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# THE INFLUENCE OF GKP-305 ON THE CULTURAL PROPERTIES OF M. BOVIS OF PATHOGENIC STRAINS AND DISSOCIATIVE FORMS

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*GKP-305 drug at 1% and 0.5% concentrations partially inhibits the growth and development of pathogenic strains of M. bovis, cultivated through the medium with pH 7.1 at 37° C. The growth of colonies of investigated strain of mycobacteria through the medium with pH 6.5 at 0.5 and 1.0% concentrations of the compound has not been observed within 90 days that means the tuberculostatic effect of the compound.*

*The received data can be used to improve bacteriological diagnosis of bovine tuberculosis, selection of vaccine strains for designing drugs with specific prevention of disease*

***Dissociative forms, low positive temperature, M. bovis, tuberculostatic effect***

**1. Introduction.** Despite the experience gained in the control and prevention of tuberculosis, this disease still continues to be an important medical, veterinary and social problem, probably, due to the variety of biological properties (conversion and reversion) of its pathogenic agent. The early detection of the disease and effective control of its agent becomes the more urgent issue today [1-3].

One of the forms of variability of tuberculosis mycobacteria is the formation of resistance to antibacterial compounds. Mycobacteria acquire the ability to survive and reproduce at the following concentrations of antibacterial agents that are typically not compatible with their life. Antibacterial drugs have no effect on the process of synthesis of modified forms of mycobacteria. Variability in different strains varies widely [4-7].

Antibacterial drugs, stains, acids and other chemicals are the variability factors of Mycobacterium bovis. They cause the pigment appearance; decrease an amount of bacilli with bacterial cell wall defect (L-forms); rapid formation of granular forms; the loss of acid-fast in microorganisms' cultures [8].

Due to the massive use of antibiotic therapy strains resistant to ftivazid, streptomycin, 4-aminosalicylic acid (PAS), cycloserine have been appeared. Mycobacteria can have double and even triple resistance [1,5-7,9].

The complex evolutionary formed structure, and hence the physiological mechanisms that develop in the process of life, provide extremely high adaptive capacity to a particular medium. This is accompanied by appearance of mycobacteria that are unusual, different from traditional forms.

The biological properties of mycobacteria, in particular of M. bovis, are still need to be investigated, because there are more and more articles about the new features of some of them. It has been shown by Tkachenko et al. [10], that the following passages of modified forms of mycobacteria through the artificial nutrient medium with different pH result in the appearance of L-forms that retain some time the ability to reverse in the initial acid-fast bacillus of M. bovis. Meanwhile, same authors accented that in some cases the appearance of forms of mycobacteria, conversion and

reversion was accompanied by loss of initial ability to form colonies.

In this work the influence of drug GKP-305 and pH of medium on the cultural properties of M. bovis of pathogenic strains and dissociative forms at 3 and 37° C has been investigated.

**2. Experimental.** The cultivation and biomass accumulation of studied strains of M. bovis was performed on egg nutrient medium. GKP-305 (N'-2(-(5-((teophilin-7-il)methyl)-4-ethyl-4H-1,2,4-triazol-3-ilthio)acethyl) isonicotinic acid hydrazide) was synthesized at Zaporizhzhia State Medical University. This drug was added to the medium in concentrations of 0.1–1% depending on the purposes of experiment. The investigation was carried out with the two levels of pH: 6.5 and 7.1.

Mycobacteria of 117$^{th}$ a variant, accumulated at 3° C, were planted by two wire inoculating loops on slope nutrient medium of 10 test tubes, followed by incubation in a thermostat (TD-1/80 CPU and TDC-1/80 CPU, Russia) for three months at different temperatures (3, 37° C) with daily observation.

Tinktorial properties, morphological features, the term of the primary growth appearance, the intensity and nature of the subculture of isolated and accumulated mycobacteria have been evaluated. Analysis and evaluation were carried out by estimation of the number of colonies, its size, shape, surface texture, chromogenesis, transparency, luster and emulsification in saline. Tinktorial properties of mycobacteria was determined through preparing smears from colonies (culture), painted by Ziehl–Neelsen stain and analyzing them under a microscope immersion of SUNNY XS series with digital microscopic complex «Mykmed-2-1600» (St. Petersburg, Russia). Morphology of mycobacteria was determined by length, thickness, shape, character of cell ends, grain and location. M. bovis of pathogenic strain of 100$^{th}$ passage and dissociative forms of 117$^{th}$ a variant without adding of GKP-305 to the medium were used as reference.

**3. Results and Discussion.** 100$^{th}$ Passage of M. bovis that was cultured at 37° C through the medium with GKP-305 in above mentioned concentrations in the thermostat for three months on a medium with a pH of 6.5 and pH 7.1 (in the amount of ten tubes for each compound concentration). M. bovis of 117$^{th}$ a variant was cultured at 3° C through the medium with GKP-305 in same concentrations in thermostat with cooling effect for three months on a medium with an anologous pH.

