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In Vitro Activities of Ramoplanin and Four Glycopeptide Antibiotics against Clinical Isolates of *Clostridium difficile*

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Seventy strains of *Clostridium difficile*, all isolated from symptomatic patients, were found to be uniformly susceptible to ramoplanin, a new glycolipodepsipeptide antibiotic, and to four glycopeptides (vancomycin, teicoplanin, and two semisynthetic teicoplanin derivatives). Ramoplanin is recommended for further evaluation in the treatment of *C. difficile*-associated disease.

The role of toxin-producing strains of *Clostridium difficile* in antibiotic-associated diarrhea and colitis was discovered in the late 1970s (4, 11, 16). Extensive studies carried out during the 1980s have provided a substantial data base for the organism and the disease it causes. In particular, antimicrobial susceptibilities of *C. difficile* have been widely investigated both in vitro and in vivo. Generally, oral vancomycin is considered the treatment of choice for *C. difficile*-associated disease (2). Even more encouraging results have been recently reported with teicoplanin, the newest drug in the family of glycopeptides (8). Alternative agents, including metronidazole, fusidic acid, and bacitracin, can be considered, especially for patients with less serious forms of the disease (3).

In this study, we have examined ramoplanin and four glycopeptide antibiotics comparatively for their in vitro activity against clinical strains of *C. difficile*. Ramoplanin (formerly A-16686) is a new antimicrobial agent obtained from the fermentation of an *Actinoplanes* strain and is active against gram-positive aerobic and anaerobic bacteria (5, 21). The four glycopeptides tested included the two clinically available drugs of this family, namely vancomycin and teicoplanin, and two investigational amide derivatives of teicoplanin A2 complex (CTA-A1, also known as MDL 62211 or compound 21) and teicoplanin aglycone (TD-A3, also known as MDL 62208 or compound 62) (17). Vancomycin was obtained from Eli Lilly Italia, Sesto Fiorentino, Italy. Ramoplanin, teicoplanin, and the two semisynthetic teicoplanin derivatives were supplied by the Lepetit Research Center, Gerenzano, Italy.

A total of 70 clinical strains of *C. difficile*, isolated in various Italian hospitals, were used. The strains were obtained from fecal samples of different symptomatic patients with antibiotic-related intestinal disturbances, ranging from mild, watery diarrhea to severe pseudomembranous colitis and toxic megacolon. The organisms were cultured and identified by conventional laboratory methods (13). Gas-liquid chromatography was used to confirm the identification of some isolates.

MICs were determined by the reference agar dilution procedure recommended for anaerobic bacteria (18), using an automatic replicating device (Titertek; Flow Laboratories, Rockville, Md.) for plate inoculation and GasPak jars (Becton Dickinson Microbiology Systems, Cockeysville, Md.) for anaerobic incubation (48 h at 35°C). Quality controls of the medium and the test procedure were performed with *C. perfringens* ATCC 13124 as the reference strain. Serial twofold dilutions of the five antimicrobial agents were used to prepare test plates of Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.) with antibiotic concentrations ranging from 0.03 to 8 μg/ml.

All strains were inhibited by concentrations not exceeding 2 μg/ml for vancomycin and 1 μg/ml for the other antibiotics, with MICs distributed over a narrow range (Table 1). In fact, in agreement with previous findings (28), the MICs of teicoplanin ranged from 0.25 to 1 μg/ml, generally being two to four times lower than those of vancomycin, which ranged from 1 to 2 μg/ml. When compared with the MICs of teicoplanin, those of teicoplanin derivatives were generally identical or twice as high, the latter condition being more frequently encountered with TD-A3 than with CTA-A1. Ramoplanin MICs were comparable to those of teicoplanin, occasional differences never exceeding one twofold dilution.

