

Kladnitskii V. Y., Galkin M. B.

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine; E-mail: quarzoniam@gmail.com

AMIXIN EFFECT ON DIFFERENT STRAINS OF *PSEUDOMONAS AERUGINOSA* WHICH HAVE DIFFERENT ABILITIES FOR BIOFILM FORMATION

Abstract

The study of how tilorone in different concentrations interact with collection strains of *Pseudomonas aeruginosa*, which have different ability to form the biofilm was performed. For the experiment were prepared five dilutions of tilorone 25, 50, 100, 250 and 500 mcg/ml. To determine the effect of tilorone on the biofilm formation used method for cultivation of the biofilms in the plates for cell culture, using the medium LB. The presence of the biological activity of the compounds judged by the presence of optical density difference between the experimental and control samples. The results showed that the different concentrations of test compound act differently on growth and biofilm formation of test strains *Pseudomonas aeruginosa*, causing stimulation or inhibition, or nothing changes at all.

Introduction

Today, epidemics of many diseases are less common now; infections caused by conditionally pathogenic microorganisms are becoming more widespread. In the twentieth century, antimicrobial agents were created which almost immediately entered clinical practice and produced high results.

However, through the widespread use of antibiotics, science and medicine have faced a rather complex problem of resistance to various antimicrobial drugs.

Particular attention should be paid to conditionally pathogenic bacteria that very quickly acquire polyresistance to a wide range of antimicrobial agents; in particular, *P. aeruginosa* is a microorganism that very often causes nosocomial infections. An additional factor of danger is that it is very resistant to disinfectants. This makes it possible to reproduce on medical instruments, which must be sterile.

Thus, the development and introduction of the medical practice of new approaches for combating opportunistic pathogenic microorganisms are relevant.



Materials and methods

The experiment was conducted on a several strains of *Pseudomonas aeruginosa* PA01 (wild type) and its derivatives *P. aeruginosa* *wspF1* carrying a mutation in the gene *wspF1* and has a high ability to form biofilms, and *P. aeruginosa* pJN2133 carrying plasmid pJN2133 and has reduced ability to form biofilms.

In work used the method of culturing microorganisms in static conditions using sterile 96-well polystyrene flat-bottomed plates. To determine the intensity of biofilm formation was used the method of staining by crystal violet with some modifications. Formation of biofilm determine spectrophotometrically [1].

Results and discussions

The influence of tilorone on the formation of biofilms by *P. aeruginosa* PA01, *P. aeruginosa* *wspF1*, and *P. aeruginosa* pJN2133 was performed after 24 hours of incubation with different concentrations of test compound. Comparison of control culture and culture with Tilorone showed that stimulation of biofilm formation at all concentrations was observed only in the strain *P. aeruginosa* PA01 (Figure 1).

There was inhibition of biofilm formation at all concentrations only in strain of *P. aeruginosa* *wspF1* (Figure 2), the highest inhibition effect found at tilorone concentration of 100 mcg/ml and was 31, 29% compared with the control.

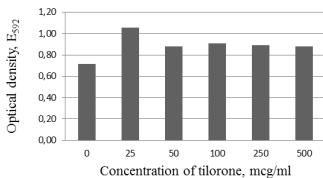


Fig. 1. Indicators of biofilm formation by *P. aeruginosa* PA01 in the presence of tilorone

Note: * - significant difference compared with control

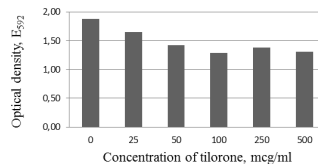


Fig. 2. Indicators of biofilm formation by *P. aeruginosa* *wspF1* in the presence of tilorone

Note: * - significant difference compared with control

Different concentrations of test compound affected differently as inhibiting or stimulating the formation of biofilm only for strain of *P. aeruginosa* pJN2133, but mainly observed stimulation and maximum gain was 162, 81% at concentration of tilorone 500 mcg/ml (Figure 3).

The results showed that the different concentrations of test compound act differently on growth and biofilm formation of test strains

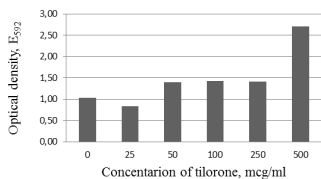


Fig. 3. Indicators of biofilm formation by *P. aeruginosa* pJN2133 in the presence of tilorone

Note: * - significant difference compared with control

Pseudomonas aeruginosa, causing stimulation or inhibition, or nothing changes at all.

But, at certain concentrations in specific strains experienced a pronounced stimulatory and/or inhibition effect on the intensity growth and formation of biofilm of microorganism. In this regard *P. aeruginosa* strain *wspF1* was

chosen as the model of inhibition effect of tilorone on ability to form biofilm, and strain *P. aeruginosa* pJN2133 as model of stimulation.

Conclusions

After comparison with other synthetic low-molecular inducers of interferon, for example, a derivative of acridine acetic acid, which have a pronounced antibacterial activity and suppress a number of opportunistic microorganisms in vitro, we can speak of the effectiveness of tilorone as an antibacterial drug; however, this efficiency depends on its concentration and microorganism characteristics. The conducted study of the antimicrobial properties of this compound may be the foundation for further study of its mechanisms of action on a bacterial cell or influence on the processes associated with the formation of biofilms.

References

1. Christensen G. D. Adherence of coagulase-negative Staphylococci to plastic tissue culture plates: a quantitative model for the adherence of Staphylococci to medical devices // J. clin. microbiol. – 1985. – V. 22. – № 6. – P. 996-1006.