

C. Miossec, H. Merdjan, J. Hodgson
 Novexel SA, Romainville, France

INTRODUCTION

β -lactam antibiotics are one of the most frequently used classes of antimicrobial agents. They offer clinical efficacy against a wide range of bacterial pathogens. However, with the emergence and spread of Extended Spectrum β -Lactamase (ESBL)-producing gram-negative bacteria, non-carbapenem β -lactams have become less useful in the clinic, including when combined with the currently available β -lactamase inhibitors.

NXL104 (formerly AVE1330A) is the first of a new class of non- β -lactam inhibitors of β -lactamases, and the first potent inhibitor of class C β -lactamases. It displays a broad spectrum inhibition profile for both class A and class C enzymes, that are inactivated very efficiently at low IC_{50} values, with low turn-over numbers, and long covalent intermediate half-lives (1). NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects β -lactams from hydrolysis in a variety of class A and class C producing

strains, including ESBL producers (2). Protection against acute lethal infections has been demonstrated in murine models (3). NXL104 appears to be a promising compound to treat clinical resistance mediated by production of β -lactamases in gram negative pathogens, notably to combat class A and class C mediated resistance.

The aims of the study are

1. to explore the general toxicity of NXL104 following daily intravenous administration (30-minute infusion) to the rat for 7 days.
2. to measure its plasma levels.

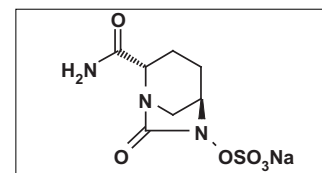


Figure 1: chemical structure of NXL104

MATERIALS AND METHODS

Animals

94 spague-dawley rats, divided as follows:

	PARALLEL TREATMENT GROUPS				BODY WEIGHT (g)
	vehicle	167	500	1500	
40 MAIN STUDY ANIMALS:	5M	5M	5M	5M	M: 294 to 320
TOXICOLOGY:	5F	5F	5F	5F	F: 195 to 225
54 SATELLITE ANIMALS:	9M	9M	9M	9M	M: 289 to 318
TOXICOKINETICS:	9F	9F	9F	9F	F: 205 to 218

Age: approx. 8 weeks at study initiation.
 M = males
 F = females

Dosing

- 30-min daily intravenous infusion into a tail vein using an infusion pump.
 - volume: 5 mL/kg.

Toxicology assessment

Examination	Frequency
Morbidity/mortality checks	at least twice daily
Clinical examinations	daily
Full clinical examination	days 1 and 7
Individual body weights	3 times weekly
Food consumption	weekly for each cage of animals.
Clinical laboratory determinations	at termination
Organ weights	at necropsy (main study animals)
Histopathology	in every main study animal
Serum clinical chemistry	day 7
Coagulation	day 7
Haematology	day 7
Urine analysis	day 7
Toxicokinetics	days 1 and 7 in the satellite animals

Toxicokinetic evaluation

- Composite blood sampling matrix involving 3 blood samples (~1mL)/24h/animal in 3 males + 3 females per time-point.

	After the start of the 30-minute infusion period					
	+30 min	+1h	+2h	+4h	+7h	+24h
3 M + 3 F	~1mL	~1mL	~1mL	~1mL	~1mL	~1mL
3 M + 3 F	~1mL	~1mL	~1mL	~1mL	~1mL	~1mL
3 M + 3 F	~1mL	~1mL	~1mL	~1mL	~1mL	~1mL

- NXL104 was analyzed in plasma by LC-MS/MS after protein precipitation and supernatant cleanup.
 - The LLOQ (lower limit of quantitation) was 0.010 μ g/mL. Precision was within 6% and inaccuracy was within \pm 5%.
 - Toxicokinetic data interpretation was conducted on pooled male and female data, in addition to the interpretation on separate genders.
 - Parameters C_{max} , AUC_{0-24} , AUC , CL , V_{ss} and $t_{1/2}$ were determined from average concentration-time profiles using non-compartmental analysis.
 - Pharmacokinetic software: WinNONLIN Enterprise® version 3.3.

Statistical analysis

- Variables: body weight gains over days 1 to 4, 4 to 7 and 1 to 7, haematology, coagulation, serum clinical chemistry, urinary volume and specific gravity on day 7, terminal body weights, absolute and relative organ weights at termination.
 - Levene's test for homogeneity of variances
 - Shapiro-Wilk's test for normality
 - If both homogeneity of variances and normality are verified, ANOVA, possibly followed by pairwise comparisons between groups
 - Otherwise: Kruskal-Wallis test, possibly followed by the Wilcoxon's rank sum test.

