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# In Vivo Activities of U-100592 and U-100766, Novel Oxazolidinone Antimicrobial Agents, against Experimental Bacterial Infections

CHARLES W. FORD,* JUDITH C. HAMEL, DEBBIE M. WILSON, JUDY K. MOERMAN, DOUGLAS STAPERT, ROBERT J. YANCEY, JR., DOUGLAS K. HUTCHINSON, MICHAEL R. BARBACHYN, AND STEVEN J. BRICKNER

*Pharmacia & Upjohn Inc., Kalamazoo, Michigan 49001*

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**The Upjohn oxazolidinones, U-100592 and U-100766, are orally bioavailable synthetic antimicrobial agents with spectra of activity against antibiotic-susceptible and -resistant gram-positive pathogens. In several mouse models of methicillin-resistant *Staphylococcus aureus* infection, U-100592 and U-100766 yielded oral 50% effective doses ($ED_{50}$) ranging from 1.9 to 8.0 mg/kg of body weight, which compared favorably with vancomycin subcutaneous $ED_{50}$ values of 1.1 to 4.4 mg/kg. Similarly, both compounds were active versus a *Staphylococcus epidermidis* experimental systemic infection. U-100592 and U-100766 effectively cured an *Enterococcus faecalis* systemic infection, with $ED_{50}$ values of 1.3 and 10.0 mg/kg, and versus a vancomycin-resistant *Enterococcus faecium* infection in immunocompromised mice, both drugs effected cures at 12.5 and 24.0 mg/kg. Both compounds were exceptionally active in vivo against penicillin- and cephalosporin-resistant *Streptococcus pneumoniae*, with $ED_{50}$ values ranging from 1.2 to 11.7 mg/kg in systemic infection models. In soft tissue infection models with *S. aureus* and *E. faecalis*, both compounds exhibited acceptable curative activities in the range of 11.0 to 39.0 mg/kg. U-100766 was also very active versus the *Bacteroides fragilis* soft tissue infection model ($ED_{50}$ = 46.3 mg/kg). In combination-therapy studies, both U-100592 and U-100766 were indifferent or additive in vivo against a monomicrobic *S. aureus* infection in combination with other antibiotics active against gram-positive bacteria and combined as readily as vancomycin with gentamicin in the treatment of a polymicrobic *S. aureus-Escherichia coli* infection. U-100592 and U-100766 are potent oxazolidinones active against antibiotic-susceptible and -resistant gram-positive pathogens in experimental systemic and soft tissue infections.**

The discovery of a new, chemically distinct class of antimicrobial agents, the (*S*)-3-aryl-5-acetamidomethyl-2-oxazolidinones, was announced by researchers at E. I. du Pont de Nemours and Co., Inc. in 1987 (16). Two leading compounds, DuP-105 {(*S*)-[(3-(4-(methylsulfinyl)phenyl)-2-oxo-5-oxazolidinyl)methyl]-acetamide} and DuP-721 {(*S*)-[(3-(4-acetylphenyl)-2-oxo-5-oxazolidinyl)methyl]-acetamide}, which were the result of structure-activity relationship research, were described (17). DuP-721 in particular demonstrated potent in vitro and in vivo experimental activities versus gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) (1, 4–6, 15–17), and was both orally and parenterally bioavailable (18, 19). Among many desirable characteristics, the du Pont oxazolidinones possessed a unique mechanism of action among commercially available antimicrobial agents as they inhibited initiation of bacterial protein synthesis (5, 6, 8, 9). As might be expected from a new antimicrobial class of compounds with a unique mechanism of action, the in vitro and in vivo activities of the oxazolidinones were unaffected by existing staphylococcal resistance to commercial antibiotics (4–6, 17) and laboratory efforts aimed at developing resistant mutants by conventional methodology were unsuccessful (4, 6, 17). Clearly, the du Pont oxazolidinone agents hold exciting promise for human antimicrobial therapy, but the compounds have not been developed, reputedly because of toxicity concerns.

Pharmacia & Upjohn Inc. (Kalamazoo, Mich.) initiated an oxazolidinone synthetic effort which most recently culminated in the synthesis of U-100592 {(*S*)-*N*-[[3-[3-fluoro-4[4-(hydroxyacetyl)-1-piperazinyl]-phenyl]-2-oxo-5-oxa-zolidinyl] methyl]-acetamide}, and U-100766 {(*S*)-*N*-[[3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]-methyl]-acetamide} (Fig. 1). The goal of the discovery program was the identification of oxazolidinone antibiotics with acceptable safety profiles and in vitro and in vivo experimental levels of activity roughly equivalent to those of vancomycin. Both U-100592 and U-100766 are completely synthetic antimicrobial agents and are readily prepared by a practical asymmetric synthesis (2). In vitro studies of both compounds have to date demonstrated excellent activities versus antibiotic-susceptible and -resistant staphylococci, enterococci, and streptococci (19). In this report we describe the in vivo antibacterial activities of U-100592 and U-100766 in experimental infections in mice and compare the two oxazolidinone candidates with vancomycin and other antibiotics appropriate to specific experimental infections.

