In Vitro Activity of Teichomycin Compared with Those of Other Antibiotics

HAROLD C. NEU* AND PORNOPEN LABTHAVIKUL

Departments of Medicine and Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received 21 March 1983/Accepted 16 June 1983

The glycopeptide antibiotic teichomycin had in vitro activity comparable to that of vancomycin against most gram-positive species, and it inhibited methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis. It was twofold more active against many S. aureus and S. epidermidis isolates than was vancomycin. Teichomycin had activity comparable to that of vancomycin against Listeria monocytogenes and Streptococcus faecalis. It was generally more active against streptococci than was vancomycin. There were no major differences between minimal inhibitory concentrations and minimal bactericidal concentrations of these drugs. Teichomycin acted synergistically with gentamicin against some bacteria.

Staphylococcal infections produced by Staphylococcus aureus and Staphylococcus epidermidis have been on the increase in the past 5 years (12). S. epidermidis is an important cause of infection in patients after cardiac surgery, after implantation of prosthetic joints, and, recently, in cancer patients also (13).

As many as 20% of S. epidermidis strains are methicillin resistant in some institutions, and methicillin resistance in S. aureus strains has also become a problem in some areas of the country (12). Only vancomycin has proved to be effective therapy for treatment of infections caused by methicillin-resistant bacteria (11). Furthermore, among new gram-positive species, group JK corynebacteria have become important as the cause of infection in immunocompromised patients (7, 9, 13) and those with prosthetic devices (4).

Teichomycin is a bactericidal antibiotic produced by Actinoplanes teichomyceticus (1, 2). Biochemically, it is related to the glycopeptide class of antibiotics to which vancomycin belongs. It resembles vancomycin in the manner in which it interferes with cell wall synthesis in bacteria (10). The purpose of this study was to determine the antibacterial activity of teichomycin compared with those of other anti-gram-positive agents.

Teichomycin was a gift of Gruppo Lepetit S.P.A. Research Laboratories; vancomycin was a gift of Lilly Research Laboratories, Eli Lilly & Co.; methicillin and nafcillin were provided by Beecham Laboratories; erythromycin was provided by Abbott Laboratories; and gentamicin was provided by Schering Corp.

The majority of the organisms used were from patients who had been hospitalized at the Columbia-Presbyterian Medical Center, New York. Other organisms had been provided as gifts from other investigators in New York because of their known resistance of the organisms to β-lactam antibiotics. Identification was done by standard methods (5, 8).

Minimal inhibitory concentrations (MICs) were determined for staphylococci by the agar dilution method with a multiple-point inoculator. The inoculum size was 10^5 CFU/ml. Incubation took place at 35°C for 24 h on Mueller-Hinton agar. Methicillin resistance was determined by incubation for 48 h at 30°C. Minimal bactericidal concentrations (MBCs) were determined in Mueller-Hinton broth in a volume of 1 ml with a final inoculum of 10^5 CFU. MBCs were determined by plating 0.1 ml of clear broth on sheep blood agar. MICs for streptococci and Listeria monocytogenes were determined with brain heart agar containing 5% sheep blood.

Synergy studies were performed with equal concentrations of drugs in twofold dilution steps in Mueller-Hinton agar. Synergy was defined as a fractional inhibitory concentration index of <0.5, and antagonism was defined as a fractional inhibitory concentration index of >2. Broth dilution synergy tests were performed in Mueller-Hinton broth with the drugs combined at one-fourth the MIC of the drugs used singly.

The results of the comparative study are shown in Table 1. Teichomycin was twofold more active than vancomycin against S. aureus. For example, many strains had teichomycin MICs of 0.8 and 1.6 μg/ml, whereas the compa-
TABLE 1. Comparative activity of teichomycin and other antimicrobial agents