GENDER-EFFECT ON PHARMACOKINETIC PARAMETERS OF NXL104

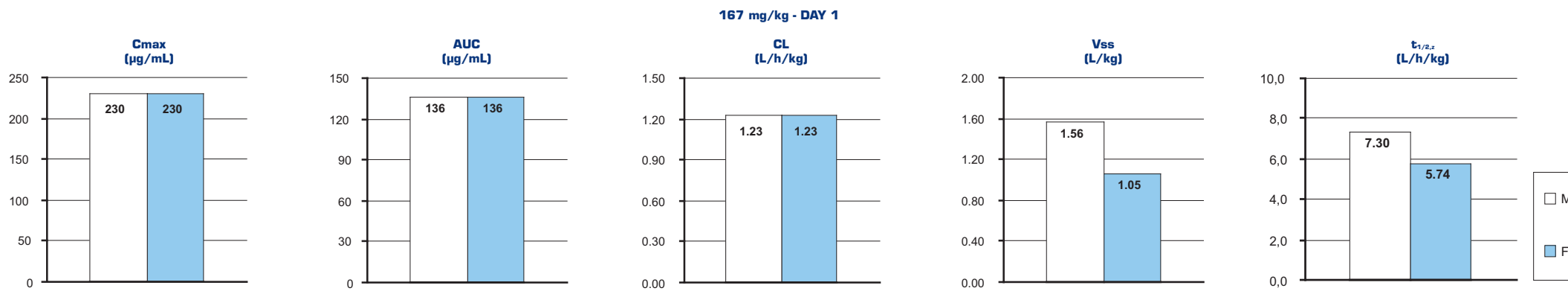


Figure 2: Pharmacokinetic parameters of NXL104 after a single intravenous administration of 167 mg/kg in male rats (white bars) or female rats (blue bars)

- After a single intravenous administration of NXL104 at 167 mg/kg, exposure parameters in male and females animals were exactly identical.
 - The clearance value (1.23 L/h/kg) was low in comparison to hepatic blood flow, suggesting good metabolic stability *in vivo*.
 - When considering pooled concentration data in male and female animals, the steady-state volume of distribution was low (1.29 L/kg) and the terminal half-life was 6.5 h.

DOSE-EFFECT ON EXPOSURE TO NXL104

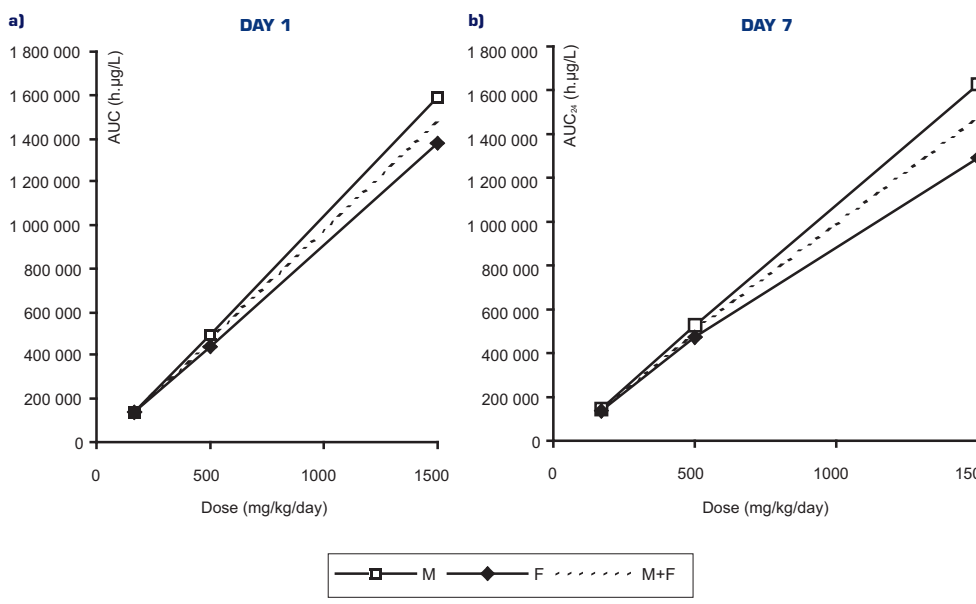


Figure 3: Influence of dose on exposure to NXL104 in rats following (a) a single (Day 1) or (b) 7 once-daily (Day 7) intravenous administrations

- Exposure to NXL104 was slightly higher in males than in females at 500 and 1500 mg/kg/day.
 - Overall, single dose AUC increased 11.7-fold in males and 10.1-fold in females, as compared to a 9-fold difference between lowest and highest doses.
 - A parallel trend towards over-proportional dose-changes was seen on parameter C_{max} , which increased 10.7-fold in males and 9.4-fold in females compared to a 9-fold difference between extreme doses (not shown).
 - The exposure (AUC_{24}) either decreased by 6%, or increased by no more than 8%, as compared to a predicted increase of up to 1%.