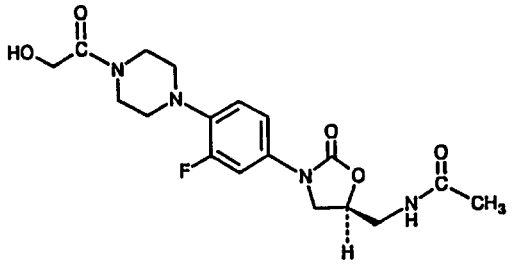
(This study was presented in part at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 1995.)

## MATERIALS AND METHODS

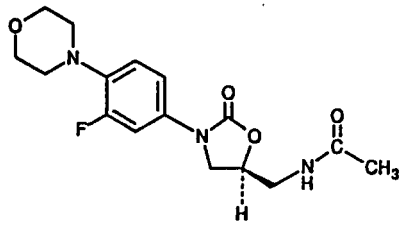
**Antimicrobial agents.** U-100592, a hydroxyacetyl piperazine oxazolidinone, and U-100766, a morpholinyl fluorophenyl biostere of the piperazine subclass, were synthesized at Pharmacia & Upjohn Inc. Clindamycin was obtained internally, and amoxicillin, gentamicin, penicillin G, rifampin, and vancomycin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Aztreonam (E. R. Squibb & Sons, Inc., Princeton, N.J.), cefaclor (Eli Lilly & Co., Indianapolis, Ind.), ciprofloxacin (Miles Inc., West Haven, Conn.), and imipenem-cilastatin (Merck & Co., Inc., West Point, Pa.) were purchased from local commercial sources.

**Bacterial strains.** The bacterial strains employed were all clinical isolates from a variety of hospital laboratories and were maintained in the clinical culture collection of Pharmacia & Upjohn Inc. Bacteria were stored frozen in the vapor phase of a liquid nitrogen freezer in brain heart infusion (BHI) broth (Difco

* Corresponding author. Mailing address: Cancer and Infectious Diseases Research, Pharmacia & Upjohn Inc., 301 Henrietta St., Kalamazoo, MI 49001. Phone: (616) 833-7973. Fax: (616) 385-6492.



U-100592



U-100766

FIG. 1. Structures of the oxazolidinones U-100592 and U-100766.

Laboratories, Detroit, Mich.) which contained 20% (vol/vol) glycerol (Sigma Chemical Co.).

**In vitro tests.** MICs were determined by agar dilution or a broth microdilution methodology, corresponding to the protocol of the National Committee for Clinical Laboratory Standards (13). MICs for streptococcal strains were determined in Mueller-Hinton broth (Difco) supplemented with divalent cations and containing 5% lysed horse blood (Becton Dickinson Co., Cockeysville, Md.). MIC determinations for anaerobic bacteria were conducted with Wilkins-Chalgren agar (Difco) (14). Serial twofold dilutions were used for all determinations of MICs, and the 128 dilution scale was utilized throughout.

**In vivo tests.** Tests to determine the 50% effective doses ($ED_{50}$s) of U-100592 and U-100766 were conducted as follows. Briefly, CF1 female mice (Harlan Sprague-Dawley, Indianapolis, Ind.) weighing 19 to 21 grams were injected intraperitoneally with sufficient bacteria to kill 100% of the untreated animals for all methicillin-sensitive *S. aureus* and MRSA strains, all *Streptococcus pneumoniae* strains, *Streptococcus pyogenes* UC152, *Escherichia coli* UC9451, and *Klebsiella pneumoniae* UC12081. In all cases, except those to determine 50% lethal doses ($LD_{50}$), six mice per group were used. C3H/HeN female mice (Charles River Laboratories, Raleigh, N.C.) weighing 12 to 14 grams were utilized in the tests with MRSE UC12084, *Enterococcus faecalis* UC12379, *Enterococcus faecium* UC15090, and *Moraxella catarrhalis* UC15095. Thawed bacterial cultures were suspended in BHI broth which contained 4 to 8% (wt/vol) dried brewer's yeast (Champlain Industries Inc., Clifton, N.J.) The exception was the experiment with *S. pyogenes*, for which only BHI broth was used as the suspending fluid. The infecting inoculum (0.2 ml) was adjusted to yield approximately 100 times the $LD_{50}$. Concurrently with each trial, the challenge $LD_{50}$ was validated by inoculating untreated animals with log dilutions of the bacteria. Five dosage levels representing a 5-log-unit dilution range were employed per antibiotic determination, with 10 mice utilized at each level. Mice were dosed with antibiotic at 1 and 5 h postinfection for intraperitoneally injected lethal systemic infections. A mortality rate of 90 to 100% was produced in all groups of untreated mice with the 100× $LD_{50}$ challenge inoculum.

