

## In Vivo Efficacy of a Novel Oxazolidinone Compound in Two Mouse Models of Infection<sup>∇</sup>

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**A novel oxazolidinone, AM 7359, was evaluated in two mouse models of *Staphylococcus aureus* infection. AM 7359 and linezolid were equally efficacious in a methicillin-susceptible *S. aureus* organ burden model and a methicillin-resistant *S. aureus* localized infection model. However, AM 7359 was eightfold more efficacious than linezolid against a linezolid- and methicillin-resistant *S. aureus* strain in this localized (thigh) infection model.**

The emerging resistance of *Staphylococcus aureus* and *Enterococcus* spp. to presently available antibiotics has reduced the options for antimicrobial therapy (3, 5, 7).

The oxazolidinones are a new therapeutic class of drugs that inhibit the initiation of protein synthesis (8, 16). The widely used oxazolidinone linezolid (Zyvox) is active against most gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* spp., and penicillin-resistant *Streptococcus pneumoniae* (14, 15). However, linezolid is limited by its inhibition of monoamine oxidase and the potential for myelosuppression (2, 11; linezolid package insert [Pharmacia]).

AM 7359 is a novel oxazolidinone which exhibits improved efficacy and activity against a broader spectrum of organisms, including *Haemophilus influenzae* and *Moraxella catarrhalis* (for which the MIC<sub>90</sub>s are 2 and 0.5 µg/ml, respectively) (10). We report here the preliminary evaluation of AM 7359 against methicillin-susceptible *S. aureus* (MSSA) strain Smith, MRSA strain COL, and linezolid-resistant MRSA (LMRSA) isolates in two mouse models of infection. In vitro MICs of AM 7359 and linezolid for these isolates were determined by procedures recommended by the Clinical and Laboratory Standards Institute (4).

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Efficacy in the organ burden model was assessed by intraperitoneally challenging C3H mice (Harlan Laboratories, Indianapolis, IN) with  $2 \times 10^3$  CFU of MSSA Smith suspended in 0.5 ml of 5% hog gastric mucin. Within 15 min of challenge, AM 7359 and linezolid were given as single doses either orally (p.o.) by gavage or intravenously (i.v.). One day after challenge, paired kidneys from euthanized mice were removed aseptically, weighed, and placed in sterile Whirl-Pak bags (Fisher Scientific, Springfield, NJ) containing 5 ml of sterile saline. Kidneys were homogenized in the bags and serially diluted in saline, and aliquots were plated onto mannitol salt

agar plates (BBL, Cockeysville, MD). Plates were incubated at 35°C for 48 h, and the numbers of CFU of the challenge organism were determined.

In the localized infection model, 0.2 ml of Trypticase soy broth containing  $3.6 \times 10^8$  CFU of MRSA COL was injected intramuscularly into the right thighs of DBA/2 mice (Taconic Laboratories, Germantown, NY). These mice are deficient in complement component 5, which renders them more susceptible to challenge with MRSA. For p.o. dosing, the first antibiotic treatment was administered 2 h after challenge. Mice

TABLE 1. Comparative efficacies of AM 7359 and linezolid against MSSA Smith in an organ burden assay

| Compound (mode of administration) and MIC (µg/ml) | Dose (mg/kg/day) | % Mortality <sup>a</sup> | Log CFU/g of kidney <sup>b</sup> (% eradication) | Reduction (SD) <sup>c</sup> |
|---|------------------|--------------------------|--|-----------------------------|
| AM 7359 (p.o.),<br>0.125                          | 25               | 0                        | 3.11* (0)  | <b>5.23 (0.58)</b>          |
|   | 12.5             | 0                        | 5.41* (0)  | <b>2.93 (0.59)</b>          |
|   | 6.25             | 0                        | 5.71* (0)  | <b>2.63 (0.80)</b>          |
|   | 3.12             | 0                        | 8.60 (0)   | −0.26 (0.37)                |
| Linezolid (p.o.),<br>2.0                          | 25               | 0                        | 2.27* (100)                                      | <b>6.07 (0.04)</b>          |
|   | 12.5             | 0                        | 5.63* (0)  | <b>2.71 (0.37)</b>          |
|   | 6.25             | 0                        | 6.90* (0)  | <b>1.44 (0.59)</b>          |
|   | 3.12             | 0                        | 8.87 (0)   | −0.53 (0.10)                |
| AM 7359 (i.v.),<br>0.125                          | 25               | 0                        | 3.81* (0)  | <b>4.59 (0.15)</b>          |
|   | 12.5             | 0                        | 3.94* (0)  | <b>4.46 (0.16)</b>          |
|   | 6.25             | 0                        | 6.37* (0)  | <b>2.03 (0.61)</b>          |
|   | 3.12             | 0                        | 8.34 (0)   | 0.06 (0.15)                 |
| Linezolid (i.v.),<br>2.0                          | 25               | 0                        | 4.13* (0)  | <b>4.27 (0.70)</b>          |
|   | 12.5             | 0                        | 6.96* (0)  | <b>1.44 (1.62)</b>          |
|   | 6.25             | 0                        | 8.52 (0)   | −0.12 (0.20)                |
|   | 3.12             | 0                        | 8.67 (0)   | −0.27 (0.11)                |
| Control (distilled H <sub>2</sub> O; p.o.)        |                  | 20                       | 8.34 (0)   |                             |
| Control (distilled H <sub>2</sub> O; i.v.)        |                  | 0                        | 8.40 (0)   |                             |

<sup>a</sup> Calculated as the number dead divided by the total number in the group.