TIME-EFFECT ON PHARMACOKINETICS OF NXL104

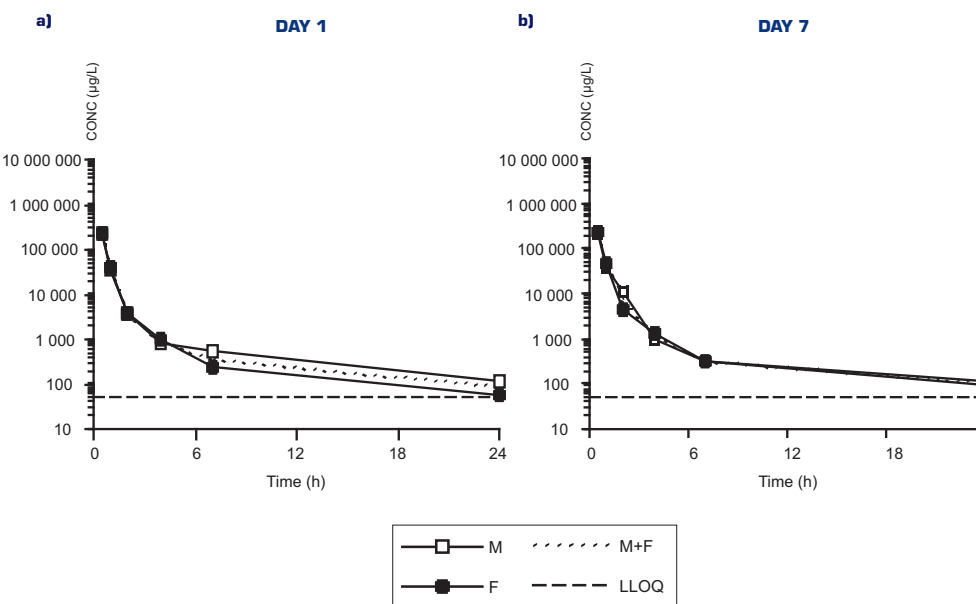


Figure 4: Mean plasma concentrations of NXL104 in rats following (a) a single (Day 1) or (b) 7 once-daily (Day 7) intravenous administrations at 167 mg/kg/day

- Pharmacokinetics is time-invariant: after repeated intravenous administration of NXL104, the extent of accumulation was predictable from the single dose data.

RESULTS

- Mortality, morbidity and clinical signs during treatment with NXL104**
 - No animal died during the study.
 - No clinical signs observed in animals of the main study.
 - Some satellite animals given 1500 mg/kg/day had damaged tails from day 3 due to repeated intravenous injection of the hyperosmolar formulation.
- Food consumption and body weight gain**
 - Food consumption slightly lower in all treated groups as compared with control group, except in the low dose female group.
 - Body weight gain slightly lower in males and females given 1500 mg/kg/day.
- Haematology**
 - Statistically significant reduction of red blood cell count on day 7 in females given 1500 mg/kg/day or 500 mg/kg/day ($P < 0.05$ for mean values). Decrease also observed in males treated with 1500 mg/kg/day without statistical significance.
 - Haemoglobin and packed cell volume decreased in animals treated with 1500 mg/kg/day; statistical significance for mean values in female group ($P < 0.01$) but not in male group.
- Serum clinical chemistry**
 - Statistically significant increase of urea concentration in males given 1500 mg/kg/day ($P < 0.01$), with all individual values remaining within normal range.
- Pathology**
 - Mean absolute kidney weight and heart weight lower for females treated at 1500 mg/kg/day ($P < 0.05$); no correlation with histological finding.
 - Site of injection: minimal to moderate perivascular subacute inflammation, minimal to marked mural thrombi, in the 1500 mg/kg/day treated groups. Findings considered to be related to irritant effect of the test item in line with the formulation used (hyperosmolarity).

CONCLUSIONS

- NXL104 produced minimal or no toxic effects when administered as repeated 7-day once-daily intravenous doses in the rat.
 - The maximum tolerated dose of NXL104 was considered to be 1500 mg/kg/day.
 - The dose level of 500 mg/kg/day was associated with very minor effects on body weight, food consumption and red cell parameters.
 - The 167 mg/kg/day could be considered as the NOAEL (no observed adverse effect level).
- Toxicokinetic measures showed no difference in exposure between male and female animals. Exposure increased almost in proportion to the dose, and was similar on the first and last days of dosing.

References

1. Bonney A. Journal of Antimicrobial Chemotherapy (2004). 54:410-417
2. Shackcloth J. 15th European Congress of Clinical Microbiology and Infectious Diseases (2005). Poster # 1348
3. Lévassseur P. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy (2005). Communication # F-1164