

AM-112, a novel enhanced-spectrum oxapenem β -lactamase inhibitor: Oral and parenteral pharmacokinetics, with and without ceftazidime, in rats and mice

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INTRODUCTION

- AM-112 is an oxapenem with broad spectrum inhibitory activity against Class A, C and D β -lactamases and antibacterial activity against Gram-positive bacteria.
- This poster reports the pharmacokinetic profiles of AM-112 in rats and mice, following oral and intravenous administration.

MATERIALS & METHODS

Assay of AM-112

AM-112 levels in serum were assayed by high-performance liquid chromatography (HPLC). The stationary phase was Hypersil 5 ODS (4.6 x 100 mm) and the mobile phase Methanol: water: phosphoric acid (25:74:1) pumped at 1.5 mL/min. Detection was by UV absorption at 280 nm. Frozen samples were thawed on ice and processed within 15 minutes of thawing as follows: Sample (50 to 100 μ L) was mixed with an equal volume of methanol, left on ice for 5-15 minutes then centrifuged (1000 g) to sediment precipitated protein and 10 μ L of supernatant was injected. Quantitation was by external standardisation by peak height, using calibrators prepared in serum.

Assay of ceftazidime

Ceftazidime levels in serum were assayed by HPLC (In-house method of the Regional Antimicrobial Reference Laboratory, Southmead Hospital, UK). The stationary phase was Hypersil 5 ODS (4.6 x 100 mm) and the mobile phase Methanol: water: phosphoric acid (20:79:1) pumped at 1 mL/min. Detection was by UV absorption at 254 nm. Sample (50 to 100 μ L) was mixed with an equal volume of 7% perchloric acid then centrifuged (1000 g) to sediment precipitated protein and 10 μ L of supernatant was injected. Quantitation was by external standardisation by peak height.

Stability at -70°C

Solutions of human serum containing 70, 14 and 1.4 mg/L AM-112 were dispensed in 1 mL amounts and placed at -70°C. Bottles were removed after 7, 14, 21 and 28 days and assayed as described below using serum calibrators freshly prepared on each occasion from a vial of freeze-dried AM-112 containing a known weight of powder.

Serum protein binding of AM-112

Protein binding studies were performed in serum from:-

- Man (UK Blood Transfusion Service)
- Mouse (Sigma and Huntingdon Research Centre [HRC])
- Monkey (Marmoset, HRC)
- Rat (purchased from Sigma and HRC).

All solutions were kept on ice and processed as quickly as possible to minimise loss by thermal degradation. In addition calibrators and tests were processed side by side.

Binding was determined by ultrafiltration using pharmacokinetically relevant serum concentrations (10 and 100 mg/L).

Filtration of 1 mL volumes was performed in Centrifree Micropartition Devices (Millipore) centrifuged for 10 minutes at 1000 g. The filters were 14 mm YMT membranes with a cut off at 30,000 molecular weight. All binding experiments were repeated twice on different days. Calibration was against 10, 20 and 100 mg/L solutions prepared in phosphate-buffered saline (PBS) ultrafiltered at the same time as the serum samples. Drug concentration was measured in the supernatant and percentage binding was calculated from

$$\left(\frac{\text{Concentration in PBS ultrafiltrate} - \text{Concentration in serum ultrafiltrate}}{\text{Concentration in PBS ultrafiltrate}} \right) \times 100$$

Animal experiments

AM-112 powder was dissolved in water to a concentration such that the required quantity of drug and administered to male ICR mice (26-30 g) was 10 mL/kg or SD rats (225-249 g) was 2 mL/kg. AM-112 was administered by iv injection via the caudal vein or orally with a syringe. In some experiments animals were similarly co-administered ceftazidime. Groups of 3 animals were bled pre dose and at various intervals up to 2 hours post dose. Blood collection from mice was performed by intra-cardiac puncture and from rats via the retro-orbital sinus into heparinised tubes, both under light halothane anaesthesia. At the end of the experiment animals were killed by asphyxiation in CO₂. Samples were stored at -70°C and assayed within 28 days of being collected. Pharmacokinetic parameters were determined using WinNonLin (Pharsight Corporation, California).

RESULTS

Stability at -70°C

- There was insignificant loss of AM-112 in human serum over the 28 day period (Figure 1). Therefore samples from the animal experiments were all placed at -70°C and assayed within 28 days of being collected.