Oxazolidinones were formulated in water or saline, with gentle heating at higher concentrations, and were administered orally, subcutaneously (s.c.), or intravenously (i.v.) at 1 and 5 h postinfection. Other antibiotics were formulated by following the manufacturer's directions. At least five dosage levels of antibiotic utilizing serial twofold dilutions were employed for each $ED_{50}$ determination. One treatment group of six mice was used for each antibiotic dosage level. Deaths in each group following infection and treatment were monitored daily for at least 6 days. Following this observation period, cumulative mortality figures were used to calculate by probit analysis the amount of drug in milligrams per kilogram of body weight per dose required to protect 50% of the lethally infected mice.

For experiments using the *E. faecium* model, C3H/HeN mice were rendered neutropenic by two intraperitoneal injections of 200 mg of cyclophosphamide (Sigma Chemical Co.) per kg of body weight separated by an interval of 40 h. Mice were infected intraperitoneally with *E. faecium* 14 h following the second cyclophosphamide dose. In the neutropenic mouse model, antibiotic was administered 1 and 5 h postinfection by oral intubation and twice a day thereafter (8:00 a.m. and 4:00 p.m.) for 4 days.

Tests with soft tissue infections and abscesses were conducted as previously described (10). Briefly, CF1 female mice weighing 19 to 21 grams were injected s.c. in the inguinal region with $2 \times 10^6$ *S. aureus* UC9271 organisms contained in 0.2 ml of a 50:50 (vol/vol) mixture of BHI broth and Cytodex-1 (Sigma Chemical Co.) microcarrier beads. In soft tissue infection experiments, antibiotic was administered immediately following the infecting dose, approximately 8 h later, and twice a day for four subsequent days at 8:00 a.m. and 4:00 p.m. Oxazolidinones were given orally, as was clindamycin; vancomycin was given s.c. Animals were sacrificed on day 6, and the soft tissue or abscess contents were excised sterilely and were liberally swabbed onto BHI agar (Difco). Untreated control infections typically had $2 \times 10^8$ *S. aureus* organisms on day 6 following infection. The plates were incubated at 35°C for 18 h. The appearance of any *S. aureus* colonies was taken as a treatment failure. The $ED_{50}$, or the amount of drug in milligrams per kilogram of body weight per dose required to microbiologically cure 50% of the soft tissue infections or abscesses, was calculated by probit analysis. In each test, a control antibiotic treatment $ED_{50}$ determination and non-antibiotic-treated control were employed to assure test validity and adequate bacterial replication in the abscesses. Soft tissue infection experiments conducted with *E. faecalis* UC15060, with an infecting inoculum containing $4.8 \times 10^7$ viable organisms, were conducted in the same fashion.

The *Bacteroides fragilis* soft tissue infection was established similarly with $3 \times 10^8$ viable *B. fragilis* UC12199 organisms contained in a 0.2-ml solution of prereduced Schaedler broth (Difco) and 50% (vol/vol) Cytodex-1 microcarrier beads (Sigma) as the infecting inoculum. Antibiotic treatment followed the same scheme as that of the *S. aureus* regimen except that a 6-day therapy was conducted, with microbiological sampling on day 7. Soft tissue infection contents were swabbed liberally onto prereduced Wilkins-Chalgren agar (Difco) and incubated anaerobically for 48 h. Control antibiotic $ED_{50}$ determinations with clindamycin were conducted in each test, and an untreated control group assured a 2- to 3-log-unit increase in *B. fragilis* numbers in each experiment.

*S. aureus* UC15083 was used as a representative MRSA isolate against which to test combination antibiotic therapy in vivo. Fixed antibiotic combinations were established by first determining the $ED_{50}$ of each antibiotic alone against UC15083 and then combining the two drugs at the ratio of their $ED_{50}$s. $ED_{50}$ determinations were then performed with the combined drugs.

Combination drug therapy was also undertaken for a polymicrobic *S. aureus* UC15081 (MRSA and gentamicin resistant)-*E. coli* UC9451 infection in mice. The object of this work was to determine if the oxazolidinones in combination with a gram-negative agent would successfully eradicate the mixed infection. The UC15081 strain was selected because it would be susceptible only to the gram-positive active antibiotic partner, not the aminoglycoside, thereby requiring that both antibiotics be effective in the infection to achieve a cure. The infection was established as previously noted except that the infecting milieu contained $2.5 \times 10^7$ UC15081 organisms and $2.5 \times 10^4$ UC9451 organisms (the 100× $LD_{50}$ of each bacterium). Fixed antibiotic combinations for testing in this model were established on the basis of the ratio of $ED_{50}$s of each antibiotic against *S. aureus* and *E. coli.*

## RESULTS

**Effect of route of administration of U-100592 or U-100766 on efficacy.** The $ED_{50}$s of U-100592 and U-100766 against *S. aureus* UC9213 (MRSA) are contained in Table 1. The compounds were dosed s.c., orally, or i.v. and directly compared with vancomycin in each test. Vancomycin was chosen as the comparator drug in these studies because of its spectrum of activity, which is similar to those of the oxazolidinones, and the wealth of human experience with vancomycin and gram-positive infections. Because of vancomycin's oral inactivity, orally dosed U-100592 and U-100766 were compared with s.c. dosed vancomycin. Via s.c. administration U-100592, U-100766, and vancomycin yielded $ED_{50}$s of 0.9, 2.0, and 3.9 mg/kg, respectively. Changing the route of administration to oral yielded an $ED_{50}$ for U-100592 of 1.9 mg/kg and an $ED_{50}$ of 5.6 mg/kg for U-100766. Administration i.v. yielded an $ED_{50}$ of 5.8 mg/kg for both U-100592 and U-100766. Vancomycin administered i.v. exhibited an $ED_{50}$ of 1.1 mg/kg. U-100592 and U-100766 given by either an oral or s.c. route of administration compared very favorably with s.c. administered vancomycin. Both compounds

