Journal of Antimicrobial Chemotherapy (1987) **19**, 659–662

Teicoplanin, vancomycin, rifampicin: in-vivo and in-vitro studies with *Staphylococcus aureus*

Holliday T. Carper, Gail W. Sullivan, Gerald L. Mandell

Division of Infectious Disease, Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908, U.S.A.

Groups of mice infected with $3{\cdot}3 \times 10^8$ *Stapyhlococcus aureus* via the tail vein were treated three days later with rifampicin (13 mg/kg), vancomycin (33 mg/kg), or teicoplanin (33 mg/kg). Rifampicin was the most effective agent (28 out of 29 survivors). Vancomycin and teicoplanin were of equivalent efficacy (21 of 29 and 24 of 29 survivors, respectively).

When intraleucocytic staphylococci were incubated with rifampicin (1 or 20 mg/l), vancomycin (100 mg/l), or teicoplanin (100 mg/l), rifampicin was the most active drug. Vancomycin and teicoplanin were similar.

Introduction

Teicoplanin is a glycopeptide antibiotic derived from *Actinoplanes teicomyceticus* and is chemically related to vancomycin. It has potent in-vitro activity against Gram-positive organisms and has slightly greater activity against *S. aureus* than vancomycin. Teicoplanin has a longer half-life than vancomycin, can be administered both intravenously and intramuscularly, and may be less toxic than vancomycin (Glupczinsky *et al*, 1986).

Studies were undertaken to compare the in-vivo efficacy of teicoplanin with rifampicin and vancomycin using a mouse model of intravenously induced sub-acute *S. aureus* infection. In addition, an in-vitro system was used to determine whether teicoplanin was able to kill intraleucocytic *S. aureus*.

Methods

Assays of MIC and MBC for rifampicin (Sigma Chemical Co., St. Louis, Mo.), teicoplanin (Merrell Dow Pharmaceuticals, Inc., Cincinnati, Ohio) and vancomycin (Sigma Chemical Co., St. Louis, Mo.) were performed by a serial dilution technique in Mueller Hinton broth with an inoculum of 10^5 cfu/ml (*S. aureus*, Wood 46, ATCC 10832). The MIC was defined as the lowest concentration of antibiotic in the first clear tube after 24 h incubation at 37°C. The MBC was defined as the lowest concentration of antibiotic resulting in no growth in a 0·1 ml subculture sample plated on blood agar (Table I). Standard 24 h time-kill curves were performed for rifampicin, vancomycin, and teicoplanin (Schoenknecht, Sabath & Thornsberry, 1985). Concentrations of the antibiotics used were: rifampicin 10 mg/l, vancomycin 20 mg/l, and teicoplanin 10 mg/l.

0305–7453/87/050659+04 $02.00/0

Male mice (30–35 g, strain ICR/Dom, Dominion Laboratories, Dublin Virginia) were injected with $3{\cdot}3 \times 10^8$ cfu Wood 46 *S. aureus* in 0·1 ml 0·9% saline via the tail vein. Infection by this route results in deep visceral infections with abscesses in liver, kidney, lungs and spleen (Lobo & Mandell, 1972). Three days after inoculation, mice were randomly divided into groups, and intraperitoneal injections of antimicrobial agents were given. Each mouse received either 0·4 mg rifampicin, 1·0 mg vancomycin hydrochloride, or 1·0 mg teicoplanin. The doses were similar to those recommended for paediatric administration (by weight) and those employed in previous studies of the therapy of experimental staphylococcal infections in mice (Lobo & Mandell, 1972). Antimicrobial agents were administered once daily for ten days. Control groups remained untreated. Cages were coded and in accordance with policies of our animal experimentation committee, mice judged by a veterinarian to be moribund (unresponsive and unable to walk, eat or drink) were killed.

Intraleucocytic activity of the antibiotics was studied in an in-vitro assay. Human neutrophils were obtained from heparinized venous blood by ficoll-hypaque separation. Approximately 10^7 neutrophils were tumbled with 10^7 cfu *S. aureus* (Wood 46 strain) for 60 min. Non-phagocytosed bacteria were removed by centrifugation at 150 **g** for 5 min and decanting the supernatant fluid. The neutrophils with phagocytosed bacteria ($7{\cdot}1 \times 10^5$ cfu/ml) were resuspended with either rifampicin (1 or 20 mg/l), teicoplanin (100 mg/l), or vancomycin (100 mg/l), in 1 ml Media 199 and then tumbled for 2 h with the antibiotics. At the end of this incubation period, the samples were centrifuged at 150 **g** for 5 min. The sediments were washed twice with Hank's Balanced Salt Solution and lysed with sterile water. Supernatants and sediments were serially diluted and plated to determine counts of viable cell-associated and cell-free bacteria.