Stability at ambient temperature

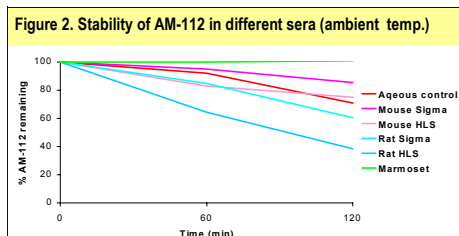
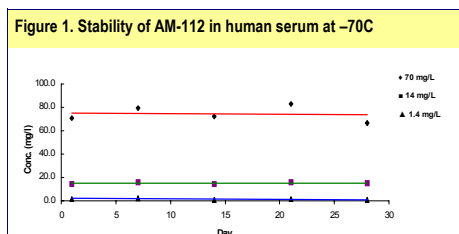
- Stability of AM-112 in various animal sera was tested at ambient temperature (Figure 2).
- AM-112 lost 8% and 30% assayable peak height over 60 and 120 min respectively, in the aqueous control.
- Mouse sera appeared to have no significant effect upon AM-112 stability (15 - 25% loss in 2 hours), compared to the aqueous control.
- AM-112 was less stable in rat sera (40 - 60% loss in 2 hours), compared to the aqueous control.
- AM-112 was very stable in marmoset serum, exhibiting no loss over 2 hours.

Serum protein binding of AM-112

- Protein binding was lowest in the monkey (-9%) and human (-13%) and highest in the mouse (-22%) rat (-27%) (Table 1).

Blood pharmacokinetics in rats and mice

- Blood levels of AM-112 after oral administration were negligible (<1 mg/L) indicating very low oral bioavailability.
- Data for iv administration of AM-112, with or without ceftazidime at double the AM-112 dose, are summarised in Figures 3-8. Co-administration of ceftazidime had little if any effect on the blood levels of AM-112.
- The pharmacokinetic profiles were broadly comparable in both rats and mice (Figures 9 and 10). AM-112 gave similar pharmacokinetics to ceftazidime in the mouse, but a slightly shorter half life in rats.
- Pharmacokinetic parameters are summarised in Table 2.



Species	At 10 mg/L (%)	At 100 mg/L (%)	Mean (%)
Mouse	23.6	20.6	22.1
Monkey	9.3	9.7	9.5
Rat	27.9	26.4	27.2
Human	14.9	10.9	12.9

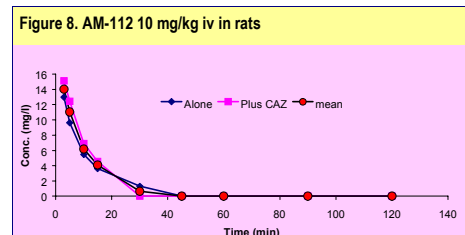
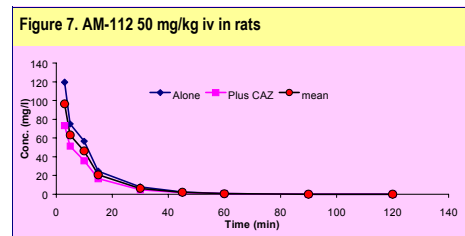
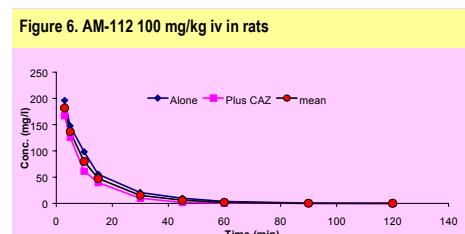
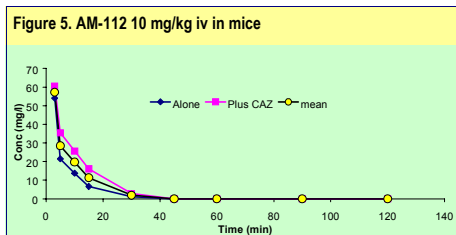
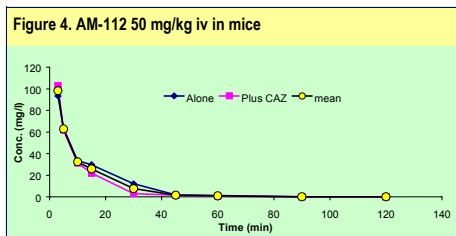
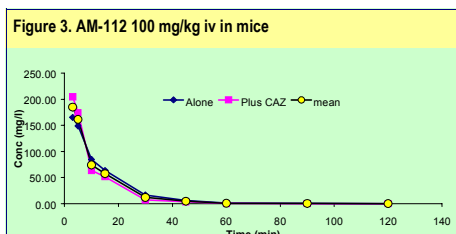
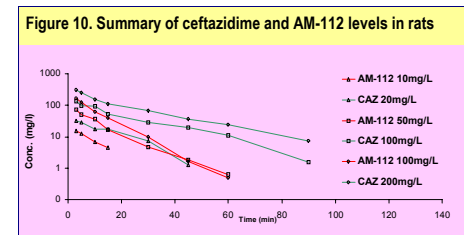
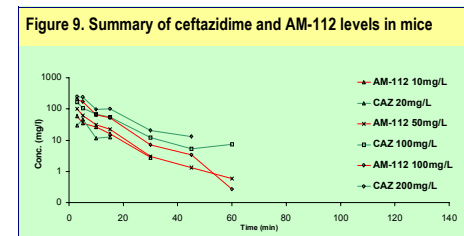


