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# THE STUDY OF SOME PROMISING PHARMACEUTICAL COMPOSITIONS WITH UREASE INHIBITORY ACTIVITY FOR THE TUBERCULOSIS REACTIVATION PREVENTION

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Mycobacterial urease is one of the pathogenetic factors inhibiting the phagocytosis completion [1,2]. Urea hydrolysis and forming ammonia, urease alkalinizes the lysosomes inside and blocks acidic proteolytic enzymes. If urease inhibitors are used in combination with the phagocytosis activators, there is a great chance to prevent reactivation of tuberculosis into acute open forms [3].

The aim of the study was to examine the urease inhibition ability of some pharmaceutical compositions that are promising in the prevention of tuberculosis reactivation [4]. In particular, according to a number of references sources, quercetin is able to successfully inhibit urease by a non-competitive mechanism [5]. Another compound - dipyrone (metamizol sodium) according to preliminary molecular modeling has a pharmacophore structure similar to urea. Accordingly, metamizole sodium is able to competitively inhibit urease. For greater effectiveness of the composition, it is necessary to combine non-competitive and competitive urease inhibitors. In this case, urease inhibitors should not be inactivated by phagocytosis activators, such as bis-succinillizine and cholecalciferol. For this purpose, the inhibitory ability for both individual compounds and their mixtures to inhibit urease activity was studied.

**Materials and methods**. As the objects of research, the pharmaceutical compositions and substances presented in Table 1 were used.

In previous studies, the highest activity against urease was found in substances such as quercetin and dipyrone. In addition, for confirm the statistical hypothesis about their high inhibitory activity against control, a series of biochemical studies of these substances effect on urease was carried out.

The main disadvantage of KV is its complete hydrophobicity and insolubility in aqueous solutions. We have developed a unique system, which in liquid form is able to store KV concentrate even at elevated temperature. The introduction of such concentrate to aqueous solutions does not lead to the KV precipitation for a long time.

**Determination of urease activity**

The study used vegetable urease from standard kits produced by Ukrmedsnab (Ukraine, Kiev) for urea determination in the blood serum. The declared enzyme activity was 5000 U / ml. The standard urease solution, when added to the reaction medium, was diluted 1000 times by distilled water. As the substrate a 0.5% aqueous urea solution was used. The reaction was carried out at a temperature of 37 ° C, the incubation time was 10 minutes. The urease activity was determined by the color reaction with the hypochlorite reagent. Photometry was performed on a FEK-3M photoelectric colorimeter at a wavelength of 590 nm.

When working with this enzyme, graphs were made for the dependence from activity and reaction time, on the enzyme concentration and on the substrate concentration. On basis of these data, the optimal composition of the reaction medium and the reaction time were selected, which made it possible to carry out investigations on linear sections the curves. The Michaelis constant (Km) for urease catalysis reactions is estimated as the main characteristic of its activity. Km was 4.2 mg / ml (or 70 mm). This indicator is consistent with the available literature data on the activity of beans urease (from 1 to 130 mm) [6,7].

The studied urease inhibitors were added to the reaction medium at various dilutions. The degree of inhibition (DI) of urease activity was calculated as follows:

$$DI = \frac{Ey - Ei}{Ey} \cdot 100\%,$$

where Ey - urease activity without inhibitor; Ei - activity of urease with the addition of the inhibitor.

Then, the degree of inhibition was recalculated taking into account the dilution of the inhibitor and the semi-inhibitory concentration of $IC_{50}$- inhibitor concentration, which inhibited urease activity by 50%, was calculated graphically.

*Investigation of biological activity of the most promising urease inhibitors*

The results of evaluating these compounds as urease activity inhibitors (the value of semi-inhibitory concentrations) are presented in Table. 1.

**Table 1. Inhibitory activity of various compounds to urease, (M ± σ)**

| The pharmaceutical compositions / substances | $IC_{50}$, mg/mL |
|---|---|
| 1. Quercetin + metamizol sodium | 1,27 ± 0,52 |
| 2. Formylpeptide 3 | 19,6 ± 6,5 |
| 3. Succinylformyllysine | 8,52 ± 5,11 |
| 4. Bisformylarginine | 6,0 ± 3,8 |
| 5. Chlorophyll + quercetin (1:1) | 0,7 ± 0,44 |
| 6. Quercetin + cholecalciferol (vit. D3) | 1,3 ± 0,85 |
| 7. Succinylformylarginine | 13,8 ± 3,57 |
| 9. Phenylimideofaconiticacid | 5,8 ± 1,22 |
| 10. a-bromophenylimide of aconitic acid | 5,7 ± 0,84 |
| 11. Metformin | 0 |
| 12. Metamizol sodium | 2,6 ± 0,43 |

From the data presented, it follows that quercetin and metamizole sodium have the strongest inhibitory properties, since the lowest values of semi-inhibitory concentrations are obtained for them. In the presence of

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chlorophyllipt, the inhibitory activity of quercetin against urease is not suppressed. Metformin showed no inhibitory activity against urease, and it was low for other substances.

