic increase in VRE prevalence, much of the epidemiology of VRE colonization and infection remains unexplained (1, 12). In the United States, it is now well recognized that the major hospital reservoir of the pathogen consists of inpatients with VRE rectal colonization. Numerous studies investigating nosocomial VRE rectal colonization have consistently identified prior administration of antianaerobic and expanded-spectrum cephalosporin antibiotics as risk factors (4, 5, 11, 14). The impact of prior intravenous (i.v.) and oral vancomycin therapy remains controversial (2, 4, 5, 8, 11, 13, 14). In addition, the role of i.v. vancomycin has remained obscure, given that it is widely accepted that there is poor bowel penetration of the drug when it is administered i.v. and that the drug is considered to be almost exclusively eliminated by renal excretion (10). Reports from investigations of single-dose i.v. vancomycin pharmacokinetics that date to the introduction of vancomycin noted that only minimal levels of drug excreted in bile were detected and that measurable concentrations of excreted drug in stool could not be detected (7, 9). Only one study assessing standing i.v. vancomycin therapy of adult patients at 500 mg every 6 h (q6h) exists (7). This investigation documented stool vancomycin levels of 0 to 110 μg/ml, but samples were randomly collected, with no attempt being made to correlate the presence of stool vancomycin levels with duration of therapy. The presence of vancomycin in stool was concluded to be an infrequent event and not likely to be clinically significant (10).

Yet recent data from prospective murine models have confirmed that subcutaneous vancomycin administration is a risk factor for the persistence of high-density *vanB* VRE intestinal colonization (6). Subcutaneously administered vancomycin maintained levels of colonization density as high as those seen with oral vancomycin and higher than those achieved with expanded-spectrum cephalosporin antibiotics. Limited data also suggest that i.v. vancomycin monotherapy may increase the density of human VRE rectal colonization (5). At our own institution, a cross-sectional study of the prevalence of *vanA* VRE rectal colonization identified a significant difference in prevalence between those patients receiving i.v. vancomycin for <5 days and those receiving it for ≥5 days, 28 and 64% ($P < 0.001$), respectively (4). This temporal variation in VRE rectal colonization prevalence was not present in patients who were administered i.v. antibiotics other than vancomycin or in patients who received no antibiotics, despite comparable lengths of stay. These studies suggested the hypothesis that biliary excretion of i.v. vancomycin occurred and that it was potentially related to duration of therapy. Our investigation sought to evaluate both possibilities.

Study patients were enrolled from among all patients who had newly initiated i.v. vancomycin therapy. Patients with a calculated creatinine clearance of <50 ml/min or evidence of active bowel pathology, including diarrhea, inflammation, and malignancy, were excluded. Thirty-one hospitalized patients were enrolled. Twenty patients provided single or multiple stool samples according to two protocols: all samples were acquired <5 days into therapy or all were acquired after ≥5 days of therapy. Serial stool samples were collected from seven patients, both before and after day 5 of therapy. Any guaiac-positive stool sample or any sample potentially contaminated with urine was excluded. Five patients receiving doses of 1 g q24h provided nine stool samples. Twenty-six patients receiving 1 g ql2h provided 54 stool samples (inclusive of all seven patients with serial stool sample collections). Bile samples were obtained from two additional patients receiving i.v. vancomycin who had undergone external biliary stenting. One patient receiving doses of 1 g q12h was sampled on days 7 and 9 of therapy, and a second patient receiving 1 g q24h was sampled on days 17, 20, and 23 of therapy.

Twenty-two patients received only i.v. vancomycin therapy, three received i.v. vancomycin and gentamicin, and six received i.v. vancomycin in combination with other antibiotics. Most patients were monitored for serum drug levels, and although serum sample testing could not be reliably arranged to coincide with timing of stool or bile sampling, there was no evidence

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TABLE 1. Vancomycin levels in the stools of 54 patients receiving i.v. vancomycin at 1 g q12h

Day of therapy	No. of samples	% Positive for detectable vancomycin	Range (μg/ml)
1	6	0	<2
2	6	0	<2
3	6	0	<2
4	8	62.5	<2–3.9
5	7	85.7	<2–94.8
6	8	87.5	<2–54.3
7	5	100	4.6–24.4
8	3	100	3.3–7.3
9	2	100	4.2–13.9
10	1	100	4.1
11	1	100	5.1
12	1	100	5.6

that measured values in serum exceeded the upper limit of the therapeutic range for any patient.