Although the leading role of oral vancomycin for the treatment of *C. difficile*-associated disease is well established, relapses characterized by recurrence of symptoms with positive results for toxin assays occur in more than 20% of patients after discontinuation of therapy (3). Even more common is posttreatment asymptomatic carriage of *C. difficile* (10). Preliminary clinical trials with teicoplanin seem to be particularly encouraging: in two consecutive series of patients, treated orally with vancomycin (first series) or teicoplanin (second series), no relapse was observed in the teicoplanin group, in contrast to an incidence of 13% in the vancomycin group (8). Therefore, the development of teicoplanin analogs with better microbiological and pharmacological properties (17), such as CTA-A1 or TD-A3, may greatly contribute to widening and improving of the resources available against such an important and insidious pathogen as *C. difficile*. It is worth noting that Jorgensen et al. (14) have shown that another newly synthesized glycopeptide antibiotic seems to be more active in vitro than vancomycin and teicoplanin against *C. difficile*.

However, the recent emergence of vancomycin resistance, mostly inducible and transferable, in enterococci (organisms which are both major human pathogens and normal inhabitants of the human intestinal tract) (25) induces uncertainty about the oral use of glycopeptides and leads one to question whether it is still warranted. Infections caused by vancomycin-resistant enterococci have been documented in patients who had been receiving intravenous vancomycin as therapy or prophylaxis (15, 27). The use of glycopeptides as topical intestinal agents could lead to an even greater risk

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TABLE 1. Susceptibility of 70 *C. difficile* strains to ramoplanin and glycopeptides

Antibiotic	MIC (μg/ml)[a]			
	Range	50%	75%	90%
Ramoplanin	0.125–1	0.25	0.5	0.5
Vancomycin	1–2	1	1	2
Teicoplanin	0.25–1	0.5	0.5	0.5
CTA-A1	0.25–1	0.5	0.5	1
TD-A3	0.25–1	0.5	1	1

[a] 50%, 75%, and 90%, MIC for 50, 75, and 90% of isolates, respectively.

that resistant enterococci will develop and possibly spread, even though it must be pointed out that vancomycin-resistant enterococcal strains are not always cross-resistant to teicoplanin and appear to be highly susceptible to teicoplanin analogs and other newly synthesized glycopeptides (24).

Of great potential interest is our finding of the excellent activity of ramoplanin against *C. difficile*, which confirms and extends preliminary results of previous studies (19–21). In fact, ramoplanin appears to be a very good candidate as a first-line therapeutic option for the treatment of *C. difficile*-associated disease, particularly in view of possible limitations of the oral use of glycopeptides which could arise from the emergence of resistant enterococci. On the other hand, the structure of ramoplanin, recently elucidated, has proved to be different from that of glycopeptides: it is a cyclic depsipeptide with fatty acid and dimannosyl residues directly attached to amino acids of the cycle (7), relatively similar to two other glycolipodepsipeptides of microbial origin, i.e., herbicolin (1a) and pantomycin (12). Furthermore, ramoplanin inhibits cell wall synthesis at a site different from that inhibited by glycopeptides, probably acting on the second stage of the biosynthetic pathway at the level of lipid intermediate formation or utilization (26). Ramoplanin is unlikely to be developed for systemic use because it is poorly tolerated after intravenous or intramuscular administration (1), but it is considered very promising for topical use, especially as an antiplaque (23) or an antiacne (22) agent, for clearing staphylococci from carriage sites (9), or for treating other superficial infections in which gram-positive bacteria are involved (6). Of course, since the National Committee for Clinical Laboratory Standards reference method is not designed for testing topical agents, caution is necessary in extrapolating the results of MIC assays when interpreting topical activity. However, several properties of ramoplanin are consistent with its promising future use as a topical agent, including good local tolerability in animal models, narrow spectrum of activity, rapid bactericidal action, no selection of resistant mutants, no cross-resistance with clinically used antibiotics, no oral absorption, and favorable comparison with mupirocin and other topical antibacterial agents (6, 9, 23). Prospective clinical trials for the evaluation of ramoplanin in the treatment of *C. difficile*-associated disease are warranted and strongly urged.

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