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Comparison of the In Vitro Activities of Teicoplanin and Vancomycin against *Clostridium difficile* and Their Interactions with Cholestyramine

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The in vitro activity of teicoplanin was compared with that of vancomycin against fecal isolates of *Clostridium difficile*. All strains were susceptible to both antibiotics, but teicoplanin was fourfold more active than vancomycin. Cholestyramine was found to bind teicoplanin almost completely, reducing its activity to nondetectable levels.

Vancomycin is considered the antibiotic of choice for the treatment of *Clostridium difficile*-induced pseudomembranous colitis (PMC) (6). If orally administered, vancomycin reaches in feces a concentration largely exceeding the MIC for this microorganism (9). Also, anion-exchange resins such as cholestyramine have proved useful in the treatment of PMC, due to direct inactivation of *C. difficile* toxin (8). These different modalities of action suggest the possibility of a combined therapy; however, the binding of vancomycin by anion-exchange resins and the following decrease in its antibacterial activity raises some concern (3, 8). Teicoplanin (teichomycin A_2) is a new glycopeptide antibiotic closely associated with the group vancomycin-ristocetin (1), which has shown remarkable in vitro activity against gram-positive aerobes, such as staphylococci and enterococci (10), and also against gram-positive anaerobes, including strains of *C. difficile* (4, 11). The purpose of our study was (i) to compare the activity of teicoplanin and vancomycin against a large number of *C. difficile* strains recently isolated from patients with PMC or antibiotic-associated diarrhea and (ii) to examine the interaction between cholestyramine and teicoplanin and to evaluate whether a combined therapy would be compatible.

Seventy-five strains of *C. difficile* recently isolated from stool samples of patients with PMC or antibiotic-associated diarrhea were studied. The antimicrobial agents evaluated were provided as standard laboratory powder. Vancomycin was manufactured by United States Biochemical Corp., Cleveland, Ohio, and teicoplanin was manufactured by Lepetit Research Laboratories, Milan, Italy.

The MIC was determined by the agar dilution method (7) with Wilkins-Chalgren agar (Oxoid Ltd.) as the test medium. Inocula were prepared from overnight cultures in Wilkins-Chalgren broth adjusted to a concentration of approximately 10^7 organisms per ml. A multipoint inoculator (model A400; Denley Instruments) was used to deliver 10^4 organisms per spot to the test plates. Incubation was carried out in an anaerobic cabinet (P.A.C.E.; Lab-Line Instruments, Inc., Melrose Park, Ill.) for 48 h. The MIC was read as the lowest antibiotic concentration which allowed no visible growth.

The interaction between cholestyramine and vancomycin or teicoplanin was studied by experiments modified from King and Barriere (5). Cholestyramine standard powder (kindly provided by Bristol Italiana, Rome, Italy) was suspended in 0.1 M phosphate-buffered saline (pH 7), and vancomycin and teicoplanin were dissolved in phosphate-buffered saline. The following samples were prepared: cholestyramine alone (12 mg/ml), vancomycin alone (2 mg/ml), teicoplanin alone (2 mg/ml), cholestyramine (12 mg/ml) plus vancomycin (2 mg/ml), and cholestyramine (12 mg/ml) plus teicoplanin (2 mg/ml). The samples were incubated in a water bath, with agitation at 37°C for 1 h, and then centrifuged at 8,000 × *g* for 15 min. The decanted supernatants, appropriately diluted, were assayed for antibacterial activity by an agar diffusion technique with *Bacillus subtilis* ATCC 6633 as the test organism; standard solutions of vancomycin and teicoplanin were tested in the same assay to determine the concentration of these drugs in the samples. All samples were tested in triplicate.

All strains tested were highly susceptible to both vancomycin and teicoplanin, with MICs distributed over a narrow range; however, MICs of teicoplanin were found to be fourfold lower than those of vancomycin. Vancomycin MICs for 50 and 90% of strains were 0.5 and 1 μg/ml, respectively, and the MIC range was 0.25 to 1 μg/ml. The teicoplanin MIC for both 50 and 90% of strains was 0.25 μg/ml, and the MIC range was 0.12 to 0.5 μg/ml. The results of the experiment to determine the binding of the two antibiotics by cholestyramine are summarized in Table 1. The presence of cholestyramine produced a fall in the active vancomycin concentration of approximately 20%, that is, 80% binding. With teicoplanin, the fall in active concentration was more striking. This antibiotic appeared to be almost entirely bound by cholestyramine, and its activity was hardly detected by plate assay.

Vast clinical experience has proven vancomycin to be highly effective against *C. difficile*-induced PMC and diarrhea. However, its cost and the high incidence of relapses

TABLE 1. Effect of cholestyramine on the antibiotic activity of vancomycin and teicoplanin

Sample (concn [mg/ml])	Antibiotic concn (mg/ml)	Antibiotic activity (%)
Cholestyramine (12)	0	
Vancomycin (2)	2.17	100
Vancomycin (2)-Cholestyramine (12)	0.41	18.9
Teicoplanin (2)	1.82	100
Teicoplanin (2)-Cholestyramine (12)	0.005	0.3

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have prompted researchers to investigate alternative antibiotic regimens (2). Teicoplanin, similarly to vancomycin, is not absorbed when taken orally (G. Buniva, personal communication), so it is virtually nontoxic and can reach high levels in the gut. Our in vitro results suggest that teicoplanin could be an interesting substitute for vancomycin against *C. difficile*. We have shown that it is more inhibitory than vancomycin against this microorganism, confirming previous studies (4). Its higher activity could possibly help in eradicating *C. difficile* from the gut without permitting spore formation; if so, the relapse rate could be lower than with vancomycin. In our study, cholestyramine showed a higher affinity for teicoplanin than for vancomycin. For vancomycin, we found an approximately 80% loss of activity after 1 h of incubation with cholestyramine, which is not dissimilar from data found by other workers (2, 8). For teicoplanin, the activity was reduced to hardly detectable levels. This finding discourages the contemporaneous use of teicoplanin and an anion-exchange resin. Since a course of cholestyramine after a course of vancomycin has been recently suggested, especially for the treament of relapses (2), the same therapeutic schedule could be attempted with teicoplanin. Our in vitro studies confirm the potential use of teicoplanin in *C. difficile*-associated diarrhea and PMC. Clinical experience is needed to assess the value of this antibiotic in human diseases.

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