As one can conclude there is no growth of the culture of 100$^{th}$ passage of M. bovis if GKP-305 presents at 1% concentration only (Table 1). The absence of growth has been marked during the all experiment (90 days). This indicates the tubercu-

Table 1. **Cultural properties of 100 passage of M. bovis cultivated at 37° C through the medium with different pH**

<table>
<tr><td rowspan="3">Term, days</td><td colspan="4">pH 7.1</td><td colspan="4">pH 6.5</td></tr>
<tr><td rowspan="2">Reference</td><td colspan="3">Drug concentration GKP-305, %</td><td rowspan="2">Reference</td><td colspan="3">Drug concentration GKP-305, %</td></tr>
<tr><td>0.1</td><td>0.5</td><td>1</td><td>0.1</td><td>0.5</td><td>1</td></tr>
<tr><td>7</td><td>Rough film</td><td colspan="3" rowspan="2">No growth</td><td>Single colonies on the sow line</td><td>No growth. Rough film on the sow line</td><td colspan="2" rowspan="6">No growth</td></tr>
<tr><td>14</td><td>Rough film, single white colonies on the sow line</td><td rowspan="4">The continuous growth. Small, white, smooth colonies</td><td>Single small colonies on the sow line</td></tr>
<tr><td>30</td><td rowspan="2">The continuous growth. Smooth, small whitish colonies</td><td colspan="2" rowspan="3">Rough film</td><td colspan="2" rowspan="3">No growth</td><td>Growth of white and yellow dewy colonies</td></tr>
<tr><td>60</td><td>Growth of single small colonies amount</td></tr>
<tr><td>90</td><td>The continuous growth</td><td>The growth of the rough large R-forms, which are then converted into a smooth whitish S-forms</td></tr>
</table>

lostatic effect of GKP-305 at 1% amount. At 0.1% and 0.5% concentration a gradual growth of the colonies, starting from the 30th day of the experiment - rough film on the sow line has been marked, that during 90 days of observation has not changed. Up to 7th day of the experiment the culture growth of 100th passage of M. bovis in the reference group both on the medium with pH 7.1 and 6.5 at 37° C was not marked.

Effect of the compound at different concentrations in the medium with a pH of 6.5 is slightly different than with pH 7.1. On the 7th day at 0.1% concentration of GKP-305 the growth of the colonies was not observed, but there is rough film on the sow line (in the reference - single colonies on the sow line), then on 14th day single small colonies on the sow line appears (in the reference - continuous growth, colonies are small, white; smooth (S-forms). Over the next observation period for 0.1% concentration of GKP-305 drug the growth of white and yellow dewy colonies (30th day of the experiment); increasing both of number of small isolated colonies (60th day of the experiment) and rough R-forms, which are then converted into a smooth whitish S-form colonies (90th day of the experiment) has been observed, but the number of colonies is less compared with the reference.

As concerns the 0.5% and 1.0% concentrations GKP-305 drug there was no growth of pathogenic strains of 100th passage of M. bovis throughout the observation period (90 days). Thus, we can conclude that the GKP-305 drug at 0.1% concentration has no effect on cultural properties of the pathogenic strain of M. bovis, cultivated through a medium with a pH of 6.5 at 37° C, when at 0.5% and 1.0 % concentration it has tuberculostatic effect.

As one can conclude, the growth of culture of 117th a variant of dissociative forms of M. bovis through the medium with pH 7.1 at a temperature 3° C is observed on the 3rd day of the experiment (Table 2). Within 30 days of observation at 0.1 and 0.5% concentrations a growth of the culture occurs: a mucous film on the sow line on the 7th day; rough film on the sow line with grayish (0.1%), orange (0.5%) color on the 14th day of experiment and continuous growth on the 30th day. Meanwhile at the 1% concentration during the first 7 days of the experiment there was no growth of colonies. During the follow-up observation mucoid film on the sow line appears on the

Table 2. **Cultural properties of 117 a variant of M. bovis cultivated at 3° C through the medium with different pH**

<table>
<tr><td rowspan="3">Term, days</td><td colspan="4">pH 7.1</td><td colspan="4">pH 6.5</td></tr>
<tr><td rowspan="2">Reference</td><td colspan="3">Drug concentration GKP-305, %</td><td rowspan="2">Reference</td><td colspan="3">Drug concentration GKP-305, %</td></tr>
<tr><td>0.1</td><td>0.5</td><td>1</td><td>0.1</td><td>0.5</td><td>1</td></tr>
<tr><td>3</td><td>Mucous grayish film</td><td colspan="3">No growth</td><td>Mucous grayish film</td><td colspan="3">No growth</td></tr>
<tr><td>7</td><td>Mucous yellow-grayish film</td><td>Mucous grayish film</td><td>No growth. Rough film on the sowing line</td><td>No growth</td><td>Mucous yellow-grayish film</td><td colspan="2">No growth. Rough film on the sow line</td><td>No growth</td></tr>
<tr><td>14</td><td rowspan="2">The continuous growth of orange colonies</td><td>Rough gray film on the sow line</td><td>Rough orange film on the sow line</td><td>Mucous grayish film</td><td rowspan="2">The continuous growth of orange colonies</td><td>Single small pale pink colonies on the sow line</td><td>Rough gray film on the sow line</td><td>No growth. Rough film on the sow line</td></tr>
<tr><td>30</td><td>The continuous growth of orange colonies</td><td>Single small orange colonies on the sow line</td><td>Rough gray film on the sow line</td><td>The continuous growth of orange colonies</td><td colspan="2">Rough gray film on the sow line</td></tr>
</table>

14th day, and rough gray film on the sow line on the 30th day has been observed.

