In Vivo and In Vitro Efficacy of Novel Extended Spectrum Pleuromutilins Against S. aureus and S. pneumoniae

S. Paukner, W. W. Wicha, K. Thirring, H. Kollmann and Z. Ivezic-Schoenfeld

Nabriva Therapeutics AG, Vienna, Austria

ABSTRACT

Objective: Resistance development to antibiotics is gaining a growing public health threat worldwide. In Europe particularly the resistance of E. coli and K. pneumoniae against cephalosporins, fluoroquinolones and aminoglycosides increased significantly while the percentage of MRSA was decreasing during the last few years. Nevertheless, MRSA isolates above 25% in one third of the reporting countries and is one of the most frequent causes of antibiotic resistance, particularly in healthcare-associated infections (HCAIs). The need for new antibiotics acting against those pathogens is evident.

Methods: Cytotoxicity and antibacterial activity of novel extended spectrum pleuromutilins (ESPs) address this problem by being active against Gram-negative and positive bacteria. This study reports new findings on the in vivo and in vitro antibacterial activity of ESP derivatives against S. aureus (ESP, MRSA), and S. pneumoniae including multi-resistant isolates. Furthermore, these antibiotics were evaluated in single hit path infection model, in vivo efficacy model in mice infected with S. aureus to proof their therapeutic potential in comparison to tigecycline.

INTRODUCTION

Extended spectrum pleuromutilins (ESPs) are a new generation of semi-synthetic pleuromutilin derivatives. By the substitution at the C-14 side chain and the trixylic pleuromutilin core (Figure 1), broad-spectrum activity was achieved against a wide range of Gram-negative and Gram-positive bacterial pathogens including Enterobacteriaceae, staphylococci, streptococci and fastidious Gram-negative bacteria. ESPs are potent inhibitors of the bacterial protein translation by specific blocking of the polyrhamny transferase center and thereby overcome major resistances. 1, 3

Four novel ESP derivatives from Nabriva’s drug discovery program have been selected for this study based on their potent in vitro activity against E. coli, low potential for cytotoxicity and high metabolic stability 1 to investigate the antibacterial activity of these new derivatives against larger sets of S. aureus (ESP, community-acquired methicillin-resistant S. aureus (CA-MRSA) and S. pneumoniae in addition to E. coli. Furthermore, the in vivo activity against S. aureus was explored in a bacteremia model in mice.

METHODS

MIC determination and bacterial isolates

The minimal inhibitory concentration (MIC) was determined by both microbroth dilution using CA-MHB according to CLSI M7-A9 (2012). Bacterial isolates were provided by various sources: MSSA isolates by ATCC and the general hospital (AKH) Vienna, Austria; CA-MRSA and S. pneumoniae by JM Laboratories (North Little Rock, AR, USA); E. coli isolates by D. Livernose (Health Protection Agency, UK) and F.J. Schmitz (Klinikum Minden, D).

In vivo bacteremia model in mice

The in vivo antibacterial activity of the ESP and tigecycline was determined in a sepsis model in C57Bl/6 mice (n = 15 per treatment group). One single hit of infection was performed by intravenous injection of 1·106 colony forming units (BCFU) of S. aureus ATCC49951 into the tail vein of the mice. In vivo efficacy model in mice

The in vivo activity of the ESPs and tigecycline was determined in a single-hit path infection model in C57Bl/6 mice (n = 15 per treatment group). One single hit of infection was performed by intravenous injection of 1·106 colony forming units of S. aureus infected with S. aureus to proof their therapeutic potential in comparison to tigecycline.

RESULTS

- All selected ESP (BC-7634, BC-9563, BC-9529 and BC-9563) having distinct substitutions at R1 and R2 demonstrated potent antibacterial activity against the tested clinical S. aureus isolates including both MSSA and CA-MRSA (Table 1).
- The MIC90 of ESP against CA-MRSA including isolates resistant to clindamycin, tetracyclines and fluoroquinolones ranged between 0.12 μg/mL and 0.25 μg/mL, which was as potent as tigecycline (MIC90 0.06 μg/mL) and significantly more active than vancomycin (MIC90 1 μg/mL).
- Against S. pneumoniae the ESP derivatives were active with 0.25 - 0.5 μg/mL similar potent as cefotaxime (MIC90 0.25 μg/mL) and vancomycin (MIC90 1 μg/mL).
- No cross-resistance was observed with macrolides, tetracyclines, 8-lactam antibiotics or fluoroquinolons.

Table 1. Antibacterial activity (µg/mL) of novel ESP derivatives against clinical S. aureus and S. pneumoniae isolates

Table 2. In vivo efficacy and antibacterial activity of ESP and comparisons against S. aureus ATCC 49951

CONCLUSIONS

- The presented extended spectrum pleuromutilins (ESP) having various distinct side chains at R1 and R2 demonstrated potent activity against staphylococci and streptococci including CA-MRSA and resistant clinical isolates.
- ESP maintained their activity against Gram-positive organisms while their antibacterial spectrum was extended by Enterobacteriaceae.
- The good in vitro antibacterial activity against S. aureus could be fully translated into good in vivo activity in the bacteremia model in mice demonstrating good drug absorption.
- Based on the data presented, ESP will be further explored for development as potent broad-spectrum antibiotics.

REFERENCES


ACKNOWLEDGMENTS

The authors gratefully acknowledge the practical work of A. Drusch, C. Mücke, A. Bauchinger, E. Fischer and E. Kappes. This project was partly funded by Z1T (Vienna, Austria) as part of the program “From Science to Products 2013”.

Figure 1. ESP Structure

Figure 2. Efficacy of novel extended spectrum pleuromutilins against MRSA and S. pneumoniae.

Figure 3. Efficacy of novel extended spectrum pleuromutilins against MRSA and S. pneumoniae.

Figure 4. Efficacy of novel extended spectrum pleuromutilins against MRSA and S. pneumoniae.