

## Pharmacodynamics of Ceftazidime plus the Serine $\beta$ -Lactamase Inhibitor AM-112 against *Escherichia coli* Containing TEM-1 and CTX-M-1 $\beta$ -Lactamases

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**A strain of *Escherichia coli* containing TEM-1 and CTX-M-1 was tested in an in vitro pharmacokinetic model against ceftazidime with and without AM-112, a serine  $\beta$ -lactamase inhibitor. Ceftazidime alone was less effective than ceftazidime plus AM-112, and a single dose was more effective than three fractionated doses.**

AM-112 is one of a series of oxapenam serine  $\beta$ -lactamase inhibitors which has good activity against a range of  $\beta$ -lactamases, including extended-spectrum  $\beta$ -lactamases (3, 4). It is more active than clavulanic acid against class C and D  $\beta$ -lactamases but, like clavulanic acid, it has no activity against class B metallo- $\beta$ -lactamases. The minimal inhibitory concentrations (MICs) for aerobic gram-negative rods with known enzymes are lowered by a combination of ceftazidime plus AM-112 compared to ceftazidime alone, and this difference is also reflected with mouse models.

A dose-response effect has been noted for AM-112, with the greatest MIC reductions being with the highest AM-112 concentrations (D. M. Livermore, L. Tysall, M. Warner, and I. N. Simpson, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-380, 2001). Little is known of the pharmacokinetics of AM-112, but it has been found to have human serum protein binding of 11 to 13% and it is not absorbed by experimental animals. The half-life of AM-112 in rats and mice is around 10 min, which is similar to that of ceftazidime (L. O. White, L. Brevik, R. Owens, I. Simpson, and M. Cavaleri, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-386, 2001).

The aim of the present study was to assess the antibacterial effect of ceftazidime alone and in combination with AM-112 against a  $\beta$ -lactamase-producing *Escherichia coli* and to assess the impact of different inocula and of dose fractionation of AM-112 on its activity. Preliminary findings on the activity of AM-112 have been reported previously (K. E. Bowker, A. R. Noel, T. R. Walsh, and A. P. MacGowan, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-1159, 2003). Although human pharmacokinetic data for AM-112 are not available, we elected to model ceftazidime and AM-112 at a 1:1 ratio and assumed that the half-life of AM-112 in serum was similar to that of ceftazidime, as is the case with animals.

Ceftazidime was obtained from Eli Lilly & Co., Basingstoke, United Kingdom. AM-112 was supplied by SynProTec Ltd., Manchester, United Kingdom. Experiments were performed with *E. coli* containing a *bla* TEM-1 and CTX-M-1 gene, for which both  $\beta$ -lactamases are plasmid encoded, from the collection of the Department of Pathology and Microbiology, University of Bristol. L1  $\beta$ -lactamase as previously described (8) was used to neutralize AM-112 and ceftazidime.

The ceftazidime MIC was determined by using British Society for Antimicrobial Chemotherapy guidelines (1), except that 10% Mueller-Hinton broth was used with fixed concentrations of 4 and 16 mg of AM-112/liter with an inoculum of approximately  $10^6$  CFU/ml.

The in vitro pharmacokinetic model as previously described (5) was used for all kill curve experiments. The  $10^6$  CFU/ml inoculum was prepared by adding 720  $\mu$ l of overnight broth culture of the test strain to the culture chamber and allowing the model to run for 45 min. The larger inoculum of  $10^8$  CFU/ml was prepared by inoculating the culture chamber with 2 ml of a 0.5 McFarland standard equivalent suspension of the test strain and allowing the model to run overnight (18 h) so that a steady state could be achieved.

The pharmacokinetics simulated for both ceftazidime and AM-112 were a maximum concentration of 200 mg of serum/liter and a half-life of 2 h. Ceftazidime was added to the central chamber at time zero and at 8 and 16 h. AM-112 was added to the chamber either as one dose at time zero or fractionated into three 8-hourly doses. Samples were collected from the model throughout the 24-h time period (time zero and 1, 2, 3, 4, 5, 6, 7, 8, 16, and 24 h) for assessments of the viable counts and determinations of ceftazidime concentrations by bioassays as previously described (2). The measures of antibacterial effects calculated for each inoculum were the log changes in viable counts at 8, 16, and 24 hours; the median times for the initial inoculum to be reduced by 99.9%; and the areas under the bacterial kill curve from 0 to 24 h (AUBKC24). All experiments were performed in triplicate. Statistical analysis was done by analysis of variance (ANOVA), and model assumptions were assessed graphically. Results are presented as differences in mean responses, with 95% confidence intervals.

