Poster F1-1485

49th ICAAC Meeting
San Francisco, CA
September 12-15, 2009

Efficacy of BAL30072 in Experimental Respiratory Tract Infections

W. J. WEISS[1]*, M. E. PULSE[1], P. NGUYEN[1], J. PIERCE[1], J. SIMECKA[1], M. G. P. PAGE[2]
[1]UNT Health Science Center, Ft. Worth, TX, [2]Basilea Pharmaceutica Intl. Ltd, Basel, Switzerland

Contact Information:
UNT Health Science Center
3500 Camp Bowie Blvd.
Fort Worth, TX 76107
wweiss@hsc.unt.edu
www.hsc.unt.edu/preclinical

Abstract

Background: BAL30072 (BAL), a novel siderophore monobactam, exhibits potent activity against gram-negative non-fermentors. The current study was performed to determine the efficacy of BAL in murine lung infection models with isolates of *K. pneumoniae* (Kpn) and *P. aeruginosa* (Psa). **Methods:** Female CD-1 mice were infected intranasally with Kpn or Psa and treated IP with either single or multiple doses (5 – 100 mg/kg) of BAL, Aztreonam (Azt), Meropenem (Mero), Imipenem (Imi) or BAL:Mero (at a ratio of 1:1). Bacterial lung counts were determined at 24 hr following a single dose (+4 hrs post-infection) and survival curves were tracked following a multiple dosing regimen (bid x 3 days). **Results:** Within Kpn infected animals, single doses of 25 – 100 mg/kg exhibited lung $\log_{10}$ cfu reductions vs untreated controls of 0.3 – 2.1 for BAL, 2.2 – 2.9 for Mero, 2.2 – 3.6 for Azt and 2.2 – 3.9 for BAL:Mero. Multiple dosing within Kpn infected animals resulted in 0% survival at 25 mg/kg for BAL and Mero and 40% survival for BAL and Mero at 50 and 100 mg/kg, 80% survival for Azt at 100 mg/kg, and 100% survival for BAL:Mero at 100 mg/kg. Single doses of 5 – 50 mg/kg within Psa infected animals resulted in lung log10 cfu reductions vs untreated controls of 0.2 – 0.3 for BAL, 1.3 – 2.2 for Mero, 1.5 – 2.1 for Imi and 0.8 – 1.7 for BAL:Mero. Survival curves following multiple dosing within Psa infected animals resulted in 40% and 60% survival for BAL at 25 and 50 mg/kg; 40% to 80% survival for Mero at 5, 25 and 50 mg/kg; 100% survival for Imi at 25 and 50 mg/kg; and 40%, 40% and 100% survival for BAL:Mero at 5, 25 and 50 mg/kg, respectively. **Conclusions:** BAL30072 exhibits comparable efficacy to that of Meropenem during multiple dosing of chronic Kpn and Psa lung infections, and efficacy appears to be enhanced over either agent alone when the two compounds are combined for treatment. The utility of BAL for the treatment of these difficult to treat respiratory infections warrants further investigation.

Introduction

BAL30072 is a novel siderophore monobactam that exploits the natural nutrient uptake like a trojan horse. Its unique pattern of penicillin-binding-protein inhibition and its bactericidal mode of action confer potent *in vitro* activity against Gram-negative fermentors and non-fermentors. These properties enable BAL30072 to overcome most of the genetically defined factors of beta-lactam resistance. In addition to the potent inhibition of *Pseudomonas, Acinetobacter spp.* and *Enterobacter spp.* BAL30072 also exhibits strong activity against *Burkholderia spp.* and *Stenotrophomonas,* which are two of the more difficult to treat Gram-negative pathogen.

New *in-vitro* data presented at ECCMID show that the already low rate of resistance development to BAL30072 in difficult-to-treat bacilli such as *P. aeruginosa* and *Acinetobacter spp.* is further decreased when BAL30072 is combined with currently marketed Gram-negative antibiotics such as carbapenems. Concomitantly, the same combination also increased the bactericidal activity of BAL30072.

The current study was performed to evaluate the efficacy of BAL30072 in murine models of gram-negative respiratory tract infections both alone and in combination with a carbapenem antibiotic.

Methods and Materials

Bacteria: *K. pneumoniae* ATCC43816 and *P. aeruginosa* ATCC39324.

Animals: Female 5 - 6 week old CD-1 mice, 18 – 22 gms.

Infection: Strains were grown overnight in MHB (Mueller-Hinton Broth) at 37°C. Overnight cultures were diluted into fresh MHB (1:10 dilution) and incubated under the same conditions for 4 hrs. The cell suspensions of 4-hr cultures were adjusted to an optical density of 1.0 at 600_{nm} (10^9 CFU/mL) and diluted 1:3 in MHB ($10^{8.9}$ CFU/mL). The mice were anesthetized w/ Ketamine HCl @ 40 mg/kg b.w. + Xylazine @ 6 mg/kg b.w. injected intraperitoneally. Each animal was inoculated intranasally with 0.05 mL of an inoculum, which yielded a final input CFU of 105 per mouse. Mice were placed on their backs in the cage and allowed to recover from the anesthesia.

