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In vitro Antibacterial Activity and *in vivo* Efficacy of Sulbactam-Durlobactam (ETX2514) against Pathogenic *Burkholderia* Species

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Abstract

Background

The genus Burkholderia contains several pathogenic species with distinct etiologies, including *Burkholderia pseudomallei* (*Bp*), the biothreat pathogen responsible for melioidosis, and *Burkholderia mallei* (*Bm*), which causes glanders. β-lactams, such as ceftazidime and meropenem, are important therapeutic options for these infections. However, clinical resistance to β-lactams, which is primarily mediated by multiple types of β-lactamases in these species, is a growing concern. Durlobactam (ETX2514, DUR) is a novel β-lactamase inhibitor with broad spectrum activity against Ambler class A, C and D β-lactamases. Sulbactam (SUL) is an Ambler Class A β-lactamase inhibitor with intrinsic antibacterial activity against a limited number of species, including *Acinetobacter* spp. SUL-DUR is currently in Phase 3 clinical testing for the treatment of carbapenem-resistant infections caused by *Acinetobacter* spp. In this study, SUL-DUR was tested for *in vitro* antibacterial activity against *Bp* and *Bm,* as well as for *in vivo* efficacy in a preclinical model of melioidosis.

Methods

The antibacterial activity of SUL alone or in combination with DUR (fixed at 4 mg/L) against *Bm* (n = 30) and *Bp* (N = 28) was determined following CLSI guidelines. *In vivo* efficacy was tested in an acute murine model of melioidosis in which 4 x 10^4 cfu *Bp* K96423 (SUL-DUR MIC = 1 mg/L) was administered intranasally to BALB/c mice. SUL-DUR (100/200 or 400/200 mg/kg) was administered q4h subcutaneously 4 hours post-challenge for 6 days, and murine survival was monitored for 45 days. Doxycycline (DOX) and ciprofloxacin (CIP) were dosed as positive controls at 40 mg/kg q12 h for 6 days.

Results

The addition of DUR effectively lowered the SUL $MIC_{50/90}$, from 8/16 to 0.25/0.5 mg/L vs. *Bm,* and from 8/8 to 1/2 mg/L for *Bp.* All untreated mice in the melioidosis model succumbed to infection within 3 days of challenge. 60% survival was observed for both dose arms of SUL-DUR at 45 days post-infection, compared to 40% survival observed for both CIP and DOX.

Conclusions

Preliminary preclinical data demonstrating robust *in vitro* and *in vivo* antibacterial activity of SUL-DUR against *Burkholderia* spp. suggests this combination may be an effective new therapy for the treatment of these challenging pathogens.

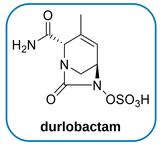
Burkholderia spp. Classified as Select Agents

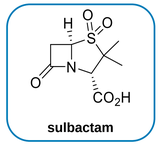
Burkholderia pseudomallei (*Bp*) is an aerobic, Gram-negative, soil-dwelling pathogen that causes melioidosis, an important cause of severe sepsis in Southeast Asia. This organism is considered a biothreat pathogen due to its high mortality rate, intrinsic antibiotic resistance, potential for aerosol spread, and prevalence in the environment in endemic areas[1].

Burkholderia mallei (*Bm*), the etiologic agent of glanders disease, is a Gram-negative, nonmotile, facultative intracellular pathogen. Unlike most known members of the family *Burkholderiaceae, Bm* is an obligate mammalian pathogen. While glanders is primarily an equine disease, *Bm* is also considered a select agent as it is intrinsically drug-resistant, can be highly infectious to humans in aerosol form, and infection requires very few organisms. The use of *B. mallei* as a biological warfare agent during World Wars I and II and the Russian invasion of Afghanistan has been reported[2].