The ceftazidime MIC for the *E. coli* was 64 mg/liter alone

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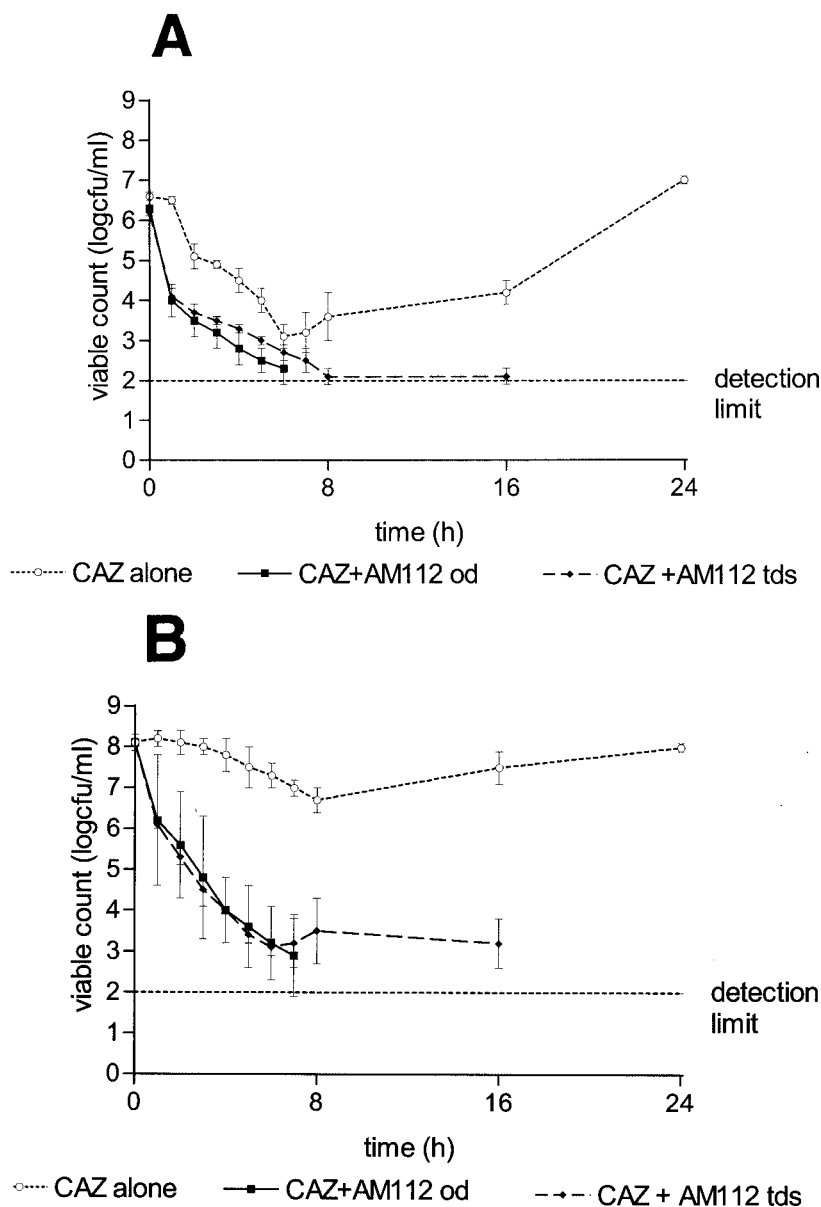


FIG. 1. Antibacterial activity of ceftazidime alone and in combination with AM-112 as a single 24-hourly dose and three divided doses. (A) Inoculum of  $10^6$  CFU/ml; (B) inoculum of  $10^8$  CFU/ml.

and 0.25 mg/liter when AM-112 was present at a fixed concentration of either 4 or 16 mg/liter.

Ceftazidime on its own produced a 3.0 to 3.5  $\log_{10}$  reduction in viable count up to 16 h with the  $10^6$ -CFU/ml inoculum and

a 1.0  $\log_{10}$  reduction in viable count with the  $10^8$ -CFU/ml inoculum. Ceftazidime plus AM-112 resulted in bacterial clearance from the model at 24 h with both inocula and at both AM-112 dosing regimens. The three-times-a-day (TDS) ad-

TABLE 1. Antibacterial effects of ceftazidime alone and in combination with AM-112