Bile and stool samples were assayed for vancomycin concentrations with an AxSym II fluorescence polarization immunoassay. The serum assay protocol was modified for stool samples to include an extraction of the sample in dilute ammonia to prevent nonspecific binding of vancomycin to protein in the stool. The modified assay was calibrated by comparing vancomycin-spiked stool samples of known concentration to corresponding known concentrations of vancomycin in water. There was excellent correlation across a range of values from >2 to 62.5 μg/ml, and the assay was sensitive to within 2 μg of vancomycin per ml. Levels of <2 μg/ml were considered negative and were included as a level of 0 μg/ml for the calculation of mean concentrations.

For each sample, 1 g of stool was vortexed in 3 ml of dilute ammonia solution and then centrifuged to remove particulate matter. One-hundred-fifty-microliter aliquots of the supernatant were assayed; 150-μl aliquots of bile were directly assayed.

Results for the 46 stool samples analyzed from 23 patients receiving doses of 1 g of vancomycin q12h are summarized in Table 1. Results for the nine stool samples from patients receiving 1 g q24h are summarized in Table 2.

Among patients receiving doses of 1 g ql2h, 21 of 26 samples (80.8%) collected <5 days into therapy did not have detectable vancomycin levels and thus were considered negative. The five positive samples all occurred on day 4 of therapy, with measured values of ≤3.9 μg/ml. Among patients receiving ≥5 days of therapy, 26 of 28 samples (92.9%) had detectable levels of vancomycin that ranged from 3.3 to 94.8 μg/ml, with 28.6% of samples containing vancomycin concentrations of >10 μg/ml. The proportion of positive cases and the mean measured vancomycin concentration were significantly higher among samples obtained from patients who had received ≥5 days of therapy than among those who had received <5 days of therapy (92.9% versus 19.2% and 12.1 μg/ml versus 0.7 μg/ml [$P < 0.005$]). Samples from the seven patients with serial stool samples were all negative before day 5 and had measurable levels of vancomycin on or after day 5 (range, 3.0 to 20.8 μg/ml; mean, 7.6 μg/ml; 71.4% of samples had >5.05 μg/ml).

TABLE 2. Vancomycin levels in the stools of nine patients receiving i.v. vancomycin at 1 g q24h

Day of therapy	No. of samples	% Positive for detectable vancomycin	Range (μg/ml)
1	3	0	<2
2	1	0	<2
3	1	0	<2
4	1	0	<2
5			
6	2	0	<2
7	1	100	8.8

Among patients receiving doses of 1 g q24h, only one sample, obtained on the seventh day of therapy, was positive at 8.8 μg of vancomycin per ml.

All bile samples had detectable levels of vancomycin. The patient receiving doses of 1 g ql2h had vancomycin concentrations of 8.2 and 10.9 μg/ml on days 7 and 9 of therapy, respectively. The patient receiving doses of 1 g q24h had 11.4, 10.8, and 12.5 μg of vancomycin per ml in bile on days 17, 20, and 23 of therapy, respectively.

The results demonstrate that biliary excretion of vancomycin can occur during i.v. administration of the drug and can result in detectable levels of vancomycin in stool. The influence of stool vancomycin on VRE rectal colonization at the concentrations we report remains largely uninvestigated. Evidence supporting the clinical significance of our results includes (i) a single prior study which documented the elimination of *Clostridium* species and the elimination or reduction of vancomycin-sensitive *Enterococcus faecalis* concentrations with stool vancomycin concentrations of 0 to 110 μg/ml and (ii) limited data suggesting that i.v. vancomycin monotherapy can increase human VRE rectal colonization density (5, 7). These studies suggest that the concentrations of vancomycin in stool samples reported in this investigation are capable of disruption of the natural bowel anaerobic flora (a process linked with the establishment of VRE colonization) and possibly enrichment of existing VRE in the bowel. Further research into the microbiological impact of low concentrations of vancomycin in stools is required, but the present findings are suggestive of a mechanism by which i.v. vancomycin therapy could influence VRE rectal colonization.

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