<sup>b</sup> \*, significant reduction compared to sham-treated control as determined by Student's *t* test.

<sup>c</sup> Reduction in log number of CFU compared to sham-treated control. Significant reductions are shown in bold. SD, standard deviation of results for the four mice per group.

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TABLE 2. ED<sub>99</sub> of AM 7359 and linezolid in mouse efficacy models

| Model (organism)           | ED <sub>99</sub> (mg/kg) (95% CI) of: |                 |               |               |
|----------------------------|---------------------------------------|-----------------|---------------|---------------|
|                            | AM 7359                               |                 | Linezolid     |               |
|                            | p.o.                                  | i.v.            | p.o.          | i.v.          |
| Kidney (MSSA) <sup>a</sup> | 4.9 (3.1–7.6)                         | 6.3 (4.2–9.5)   | 7.0 (5.3–9.2) | 12 (7.8–18.5) |
| Thigh (MRSA) <sup>b</sup>  | 4.2 (2.8–6.1)                         | 5.6 (2.3–∞)     | 5.2 (4.2–6.6) | 6.9 (4.5–∞)   |
| Thigh (LMRSA)              | 10.2 (4.3–24)                         | NT <sup>c</sup> | 85 (50.2–∞)   | NT            |

<sup>a</sup> Values are based on mice receiving a single dose 15 min postchallenge; therefore, ED<sub>99</sub> values are equal to the total mg/kg/day given each mouse.

<sup>b</sup> Orally dosed mice received a total of eight doses in 5 days and i.v. dosed mice a total of four doses in 24 h; therefore, the ED<sub>99</sub> values represent the value of each individual dose given in each model.

<sup>c</sup> NT, not tested.

were treated again at 6, 10, 24, 48, 72, 96, and 120 h postinfection (eight doses in 5 days). Due to difficulty encountered in multiple i.v. dosing in the lateral tail veins, the MRSA-challenged mice treated i.v. received only the first four antibiotic doses (0.2 ml at 2, 6, 10, and 24 h post challenge). C3H mice were challenged with 10<sup>9</sup> CFU of LM RSA/thigh, and compounds were administered only p.o. as described above. Two days after the final antibiotic dose, mice were euthanized and denuded thighs were surgically removed, placed into tubes containing 4 ml of phosphate-buffered saline, and ground with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Serial dilutions in saline were plated onto mannitol salt agar, the plates were incubated at 35°C for 48 h, and the numbers of CFU of the organisms per thigh were determined.

The mean log<sub>10</sub> numbers of CFU of the organisms per gram of kidneys or per thigh from the drug-treated groups were compared to those from the sham-treated control by using Student's *t* test (two-tailed and unpaired) on Microsoft Excel. Comparisons were deemed significant at a level of  $\alpha$  of 0.05. Inverse regression was subsequently used for both tests to estimate 99% effective doses (ED<sub>99</sub>) defined as the doses (in milligrams per kilogram of body weight) that reduced the number of CFU per organ by 99% compared to the number for the sham-treated control (9, 12).

In the organ burden model, p.o. or i.v. treatment with AM 7359 or linezolid at 25 or 12.5 mg/kg significantly reduced

kidney burdens relative to those of sham-treated mice (Table 1). While both compounds were effective in lowering the bacterial tissue burden, renal sterilization was seen only at the 25-mg/kg p.o. dose of linezolid. Based on the ED<sub>99</sub>, AM 7359 was equivalent to linezolid in efficacy against the MSSA strain in this model when administered p.o. (ED<sub>99</sub>, 4.9 versus 7.0 mg/kg, respectively) or i.v. (ED<sub>99</sub>, 6.3 versus 12 mg/kg) (Table 2).

In the localized MRSA infection model, AM 7359 and linezolid administered p.o. were equally efficacious at 10 mg/kg (decrease, log<sub>10</sub> ~3.7 CFU) and 5 mg/kg (decrease, log<sub>10</sub> ~2.0 CFU) (Fig. 1). When given i.v., AM 7359 and linezolid reduced the burden by approximately log<sub>10</sub> 2.4 and 1.5 CFU at 10 and 5 mg/kg, respectively, compared to the burden in the sham-treated control. The ED<sub>99</sub> of AM 7359 and linezolid in these localized assays are listed in Table 2. The better efficacy of approximately log<sub>10</sub> 1 CFU achieved when these compounds were administered p.o. rather than i.v. may be explained by the difference in dosing regimens or perhaps the greater time of exposure to the antibiotics, with only four doses given in the i.v. assay compared to eight doses (four in the first 24 h) in the p.o. assay. As others have shown previously, the best pharmacokinetics or pharmacodynamics parameter to correlate with the efficacy of this class of compounds against *S. aureus* is the ratio of the area under the concentration-time curve to the MIC (1). Interestingly, the greater in vitro activity of AM 7359 did not result in better efficacy than that of linezolid in these models. While the normalized areas under the concentration-time curves obtained at a 25 mg/kg dose were similar, 1.16 and 0.78  $\mu\text{g} \cdot \text{h/ml}$  for AM 7359 and linezolid, respectively, the levels of plasma protein binding of these two compounds were significantly different, with 19 and 68% free compound for AM 7359 and linezolid, respectively. This difference in protein binding was likely enough to bridge the difference in MICs and thereby equalize the ED<sub>99</sub> data. Other factors such as lower bioavailability, 30% for AM 7359 versus 100% for linezolid, and a lower plasma clearance rate may also play a role in the discrepancy between these in vitro and in vivo results.