TABLE 1. $ED_{50}$s of U-100592 and U-100766 against *S. aureus* UC9213

| Route | Compound | MIC (μg/ml) | $ED_{50}$ (mg/kg/day)[a] |
|---|---|---|---|
| s.c. | U-100592 | 4.0 | 0.9 (0.01–2.3) |
| s.c. | U-100766 | 4.0 | 2.0 (0.9–2.6) |
| s.c. | Vancomycin | 1.0 | 3.9 (2.5–6.4) |
| Oral | U-100592 | 4.0 | 1.9 (1.4–3.8) |
| Oral | U-100766 | 4.0 | 5.6 (2.9–8.5) |
| s.c. | Vancomycin | 1.0 | 3.9 (2.5–6.4) |
| i.v. | U-100592 | 4.0 | 5.8 (3.6–12.2) |
| i.v. | U-100766 | 4.0 | 5.8 (3.4–9.6) |
| i.v. | Vancomycin | 1.0 | 1.1 (1.1–2.8) |

[a] $ED_{50}$ is the amount of antibiotic in milligrams per kilogram of body weight per day needed to cure 50% of the infected animals. Numbers in parentheses are 95% confidence ranges.

were somewhat less active than vancomycin by the i.v. route of administration.

**Efficacies of U-100592 and U-100766 versus staphylococcal pathogens.** U-100592 and U-100766 were tested in vivo versus nine additional strains of *S. aureus* (Table 2). Both compounds displayed activities roughly equivalent to those of vancomycin versus methicillin-susceptible strains UC9271 and UC12454 (a quinolone-resistant strain). The $ED_{50}$s for U-100592 were 6.9 and 5.6 mg/kg, respectively, and those for U-100766 were 2.9 and 3.7 mg/kg. The oxazolidinones yielded evidence of in vivo experimental activities versus seven MRSA strains, with $ED_{50}$s ranging from 1.9 to 8.0 mg/kg for U-100592 and from 2.8 to 15.0 mg/kg for U-100766. Similarly, the $ED_{50}$s for the control compound, vancomycin, ranged from 1.5 to 7.0 mg/kg. U-100592 and U-100766 also displayed antibacterial activities versus an MRSE in vivo model (UC12084) with $ED_{50}$s of 1.9 and 4.7 mg/kg, while that of vancomycin was 1.8 mg/kg. Clearly, both U-100592 and U-100766 possess in vivo experimental activities versus MRSA strains which are comparable to those of vancomycin.

TABLE 2. $ED_{50}$s of U-100592 and U-100766 against staphylococcal infections

| Bacterium | Compound[a] | MIC (μg/ml) | $ED_{50}$ (mg/kg/day)[b] |
|---|---|---|---|
| *S. aureus* UC9271[c] | U-100592 | 4.0 | 6.9 (3.8–10.8) |
| | U-100766 | 4.0 | 2.9 (1.8–4.9) |
| | Vancomycin | 1.0 | 13.2 (7.9–32.5) |
| *S. aureus* UC12454[c] | U-100592 | 2.0 | 5.6 (3.1–12.3) |
| | U-100766 | 2.0 | 3.7 (3.2–6.6) |
| | Imipenem-cilastatin | 0.25 | 0.2 (0.1–9.9) |
| *S. aureus* UC6685[d] | U-100592 | 1.0 | 2.8 (1.4–4.2) |
| | U-100766 | 2.0 | 3.8 (2.2–5.6) |
| | Vancomycin | 2.0 | 2.6 (1.4–5.0) |
| *S. aureus* UC15081[d] | U-100592 | 8.0 | 4.0 (2.5–6.4) |
| | U-100766 | 4.0 | 2.8 (1.8–4.4) |
| | Vancomycin | <0.5 | 2.0 (1.8–4.5) |
| *S. aureus* UC15082[d] | U-100592 | 4.0 | 2.0 (1.0–2.9) |
| | U-100766 | 4.0 | 5.7 (3.5–8.8) |
| | Vancomycin | 1.0 | 2.5 (1.4–3.5) |
| *S. aureus* UC15083[d] | U-100592 | 4.0 | 5.0 (2.9–7.0) |
| | U-100766 | 4.0 | 7.0 (3.9–11.1) |
| | Vancomycin | 1.0 | 3.2 (1.8–4.5) |
| *S. aureus* UC15084[d] | U-100592 | 4.0 | 6.3 (4.0–10.0) |
| | U-100766 | 4.0 | 2.9 (1.8–4.4) |
| | Vancomycin | 1.0 | 4.4 (2.5–6.3) |
| *S. aureus* UC12673[d] | U-100592 | 4.0 | 8.0 (3.1–13.3) |
| | U-100766 | 4.0 | 15.0 (9.8–17.0) |
| | Vancomycin | 1.0 | 7.0 (4.9–8.0) |
| *S. aureus* UC15080[d] | U-100592 | 0.5 | 1.9 (0.5–2.1) |
| | U-100766 | 0.5 | 3.8 (2.2–5.6) |
| | Vancomycin | 1.0 | 1.5 (0.8–2.6) |
| *S. epidermidis* UC12084[e] | U-100592 | 0.5 | 1.9 (1.1–2.8) |
| | U-100766 | 1.0 | 4.7 (3.1–7.8) |
| | Vancomycin | 2.0 | 1.8 (1.1–3.0) |

