Секция «Микробиология»

The new serine proteinase PAPC from Aspergillus ochraceus VKM-F4104D for targeting Staphylococcus aureus biofilms

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Background

Biofilms, complex microbial communities attached to surfaces and surrounded by a matrix of extracellular polymeric substances (EPS). Biofilm formation is considered an important element determining the pathogenicity and antimicrobial resistance of bacteria via decreasing the penetration of antibiotics. Many opportunistic bacteria form biofilms on chronic and acute dermal wounds impeding their healing and causing recurrent infections. The prevention of biofilm formation and disruption of already established biofilms is crucially important for the clinical treatment of infectious diseases. Enzymatic hydrolysis of biofilms could facilitate the penetration of antimicrobials to cells and increase the efficiency of antimicrobial treatment.

Materials and methods

The recombinant fibrinolytic protease-activator of protein C of blood plasma from micromycete *Aspergillus ochraceus* VKM-F4104D (PAPC) was expressed in *E. coli* BL21 (DE3) and purified on Ni-NTA resin. Two-days old biofilms of *Staphylococcus aureus* (ATCC 29213) were treated with PAPC protease for 24 hours in the presence or absence of antimicrobials. To assess the suppression of biofilm formation by PAPC, a CV-stain and Congo-Red stain were applied. PAPC-treated biofilms were quantified in the presence or absence of antimicrobials by CFUs count and metabolic assays including Resazurin and MTT assays. Additionally, the Live/dead BacLight assay was applied to assess the bacterial viability.

Results

The treatment of 48-h old *S. aureus* biofilms with PAPC protease (100 μg/ml) reduced the biomass by half in both CV- and Congo-Red stain. The combination of PAPC with vancomycin and amoxicillin increased the efficiency of the latter by 10-fold as judged by MTT-assay, Resazurin stain, and CFUs count. The results of Live/dead BacLight assay have matched the results of the previous methods.

Conclusion

The serine protease PAPC appears as a promising agent for combined antibiotic-enzymatic therapies for the external treatment of S. aureus biofilm associated infections.

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Illustrations

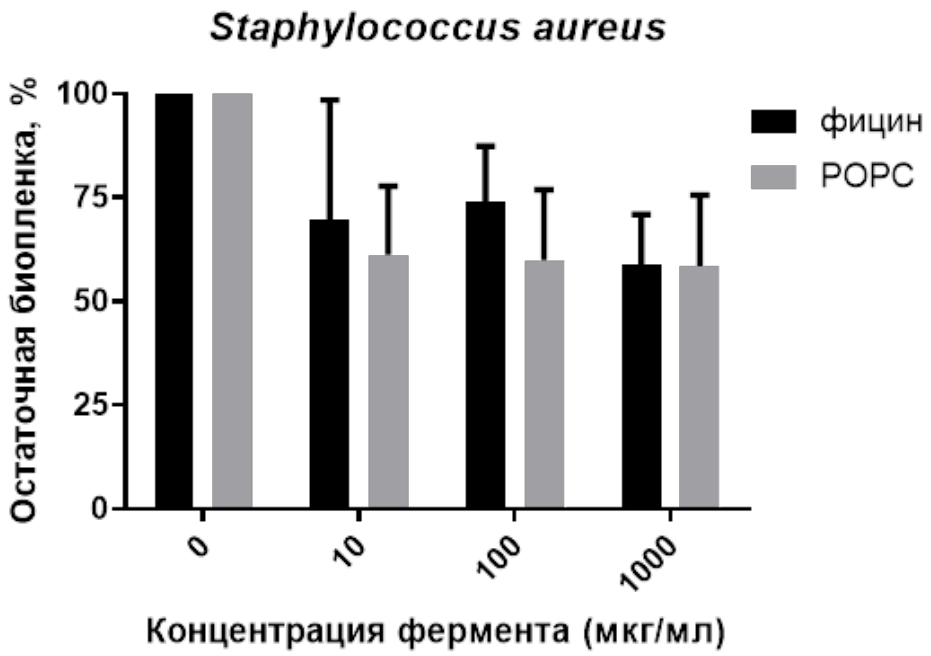

Рис. 1. Оценка разрушения биопленок золотистого стафилококка Фицином и протеазой PAPC, с помощью окраски кристаллическим фиолетовым.

