In vivo efficacy of ceftobiprole medocaril in two rabbit models of methicillin-resistant *Staphylococcus aureus* necrotizing pneumonia.

D. Hayez[1], S. Albac[1], C. Badiou[2], F. Vandenesch[2-3], G. Lina[2-3], M . Dumitrescu[2-3], A. Santerre-Henriksen[4], A. Schmitt-Hoffmann[4], N. Anzala[1], P. Chavanet[1,5] D. Labrousse[1], D. Croisier[1].

[1]Vivexia, Dijon, France. [2]Centre International de Recherche en Infectiologie, Inserm U1111, Université Lyon 1, Ecole Normale Supérieure de Lyon, CNRS UMR 5308, France. [3]Centre National de Référence des Staphylocoques, Hospices Civils de Lyon, France. [4]Basilea Pharmaceutica International Ltd, Basel Switzerland. [5]Infectious Diseases Department, University Hospital, Dijon, France.

Introduction

- Survival outcomes for patients suffering from necrotizing pneumonia caused by methicillin-resistant *Staphylococcus aureus* (MRSA), belonging to hospital-acquired (HA) or community-acquired (CA) lineages, is poor. Ceftobiprole (BPR) (active form of the prodrug ceftobiprole medocaril) is an advanced-generation cephalosporin with MRSA activity (including the major clones), currently approved in a number of countries for the treatment of community-acquired pneumonia and hospital-acquired pneumonia (excluding VAP).
- In this study, the efficacy of BPR was compared to vancomycin (VAN), clindamycin (CLI) and linezolid (LZO) in two rabbit models of necrotizing pneumonia: one induced by a Panton-Valentine-Leukocidine (PVL)-positive MRSA isolate, the other by a PVL-negative but alpha hemolysin (Hla) high producing MRSA isolate. All antibiotics were administered using human-equivalent dosing.

Materials and Methods

Bacterial Strains and Antimicrobials

Strain	Toxinic profile	Susceptibility to antibiotics	Source
SF8300	PVL positive	Fully susceptible	CA-MRSA pulsed-field type USA300-0114 (B. Diep)
ST20120426	PVL negative / Hla +++	Fully susceptible, except an inducible MLSb (*ermA*) resistance	HA-MRSA (clinical strain from a patient suffering from a lethal necrotizing pneumonia, CNR Lyon)

- Tested antimicrobials and sources were as follow : BPR for *in vitro* or *in vivo* use (Basilea Pharmaceutica International Ltd), VAN 500 mg for commercial use (Mylan), CLI 600 mg (Pfizer) and LZO 600 mg (Pfizer). Test materials were reconstituted according to the manufacturer instructions.
- For *in vivo* experiments, bacteria were grown on casein hydrosylate and yeast extract (CCY) supplemented with pyruvic acid as culture medium to optimize the PVL levels.

Contact : delphine.croisier-bertin@vivexia.fr

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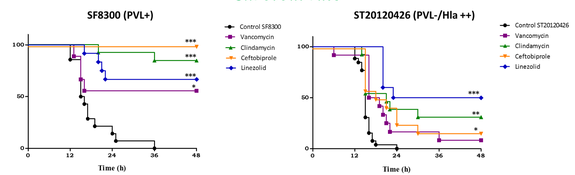
In vivo experimental necrotizing pneumonia model

- 24h after the jugular installation of a 2-lumen catheter, necrotizing pneumonia was induced by endobronchial bacterial challenge of 2.8-3kg New Zealand male rabbits with 0.5mL of saline containing 3.5x10^9 log CFU/mL.
- 5h after bacterial challenge (SF8300 PVL+ or ST20120426 PVL-), rabbits were randomly assigned to control (no treatment) or one of the tested antibiotic treatments at infusion rates controlled by a computerized electric pump and in doses to simulate the antibiotic kinetics observed in human serum : VAN continuous infusion (objective 30mg/L at the steady-state), CLI 600mg /8h, LZO continuous infusion (objective 8mg/L at the steady-state) and BPR 500mg/8h.
- Antibiotics concentrations were determined on iterative blood samples : VAN concentrations were determined by an immunofluorescence polarization method, CLI and LZO concentrations by HPLC and BPR concentrations by HPLC-MS-MS.
- 9 to 12 animals per group were used for the study
- Overall efficacy of treatment was assessed by survival rate, bacterial load in lungs and spleen, residual amount of toxins PVL & Hla (Elisa assay), and extent of lung damage by macroscopic score (sum of scores for individual pulmonary lobes, with 2 points added for purulent pleural effusion). For statistical analysis, animals for which an early death (<24h) was recorded were separated from the animals for which the autopsy was programmed after a 48h treatment. **p*<0.05, ***p*<0.001 & ****p*<0.0001 vs controls.