Results

The MICs and MBCs are shown in Table I. Time-kill studies showed that the percentages of *S. aureus* killed at 8 h were as follows: rifampicin 69·4%, vancomycin 46·0%, and teicoplanin 11·4%. At 24 h, the percentages killed were: rifampicin 94·6%, vancomycin 94·2%, and teicoplanin 67·6%.

Table I. Intraleucocytic activity of antibiotics [a]

Antibiotic	MIC (mg/l)	MBC (mg/l)	*S. aureus*/ml in supernatants	*S. aureus*/ml in sediments
None	...	...	$3{\cdot}72 \pm 1{\cdot}14 \times 10^4$	$2{\cdot}40 \pm 1{\cdot}23 \times 10^5$
Vancomycin 100 mg/l	1·17	12·5	$1{\cdot}26 \pm 1{\cdot}35 \times 10^4$*	$2{\cdot}00 \pm 1{\cdot}15 \times 10^5$
Teicoplanin 100 mg/l	0·78	25·0	$3{\cdot}39 \pm 1{\cdot}35 \times 10^4$	$1{\cdot}66 \pm 1{\cdot}26 \times 10^5$
Rifampicin 1 mg/l	0·0078	0·031	$5{\cdot}89 \pm 1{\cdot}48 \times 10^3$*	$8{\cdot}91 \pm 1{\cdot}55 \times 10^4$*
Rifampicin 20 mg/l	0·0078	0·031	$1{\cdot}95 \pm 2{\cdot}19 \times 10^1$*	$5{\cdot}37 \pm 1{\cdot}26 \times 10^4$*

[a]Human neutrophils were incubated with *S. aureus* Wood 46 for 60 min resulting in $7{\cdot}1 \times 10^5$ cell associated *S. aureus*/ml. Preparations were then incubated with antibiotics for 2 hours. Results are reported as geometric means *S. aureus*/ml ± S.E.M.

*P value vs. control $< 0{\cdot}05$, Student t-test.

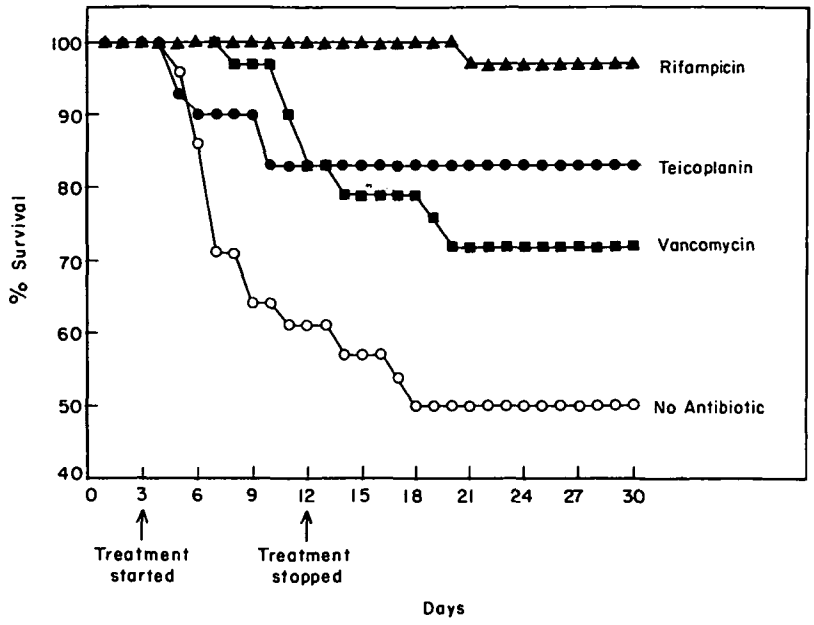

Figure 1. Effect of antibiotics on survival of mice infected with staphylococci. One hundred and twenty mice were injected intravenously with $3{\cdot}3 \times 10^8$ cfu *S. aureus* (Wood 46). On the third day, the surviving 115 mice were divided into four treatment groups. Groups of 29 mice were treated with either rifampicin (0·4 mg/mouse), teicoplanin (1·0 mg/mouse), or vancomycin (1·0 mg/mouse) daily for ten days; 28 mice were left untreated. Rifampicin therapy and teicoplanin therapy were significantly more effective than no therapy ($P<0{\cdot}05$, chi-square).

The figure 1 shows the results of antibiotic therapy of infected mice. At 30 days survival was as follows: 28 of 29 rifampicin treated animals; 24 of 29 teicoplanin treated animals; 21 of 29 vancomycin treated animals; and 14 of 28 untreated animals. Survival of rifampicin treated mice at 30 days was significantly greater than survival of untreated mice ($P=0{\cdot}001$; chi-square). Survival after vancomycin therapy was not significantly greater than for untreated mice ($P=0{\cdot}162$). Therapy with teicoplanin was significantly more effective than no treatment ($P=0{\cdot}017$) but was not significantly different from vancomycin treatment ($P=0{\cdot}50$).