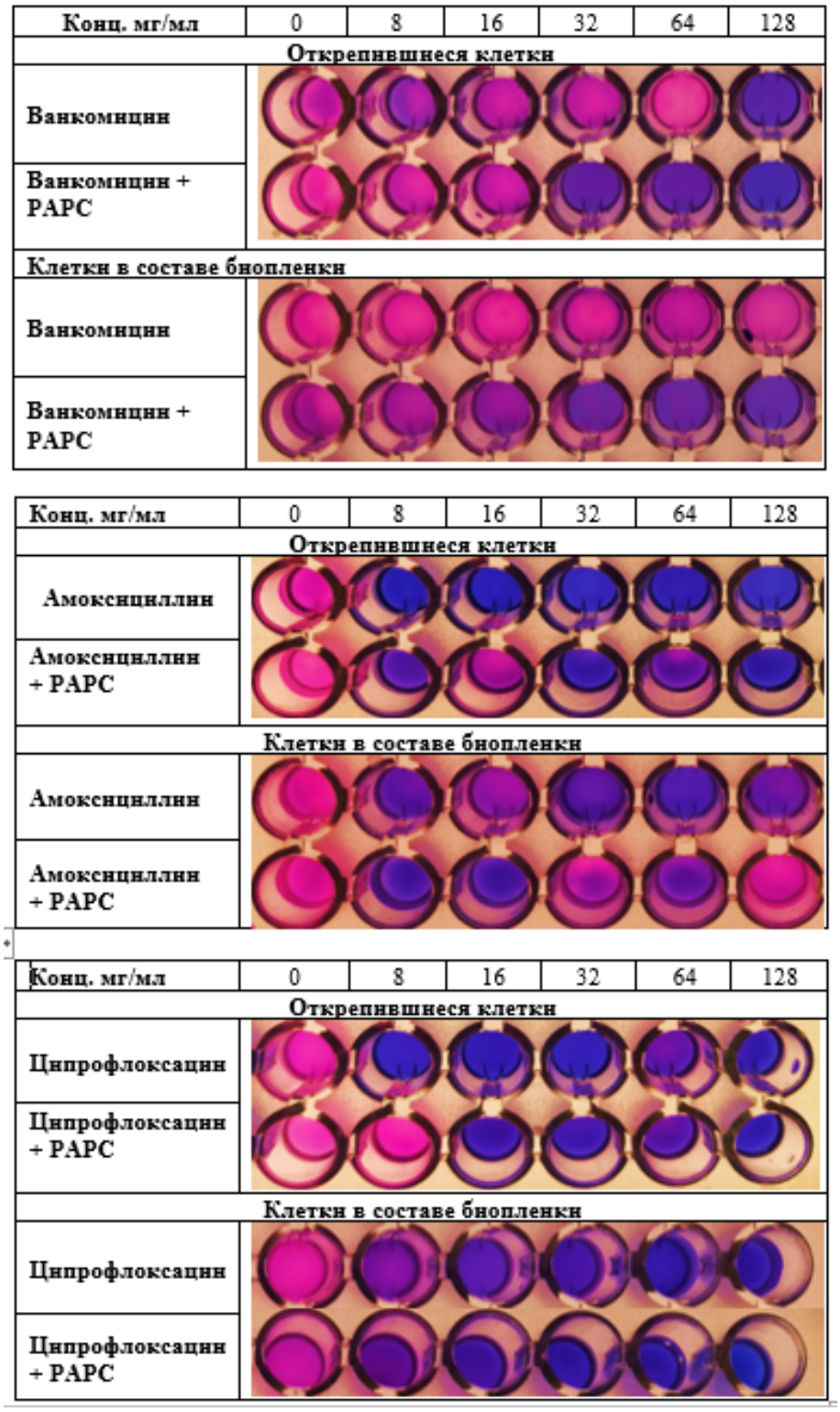

Рис. 2. Оценка синергизма протеазы PAPC с антибиотиками в отношении клеток S. aureus в составе биопленки (А) и открепившихся клеток (Б) в резазуриновом тесте.