| Organism (no.)             | Agent   | Range       | MIC (µg/ml)* |
|----------------------------|---------|-------------|--------------|
|                            |         |             | 50% | 90% |
| S. aureus (90)             | Teichomycin | 0.2–6.3     | 0.8 | 1.6 |
|                            | Vancomycin | <0.1–6.3    | 1.6 | 3.1 |
|                            | Nafcillin  | 0.1–3.1     | 0.8 | 1.6 |
|                            | Methicillin | 0.8–12.5  | 3.1 | 12.5 |
| Methicillin-resistant S. aureus (22) | Teichomycin | 0.2–6.3     | 0.8 | 1.6 |
|                            | Vancomycin | 0.2–6.3     | 1.6 | 3.1 |
|                            | Methicillin | 25->100    | 50  | >100 |
| S. epidermidis (50)        | Teichomycin | 0.2–12.5    | 1.6 | 6.3 |
|                            | Vancomycin | <0.1–6.3    | 1.6 | 6.3 |
|                            | Nafcillin  | 0.1–6.3     | 0.8 | 6.3 |
|                            | Methicillin | 0.2–12.5   | 6.3 | 12.5 |
| Methicillin-resistant S. epidermidis (27) | Teichomycin | 0.2–12.5    | 1.6 | 3.1 |
|                            | Vancomycin | 0.8–6.3     | 1.6 | 3.1 |
|                            | Methicillin | 25->100    | 50  | >100 |
| S. faecalis (32)           | Teichomycin | 0.1–3.1     | 1.6 | 3.1 |
|                            | Vancomycin | 0.2–3.1     | 1.6 | 3.1 |
|                            | Ampicillin | 0.4–6.3     | 0.8 | 3.1 |
| L. monocytogenes (26)      | Teichomycin | 0.2–1.6     | 0.8 | 0.8 |
|                            | Vancomycin | 0.1–1.6     | 0.8 | 1.6 |
|                            | Ampicillin | 0.05–1.6    | 0.4 | 1.6 |
|                            | Gentamicin | 0.8–25      | 1.6 | 3.1 |
| Group JK corynebacteria (7) | Teichomycin | 1.6–3.1     | 1.6 | 1.6 |
|                            | Vancomycin | 0.8         | 0.8 | 0.8 |

* 50% and 90%, MIC at which 50 and 90% of the strains, respectively, were inhibited.

Rable vancomycin MIC was 3.1 µg/ml. Teichomycin inhibited all 14 of the methicillin-resistant S. aureus isolates at the same concentrations as those required for it to inhibit the methicillin-susceptible isolates. The MICs of both teichomycin and vancomycin for S. epidermidis isolates were two- to fourfold higher than those for S. aureus isolates, and the MICs of both drugs for the methicillin-resistant S. epidermidis isolates were higher than those for the susceptible isolates. Both teichomycin and vancomycin inhibited Streptococcus faecalis isolates at concentrations comparable to those achieved with ampicillin. Individual strain differences were noted. For example, one isolate had a teichomycin MIC of 0.8 µg/ml and a vancomycin MIC of 3.1 µg/ml, whereas another isolate had a teichomycin MIC of 1.6 µg/ml and a vancomycin MIC of 0.4 µg/ml.

The agents had comparable activity against L. monocytogenes. Teichomycin inhibited group JK corynebacteria at concentrations twofold greater than those needed for vancomycin.

Table 2 shows the activity of teichomycin against other gram-positive bacteria. The various beta-hemolytic streptococci were inhibited by concentrations of ≤0.4 µg/ml, as were Streptococcus pneumoniae isolates. Streptococcus faecium isolates were inhibited at concentrations comparable to those needed to inhibit S. faecalis isolates. Not all the strains were compared, but in general, teichomycin was two- to fourfold more active than vancomycin against the various streptococcal species. For example, teichomycin and vancomycin MICs for S. faecium were 0.8 and 1.6 µg/ml, respectively; those for Streptococcus mitis were 0.1 and 1.6 µg/ml respectively; and those for Streptococcus bovis were 0.4 and 1.0 µg/ml, respectively.