Treatment Groups: All treatment groups consisted of 5 animals, and treatment was initiated for all groups 4 hrs post-infection. One set of animals received only this single treatment for each of the compounds at the dose levels tested. Another set of animals was treated b.i.d. for a period of three days. The combination of BAL30072 and Meropenem was tested at a 1:1 ratio for both the efficacy studies and MIC determinations.

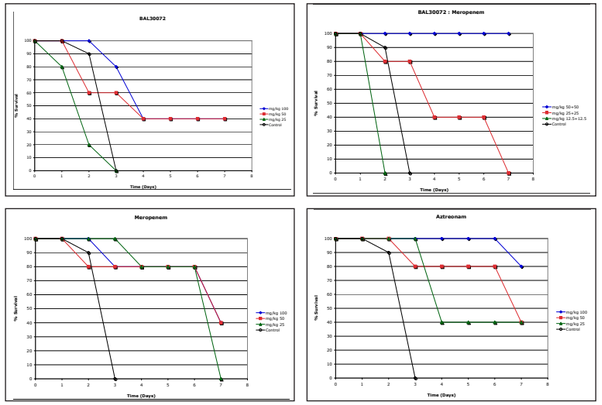
Sampling: The groups of mice receiving only a single treatment were euthanized by CO_2 inhalation +24 hrs post-infection. Lungs were aseptically removed, homogenized, serially diluted and plated onto charcoal agar to determine CFU counts. Animals receiving multiple b.i.d. treatment for 3 days were maintained and monitored for a census of survivors over a period of 5-7 days after the last dose.

Efficacy of BAL30072, Meropenem and Aztreonam following a single dose i.p. administration in the *K. pneumoniae* ATCC43816 lung infection

Compound	Dose	Geomean $\log_{10}$ CFU	SD	Mean $\log_{10}$ reduction
BAL30072	100 mg/kg	7.79	1.29	1.23
	50 mg/kg	6.89	0.15	2.13
	25 mg/kg	8.72	0.54	0.3
Meropenem	100 mg/kg	6.02	0.7	2.99
	50 mg/kg	6.27	0.46	2.74
	25 mg/kg	6.87	0.45	2.15
BAL + Mero[a]	100 mg/kg	5.17	0.16	3.85
	50 mg/kg	5.79	0.36	3.22
	25 mg/kg	6.86	0.44	2.16
Aztreonam	100 mg/kg	5.4	0.39	3.61
	50 mg/kg	5.76	0.51	3.25
	25 mg/kg	6.79	0.54	2.23
Control	-	9.02	0.14	-

[a] total dose of BAL + Mero combined at a 1:1 ratio

Survival curves following i.p. administration of BAL30072, Meropenem and Aztreonam (bid x 3 days) against *K. pneumoniae* ATCC43816



Minimum Inhibitory Concentration (MIC) of selected compounds against *K. pneumoniae* ATCC43816

MIC (ug/mL)						
BAL30072	Meropenem	BAL + Mero[a]	Imipenem	Aztreonam	Ceftazidime	Tobramycin
0.06	0.03	0.008	0.125	0.125	1	0.5

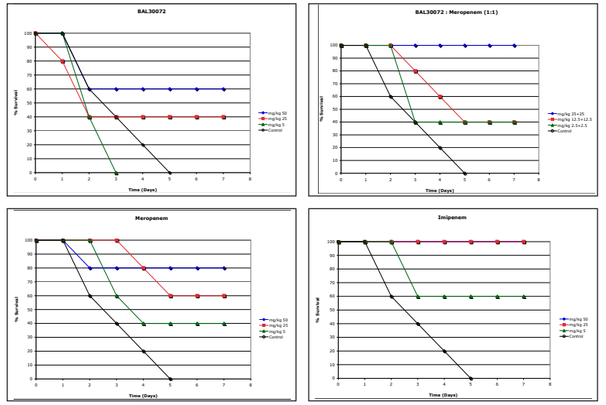
[a] BAL + Mero MIC represents combined concentration at a 1:1 ratio

Efficacy of BAL30072, Meropenem and Imipenem following a single dose i.p. administration in the *P. aeruginosa* ATCC39324 lung infection