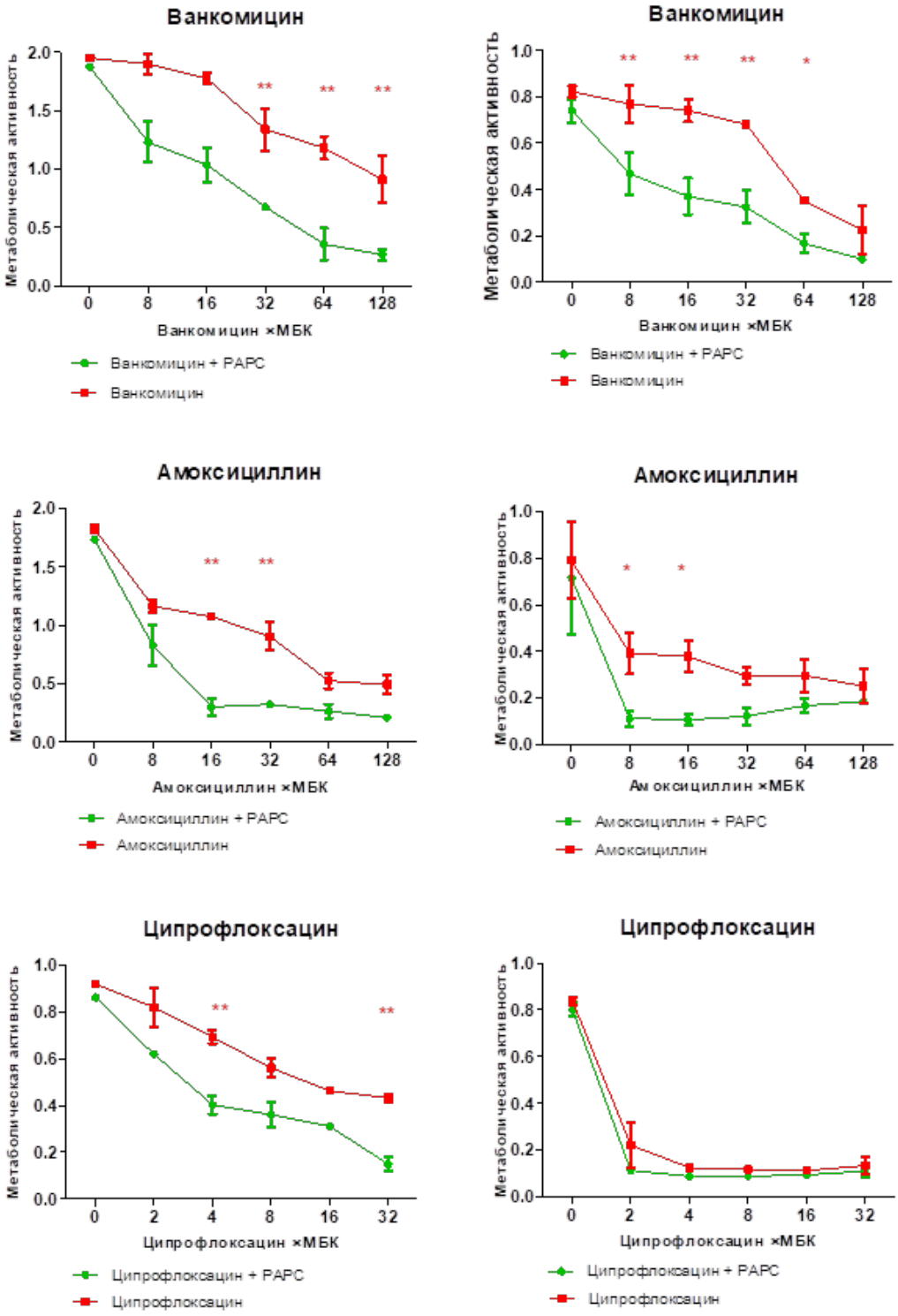

Рис. 3. Оценка синергизма протеазы PAPC с антибиотиками в отношении клеток S. aureus в составе биопленки (А) и открепившихся клеток (Б) путем оценки метаболической активности в МТТ-тесте.