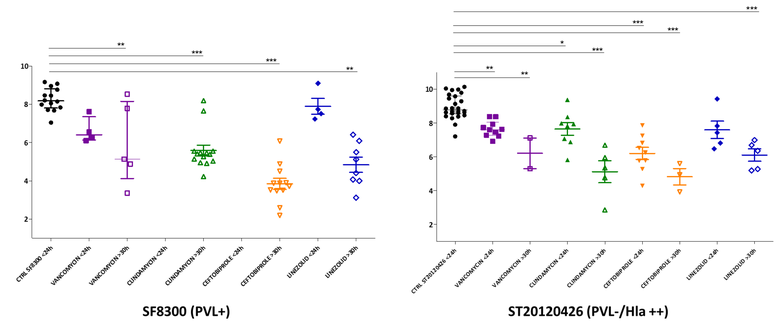
Results

Survival rate

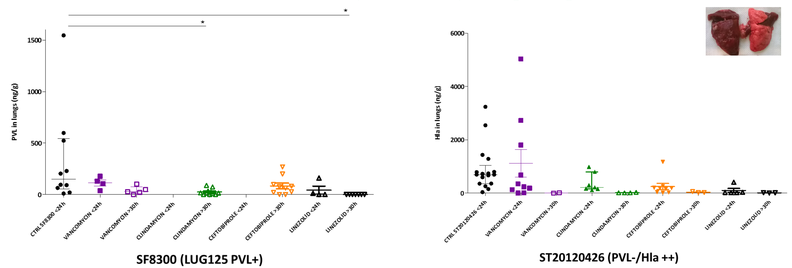


- In these two models, a rapid death was recorded in 100% of untreated animals (within 18h for strain ST20120426 versus 24h for strain SF8300).
- In the **CA-MRSA (PVL-positive) model**, the mortality rate was greatly reduced by BPR, CLI, LZO and to a lesser extent by VAN.
- In the **HA-MRSA (PVL-negative/Hla +++) model**, none of the antibiotics tested was completely effective, Highest survival was observed for LZO (continuous infusion) and then for CLI, BPR . Overall, LZO, CLI, BPR groups were significantly different to control group because of a postponed time to death.

Residual bacterial load (Log CFU/g)



Residual toxins (PVL and Hla) in lung homogenates



- In the **CA-MRSA (PVL-positive) model**, the highest pulmonary efficacy was obtained with BPR, CLI and LZO (top left panel). These antibiotics also significantly reduced the splenic bacterial load (the highest proportion of negative spleens was obtained with BPR and CLI) (data not shown).
- In the **HA-MRSA (PVL-negative/Hla +++) model**, significant antibacterial effects were observed in the lungs (top right panel) and spleens (data not shown) of both early-death and surviving animals with all treatments, with CLI and BPR being the most active agents.
- Macroscopic scores were not easily comparable because all control animals died very rapidly. However, in both models, significantly lower macroscopic scores were noted after treatment with CLI.
- In the **CA-MRSA (PVL-positive) model**, CLI and LZO significantly reduced PVL in lungs compared with controls (bottom left panel). Also, all pleural effusions collected in BPR-, LZO- or CLI-treated rabbits were PVL negative as compared to controls or VAN groups (data not shown).
- In the **HA-MRSA (PVL-negative/Hla +++) model**, reduced Hla pulmonary concentrations were observed in surviving rabbits treated by CLI, LZO or BPR (bottom right panel), but this diminution was not significant.

Conclusions

In the **PVL(+) CA-MRSA** rabbit model of necrotizing pneumonia, **ceftobiprole** and **clindamycin** displayed the highest efficacy in terms of survival rates, pulmonary and splenic bacterial reductions. Linezolid was also quite effective but did not allow to obtain a 100% survival. Clindamycin and linezolid significantly reduced the PVL amount in lung homogenates.

In the **PVL(-) HA-MRSA** model, the high alpha-toxin producing strain was highly virulent. **Linezolid** and also **clindamycin** (despite an inducible MLSb resistance) significantly attenuated the virulence of the strain, probably due to their anti-toxinic effect. Linezolid administered in continuous infusion provided a strong mortality reduction as compared to discontinuous infusion (previous data), supporting this mode of administration in patients suffering from severe pneumonia. Despite a moderate survival effect, ceftobiprole produced a rapid bactericidal effect in lungs and spleen.

Overall, these results suggest that administration of both a highly bactericidal antibiotic such as ceftobpirole and antitoxinic therapy with clindamycin/linezolid in the early life-threatening stages may improve the outcome of PVL-associated staphylococcal necrotizing pneumonia.

Croisier-Bertin D. *et al.,* AAC 2014 ; Saleh-Mghir A. *et al.,* AAC 2012; Hodille E. *et al.,* Med Mal Inf 2017; Nicholson SC. *et al.,* Int J Antimicrob Agents 2012; Diep BA. *et al.,* AAC 2017