Studying the effect of different concentrations of GKP-305 drug on cultural properties of 117th a variant of M. bovis cultivated through the medium with pH 6.5 at a temperature of 3° C the analogous tendency to the pH 7.1: during the first 3 days of observation at different concentrations the growth of M. bovis was absent; since 7th day rough grayish film has been revealed; mucous grayish-yellow film (reference), small pale pink colonies (0.1% concentration); rough film on the sow line (0.5% concentration) have been observed on the 14th day; the continuous growth of orange colonies (0.1% concentration); rough gray film on the sow line (0.5 and 1% concentrations) have been observed on the 30th day.

**4. Conclusions**

Thus, these investigations have shown that GKP-305 drug at 1% and 0.5% concentrations partially inhibits the growth and development of pathogenic strains of M. bovis, cultivated through the medium with pH 7.1 at 37° C. The growth of colonies of investigated strain of mycobacteria through the medium with pH 6.5 at 0.5 and 1.0% concentrations of the compound has not been observed within 90 days that means the tuberculostatic effect of the compound.

The received data can be used to improve bacteriological diagnosis of bovine tuberculosis selection of vaccine strains for designing drugs with specific prevention of disease.

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## ВЛИЯНИЕ ПРЕПАРАТА ГКП-305 НА КУЛЬТУРАЛЬНЫЕ СВОЙСТВА M. BOVIS ПАТОГЕННЫХ ШТАММОВ И ДИССОЦИАТИВНЫХ ФОРМ

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*В данной работе было исследовано влияние препарата ГКП-305 и pH среды на культуральные свойства M. bovis патогенных штаммов и диссоциативных форм при 3 и 37° С.*

*Установлено, что препарат ГКП-305 в концентрациях 1 и 0,5% частично угнетает рост и развитие патогенных штаммов M. bovis, культивированных через среду с pH 7,1 при температуре 37° С. При культивировании через среду с pH 6,5 при 0,5 и 1,0% концентрации препарата в течение 90 дней (период эксперимента) рост колоний данного штамма отсутствовал, что свидетельствует о туберкулостатическом действии препарата.*

*Полученные результаты могут быть использованы для повышения эффективности бактериологической диагностики туберкулеза животных, селекции вакцинных штаммов для конструирования препаратов специфической профилактики заболевания*

***Диссоциативные формы, низкая плюсовая температура, M. bovis, туберкулостатический***

## ВПЛИВ ПРЕПАРАТУ ГКП-305 НА КУЛЬТУРАЛЬНІ ВЛАСТИВОСТІ M. BOVIS ПАТОГЕННИХ ШТАМІВ ТА ДИСОЦІАТИВНИХ ФОРМ

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*В даній роботі було досліджено вплив препарату ГКП-305 і pH середовища на культуральні властивості M. bovis патогенних штамів і дисоціативних форм за 3 та 37° С.*

*Встановлено, що препарат ГКП-305 за 1% і 0,5% концентрацій частково пригнічує ріст і розвиток патогенного штаму M. bovis, культивованого на середовищі з pH 7,1 за температури 37 °С. На середовищі з pH 6,5 за 0,5 та 1,0% концентрацій препарату протягом 90 діб (період експерименту) ріст колоній даного штаму мікобактерій був відсутній, що свідчить про туберкулостатичну дію препарату.*

*Низька концентрація (0,1 та 0,5%) препарату ГКП-305 на середовищі з pH 7,1 та 0,1% з pH 6,5 за температури 37°С ріст патогенного штаму M. bovis 100-го пасажу не стримує.*

*Різні концентрації препарату ГКП-305 (0,1, 0,5%) на середовищі з pH 6,5 та 7,1 за температури 3°С не впливають на характер росту колоній дисоціативних (апатогенних) форм M. bovis 117а варіанта: ріст відбувається так само, як і в контролі; 1% концентрація препарату частково затримує ріст і розвиток колоній впродовж 7 діб.*

*Отримані результати можуть бути використані для підвищення ефективності бактеріологічної діагностики туберкульозу тварин, селекції вакцинних штамів для конструювання препаратів специфічної профілактики хвороби.*

***Дисоціативні форми, низька плюсова температура, M. bovis, туберкулостатичний ефект***