POST-ANTIBIOTIC EFFECT OF ALX-009 AND ITS COMPONENTS ON CLINICAL STRAINS ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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INTRODUCTION

The post-antibiotic effect (PAE) of an antimicrobial agent is a Pharmacokinetic/Pharmacodynamic (PK/PD) in vitro test defined as the period of time after short-term exposure of the bacterial population to an antibiotic that elapses between the removal of the drug and the point at which the affected bacterial population recovers and resumes growing. Thus, the PAE may describe the recovery period of surviving cells of a bacterial population after exposure to an antimicrobial drug. A second PK/PD parameter, the post-antibiotic sub-MIC effect (PA-SME), describes the suppression of re-growth of populations in the post-antibiotic phase caused by a drug that is present at sub-inhibitory concentrations. Both parameters may be important since the Minimum Inhibitory Concentration does not reflect the in vivo scenario, where bacteria are being exposed to constantly changing antibiotic concentrations. In particular, the PA-SME is likely to mimic the clinical situation in the patient since concentrations of antimicrobial agents in serum and tissue may slowly fall below inhibitory concentrations towards the end of the interval between drug doses.

The goal of this study was to define if the ALX-009 combination induces a post-antibiotic effect and its extent. Obtained results may contribute to better characterize the pharmacological activity of this drug candidate.

METHODS

BACTERIAL CLINICAL ISOLATES

A clinical isolate of A. xylosoxidans, B. cepacia, P. aeruginosa, P. aeruginosa multi-drug resistant and S. maltophilia each was assayed. The strains were obtained from CF centers in Europe and USA.

TEST COMPOUNDS

OSCN: was produced by enzymatic reaction with Alaxia’s proprietary technology, bLF is a pharma grade bovine Lactoferrin produced by Alaxia.

MICROBIOLOGICAL METHODS

Bacterial isolates were cultured in Müller Hinton growth medium at 37°C overnight and diluted to a density of 10⁶-10⁷ CFU/ml. Cultures were exposed to 2-fold for OSCN and 10-fold the MIC for bLF alone or in the combination. The exposure time of the bacterial populations to the product was 10 min for OSCN and 2 hours for bLF when tested alone. In the combination, and due to the high antibacterial potential of OSCN that reduces drastically the bacterial populations below the limit of quantification within 1-2 hours, the exposure time was limited to 10 min. After exposure to the drugs, cultures were harvested by centrifugation and resuspended in fresh media to a DO₉₀₉ = 0.1. This standardized bacterial inoculum was then diluted 10 times without drugs (for PAE) or with drugs at 0.1, 0.2 or 0.3-fold the MIC for PA-SME). Cultures were then incubated at 37°C with orbital shaking. For each experiment, a control culture without drugs that was incubated, harvested and diluted identically to the test cultures was included.

POST-ANTIBIOTIC EFFECT MEASUREMENTS

PAE and PA-SME measurements were done by spectrophotometry as described by Dominguez et al. (2001). Antimicrob Chemother. 2001; 47: 391). Growth kinetics was followed at DO₉₀₉ every hour until the culture reaches DO₉₀₉ = 0.1. Post-antibiotic effects were measured as the difference of time of the test culture compared to the control culture to reach DO₉₀₉ = 0.1.

RESULTS

OSCN: PAEs ranging from 1.1 to 4.5 hours were induced in the tested isolates. The PA-SME testing revealed that delay of growth is not prolonged by the presence of sub-inhibitory concentrations of OSCN at either 0.1, 0.2 or 0.3-fold the MIC.

bLF: Lactoferrin demonstrates almost no post-antibiotic effect. Indeed, PAEs less than one hour (0.1 – 0.9) were induced for the A. xylosoxidans, B. cepacia, P. aeruginosa, S. maltophilia. Striking, for the P. aeruginosa MDR isolate, the post-antibiotic effect induced by bLF was of 9h. The PA-SME testing revealed that delay of growth is prolonged by the presence of sub-inhibitory concentrations of lactoferrin at 0.3 fold the MIC. At this dose, PA-SMEs ranging from 0.8 – 1.2 hours were obtained for the A. xylosoxidans, B. cepacia, P. aeruginosa, S. maltophilis, and 14.4h for the P. aeruginosa MDR strain.

ALX-009: The results demonstrated that the PAE effect observed with ALX-009 is similar to the effect induced by OSCN: alone and ranged from 1.4 – 3.5 hours. The PA-SME tests with 0.2 or 0.3-fold the MIC of ALX-009 prolonged the delay of re-growth for all isolates. The maximal PA-SME effect was observed at the 0.3 MIC dose level with values that ranged from 2.6 – 6.4 hours according to the species.

CONCLUSIONS

Like other antibiotics, PAE and PA-SME effects of ALX-009 and its components are product and strain dependent:

- OSCN induces a PAE effect in all strains but no PA-SME effect
- bLF shows a short PAE effect and the effect is increased by the presence of sub-inhibitory concentrations of this compound
- ALX-009 has a PAE effect similar to this of OSCN alone with no apparent impact of bLF. However, the effect is increased by the presence of sub-inhibitory concentration of bLF

These results confirm the interest of the ALX-009 combination to fight bacterial infections in CF patients.

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ALX-009 IS A FIRST IN CLASS ANTIMICROBIAL PRODUCT COMPOSED OF HYPOCHLORITE (OCl⁻) AND BOVINE LACTOFERRIN (bLF)

IN PREVIOUS REPORTS, WE HAVE DEMONSTRATED THAT ALX-009:

1. Is active against gram negative multi-drug resistant (MDR) bacteria infecting the lung of cystic fibrosis (CF) patients such as Achromobacter xylosoxidans, Burkholderia cepacia, Pseudomonas aeruginosa, P. aeruginosa multi-drug resistant and Stenotrophomonas maltophilia

2. Is active against bacterial populations embedded in biofilms or sputum of CF patients

3. Does not induce development of resistance on gram negative bacteria nor cross resistance to major class of antibiotics used in the clinic.

Safety pharmacology and general toxicological tests demonstrate that ALX-009 exhibits a favorable safety profile to support administration by inhalation to healthy volunteers and cystic fibrosis patients.

Table 1. PAE and PA-SME of OSCN, bLF and ALX-009 for CF clinical isolates.
Results are the average of three individual experiments, standard deviation in parenthesis.