Table 2. Pharmacokinetic parameters for AM-112 (ceftazidime) in rats and mice dosed intravenously with AM-112 with or without ceftazidime in a 1:2 ratio

Rodent	AM-112 (ceftazidime) (mg/kg)	T _{1/2} (min)	AUC _{0-∞} (mg.min/L)	Cl (mL/min/kg)	MRT (min)	V _{ss} (L/kg)
Mouse	10 (0)*	5.8	327.2	30.6	8.7	0.3
Mouse	10 (20)	6.2 (7.6)	765.8 (410.2)	13.1 (48.8)	8.4 (10.5)	0.1 (0.5)
Mouse	50 (0)*	8.9	1330.5	37.6	11.8	0.4
Mouse	50 (100)	12.9 (13.5)	1178.6 (2586.4)	42.4 (38.7)	8.5 (15.5)	0.4 (0.6)
Mouse	100 (0)*	9.0	2513.8	39.8	12.0	0.5
Mouse	100 (200)	6.4 (10.0)	2339.3 (3757.4)	42.7 (53.2)	8.8 (13.6)	0.4 (0.7)
Rat	10 (0)*	9.8	181.8	55.0	12.1	0.7
Rat	10 (20)	6.9 (13.6)	199.4 (646.9)	50.1 (30.9)	9.9 (19.5)	0.5 (0.6)
Rat	50 (0)*	9.0	1546.4	32.3	9.7	0.3
Rat	50 (100)	9.3 (15.1)	846.4 (2634.0)	59.1 (38.0)	11.1 (22.4)	0.7 (0.9)
Rat	100 (0)*	11.5	2963.5	33.7	13.6	0.5
Rat	100 (200)	6.8 (19.6)	2018 (6413.2)	49.5 (31.2)	9.1 (24.1)	0.5 (0.8)

* AM 112 dose without ceftazidime

T_{1/2} = Elimination half-life; AUC_{0-∞} = Area under the curve extrapolated to infinity; Cl = Clearance; MRT = Mean residence time; V_{ss} = Volume of distribution at steady state.



CONCLUSIONS

- An HPLC method, using UV detection, has been established for the assay of oxapenem AM-112 in human and animal sera. The lower limit of detection is 0.2 mg/L.
- Stability studies indicated that AM-112 was stable in serum samples stored at -70°C for at least 28 days, but was less stable at ambient temperature.
- AM-112 did not exhibit significant oral absorption.
- Following iv administration to mice and rats, at 10 mg/L, 50 mg/L and 100 mg/L, AM-112 was readily detected at intervals up to 60 min.
- AM-112 exhibited a linear dose response AUC, good volume of distribution (V_{ss}) and relatively short T_{1/2}, similar to ceftazidime in mice.
- Co-administration of ceftazidime at 2:1 ratio with AM-112 had no obvious effect on AM-112 pharmacokinetics.
- Results to date indicate that AM-112 has sufficiently similar pharmacokinetics to ceftazidime to warrant further investigation of this combination.

Poster prepared by Micron Research