[a] U-100592 and U-100766 were administered orally. Vancomycin and imipenem-cilastatin were administered s.c.
[b] $ED_{50}$ is the amount of antibiotic in milligrams per kilogram of body weight per day required to cure 50% of the infected animals. Numbers in parentheses are 95% confidence ranges.
[c] Methicillin-susceptible strain.
[d] Multidrug-resistant MRSA.
[e] Multidrug-resistant MRSE.

TABLE 3. $ED_{50}$s of U-100592 and U-100766 against streptococcal and enterococcal infections

| Bacterium | Compound[a] | MIC (μg/ml) | $ED_{50}$ (mg/kg/day)[b] |
|---|---|---|---|
| *S. pyogenes* UC152 | U-100592 | 1.0 | 5.1 (2.8–7.0) |
| | U-100766 | 2.0 | 5.0 (3.6–17.4) |
| | Clindamycin | 0.6 | 8.6 (6.3–12.0) |
| *S. pneumoniae* UC9207[c] | U-100592 | 0.5 | 11.7 (6.4–16.9) |
| | U-100766 | 2.0 | 2.5 (1.4–3.6) |
| | Amoxicillin | 4.0 | 3.4 (2.0–4.9) |
| *S. pneumoniae* UC15062[d] | U-100592 | <0.25 | 1.2 (0.2–2.1) |
| | U-100766 | 0.5 | 2.8 (1.2–3.7) |
| | Amoxicillin | 1.9 | 6.9 (3.8–10.8) |
| *S. pneumoniae* UC15088[e] | U-100592 | 0.5 | 2.0 (0.8–2.6) |
| | U-100766 | 1.0 | 2.7 (1.8–4.4) |
| | Penicillin G | 4.0 | ≥20.0 (ND) |
| | Cefaclor | >32.0 | ≥30.0 (ND) |
| *S. pneumoniae* UC15087[e] | U-100592 | 0.5 | 1.8 (0.9–2.6) |
| | U-100766 | 0.5 | 3.8 (2.3–5.5) |
| | Penicillin G | 8.0 | >20.0 (ND) |
| | Cefaclor | >32.0 | >20.0 (ND) |
| *E. faecalis* UC12379[f] | U-100592 | 2.0 | 1.3 (0.9–2.2) |
| | U-100766 | 4.0 | 10.0 (6.2–19.5) |
| | Vancomycin | 1.0 | 0.5 (0.3–0.8) |
| *E. faecium* UC15090[g] | U-100592 | 2.0 | 12.5 (8.7–42.4) |
| | U-100766 | 4.0 | 24.0 (16.3–62.7) |
| | Vancomycin | >64.0 | >100 (ND) |

[a] Antibiotics were administered orally, except vancomycin and penicillin G, which were administered s.c.
[b] $ED_{50}$ is the amount of antibiotic in milligrams per kilogram of body weight per day required to cure 50% of the infected animals. Numbers in parentheses are 95% confidence ranges. ND, not determined.
[c] Penicillin-resistant strain.
[d] Clindamycin-resistant strain.
[e] Penicillin-cephalosporin-resistant strain.
[f] Aminoglycoside-resistant strain.
[g] Vancomycin-resistant strain.

**Efficacies of U-100592 and U-100766 versus streptococcal and enterococcal pathogens.** Both U-100592 and U-100766 were active in vivo versus *S. pyogenes* UC152 (Table 3) at levels at least equivalent to that seen with clindamycin. Interestingly, both compounds were active in vivo versus four *S. pneumoniae* strains. Versus *S. pneumoniae* UC9207, the $ED_{50}$ of U-100766 was equivalent to that of amoxicillin while U-100592 yielded a somewhat higher value. Both compounds appeared only slightly more active than amoxicillin versus clindamycin-resistant *S. pneumoniae* UC15062 in vivo, and both maintained consistently low in vivo $ED_{50}$s when tested against penicillin- and cephalosporin-resistant *S. pneumoniae* UC15088 and UC15087. With the possible exception of the 11.7-mg/kg $ED_{50}$ obtained with U-100592 against UC9207, both oxazolidinones were consistent performers in vivo versus *S. pneumoniae*, displaying low $ED_{50}$s. Additionally, the oxazolidinones were ac-