Further, the type of inhibition of the two most potent urease inhibitors, quercetin and metamizole sodium, was determined. To this end, the dependence of the reaction rate on the substrate (urea) concentration without inhibitor and in its presence was determined (Table 2).

**Table 2. Dependence of urease activity on substrate concentration under the influence of quercetin and metamizole sodium, (M ± σ)**

| Inhibitor | Urease activity at substrate concentrations, mg/mL: | | |
|---|---|---|---|
| | 1 | 2 | 5 |
| Without an inhibitor | 0,481 ± 0,042 | 0,816 ± 0,075 | 1,370 ± 0,051 |
| Quercetin (0.64 mg / ml) | 0,177 ± 0,008 | 0,303 ± 0,022 | 0,484 ± 0,014 |
| Quercetin (0.25 mg / ml) | 0,343 ± 0,026 | 0,561 ± 0,017 | 0,938 ± 0,034 |
| Metamizole (6.25 mg / ml) | 0,315 ± 0,030 | 0,438 ± 0,028 | 0,602 ± 0,029 |
| Metamizole (4.17 mg / ml) | 0,366 ± 0,035 | 0,560 ± 0,037 | 0,815 ± 0,020 |

To evaluate the type of urease inhibition, the inverse substrate concentration (1 / S) and the reaction rate (1 / V) were calculated. Based on these data, plots of 1 / V versus 1 / S were made, (Fig. 1).

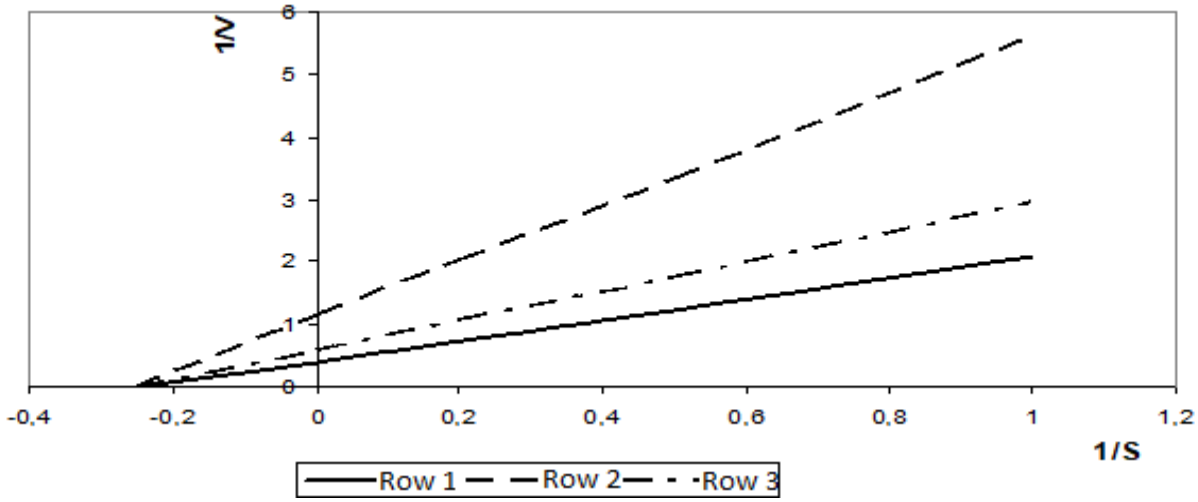


**Fig. 1 - Effect of quercetin on 1 / V versus 1 / S urease; V- reaction rate (mg / min) S-substrate concentration (mg / ml). Row 1 - urease without inhibitor, Row 2 - urease + quercetin (0.64 mg / ml), Row 3 - urease + quercetin (0.25 mg / ml).**