Compound	Dose	Geomean $\log_{10}$ CFU	SD	Mean $\log_{10}$ reduction
BAL30072	50 mg/kg	7.52	0.39	0.34
	25 mg/kg	7.7	0.51	0.17
	5 mg/kg	7.72	0.57	0.15
Meropenem	50 mg/kg	5.7	0.84	2.16
	25 mg/kg	5.8	0.51	2.06
	5 mg/kg	6.6	0.82	1.26
BAL + Mero[a]	50 mg/kg	6.28	1.15	1.6
	25 mg/kg	6.2	0.5	1.7
	5 mg/kg	7.08	0.7	0.78
Imipenem	50 mg/kg	5.8	0.52	2.06
	25 mg/kg	5.77	0.45	2.1
	5 mg/kg	6.34	0.56	1.5
Control	-	7.86	0.2	-

[a] total dose of BAL + Mero combined at a 1:1 ratio

Survival curves following i.p. administration of BAL30072, Meropenem and Imipenem (bid x 3 days) against *P. aeruginosa* ATCC39324

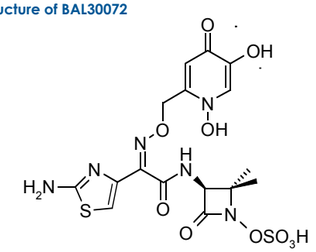


Minimum Inhibitory Concentration (MIC) of selected compounds against *P. aeruginosa* ATCC39324

MIC (ug/mL)						
BAL30072	Meropenem	BAL + Mero[a]	Imipenem	Aztreonam	Ceftazidime	Tobramycin
0.015	0.06	0.008	2	0.5	2	0.125

[a] BAL + Mero MIC represents combined concentration at a 1:1 ratio

Chemical Structure of BAL30072



Summary and Conclusions

- Single dose administration of BAL30072 or Meropenem alone reduced bacterial lung counts up to 2.1 $\log_{10}$ and 2.9 $\log_{10}$ for the *K. pneumoniae* and 0.34 $\log_{10}$ and 2.1 log10 for *P. aeruginosa,* respectively, in the experimental respiratory tract infections tested.
- Efficacy (single dose log reduction) against both these infections was enhanced when BAL30072 and Meropenem were combined in a 1:1 ratio with observed reductions of up to 3.9 and 1.7 against *K. pneumoniae* and *P. aeruginosa,* respectively.
- Single dose efficacy of the BAL30072 and Meropenem combination was equivalent, on a dose to dose basis, to that of Aztreonam in the lung tissue counts of mice infected with *K. pneumoniae.*
- Multiple dose administration of BAL30072 and Meropenem resulted in comparable survival rates of 40%, 40% and 0% for doses of 100, 50 and 25 mg/kg against the *K. pneumoniae* infection. Observed survival increased to 100% for the BAL:Mero combination at 100 mg/kg (50 + 50 mg/kg).
- Survival studies indicated that Meropenem alone was slightly more efficacious with multiple dosing than BAL30072 in *P. aeruginosa* infected animals. However, survival rates were enhanced when the BAL30072:Meropenem combination was dosed at 50 mg/kg (25 + 25 mg/kg), which was equal to the highest dose administered for each compound alone in *P. aeruginosa* infected animals.
- The use of BAL30072 for the treatment of these difficult to treat gram-negative respiratory infections, either alone or combined with a carbapenem, warrants further investigation to elaborate on it's clinical utility.

References

1. Cleeland,R. and E. Squires. 1991. Evaluation of New Antimicrobials *in Vitro* and in Experimental Animal Infections. In Antibiotics in Laboratory Medicine", 3rd. ed., edited by Victor Lorian. Willams and Wilkins Baltimore, Maryland. pp. 752 - 783.
2. Lau, H., S. Clegg, T. Moore. 2007. Identification of *Klebsiella pneumoniae* genes uniquely expressed in a strain virulent using a murine model of bacterial pneumonia. Microb. Pathog. 42(4):148-155.
3. National Research Council. Guide for the Care and Use of Laboratory Animals. National Academy Press Washington. DC. 1996.
4. Nishi, T., K. Tsuchiya. 1980. Therapeutic effects of cefotiam and cefazolin on experimental pneumonia caused by *Klebsiella pneumoniae* DT-S in mice. Antimicrob. Agents Chemother. 18:549-556.
5. Experimental chronic pulmonary infection in mice. Y. Iizawa. Micro. Immunol 1988. pp 895-906.
6. Fournier, J-L., et.al. 1996. Assessment of two penicillin plus b-lactamase inhibitors versus cefotaxime in treatment of murine *Klebsiella pneumoniae* infections. Antimicrob. Agents Chemother. 40:325-330.
7. Bakker-Woudenberg, I., *et.al.* 2006. Effect of treatment duration on PK/PD indices correlating with therapeutic efficacy of ceftazidime in experimental *Klebsiella pneumoniae* lung infections. Antimicrob. Agents Chemother. 50:2919-2925.

ACKNOWLEDGEMENT
This study was funded and supported by Basilea Pharmaceutica Intl. Ltd., Basel, Switzerland.