TABLE 4. $ED_{50}$s of U-100592 and U-100766 against gram-negative infections

| Bacterium | Compound[a] | MIC (μg/ml) | $ED_{50}$ (mg/kg/day)[b] |
|---|---|---|---|
| *E. coli* UC9451 | U-100592 | 256 | >200 |
| | U-100766 | 32 | 80 |
| | Ciprofloxacin | 0.03 | 0.4 |
| *K. pneumoniae* UC12081 | U-100592 | >64.0 | >20.0 |
| | U-100766 | >64.0 | >20.0 |
| | Ciprofloxacin | 0.06 | 5.0 |
| *M. catarrhalis* UC15095 | U-100592 | 4.0 | 40.0 |
| | U-100766 | 4.0 | >50.0 |
| | Cefaclor | 2.0 | 7.6 |

[a] Antibiotics were administered orally.
[b] $ED_{50}$ is the amount of antibiotic in milligrams per kilogram of body weight per day required to cure 50% of the infected animals. Ninety-five percent confidence ranges are not reported.

TABLE 5. $ED_{50}$s of U-100592 and U-100766 against soft tissue infections

| Bacterium | Compound | MIC (μg/ml) | $ED_{50}$ (mg/kg)[a] |
|---|---|---|---|
| *S. aureus* UC9271[b] | U-100592 | 4.0 | 12.5 |
| | U-100766 | 4.0 | 39.0 |
| | Vancomycin | 1.0 | 4.7 |
| *E. faecalis* UC15060[c] | U-100592 | 2.0 | 20.6 |
| | U-100766 | 4.0 | 11.0 |
| | Vancomycin | 2.0 | 16.3 |
| *B. fragilis* UC12199[c] | U-100592 | 16.0 | >100 |
| | U-100766 | 4.0 | 46.3 |
| | Clindamycin | 1.0 | 200 |

[a] $ED_{50}$ is the amount of antibiotic in milligrams per kilogram of body weight required to eradicate bacteria from 50% of the abscesses.
[b] *S. aureus* infections were dosed immediately upon infection. Then, animals were dosed twice daily for 4 days. Antibiotic was administered orally, except vancomycin (administered s.c.).
[c] *E. faecalis* and *B. fragilis* infections were dosed immediately upon infection. Animals were dosed twice daily for 6 days. Antibiotic was administered orally, except vancomycin (administered s.c.).

tive in vivo versus two strains of enterococci. Against aminoglycoside-resistant *E. faecalis* UC12379, U-100592 displayed promising experimental activity ($ED_{50}$ = 1.3 mg/kg) compared with vancomycin, with its $ED_{50}$ of 0.5 mg/kg, and U-100766 was definitely less active ($ED_{50}$ = 10.0 mg/kg). Versus vancomycin-resistant *E. faecium* UC15090, both oxazolidinones were curative while vancomycin was ineffective at the highest dose tested. The values of 12.5 mg/kg for U-100592 and 24.0 mg/kg for U-100766 might result in part from the fact that this experiment was conducted with neutropenic mice.

**Efficacies of U-100592 and U-100766 against gram-negative pathogens.** The oxazolidinones displayed virtually no in vivo activity versus three gram-negative bacterial infections in vivo (Table 4), consistent with MIC determinations. U-100592 was inactive versus *E. coli* UC9451 ($ED_{50}$ > 200 mg/kg), and U-100766 demonstrated only minimal activity, with an $ED_{50}$ of 80.0 mg/kg. The control antibiotic, ciprofloxacin, yielded an $ED_{50}$ of 0.4 mg/kg. Neither oxazolidinone was active versus *K. pneumoniae* UC12081, while the ciprofloxacin $ED_{50}$ was 5 mg/kg. U-100592 exhibited only slight activity versus *M. catarrhalis*, with an $ED_{50}$ of 40.0 mg/kg, compared with the $ED_{50}$ of cefaclor of 7.6 mg/kg. U-100766 was inactive ($ED_{50}$ > 50.0 mg/kg).

**Efficacies of U-100592 and U-100766 against soft tissue infections.** Results of $ED_{50}$ determinations for U-100592 and U-10076 versus three soft tissue infections in mice are contained in Table 5. U-100592 displayed promising activity against the *S. aureus* soft tissue infection, with an $ED_{50}$ of 12.5 mg/kg, and this result compared quite favorably to the measured vancomycin activity ($ED_{50}$ = 4.7 mg/kg). U-100766 was modestly active ($ED_{50}$ = 39.0 mg/kg). In the soft tissue infection caused by *E. faecalis* UC15060, the efficacies of both oxazolidinones were equivalent to that of vancomycin. The $ED_{50}$s were 20.6 mg/kg for U-100592, 11.0 mg/kg for U-100766, and 16.3 mg/kg for vancomycin. Lastly, when tested versus the *B. fragilis* UC12199 soft tissue infection, U-100592 was inactive at the highest dose tested ($ED_{50}$ > 100 mg/kg) while U-100766 performed slightly better ($ED_{50}$ = 46.3 mg/kg) than the positive control drug clindamycin ($ED_{50}$ = 200 mg/kg).