At the time of death from infection or at 30 days, the heart, lungs, liver and kidneys of the animals were cultured by swab. Only one of 29 rifampicin treated mice was positive for *S. aureus*, 20 of 29 vancomycin treated mice were positive, and four of 29 teicoplanin treated mice were positive ($P=0{\cdot}001$ for teicoplanin vs. vancomycin). None of the isolates was resistant to the antibiotics used.

When neutrophils that had ingested *S. aureus* were exposed to antibiotic for 2 h following phagocytosis, only rifampicin (1 or 20 mg/l) significantly reduced the number of viable intraleucocytic staphylococci ($P=0{\cdot}019$ and $P=0{\cdot}006$, respectively, paired *t*-test) (Table I).

Discussion

Teicoplanin has excellent antistaphylococcal activity (Williams & Gruneberg, 1984), and is a possible alternative candidate to vancomycin. Since Fietta *et al.* (1986) reported that teicoplanin, but not vancomycin, was able to enhance killing of

staphylococci ingested by neutrophils, we examined intraleucocytic bactericidal activity. Rifampicin was very active, confirming previous observations (Mandell & Vest, 1972) but neither vancomycin nor teicoplanin exhibited significant intraleucocytic bactericidal activity.

As a further test of the efficacy of teicoplanin, we used a previously employed mouse model of deep visceral staphylococcal infection (Lobo & Mandell, 1972), and found that rifampicin was clearly superior to both teicoplanin and vancomycin. Vancomycin and teicoplanin were not statistically different from each other in this model and supported our in-vitro observations of equivalent intraleucocytic activity. The time-kill study indicates that rifampicin was most rapidly bactericidal followed by vancomycin and then teicoplanin.

However there were more survivors, albeit not significantly, in the teicoplanin treated group than in the vancomycin treated group and significantly fewer positive organ cultures. This may be related to the longer half-life of teicoplanin. Pallanza *et al* (1983) using a subcutaneous dose of teicoplanin or vancomycin for mice of 50 mg/kg (we used 33 mg/kg) found blood levels of 112 mg/l at 4 h and 0·9 mg/l at 72 h for teicoplanin compared to 5 mg/l at 2 h and 3 mg/l at 4 h for vancomycin. Lobo & Mandell (1972) found that 4 h after injection with rifampicin (13 mg/kg) serum levels were 20 mg/l and 1 mg/l at 24 h. In humans, the teicoplanin plasma terminal half-life is 48 h compared with 7 h for vancomycin (Williams & Gruneberg, 1984). The longer half-life of teicoplanin may explain its relative efficacy in this model.

Acknowledgements

This study was supported in part by a grant from Merrell Dow Pharmaceuticals, Inc.

References

Fietta, A., Bersani, C., De Rose, V., Grassi, F. M. & Grassi, G. G. (1986). The effect of teicoplanin on leukocytic activity and intraleukocytic micro-organisms. *Journal of Hospital Infection* **7**, *Suppl. A*, 57–63.

Glupczynski, Y., Lagast, H., Van der Auwera, P., Thys, J. P., Crokaert, F., Yourassowsky, E., *et al.* (1986). Clinical evaluation of teicoplanin for therapy of severe infections caused by gram-positive bacteria. *Antimicrobial Agents and Chemotherapy* **29**, 52–7.

Lobo, M. C. & Mandell, G. L. (1972). Treatment of experimental staphylococcal infection with rifampin. *Antimicrobial Agents and Chemotherapy* **2**, 195–200.

Mandell, G. L. & Vest, T. K. (1972). Killing of intraleukocytic *Staphylococcus aureus* by rifampin: in-vitro and in-vivo studies. *Journal of Infectious Diseases* **125**, 486–90.

Pallanza, R., Berti, M., Goldstein, B. P., Mapelli, E., Randisi, E., Scotti, R., *et al.* (1983). Teichomycin: in-vitro and in-vivo evaluation in comparison with other antibiotics. *Journal of Antimicrobial Chemotherapy* **11**, 419–25.

Schoenknecht, F. D., Sabath, L. D. & Thornsberry, C. (1985). Susceptibility tests: special tests. In *Manual of Clinical Microbiology*, 4th edn (Lennette, E. H., Balows, A., Hauser, W. J. Shadomy, H. J., Eds), pp. 1000–8. American Society for Microbiology, Washington D.C.

Williams, A. H. & Gruneberg, R. N. (1984). Teicoplanin. *Journal of Antimicrobial Chemotherapy* **14**, 441–5.

(Manuscript accepted 22 November 1986)