The antifungal caspofungin (CAS) increases moxifloxacin (MXF) activity against Staphylococcus aureus biofilms in vitro or in a mice subcutaneous model

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Introduction

Staphylococcus aureus is an important human pathogen causing chronic infections that are difficult to treat. Biofilm contributes to the persistence of infections, by protecting bacteria from immune system and antimicrobial agents. We showed that many antibiotics are poorly active on biofilms [1], especially against clinical isolates from persistent infections [2]. In a preliminary screening of combinations of moxifloxacin (MXF) with drugs selected based on their amphiphilic character, we observed that the antifungal caspofungin (CAS) was synergistic. Our aim was now to test this combination on biofilms preformed on catheters in vitro and in vivo.

Materials and Methods

Biofilms were grown inside 1cm polyurethane catheters at 37°C for 24h (initial inoculum: 5.10^6 cells/ml).

In vitro, 7 clinical isolates strain were used. Biofilms grown on catheters were placed in 24-well plates, incubated with MXF (10mg/L); CAS (80mg/L) or MXF/CAS for 48 h. Catheters were washed, sonicated, and CFUs/catheter were counted.

In vivo, 5 catheters with pre-grown biofilms of the 2011S027 clinical isolate were implanted subcutaneously in the back of mice. Animals were treated intravenously with MXF (40 mg/kg twice daily), CAS (4 mg/kg/day) or with the MXF/CAS combination during 7 days. CFUs/catheter were counted.

Scanning electron microscopy (SEM) of in vivo biofilms; catheters were retrieved from the back of the mice after 7 days of treatment, fixed in 2% paraformaldehyde–2.5% glutaraldehyde and, after washing steps with PBS, postfixed with 1% osmium tetroxide, rinsed with PBS, and dehydrated using a series of washes with ethyl alcohol (30 to 100%). Representative SEM images were obtained as described previously [3].

Results

Effect of MXF, CAS or MXF-CAS combination on biofilms of clinical strains in catheters in vitro model

Results represent log_{10} CFU per catheter (3 catheters per treatment).

Statistical analysis (ANOVA): Differences between the MXF-treated group and the MXF+CAS-treated group were statistically significant in 6 out of 7 strains (P values 0.0002).

In vivo

Effects of the administration of MXF, CAS, and MXF+CAS on clinical isolate (2011S027) biofilms developed in subcutaneous mice model.

The horizontal lines indicate the median values for log_{10} CFU of MXF (7.12; 6.93; 6.49; 5.25 for the untreated, CAS, MXF and MXF-CAS respectively).

Statistical analysis (ANOVA): p <0.01 when comparing MXF-treated group and MXF-CAS group

Conclusions

Combining MXF with CAS proves highly synergistic in vitro and in vivo against staphylococcal biofilms of clinical strains.

This opens promising perspectives for new therapeutic strategies directed towards S. aureus biofilm-related infections.

References


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