**Combination antibiotic studies with a monomicrobic MRSA infection.** U-100592 and U-100766 were tested in vivo in combination with vancomycin, imipenem-cilastatin, gentamicin, or rifampin in the treatment of a monomicrobic MRSA systemic infection (Tables 6 and 7). In these tests, U-100592 combined with vancomycin, imipenem-cilastatin, gentamicin, and rifampin in the treatment of the MRSA infection yielded combination $ED_{50}$s ranging from 1.1 to 8.2 mg/kg (Table 6). The combination therapy appeared to behave indifferently or in a simple additive manner; that is, the combination therapy yielded $ED_{50}$s quite similar to those seen with the individual antibiotics. Those observations appeared to hold true for the U-100766 combination studies, in which observed $ED_{50}$s ranged from 1.2 to 8.6 mg/kg (Table 7). While these tests are not at all exhaustive, they do indicate that both oxazolidinones could be evaluated for use in combination therapy in humans.

**Combination antibiotic studies with the polymicrobic *S. aureus-E. coli* infection.** Combination therapy studies with U-100592 or U-100766 and gram-negative active antibiotics were undertaken versus a polymicrobic *S. aureus-E. coli* experimental infection (Table 8). In this infection model, both the gram-positive and the gram-negative bacterial components of the infection must be treated in order to observe a cure. The vancomycin-gentamicin combination cured the mixed infection very readily, with an $ED_{50}$ of 8.0 mg/kg, as did the U-100592–gentamicin and U-100766–gentamicin combination therapies, which yielded $ED_{50}$s of 10.0 and 5.6 mg/kg, respectively. The *S. aureus* strain employed in this study was gentamicin resistant, thus ensuring that the gentamicin antibiotic component did not contribute to the cure of the *S. aureus* partner in the infection. Similarly, both oxazolidinones and vancomycin in combination

TABLE 6. Combination treatment of MRSA[a] infections with U-100592 and antibiotics active against gram-positive bacteria

| Treatment | $ED_{50}$ (mg/kg)[b] |
|---|---|
| U-100592 | 5.6 |
| Vancomycin | 5.0 |
| U-100592–vancomycin | 8.2[c] |
| U-100592 | 2.0 |
| Imipenem[d] | 2.3 |
| U-100592–imipenem[d] | 3.2[c] |
| U-100592 | 4.4 |
| Gentamicin | 0.9 |
| U-100592–gentamicin | 1.3[c] |
| U-100592 | 8.2 |
| Rifampin | 0.07 |
| U-100592–rifampin | 1.1[c] |

[a] *S. aureus* UC15083, a highly methicillin-resistant strain.
[b] The amount of drug ($ED_{50}$) in milligrams per kilogram required to cure 50% of the infected animals when given at 1 and 5 h postinfection.
[c] The $ED_{50}$ of combined drugs. The ratio of the two drugs was 1:1 except in the case of rifampin, for which the U-100592-to-rifampin ratio was 4:1.
[d] Imipenem-cilastatin.

TABLE 7. Combination treatment of MRSA[a] infections with U-100766 and antibiotics active against gram-positive bacteria

| Treatment | $ED_{50}$ (mg/kg)[b] |
|---|---|
| U-100766 | 7.5 |
| Vancomycin | 10.0 |
| U-100766–vancomycin | 7.6[c] |
| U-100766 | 5.0 |
| Imipenem[d] | 10.0 |
| U-100766–imipenem[d] | 8.6[c] |
| U-100766 | 6.8 |
| Gentamicin | 6.6 |
| U-100766–gentamicin | 5.9[c] |
| U-100766 | 7.0 |
| Rifampin | 0.08 |
| U-100766–rifampin | 1.2[c] |

[a] *S. aureus* UC15083, a highly methicillin-resistant strain.
[b] The amount of drug ($ED_{50}$) in milligrams per kilogram required to cure 50% of the infected animals when given at 1 and 5 h postinfection.
[c] The $ED_{50}$ of combined drugs. The ratio of the two drugs was 1:1 except in the case of rifampin, for which the U-100766-to-rifampin ratio was 4:1.
[d] Imipenem-cilastatin.

with aztreonam readily effected cures of the mixed infection, delivering $ED_{50}$s of 5.6 mg/kg for U-100766–aztreonam, 12.0 mg/kg for U-100592–aztreonam, and 3.7 mg/kg for vancomycin-aztreonam. Aztreonam is devoid of *S. aureus* antibacterial activity, demonstrating in a similar fashion that U-100592 and U-100766 adequately dealt with the gram-positive component of the mixed infection.

