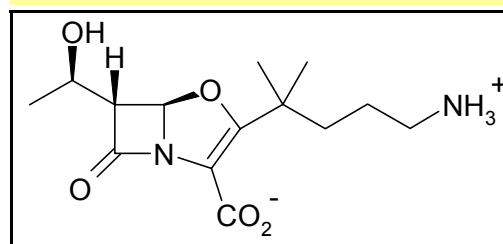


Oxapenem AM-112: *In vivo* efficacy alone and in combination with ceftazidime in animal models of infection, involving pathogens producing Class A and Class C β -lactamasesI N Simpson¹, E Di Modugno², D Jabes³ & D Nicolau⁴¹Micron Research, Cambridge, UK, ²Glaxo SmithKline, Verona, Italy, ³Biosearch Italia, Gerenzano, Italy, ⁴Hartford Hospital, Massachusetts, USAI Simpson
Micron Research
Cambridge CB3 7ES
Tel. +44 (0) 1223 262810
Fax. +44 (0) 1223 262810
iain.simpson@micron-research.com**INTRODUCTION**

Oxapenem AM-112 (Figure 1) is a novel broad-spectrum β -lactamase inhibitor with not only inhibitory activity against both Class A and Class C β -lactamases, but also *in vitro* antimicrobial activity against several genera including *Staphylococcus* spp.

We report on the efficacy of AM-112 alone and in combination with ceftazidime in three animal models of infection involving *Staphylococcus aureus*, *Enterobacter cloacae* P99 (Class C β -lactamase) and *Escherichia coli* SHV-5 (Extended spectrum Class A β -lactamase).

Figure 1. AM-112**METHODS****Antibiotics**

AM-112 was supplied by Amura Ltd, Cambridge, UK

Susceptibility tests

The susceptibility of each bacterial strain to ceftazidime, AM-112, clavulanic acid and combinations of ceftazidime with either AM-112 or clavulanic acid, was determined according to NCCLS guidelines.

***In vivo* infection models**

Experimental details of each model are given under the appropriate section.

Activity against *Staphylococcus aureus* 3816**Methods**

- Groups of ICR mice, 10 per antibiotic dose, were infected with *S.aureus* 3816, a β -lactamase positive methicillin-susceptible isolate, by intraperitoneal injection, on two occasions (Table 1).
- Antibiotics were administered by a single subcutaneous dose 15 minutes post-infection. AM-112 was also administered orally on occasion I.

Table 1. Comparative efficacy of ceftazidime, AM-112 and combinations of ceftazidime and AM-112, *in vitro* and *in vivo*, against *S.aureus* 3816

Antibiotic	MIC (mg/L)	Challenge (cfu)	Route of administration	Occasion	ED ₅₀ (mg/kg)
Ceftazidime	16	3 x 10 ⁷	subcutaneous	I	18.3
		8 x 10 ⁶	subcutaneous	II	22.6
AM-112	1	3 x 10 ⁷	oral	I	28.3
		3 x 10 ⁷	subcutaneous	I	2.7
		8 x 10 ⁶	subcutaneous	II	2.6
Ceftazidime: AM-112 (4:1)	ND*	8 x 10 ⁶	subcutaneous	II	4.8 + 1.2
Ceftazidime: AM-112 (7:1)	ND*	8 x 10 ⁶	subcutaneous	II	8 + 1

* Not determined

Denotes additivity; ie 2 fold reduction in required concentration of both components

Results

- In MIC studies, *S.aureus* 3816 was moderately susceptible to ceftazidime (16 mg/L) but fully susceptible to AM-112 (1 mg/L).
- The ED₅₀ for ceftazidime on both occasions was similar to the MIC.
- The ED₅₀ for AM-112 on both occasions was slightly higher than the MIC, but at least 6-fold lower than determined for ceftazidime. The ED₅₀ for AM-112 was 10-fold higher (28.3 mg/kg) following oral administration.
- The ED₅₀s for 4:1 and 7:1 combinations of ceftazidime and AM-112 were largely determined by the superior activity of the AM-112 component; on both occasions a dose of AM-112 at 1 mg/kg was effective.

Activity against *Enterobacter cloacae* P99**Methods**

- CD1 male mice (20-22g, C. River) were infected by intraperitoneal injection with *E.cloacae* P99, a high level producer of Class C β -lactamase (Table 2).
- Antibiotics were administered intravenously, at 6 dose levels, 1 and 5 hours post-infection to groups of 5 mice per dose level. Survival was scored over the following 4 days.