Key Features of Sulbactam-Durlobactam

Sulbactam-durlobactam is a β-lactam/β-lactamase inhibitor (BL/BLI) combination[3,4] currently in Phase 3 clinical development for the treatment of infections caused by resistant *Acinetobacter baumannii calcoaceticus* complex (ABC) organisms.





- Durlobactam (ETX2514) is a novel diazabicyclooctenone BLI with best-in-class broad spectrum activity against class A, C and D β-lactamases.[3]
- Sulbactam is an approved BLI with intrinsic antibacterial activity that is limited to only a few bacterial species. This activity in *Acinetobacter baumannii (Ab)* has been shown to be due to its inhibition of PBP3, an enzyme required for cell wall biosynthesis.[5]

Study Design

All studies were performed under a CRADA with Entasis Therapeutics by the Therapeutics Discovery Group, Division of Countermeasures at USAMRIID, and were carried out in accordance with accepted scientific practice for this type of work, which is early stage research. Nothing adverse occurred during the study. Broth microdilution susceptibility testing was conducted according to CLSI guidelines, including testing control compounds against QC organisms[6]. SUL-DUR was assayed by two-fold dilution of sulbactam in the presence of a fixed concentration of 4 μg/mL durlobactam.

Sulbactam-durlobactam and comparator compounds were evaluated for *in vivo* efficacy vs. *Burkholderia pseudomallei* in an acute murine model of meliodosis using the sentinel strain K96423 (MIC = 1 μg/mL). This strain, which was isolated from a female patient in Thailand who succumbed to the infection[7], is among the most frequently used isolates in preclinical models of meliodosis. Notably, *Bp* K96423 is multidrug resistant and encodes several β-lactamases, including *penA* and $bla_{OXA\text{-}59}$[8].

A lethal challenge of *Bp* K96423 (4 x 10^4 cfu/mouse) was administered intranasally to BALB/c mice (N = 10/group). Therapy (for six consecutive days) was initiated 4 hours post-challenge via subcutaneous injection of SUL-DUR or intraperitoneal administration of positive control compounds, doxycycline and ciprofloxacin. Animals receiving vehicle only generally succumb to the infection within the first 3 days. Survivors were monitored for 45 days after treatment for potential relapse.

In the absence of any *in vitro* PK/PD understanding of SUL-DUR against these potential biothreat pathogens, exposure for initial studies was targeted at sulbactam and durlobactam doses at which efficacy was previously demonstrated against *A. baumannii* in the mouse neutropenic thigh model.[3]

In vitro Activity of Sulbactam-Durlobactam (MIC, μg/mL)

B. mallei (N = 30)

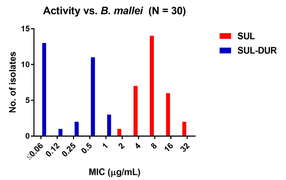
Isolate	DUR	SUL	SUL-DUR	DOX
NCTC120	4	4	≤0.03	0.06
NCTC10248 (Strain 6)	4	8	≤0.03	0.06
NCTC 10229	8	16	1	0.12
NCTC 10247	8	16	1	0.12
China 7	8	8	0.25	0.06
NCTC 3709	8	8	0.5	0.06
2000031063	4	32	≤0.03	0.03
2000031064, India 86-567-2	8	32	0.5	0.5
China 5	8	16	≤0.03	0.03
2002721276, KC237	0.25	8	0.25	0.06
2002721280, KC 1092, 52-236 Pasteur	8	8	0.5	0.03
2002734301, NCTC 10260 (strain 11)	8	8	≤0.03	0.12
FMH	8	8	0.5	0.25
NCTC 3708	8	16	0.5	0.06
ATCC 10399	8	16	0.5	0.06
Turkey 1	4	4	≤0.03	0.06
Turkey 2	4	4	≤0.03	0.06
Turkey 3	8	8	0.06	0.06
Turkey 4	8	8	0.5	0.06
Turkey 5	8	8	0.5	0.06
Turkey 6	8	16	≤0.03	0.12
Turkey 7	8	8	0.06	0.12
Turkey 8	4	4	≤0.03	0.12
Turkey 9	8	4	0.12	0.06
Turkey 10	2	4	≤0.03	0.015
2002721274	8	8	0.5	0.06
2002721278	8	8	1	0.03
2002721279	8	4	0.5	0.06
20000031065	4	2	≤0.03	0.06
ATCC 23344	8	8	0.5	0.12
Range, μg/mL	0.25 to 8	2 to 32	≤0.03 to 1	0.015 to 0.5
MIC_{50}	8	8	0.25	0.06
MIC_{90}	8	16	0.5	0.12