## DISCUSSION

The resistant staphylococci, streptococci, and enterococci cause both hospital and community infections, and an ideal antibiotic with which to treat such infections would be active both orally and i.v. The oxazolidinones U-100592 and U-100766 are effective in our mouse models of MRSA infection by oral and i.v. routes of administration. Consistent with the oral efficacies of the two oxazolidinones in infectious disease models, rat pharmacokinetic evaluations revealed a peak level in blood of 7.8 μg/ml with a 20-mg/kg oral dose of U-100766, while the peak level seen with U-100592 was 2.3 μg/ml with a 25-mg/kg dose (11, 12). Studies directed at carefully defining the relationship between efficacies in mouse models and the pharmacokinetic and pharmacodynamic parameters of the two compounds are under way. The activities of U-100592 and U-100766 in vivo versus MRSA and MRSE experimental infections demonstrate that they are comparable to vancomycin's in vivo activity and that these oxazolidinones are therefore worthy candidates for clinical evaluation.

U-100592 and U-100766 also possess in vivo activities in our models with resistant and susceptible *S. pneumoniae*, *E. faecalis*, and *E. faecium*. Versus *S. pneumoniae*, the oral $ED_{50}$s ranged from 1.2 to 3.8 mg/kg, with a single outlying value of 11.7 mg/kg. Both compounds were effective against penicillin- and penicillin- and cephalosporin-resistant *S. pneumoniae* in vivo. These compounds hold promise as clinical candidates for the treatment of *S. pneumoniae* infection. Both compounds effectively treated the vancomycin-susceptible *E. faecalis* infection, although U-100766 was less active than U-100592. The vancomycin-resistant *E. faecium* model was a more difficult test for U-100592 and U-100766 in that the host mice were immunocompromised, but both antibiotics did effect cures when administered in the range of 12.5 to 24 mg/kg. While our experience with U-100592 and U-100766 against the enterococci is limited, the oral activities of these two compounds in vivo are promising.

U-100592 and U-100766 also possessed oral activities versus soft tissue infections with *S. aureus*, *E. faecalis*, and *B. fragilis*. This is of particular note for the staphylococci, which are extremely common causes of soft-tissue infections. The values we obtained against *S. aureus*, 12.5 to 50 mg/kg, fall within an acceptable range for antibiotics active against staphylococci in our soft tissue infection models and argue that the soft tissue infection activities of these compounds should be evaluated in the clinic. Of particular note, both U-100592 and U-100766 were comparable to vancomycin in microbiologically curing the *E. faecalis* soft tissue infection model, which reinforces our view that such activities are quite promising. U-100766 did possess activity against *B. fragilis* in vivo, but whether that translates into utility for humans very much remains to be seen.

Antibiotic combination therapy is commonly being used in the treatment of MRSA infections (7) as well as of polymicrobic MRSA and *E. coli* infections (3). Our efforts to evaluate oxazolidinones in combination with antibiotics active against gram-positive bacteria in limited studies indicated that U-100592 and U-100766 might not be significantly antagonistic in vivo in combination with representative members of other drug classes of interest. The results suggested that the oxazolidinones in combination with vancomycin, imipenem-cilastatin, gentamicin, and rifampin yielded little evidence of antagonism and might possibly have additive effects, although that was not specifically demonstrated. Similarly, U-100592 and U-100766 behaved equivalently to vancomycin in combination studies with gentamicin and aztreonam when tested against a mixed gram-positive and gram-negative infection.

U-100592 and U-100766 belong to a new antimicrobial class, the oxazolidinones. As antimicrobial agents, they are active in mouse models of human infections by the oral, i.v., or s.c. route of administration. The oxazolidinones hold particular promise for meeting the needs for new antibiotics active against sensitive and resistant staphylococci, streptococci, and enterococci and in our testing efforts demonstrated impressive in vivo activities in mouse models of such infections. Additionally, both compounds effected cures when tested in soft tissue infection models in mice and both of them readily combined with existing antimicrobial agents in experimental therapies for MRSA and a polymicrobic gram-positive-and-gram-negative infection model. These oxazolidinones, at least on an experimental level, possess several desirable characteristics for a new class of compounds with promise for the treatment of problematic gram-positive infections. Both U-100592 and U-100766 are nearing

TABLE 8. Combination treatment of a polymicrobic MRSA UC15081-*E. coli* UC9451 infection with U-100592 or U-100766 and antibiotics active against gram-negative bacteria

| Drug combination[a] | $ED_{50}$[b] |
|---|---|
| Vancomycin-gentamicin | 8.0 |
| U-100592–gentamicin | 10.0 |
| U-100766–gentamicin | 5.6 |
| Vancomycin-aztreonam | 3.7 |
| U-100592–aztreonam | 12.0 |
| U-100766–aztreonam | 5.6 |

[a] None of the drugs listed was capable of curing the infection when used as monotherapy; $ED_{50}$s were >20 mg/kg. The ratio of vancomycin, U-100592, or U-100766 to the other drug was 1:1 for the gentamicin combinations and 5:1 for the aztreonam combinations.
[b] $ED_{50}$ is the amount of drug (in milligrams per kilogram) in combination required to cure 50% of the infected animals when given at 1 and 5 h postinfection. *S. aureus* UC15081 is gentamicin resistant.

completion of phase I human clinical studies, and phase II studies are planned.

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