EV0208

Efficacy of Novel Extended Spectrum Pleuromutilins Against *E. coli In Vitro* and *In Vivo*

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INTRODUCTION

Extended spectrum pleuromutilins (ESP) are a novel generation of pleuromutilin antibiotics displaying broad antibacterial profile, including multi-drug resistant (MDR) *Enterobacteriaceae*, in addition to the profile of conventional pleuromutilins. Conventional pleuromutilin derivatives such as lefamulin (BC-3781) or retapamulin display potent activity against staphylococci, streptococci, *Haemophilus* spp., *Legionella pneumophila*, *Mycoplasma* spp., *Chlamydia* spp., and *Neisseria gonorrhoeae* among others but lack activity against *Enterobacteriaceae*. [1-4]

OBJECTIVE

Among the resistant bacterial pathogens causing serious infections *E. coli* is one of most worrisome since multi-drug resistant strains continue to emerge in both the nosocomial and the community setting leaving no viable treatment options. The Extended Spectrum Pleuromutilins are a new generation of pleuromutilin antibiotics with efficacy against important Gram-negative pathogens, including multi-resistant *Enterobacteriaceae*. ESP cover a majority of bacterial pathogens imposing urgent and serious threats according to the CDC. Those include multi-drug resistant *E. coli*, *S. aureus*, *K. pneumoniae*, and *S. pneumoniae*, among others. This study investigated the *in vitro* and *in vivo* efficacy of four new ESP derivatives (BC-7634, BC-9074, BC-9529 and BC-9563) against *E. coli* in comparison to current treatment options and evaluated the metabolic stability and cytotoxicity in human hepatocytes.

METHODS

The ESP were evaluated for their *in vitro* activity against *E. coli* ($n = 32$) including ESBL (TEM, CTX-M) producing strains and carbapnem-resistant *Enterobacteriaceae* by broth microdilution according to CLSI (M7/A9). For evaluation of the metabolic stability and cytotoxicity primary human hepatocytes were used. The therapeutic potency of ESP *in vivo* was evaluated in a lethal murine sepsis model. Mice were infected intraperitoneally with an inoculum of ~10^6 CFU *E. coli* per mouse. Simultaneously, animals were treated s.c. with incrementing doses of the test compounds. Survival was recorded for 96 h. The total daily dose required for survival of 50 % of mice at 96 h post infection (ED_{50}) and 95 % confidence limits were determined by binary probit analysis.

Table 1. Antibacterial activity [µg/mL] against clinical *E. coli* isolates ($n = 32$) and carbapenem-resistant *Enterobacteriaceae* ($n = 12$)

Compound	*E. coli* ($n = 32$; 78.1 % ESBL producers)			Carbapenem-resistant *Enterobacteriaceae* [a] ($n = 12$)
	MIC_{50}	MIC_{90}	Range	Range
BC-7634	0.5	1	0.25-2	0.5-0.5 [b]
BC-9074	0.12	0.5	0.06-0.5	0.25-4
BC-9529	0.5	1	0.25-1	0.5-4
BC-9563	1	1	0.5-2	0.5-4
Amoxicillin/ clavulanic acid	**16**	**>32**	**8->32**	**>32**
Ceftriaxone	**>16**	**>16**	0.03-**>16**	**>32**
Ceftazidime	**32**	**>32**	0.12-**>32**	**>32**
Ciprofloxacin	**16**	**>16**	≤0.015-**>16**	**32->32**
Doxycycline	**8**	**32**	0.5-**>32**	**4->32**
Meropenem	ND	ND	ND	1-**>32**
Tigecycline	0.25	0.5	0.06-1	1-**16**

[a], Carbapnem-resistant *Enterobacteriaceae* include: NDM-1 producing *E. coli* (n=2), NDM-1 and KPC-2/-3 producing *K. pneumoniae* (n=3 and n=5) and NDM-1 and KPC producing *E. cloacae* (n=1 and n=1)
[b], Range includes only MIC against *E. coli*

Table 2. Metabolic stability, antibacterial activity, and *in vivo* efficacy of ESP, and tigecycline against *E. coli* ATCC25922

Compound	Metabolic stability (4h, 1 µg/mL) [% of parent compound]	*E. coli* ATCC 25922 MIC [µg/mL]	*E. coli* ATCC 25922 ED_{50} [mg/kg/day]
BC-7634	46.3	0.25	3.46
BC-9074	59.4	0.12	0.78
BC-9529	53.6	0.25	3.75
BC-9563	65.9	0.5	2.30
Moxifloxacin	100	≤0.03	0.47
Tigecycline	ND	0.125	0.45

ND = not determined

ACKNOWLEDGMENTS

The authors gratefully acknowledge the practical work of C. Muska, A. Bischinger, E. Fischer, B. Kappes, and A. Gruss.

RESULTS

All four ESP exhibited potent antibacterial activity against *E. coli* being comparable to that of tigecycline. The MIC values ranged from 0.06–2 µg/mL, with all isolates being inhibited at concentrations of ≤ 2 µg/mL. The activity of ESP was completely unaffected by the production of ß-lactamases which included TEM-, SHV-, CTX-M- and KPC- type ESBLs or metallo-ß-lactamases (NDM-1). Testing in primary human hepatocytes confirmed the metabolic stability and low cytotoxic potential (data not shown) of these new derivatives. In the murine bacteremia model all selected ESP showed good efficacy with the most active ESP being comparable to tigecycline (ED_{50} of 0.45 mg/kg/day). BC-7634, BC-9074, BC-9529, and BC-9563 displayed ED_{50} values of 3.46 mg/kg/day, 0.78 mg/kg/day, 3.75 mg/kg/day, and 2.30 mg/kg/day, respectively, correlating well with $MIC_{50/90}$: 0.5/1 µg/mL, 0.12/0.5 µg/mL 0.5/1 µg/mL, and 1/1 µg/mL.

CONCLUSIONS

- **The selected extended spectrum pleuromutilins demonstrated potent *in vitro* activity against *E. coli* including highly resistant isolates.**
- **ESP activity was completely unaffected by the production of ß-lactamases including TEM-, SHV-, CTX-M- and KPC- type ESBLs or metallo-ß-lactamases (NDM-1).**
- **ESP demonstrated good metabolic stability.**
- **The good *in vitro* antibacterial activity could be translated into successful treatment of Gram-negative infections caused by highly resistant pathogens.**

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25th European Congress of Clinical Microbiology and Infectious Diseases, Copenhagen, Denmark, 25-28 April 2015