Table 2. Comparative efficacy of ceftazidime, AM-112, clavulanic acid and combinations of ceftazidime and AM-112 or clavulanic acid, *in vitro* and *in vivo*, against *E.cloacae* P99

Antibiotic	MIC (mg/L)	Challenge (cfu)	Route of administration	ED ₅₀ (mg/kg)
Pilot Expt				
Ceftazidime	>128	2 x 10 ⁷	intravenous	>200
AM-112	16	2 x 10 ⁷	intravenous	20
CAZ+AM-112 (1:1)	2 + 2	2 x 10 ⁷	intravenous	2+2
Secondary Expts				
Ceftazidime	128	2.2 x 10 ⁷	intravenous	>100
CLAV	64	1.6 x 10 ⁷	intravenous	>100
CAZ+CLV (1:1)	128	1.6 x 10 ⁷	intravenous	44+44
CAZ+CLV (4:1)	128	9.1 x 10 ⁷	intravenous	>100+25
Ceftazidime	128	2.2 x 10 ⁷	intravenous	>100
AM-112	32	2.2 x 10 ⁷	intravenous	19
CAZ+AM-112 (1:1)	2 + 2	2.2 x 10 ⁷	intravenous	2+2
CAZ+AM-112 (2:1)	4 + 2	>2.2 x 10 ⁷	intravenous	4+2
CAZ+AM-112 (4:1)	4 + 1	>2.2 x 10 ⁷	intravenous	11 + 3

Denotes synergy; ie \geq 4 fold reduction in required concentration of both components

Results

- E.cloacae* P99 was resistant to ceftazidime (MIC \geq 128 mg/L) and clavulanic acid (MIC 64 mg/L), but did display moderate susceptibility to AM-112 (MIC 16 – 32mg/L). These MIC values were closely reflected in the ED₅₀ values determined for each agent.
- Addition of clavulanic acid did not reduce the ceftazidime MIC. There was a small reduction in the ED₅₀ of each agent when combined in a 1:1 ratio.
- Synergy was seen between ceftazidime and AM-112 in both MIC and ED₅₀ tests.
- In MIC tests, the incorporation of AM-112 as the minor component in 1:1, 2:1 or 4:1 combinations with ceftazidime resulted in at least a 8-fold reduction in AM-112 MIC and at least a 32-fold reduction in ceftazidime MIC. The most potent combination was 2 mg/L each of ceftazidime and AM-112.
- The MIC results for ceftazidime and AM-112 were highly predictive of ED₅₀ values in the animal model; the 1:1 and 2:1 combinations showing at least a 8-fold reduction in the ED₅₀ of individual agents. Even at 4:1 ratio, AM-112 reduced the ceftazidime ED₅₀ from >100 mg/kg to 11 mg/kg.

Activity against *Escherichia coli* SHV-5**Methods**

- Specific-pathogen-free, female ICR mice (Harlan Sprague Dawley, Indianapolis) were infected by intraperitoneal injection with *E.coli* SHV-5, a producer of SHV-5 extended spectrum β -lactamase (ESBL), in 6% (w/v) mucin (Table 3).
- Antibiotics were administered as series of two-fold dilutions (5 mice per dose) of test agent by a subcutaneous dose 1 hour post-infection. Survival was scored over the following 4 days.

Table 3. Comparative efficacy of ceftazidime, AM-112, and combinations of ceftazidime and AM-112, *in vitro* and *in vivo*, against *E.coli* SHV-5

Antibiotic	MIC (mg/L)	Challenge (cfu)	Route of administration	ED ₅₀ (mg/kg)
Ceftazidime	>128	6 x 10 ⁸	subcutaneous	16
AM-112	16	6 x 10 ⁸	subcutaneous	72
CAZ+AM-112 (1:1)	8 + 8	6 x 10 ⁸	subcutaneous	2+2
CAZ+AM-112 (2:1)	16 + 8	6 x 10 ⁸	subcutaneous	2+1

Denotes additivity; ie 2 fold reduction in required concentration of both components

Denotes synergy; ie \geq 4 fold reduction in required concentration of both components

Results

- E.coli* SHV-5 was resistant to ceftazidime (MIC \geq 128 mg/L) but did display moderate susceptibility to AM-112 (MIC 16 mg/L). In either 1:1 or 2:1 combination, the ceftazidime MIC was reduced to 8–16 mg/L and that of AM-112 to 8 mg/L, indicating additivity.
- E.coli* SHV-5 was only poorly virulent, achieving 80% mortality in the antibiotic free controls.
- ED₅₀ values did not reflect the corresponding MICs. The ED₅₀ for ceftazidime (16 mg/kg) was at least 8-fold lower than the MIC, whereas the ED₅₀ for AM-112 was 4-fold higher than the MIC. Despite this unexplained discrepancy, there was marked synergy between ceftazidime and AM-112 at both 1:1 and 2:1 ratios in the ED₅₀ test; the effective dose of both components reducing by at least 8-fold.

CONCLUSIONS

- The broad-spectrum β -lactamase inhibitory profile of oxapenem AM-112, previously demonstrated *in vitro*, has been confirmed in animal models of infection.
- AM-112 protects ceftazidime from inactivation by the Class C β -lactamase from *E.cloacae* P99, a ceftazidime-resistant strain, and restores ceftazidime potency in both MIC tests and an animal model of infection.
- AM-112 acts synergistically with ceftazidime in an animal model against *E.coli* SHV-5, a ceftazidime-resistant strain producing the SHV-5 ESBL.
- AM-112 has superior *in vitro* and *in vivo* activity to ceftazidime against *S.aureus*.
- Not only has AM-112 the potential to restore the antibacterial activity of ceftazidime against isolates with β -lactamase mediated resistance but also to enhance the spectrum of ceftazidime to include *S.aureus*.