There was an inoculum effect seen with teichomycin for both S. aureus and S. epidermidis, with an increase in MICs and MBCs from 0.1 µg/ml at 10^5 CFU to 0.8 µg/ml at 10^7 CFU. At all inoculum sizes, the MBCs were either identical or only twofold greater than the MICs. There was a marked difference between MICs and MBCs for L. monocytogenes. For example, an L. monocytogenes isolate with a teichomycin MIC of 1.6 µg/ml would have an MBC of 100 µg/ml.
TABLE 2. In vitro activity of teichomycin against various gram-positive bacteria

| Organism (no.) | Range (µg/ml) | MIC | 50% | 90% |
|----------------|---------------|-----|-----|-----|
| *Streptococcus agalactiae* (14) | 0.1 to 0.4 | 0.1 | 0.4 | 0.4 |
| *Streptococcus bovis* (18) | 0.1 to 0.8 | 0.2 | 0.4 | 0.4 |
| *Streptococcus faecium* (5) | 0.8 | 0.8 | 0.8 | 0.8 |
| *Streptococcus mitis* (3) | 0.1 to 3.1 | 0.1 | 3.1 | 3.1 |
| *Streptococcus pneumoniae* (14) | 0.1 to 0.8 | 0.1 | 0.2 | 0.2 |
| *Streptococcus pyogenes* (17) | 0.1 to 0.4 | 0.2 | 0.2 | 0.2 |
| Viridans group streptococci (15) | 0.1 to 0.4 | 0.1 | 0.4 | 0.4 |
| *Streptococcus, group C* (10) | 0.1 to 0.2 | 0.1 | 0.1 | 0.1 |
| *Streptococcus, group G* (5) | 0.05 to 0.2 | 0.1 | 0.2 | 0.2 |
| *Streptococcus, group F* (5) | 0.1 to 0.2 | 0.1 | 0.2 | 0.2 |
| *Staphylococcus haemolyticus* (5) | 0.8 to 3.1 | 0.8 | 3.1 | 3.1 |
| *Staphylococcus saprophyticus* (5) | 0.8 to 3.1 | 0.8 | 3.1 | 3.1 |
| *Staphylococcus simulans* (3) | 0.4 to 1.6 | 1.6 | 1.6 | 1.6 |
| *Staphylococcus hominis* (2) | 0.2 | 0.2 | 0.2 | 0.2 |
| *Corynebacterium acnes* (3) | 0.8 to 3.1 | 0.8 | 3.1 | 3.1 |
| *Clostridium difficile* (1) | 0.8 | 0.8 | 0.8 | 0.8 |
| *Clostridium perfringens* (2) | 0.8 | 0.8 | 0.8 | 0.8 |

* a 50% and 90%, MIC at which 50 and 90% of the strains, respectively, were inhibited.

The combination of teichomycin and gentamicin against methicillin-susceptible and -resistant isolates is shown in Fig. 1. There was clear synergy against both types of organisms. With teichomycin alone, there was a notable difference between the number of CFU of the methicillin-resistant isolates versus the methicillin-susceptible isolates at 24 h. There was only a 1-log decline in the number of CFU of the methicillin-resistant isolate, but a 5-log decrease in the number of CFU of the methicillin-susceptible isolate.

When gentamicin and teichomycin were tested against various gram-positive species, an additive effect was found for half of the *L. monocytogenes* isolates. However, no synergy was found when a fractional inhibitory concentration index of <0.5 was used for synergy and a fractional inhibitory concentration index of 0.5 to 1 was used to define an additive effect. Synergy of teichomycin and gentamicin was found for 42% of *S. aureus* isolates and 33% of *S. epidermidis* isolates, but not for *S. faecalis* or *S. faecium* isolates.

This study substantiates earlier data (3) on the activity of teichomycin against both streptococcal and staphylococcal species. These results are similar to those published since this manuscript was submitted (6). We saw the same differences between inhibition and killing of *S. faecalis* and *S. faecium* as have others, and we found that this was also true for *L. monocytogenes*. There are no references to an inoculum effect on MBCs in earlier studies, but this effect is rather minor compared with that seen with β-lactam antibiotics. The synergy of teichomycin and gentamicin seen in this study was similar to that seen for the combination of vancomycin and

TABLE 3. Effect of human serum on teichomycin MICs and MBCs

| Organism       | Mueller-Hinton MIC | Human serum MIC |  |
|----------------|--------------------|-----------------|---|
| *S. aureus* 3993 | 0.4                | 0.1             | 0.8 |
| *S. aureus* 3540 | 0.4                | 0.1             | 6.3 |
| *S. aureus* 4435 | 0.4                | 0.1             | 6.3 |
| *S. epidermidis* 3120 | 0.2               | 0.1             | 3.1 |
| *S. epidermidis* 3724 | 0.2               | 0.1             | 3.1 |
| *S. epidermidis* 4011 | 0.8               | 0.1             | 12.5 |

* a MICs and MBCs are given in micrograms per milliliter.

