

## Analysis of the In Vitro Interaction Between Vancomycin and Cholestyramine

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Vancomycin and cholestyramine have been utilized both alone and in combination for the treatment of antibiotic-associated pseudomembranous colitis. Previous work has demonstrated significant binding of vancomycin by the anion-exchange resin. The antibacterial activity of vancomycin was markedly reduced when the suspension was centrifuged and the supernatant was tested for antibacterial activity. This study confirmed these findings but demonstrated that there was no immediate loss of antibacterial activity of bound vancomycin. The degree of inactivation appeared to be dependent upon the duration of incubation of vancomycin and cholestyramine in the testing system.

Antibiotic-associated pseudomembranous colitis (PMC) has been linked to the presence of *Clostridium difficile* within the colonic flora of patients with the disease. The organism elaborates a cytotoxin which is capable of producing the lesions found in PMC (1, 2, 5).

Many cases of PMC may be self-limited if the offending agent is withdrawn; however, treatment with orally administered vancomycin or cholestyramine may be necessary. Vancomycin given in doses of 0.5 to 2.0 g per day has been shown to be effective in producing symptomatic improvement and a decrease in the titers of toxin in stool (5, 8). The use of anion-exchange resins, such as cholestyramine, which bind the toxin appears to be efficacious in some cases, but the results are not as dramatic as those produced by vancomycin (5, 11).

Several reports of relapse of PMC after oral vancomycin therapy have been noted and have led some authorities to suggest that the use of vancomycin plus cholestyramine or other antibiotics active against *C. difficile* might provide superior therapy (3, 6). However, potential difficulties arise with the combination since it has been demonstrated that vancomycin is approximately 90% bound by cholestyramine (4, 10).

Although the presence of vancomycin does not inhibit binding of the cytotoxin by the resin, the concentration of vancomycin present is sharply reduced (4). These studies have examined the concentration of unbound vancomycin in the supernatant of centrifuged specimens.

To our knowledge, the activity of bound vancomycin has not been examined. The purpose of this study was to examine the effects of cholestyramine binding of vancomycin in centrifuged and uncentrifuged samples.

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### MATERIALS AND METHODS

Cholestyramine powder (Mead-Johnson Laboratories) and vancomycin powder (Eli Lilly & Co.) were suspended and dissolved, respectively, in normal saline buffered to pH 7.0 with 0.1-mol/liter sodium phosphate solution. The amounts of oral vancomycin and cholestyramine usually given within 24 h (2,000 mg and 12 g, respectively) were each mixed in 1 liter of the buffered saline. Five sets of mixtures were examined as follows: A, vancomycin (2 mg/ml); B, cholestyramine suspension (12 mg/ml); and C, D, and E, vancomycin (2 mg/ml) plus cholestyramine (12 mg/ml). Each solution was tested in triplicate. All of the tubes were agitated in a water bath at 37°C for 45 min and then centrifuged for 15 min. Samples of the supernatant from A, B, and C were then immediately decanted and assayed for vancomycin activity. Tubes in D were blended with a Vortex mixer after centrifugation to resuspend the cholestyramine, and samples of the suspension were assayed for vancomycin activity. Tubes in E were also blended with a Vortex mixer after initial centrifugation, but then allowed to resettle over 72 h at 4°C with tubes from A. After 72 h, samples of the supernatant of E were assayed for vancomycin activity (designated E<sub>1</sub>), and the tubes were blended with a Vortex mixer once again to resuspend the vancomycin. Samples of this suspension were then assayed for vancomycin activity (designated E<sub>2</sub>).

Vancomycin activity was determined by an agar diffusion technique, using *Bacillus subtilis* as the test organism.

Statistical comparison of mean values were performed by using a one-tailed Student's *t* test.

### RESULTS

In Table 1 the findings of the investigation are summarized. The vancomycin and cholestyramine control solutions, as expected, had 100% and no activity, respectively. The supernatant of the centrifuged mixture that was assayed immediately demonstrated 71% loss of activity (C). However, when the centrifuged tubes were

TABLE 1. Binding of vancomycin by cholestyramine

Sample	Vancomycin concn (mg/ml)	% Vancomycin activity
A	2.27 ± 0.12 <sup>a</sup>	100
B	0	0
C	0.66 ± 0.04	29, <i>P</i> < 0.005 <sup>b</sup>
D	2.39 ± 0.11	100, NS <sup>c</sup>
E <sub>1</sub>	1.18 ± 0.11	52, <i>P</i> < 0.025 <sup>b</sup>
E <sub>2</sub>	1.52 ± 0.03	67, <i>P</i> < 0.025 <sup>b</sup>

<sup>a</sup> Mean ± standard deviation.

<sup>b</sup> Concentration of vancomycin compared with that for group A.

<sup>c</sup> NS, Not statistically significant.

blended with a Vortex mixer and the suspension was assayed for activity, there was no loss of activity (D). The supernatant of the mixture which was allowed to settle for 72 h demonstrated only 48% binding (E<sub>1</sub>), and this mixture, when resuspended, contained only 67% of the activity or 33% binding. The mean values for C, E<sub>1</sub>, and E<sub>2</sub> were significantly different from those for the control. E<sub>1</sub> and E<sub>2</sub> were also significantly different from each other (*P* < 0.05).

## DISCUSSION

Other investigators have reported binding of approximately 90% when relatively low concentrations of vancomycin (125 µg/ml) were mixed with high concentrations of cholestyramine (100 g/ml) (10). The concentrations used in this investigation were designed to represent the daily amounts of vancomycin and cholestyramine administered within a volume conservatively approximating colonic contents during moderately severe diarrhea. The oral dose was used to estimate the concentrations as neither cholestyramine or vancomycin (7) are absorbed appreciably from the gastrointestinal tract.

Our findings suggest that the binding of vancomycin to cholestyramine is less than previously reported and appears to be relatively loose since the suspension assayed immediately contained 100% activity (D). It is possible that the vancomycin may be bound strongly at sites that do not compromise antibacterial activity. Taylor and Bartlett (10) found it difficult to elute vancomycin from binding resin, which supports this possibility. Concentrations of drug in E<sub>1</sub> and C should have been similar but were higher in E<sub>1</sub> than in C because centrifugation was probably more efficient at removing bound yet active vancomycin from the supernatant.

Apparent inactivation with time is suggested by the decreased vancomycin activity of the suspension from E<sub>2</sub> compared to suspension D.

The mechanism of this inactivation is unclear. Since gastrointestinal transit time would be expected to increase during diarrhea, this latter finding is probably of little clinical importance. It is known that vancomycin retains 100% activity in normal saline stored at 5°C for 28 days (9). Thus, it is unlikely that degradation of vancomycin could explain the lower concentrations in E<sub>1</sub> and E<sub>2</sub>. The clinical significance of all of these findings is to suggest that the interaction, although documented to occur *in vitro*, is unimportant. Even if the drug is highly bound, the activity of the bound drug and the amount free will provide concentrations of vancomycin far in excess of the minimum inhibitory or bactericidal concentrations for *C. difficile*.

Therefore, it does not seem unreasonable to investigate the effects of concomitant cholestyramine and vancomycin in patients with PMC. As suggested above, this may enhance therapeutic response and prevent relapse of the disease.

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