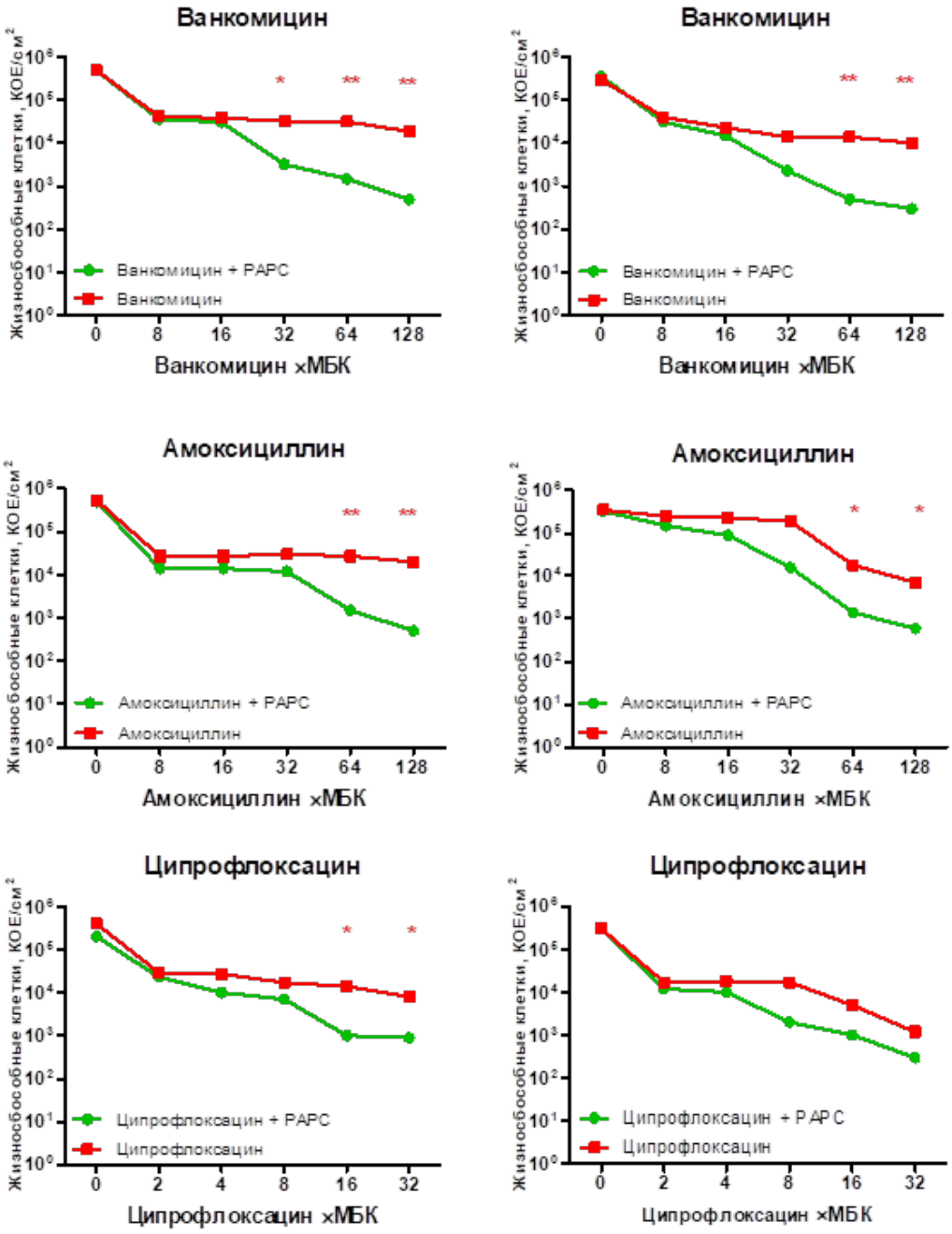

Рис. 4. Оценка синергизма протеазы PAPC с антибиотиками в отношении клеток S. aureus в составе биопленки (А) и открепившихся клеток (Б) путем оценки подсчета КОЕ.