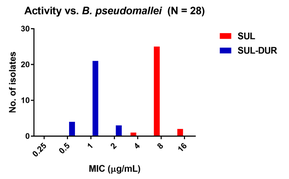
DUR = durlobactam; SUL = sulbactam; DOX = doxycycline

B. pseudomallei (N = 28)

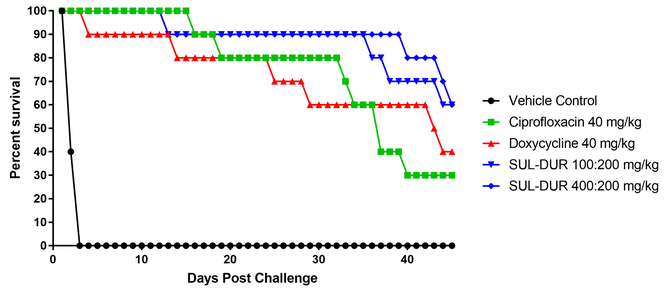
Isolate	DUR	SUL	SUL-DUR	DOX
K96243	32	8	1	1
Pasteur 52237	8	8	2	0.5
7894	8	8	1	0.25
MSHR305	16	8	0.5	4
SMHR668	32	16	2	2
NAU20B16	16	8	1	>8
NAU35A03	32	8	2	4
NCTC 13179	32	8	1	4
NCTC 13178	32	8	1	4
406e	16	8	0.5	>8
1026b	16	8	1	4
1106a	16	8	0.5	4
MSHR5855	32	8	1	>8
MSHR5848	32	8	1	4
MSHR5858	32	8	1	4
HBPUB10134a	16	8	1	4
HBPUB10303a	32	8	1	8
238	32	8	1	4
112c	32	16	1	4
465a	32	8	1	4
776	32	4	0.5	8
E8	32	8	1	8
E12	32	8	1	4
E13	16	8	1	4
316c	32	8	1	4
295	32	8	1	4
713	32	8	1	4
503	32	8	1	2
Range, μg/mL	8 to 32	4 to 16	0.5 to 2	0.25 to 8
MIC_{50}	32	8	1	4
MIC_{90}	32	8	2	8

DUR = durlobactam; SUL = sulbactam; DOX = doxycycline





In vivo Efficacy of Sulbactam-Durlobactam in a Murine Model of Melioidosis



Kaplan-Meier survival curve of mice infected with a lethal dose of *B. pseudomallei* K96243 following 6 consecutive days of treatment with ciprofloxacin (MIC = 0.5 μg/mL), doxycycline (MIC = 1 μg/mL) or sulbactam-durlobactam (MIC = 1 μg/mL).

Conclusions

- **Durlobactam restored sulbactam antibacterial activity against a collection of *B. mallei* and *B. pseudomallei* isolates.**
- **The sulbactam-durlobactam combination was efficacious in a murine model of meliodiosis.**
- **Sulbactam-durlobactam was more efficacious over time in this model than either comparator agent, with 90% vs. 60% survival at day 35 and 60% vs. ≤40% survival at day 45 for SUL-DUR or comparators, respectively.**
- **These data support further evaluation of the sulbactam-durlobactam combination for the treatment of infections caused by pathogenic *Burkholderia* species.**

References

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