In the LM RSA thigh infection model, the in vitro activity seen with AM 7359 did translate into good in vivo efficacy (Fig. 2). While a reduction of log<sub>10</sub> 2.5 CFU by linezolid at 100

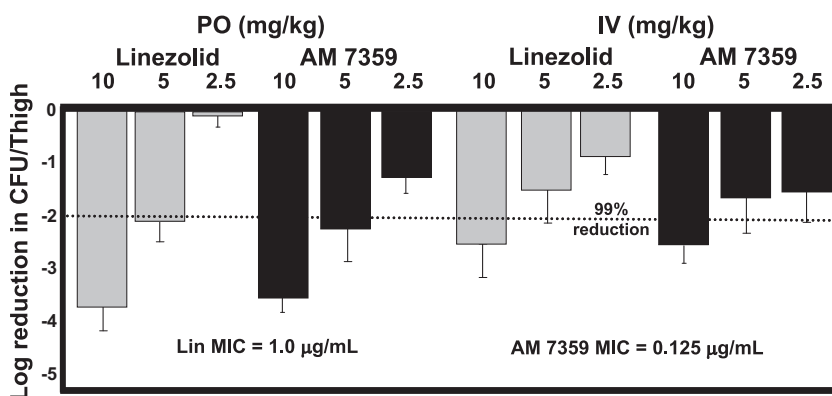


FIG. 1. Efficacy of AM 7359 and linezolid (Lin) against an MRSA COL strain in a localized infection model. DBA/2 mice (five per group) were challenged intramuscularly with  $3.6 \times 10^8$  CFU of MRSA in a 0.2-ml inoculum and treated p.o. three times a day for 1 day and once a day for 5 days or i.v. three times a day for 1 day and once a day for 5 days.

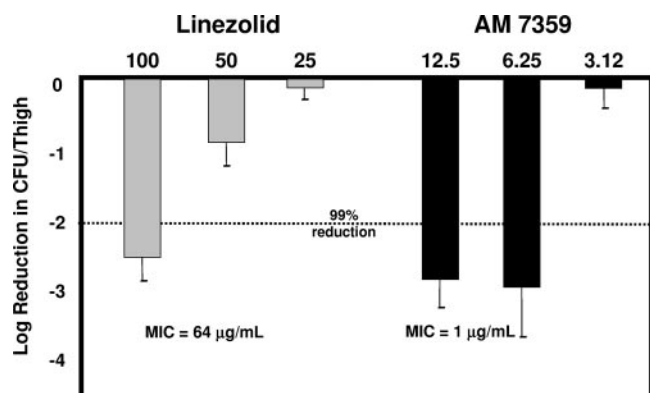


FIG. 2. Efficacy of AM 7359 and linezolid against an LMRSA strain in a localized infection model. C3H mice (five per group) were challenged intramuscularly with  $10^9$  CFU of LMRSA in a 0.2-ml inoculum and treated p.o. three times a day for 1 day and once a day for 5 days.

mg/kg compared to the sham-treated control was observed, the same or slightly better efficacy was seen for AM 7359 at 12.5 and 6.25 mg/kg. AM 7359 was eightfold ( $ED_{99}$  of 10.2 versus 85 mg/kg) more efficacious than linezolid against this LMRSA strain. To date, the complete mode of action of this series of compounds has not been elucidated. The activity against this LMRSA strain may indicate a different or supplemental biological target for AM 7359. This difference would allow AM 7359 to be used to treat infections caused by linezolid-resistant organisms.

MRSA and vancomycin-resistant *Enterococcus* infections in hospitals and, more recently, community-acquired MRSA infections have resulted in increased mortality and costs (6, 13, 17). Even with the approval of three new antibacterials, quinupristin-dalfopristin, linezolid, and daptomycin, there is still a medical need for agents to treat the multidrug-resistant pathogens. AM 7359 is an oxazolidinone compound which exhibits a broad in vitro spectrum of activity against gram-positive organisms, including MRSA, vancomycin-resistant *Enterococcus* spp., and penicillin-resistant *Streptococcus pneumoniae*, and is also active against some gram-negative respiratory tract pathogens. These properties, along with the efficacy exhibited by AM 7359 against three different *S. aureus* strains, including a linezolid-resistant strain, in two mouse models of bacterial infection, warrant further evaluation of this compound series.

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