* b Normal human serum was added at 50%.

FIG. 1. Comparative killing effect of teichomycin and gentamicin separately and in combination against methicillin-susceptible (A) and methicillin-resistant (B) *S. aureus* isolates. Teichomycin and gentamicin were added separately to Mueller-Hinton broth at concentrations four times their MICs and together at one-fourth their MICs. Samples were plated at the indicated times. Incubation took place at 35°C in a rotary shaker bath. ●, Control; ▲, teichomycin (3.2 µg/ml); △, gentamicin (1.6 µg/ml); □, teichomycin plus gentamicin.
gentamicin against certain gram-positive species. Overall, these results are encouraging, and if the reports that teichomycin can be administered intramuscularly are substantiated, this drug will be an excellent addition to our armamentarium, coming at a time when staphylococcal and enterococcal infections have shown a major resurgence.

LITERATURE CITED
1. Bardone, M. R., M. Paternoster, and C. Coronelli. 1978. Teichomycins, new antibiotics from Actinoplanes teichomyceticus nov. sp. J. Antibiot. 31:170–177.
2. Berti, M., R. Pallanza, B. P. Goldstein, and V. Arloli. 1982. Teichomycin A2, a new antibiotic from Actinoplanes: activity in vitro and in vivo, p. 342–343. In P. Periti and G. Grassi (ed.). Current chemotherapy immunotherapy, vol 1. American Society for Microbiology, Washington, D.C.
3. Cynamon, M. H., and P. A. Granato. 1982. Comparison of the in vitro activities of teichomycin A2 and vancomycin against staphylococci and enterococci. Antimicrob. Agents Chemother. 21:504–505.
4. Everett, E. D., T. C. Eckhoff, and R. H. Simon. 1976. Cerebrospinal fluid shunt infections with anaerobic diphtheroids. J. Neurosurg. 44:580–584.
5. Facklam, R. R. 1980. Streptococci and aerococci, p. 88–110. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.). Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
6. Fauststein, V., B. LeBlanc, and G. P. Bodey. 1983. Comparative in vitro study of teichomycin A2. Antimicrob. Agents Chemother. 23:497–499.
7. Gill, V. J., C. Manning, M. Lameon, P. Woltering, and P. A. Pizzo. 1981. Antibiotic-resistant group JK bacteria in hospitals. J. Clin. Microbiol. 13:472–477.
8. Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of human Staphylococcus species. J. Clin. Microbiol. 1:82–88.
9. Riley, P. S., D. G. Hollis, G. B. Utter, R. E. Weaver, and C. N. Baker. 1979. Characterization and identification of 95 diphtheroid (group JK) cultures isolated from clinical specimens. J. Clin. Microbiol. 9:418–424.
10. Somma, S., and L. Gastaldo. 1982. Mechanism of action of teichomycin A2, a new antibiotic, p. 343–345. In P. Periti and G. Grassi (ed.). Current chemotherapy immunotherapy. American Society for Microbiology, Washington, D.C.
11. Sorrell, T. C., D. R. Packham, S. Shanber, M. Goldes, and R. Mauro. 1982. Vancomycin therapy for methicillin-resistant Staphylococcus aureus. Ann. Intern. Med. 97:344–350.
12. Thompson, R. L., I. Cabezudo, and R. P. Wenzel. 1982. Epidemiology of nosocomial infections caused by methicillin-resistant Staphylococcus aureus. Ann. Intern. Med. 97:309–316.
13. Wade, J. C., S. C. Schimpff, K. A. Neilson, and P. H. Wenil. 1982. Staphylococcus epidermidis: an increasing cause of infection in patients with granulocytopenia. Ann. Intern. Med. 97:509–515.