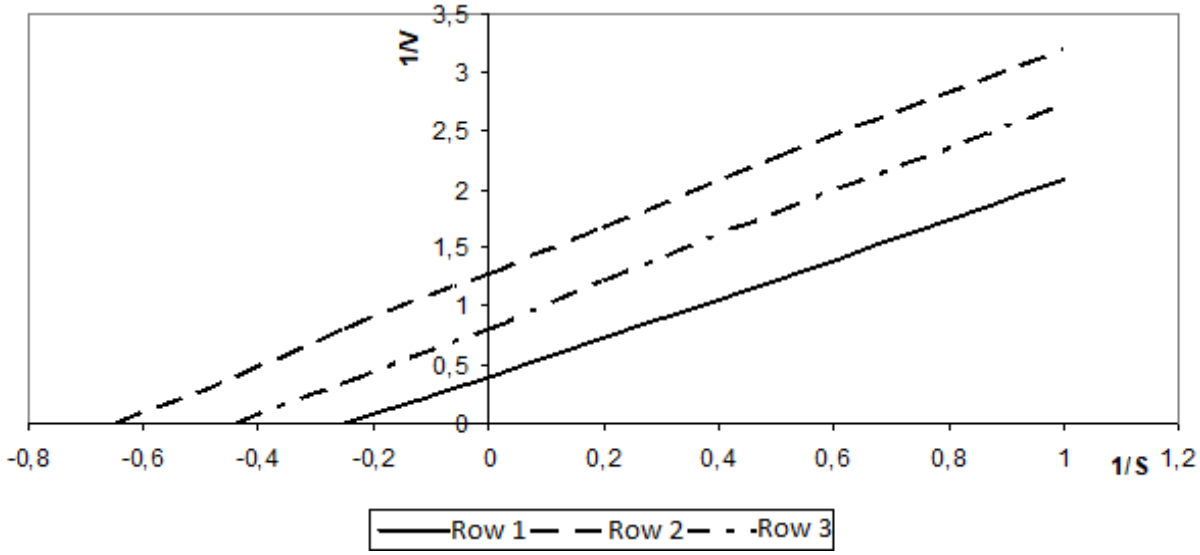


**Fig. 2 - Influence of metamizole sodium in the 1 / V dependence on 1 / S urease; V- reaction rate (mg / min) S-substrate concentration (mg / ml). Row 1 - urease without inhibitor, Row 2 - urease + metamizole (6.25 mg / ml), Row 3 - urease + metamizole 4.17 mg / ml.**

As follows from the data presented in Fig. 1 data, quercetin is characterized by a non-competitive type of urease inhibition. The degree of inhibition depends on the concentration of the inhibitor and independent of the

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substrate concentration. With this type of inhibition, inhibitor binds to the enzyme not in the substrate binding region (not in the active site).

From the data presented in Fig. 2, it can be seen that metamizole sodium is a uncompetitive inhibitor. When the substrate concentration is increased, the inhibition effect of metamizole does not decrease. As the concentration of the inhibitor increases, only the value of the segment cuted off on the ordinate axis changes. Under uncompetitive inhibition, inhibitor reversibly interacts with the enzyme-substrate complex that is created, resulting in a complex that is incapable of producing the reaction product.

The results are consistent with available references data on the effect of quercetin on urease activity. Indeed, quercetin is one of its most effective inhibitors [8,9].

**Conclusions**

The highest anti-urease activity showed a composition based on metamizole sodium and quercetin, a little less - based on quercetin and vitamin D3 (cholecalciferol). Also interesting for further research is the composition of quercetin and chlorophyllipt.

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**Introduction**. The aim of the study was to study the ability to inhibit urease by some pharmaceutical compositions that are promising in the prevention of tuberculosis reactivation . In particular, according to a number references sources, quercetin is able to successfully inhibit urease by a non-competitive mechanism. Another compound - dipyrone (metamizol sodium) according to preliminary molecular modeling has a pharmacophore structure similar to urea. **Materials and methods**. The biochemical studies (urease activity) of some substances effect on urease was carried out. As the leader substances – inhibitors was used quercetin and metamizole, another substances are not shown inhibition activity on the urease. As the substrate a 0.5% aqueous urea solution was used. The reaction was carried out at a temperature of 37 ° C, the incubation time was 10 minutes. The activity of urease was determined by the color reaction with the hypochlorite reagent. Photometry was performed on a FEK-3M photoelectric colorimeter at a wavelength of 590 nm. **Results and discussion**. Quercetin and metamizole sodium have the strongest inhibitory properties for urease, since the lowest values of semi-inhibitory concentrations are obtained for them. In the presence of chlorophyllipt, the inhibitory activity of quercetin against urease is not suppressed. Metformin showed no inhibitory activity against urease, and it was low for other substances. **Conclusion**. The highest anti-urease activity showed a composition based on metamizole sodium and quercetin, a little less - based on quercetin and vitamin D. Also interesting for further research is the composition of quercetin and chlorophyllipt.

**Keywords**: tuberculosis reactivation, urease, inhibitors, quercetine, metamizole, cholecalciferol

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