Dosing regimen	Inoculum (log CFU/ml)	Change in viable count (log CFU/ml) at:			Time to kill 99.9% inoculum (h)	AUBKC24 (log cfu/ml/h)
		8 h	16 h	24 h		
Ceftazidime	6	-3.0 ± 0.6	-2.4 ± 0.2	0.4 ± 0.1	6	113.1 ± 6.3
Ceftazidime + AM-112 OD	6	-4.2 ± 0.2	-4.3 ± 0.2	-4.3 ± 0.2	3	19.7 ± 3.1
Ceftazidime + AM-112 TDS	6	-3.8 ± 0.3	-4.1 ± 0.3	-4.2 ± 0.1	4	39.5 ± 11.6
Ceftazidime	8	-1.4 ± 0.2	-0.7 ± 0.3	-0.2 ± 0.1	>24	180.1 ± 6.2
Ceftazidime + AM-112 OD	8	-5.5 ± 0.9	-5.0 ± 0.5	-5.9 ± 0.2	2	49.1 ± 37.4
Ceftazidime + AM-112 TDS	8	-4.5 ± 0.8	-4.8 ± 0.6	-4.8 ± 0.6	3	87.8 ± 11.6

TABLE 2. Differences in drug responses for the two inocula

Dosing regimen	Inoculum (log CFU/ml)	Change in viable count (log CFU/ml) at 24 h		AUBKC24	
		Mean (95% confidence interval)	<i>P</i>	Mean (95% confidence interval)	<i>P</i>
AM-112 OD versus ceftazidime	6	-4.7 (-5.24 to -4.16)	<0.001	-93.4 (-107.9 to -78.9)	<0.001
AM-112 TDS versus ceftazidime	6	-4.67 (-5.2 to -4.13)	<0.001	-73.5 (-88.0 to -59.0)	<0.001
AM-112, OD versus TDS	6	0.03 (-0.51 to 0.57)	0.89	19.9 (5.43 to 34.4)	<0.001
AM-112 OD versus ceftazidime	8	-5.87 (-6.40 to -5.33)	<0.001	-152.6 (-168.8 to -136.4)	<0.001
AM-112 TDS versus ceftazidime	8	-4.80 (-5.33 to -4.25)	<0.001	-92.2 (-106.8 to -77.8)	<0.001
AM-112, OD versus TDS	8	1.07 (0.53 to 1.61)	0.001	60.3 (44.0 to 76.4)	<0.001

ministration of AM-112 resulted in a drop in viable count below the detection limit by 16 h; this drop occurred with once-a-day (OD) administration by 8 h (Fig. 1) (Table 1).

Statistical comparisons of the two inocula and the three dosing regimens indicated that there were significant differences in the antibacterial effect between the two inocula and between the three dosing regimens, as measured by AUBKC24 and the changes in the viable counts at 24 h.

The magnitude of the differences between the three dosing regimens also differed between the two inocula (for AUBKC24, the *P* value was <0.001; for the changes in viable counts at 24 h, the *P* value was 0.01) (Table 2). As all dosing regimens involving AM-112 resulted in pathogen clearance by 24 h, the differences in AUBKC24 noted in the OD versus TDS dosage comparisons are due to differences in the viable counts between 0 and 16 h.

These results build on those of Jamieson et al. (3), who showed that ceftazidime plus AM-112 at a ratio of 1:1 reduced the MICs for *Enterobacteriaceae* with extended-spectrum  $\beta$ -lactamases. The drug combination also reduced the 50% effective doses for ceftazidime in a murine intraperitoneal infection model with an *Enterobacter cloacae*-producing class C  $\beta$ -lactamase and *Klebsiella pneumoniae*-producing SHV-5.

Importantly, the effect of AM-112 is not greatly changed by bacterial inocula; in addition, our data indicate that administering AM-112 OD with the ceftazidime is more effective than giving the dose of AM-112 in three divided fractions. This finding is almost certainly related to the greater early AM-112 exposures with this regimen. Surprisingly, the optimal dosing regimens for  $\beta$ -lactam- $\beta$ -lactamase inhibitors based on pharmacodynamic principles are not firmly established (7). However, it is known that serine  $\beta$ -lactamase inhibitor concentrations can be low for periods between doses without reducing the antibacterial effect (9). In addition, it takes some time after  $\beta$ -lactamase inhibitor removal for the  $\beta$ -lactamase enzyme production of bacteria to return to baseline (6). These factors, combined with the AM-112 concentration-dependent reduction of the ceftazidime MIC, may explain the superiority of OD inhibitor administration (D. M. Livermore et al., 41st ICAAC).

In conclusion, the addition of AM-112 to ceftazidime at dynamic drug concentrations increases the antibacterial effect against an *E. coli* strain producing TEM-1 and CTX-M-1 enzymes. Dose fractionation with AM-112 shows that OD administration is best, indicating high early  $\beta$ -lactamase exposure; peak concentration may be an important pharmacokinetic driver for serine  $\beta$ -lactamase inhibitors.

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