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In Vivo Activity of Extended Spectrum Pleuromutilins in Murine Sepsis Model

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ABSTRACT

Objective: The emerging antimicrobial resistance development in Gram-positive and Gram-negative pathogens is one of the most serious health treats. The development of the next generation of pleuromutilins, the extended spectrum pleuromutilins (ESP), combines the good activity against Gram-positive bacteria including e.g. MRSA with the efficacy against broader range of Gram-negative pathogens including multi-resistant Enterobacteriaceae. Following the *in vitro* efficacy testing, selected ESP were evaluated in two *in vivo* efficacy models to assess activity compared to the control antibiotics linezolid and tigecycline. The selected EPS presented here were tested together with control antibiotics in mice suffering from a lethal sepsis induced by *Staphylococcus aureus* or *Escherichia coli*.

Methods: ESP were evaluated for the *in vivo* antibacterial activity in a murine septicemia infection model. A *S. aureus* ATCC 49951 (MSSA) and an *E. coli* strain ATCC 25922 were used as infectious agents. The total 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. For both pathogens groups of six to eight female NMRI mice per dose were used. Mice were infected intraperitoneally with an inoculum of approximately 4 x 10^7 CFU MSSA per mouse or approximately 10^6 CFU *E. coli* per mouse. The drugs were administered subcutaneously either one hour or simultaneously after infection. Survival of mice was recorded daily until day 10 post infection. The calculated ED_{50} values refer to the total dose given per day.

Results: In the selected bacteremia models ESP showed good efficacy when compared to linezolid (MSSA) and tigecycline (MSSA, *E. coli*). Against the MSSA, the ESP BC-9505, BC-9514, BC-9520, BC-9529 showed ED_{50} values of 0.36 mg/kg/day, 0.12 mg/kg/day, 2.08 mg/kg/day, and 0.47 mg/kg/day, respectively. Linezolid and tigecycline had ED_{50} values of 10.3 mg/kg/day and 0.99 mg/kg/day, respectively. The *in vivo* activity of the above ESP series observed in the *E. coli* sepsis model were 7.46, 3.17, 16.5, and 3.75 mg/kg/day, respectively and with this in the range of the ED_{50} obtained with tigecycline of 2.15 mg/kg/day.

Conclusion: ESP tested demonstrated excellent efficacy against severe murine bacteremia caused by *S. aureus* or *E. coli*. Based on data presented above, ESP will be further investigated in *in vivo* efficacy models including initial pre-clinical PK/PD studies.

INTRODUCTION

Staphylococcus aureus and *Escherichia coli* are leading causes of bacteremia often associated with serious complications. Nabriva's ESP program is developing a new generation of pleuromutilin antibiotics with the target to extend the conventional pleuromutilin spectrum (Gram-positive, fastidious Gram-negative and atypical pathogens), to a broader Gram-negative spectrum, primarily *Enterobacteriaceae*, addressing a significant unmet medical need.

Following *in vitro* efficacy testing, selected ESP were evaluated in two *in vivo* efficacy models to assess activity compared to the control antibiotics linezolid and tigecycline. The selected EPS presented here were tested together with control antibiotics in mice suffering from a lethal sepsis induced by *Staphylococcus aureus* or *Escherichia coli*.

METHODS

Bacterial isolates

The minimal inhibitory concentrations (MIC) were determined by CLSI broth microdilution method.

In vivo efficacy

The *in vivo* antibacterial activity of the ESP, linezolid, and tigecycline against bloodstream infections was determined in two murine sepsis infection models in immuno-competent mice.

S. aureus ATCC 49951

- NMRI Mice (~25 g) were infected intraperitoneally with an inoculum of ~ 4 x 10^7 CFU per mouse
- The drugs were dosed subcutaneously with doses between 10 and 0.06 mg/kg one hour post infection
- Mice were observed for up to 5 days for mortality

E. coli ATCC 25922

- NMRI Mice (~25 g) were infected intraperitoneally with an inoculum of ~10^6 CFU per mouse.
- The drugs were administered subcutaneously immediately after infection with single doses of 3.3 to 15 mg/kg to groups of six animals each
- Mice were observed for up to 5 days for mortality

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 the binary probit analysis.

RESULTS

- All tested ESP showed good *in vitro* and *in vivo* efficacy against *S. aureus* induced sepsis when compared to the standard antibiotics linezolid and tigecycline (Table 1)
- Linezolid and tigecycline tested against *S. aureus* showed ED_{50} values of 10.3 mg/kg/day and 0.99 mg/kg/day, respectively
- BC-9514 showed with 0.12 mg/kg/day the lowest ED_{50} dose of all tested compounds in the sepsis model caused by *S. aureus*

Table 1. Antibacterial activity and *in vivo* efficacy of ESP, linezolid and tigecycline against *S. aureus* ATCC49951

Compound	*S. aureus* ATCC 49951	
	MIC [mg/L]	ED_{50} [mg/kg/day]
BC-9505	≤0.03	0.36
BC-9514	≤0.015	0.12
BC-9520	0.06	2.08
BC-9529	≤0.015	0.47
Linezolid	4	10.3
Tigecycline	0.25	0.99

- In the murine sepsis model caused by the *E. coli* the ED_{50} values of the presented ESP ranged between 3.17 to 16.5 mg/kg/day (Table 2)
- Tigecycline showed an ED_{50} of 2.15 mg/kg/day against *E. coli*
- BC-9529 and BC-9514 showed with ED_{50} values of 3.75 and 3.17 mg/kg against *E. coli* induced sepsis , respectively, similar efficacy as tigecycline

RESULTS continued

Table 2. Antibacterial activity and *in vivo* efficacy of ESP and tigecycline against *E. coli* ATCC 25922

Compound	*E. coli* ATCC 25922	
	MIC [mg/L]	ED_{50} [mg/kg/day]
BC-9505	0.25 - 0.5	7.46
BC-9514	0.5	3.17
BC-9520	0.5	16.5
BC-9529	0.25	3.75
Tigecycline	0.125	2.15

CONCLUSIONS

- The extended spectrum pleuromutilins tested demonstrated excellent efficacy against severe murine septicemia caused by *S. aureus* and *E. coli*.
- Activity of the tested ESP against sepsis caused by *S. aureus* and *E. coli* was comparable to that of standard antibiotics linezolid or tigecycline.
- Based on the data presented above, ESP will be further investigated in *in vivo* efficacy models including initial pre-clinical PK/PD studies.

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