

## Features of experimental modeling of tuberculosis in guinea pig with the participation of *N'*-(2-(5-((theophylline-7'-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide

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Received: 20.08.2020. Accepted 26.09.2020

In this study, no toxic effects were detected after single subcutaneous injection of 40 mg/kg tadpoles. The results of macro- and microscopic examination of the internal organs 14 days after single subcutaneous administration of *N'*-(2-(5-((theophylline-7'-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide (GKP-305) at a dose of 20, 40 mg/kg showed the absence of any anatomical and morphological abnormalities in the tissue structures of the tentacles. The calculated value of the drug indicates a high degree of safety GKP-305 and its prospects for veterinary practice as an effective and safe tuberculocidal drug.

**Key words:** Tuberculosis, 1,2,4-triazole, modeling, guinea pig.

### Introduction

The advent of 1,2,4-triazole drugs has revolutionized the treatment of people with invasive and fungal infections (Neofytos, 2010; Haegler et al., 2017; Gotsulia et al., 2018; Zazhzharskiy et al., 2019). However, keep in mind that drugs with a fragment of triazole can cause various side effects and can sometimes interact with other drugs (Samelyuk & Kaplaushenko, 2015; Fard et al., 2016; Bihdan et al., 2019; Palchykov et al., 2020).

Haegler et al. (2017) noted that in HepG2 cells (human hepatocarcinoma), posaconazole and ketoconazole were cytotoxic starting at 20 and 50  $\mu$ M, and decreased cellular ATP content starting at 5 and 10  $\mu$ M. Fard et al. (2016) found a negative effect of risatriptan on hepatocytes. Hepatocyte lysosomes and mitochondria have been shown to play an important role in risatriptan-induced cell injury (Chachibaia & Hoskeri, 2016).

### Materials and methods

Acute toxicity of the preparation GKP-305 was determined on 12 nonlinear tussles of both sexes with an average mass of 250 g. There were formed 4 groups of animals of 3 animals in each group (Stephanov, 2001).

Group 1: subcutaneous administration of the drug at a dose of 20 mg/kg animal weight – a double dose of tuberculosis in the treatment of this drug.

Group 2: at a dose of 40 mg/kg animal weight.

Group 3: use of the preparation GKP-305 at a dose of 80 mg/kg animal weight.

Control group: clinically healthy animals (CDD) – subcutaneous administration of isotonic sodium chloride solution (6 ml/kg).

The evaluation of the effect of the study drug was performed on the following indicators:

- mortality (the timing of the death of animals in each group, daily);
- evaluation of manifestations of toxicity (daily), including evaluation of the appearance of the area of introduction (presence of irritation, hyperemia, edema);
- dynamics of change of body weight (in the initial state on 4, 7 and 14 days after introduction);
- macroscopy of internal organs, mass coefficients of internal organs in tadpoles (14 days).

*Chronic toxicity of the preparation GKP-305* was determined on 12 guinea pigs with an average weight of 250 g. There were formed 4 groups of animals of 3 animals in each group, which were re-injected the drug in doses, as in the experiment, studying acute toxicity for 3 months. The toxicity of GKP-305 was studied according to the indicators of irritant effect, emotional and behavioral reactions of laboratory animals and determined the coefficients of mass of internal organs for long-term administration of the drug.

We also calculated the mass ratio (MK) as the percentage of body mass to body weight, an integral indicator used to evaluate the condition of internal organs. The calculation of the mass coefficients was determined by the formula:

$$MK = \text{organ weight (g)} / \text{body weight (g)} \times 100\%$$

Animals were kept under standard conditions. Experiments conducted on live vertebrates conformed to the principles of the European Convention on the Protection of Vertebrate Animals, used for research and other scientific purposes (Strasbourg, 1986). Total serum protein was investigated by biuret method, albumin concentration by B. Dumas et al., Residual nitrogen content by color reaction with Nessler reagent, nitrogen concentration of free amino acids by GA method. Uzbekova in the modification of ZS Chulkovy, urea content - according to Marsh, creatinine concentration - according to the Popper method, activity of ALAT and AsAT - according to Reitman and Frenkel. The data in the tables are presented as  $x \pm 1.96SD$ .

## Results and discussion

After administration of the preparations, the animals were monitored for 14 days and their general condition, mortality, and body weight dynamics were evaluated. Indicators of acute toxicity of the GKP-305 with subcutaneous injection of tadpoles in different doses are presented in Table 1.

**Table 1.** Indicators of acute toxicity of the GKP-305, n = 10

| Group of animals | Dose<br>mg/kg | The number of dead<br>animals | The number of surviving<br>animals | Mortality rate, % |
|------------------|---------------|-------------------------------|------------------------------------|-------------------|
| I                | 20.0          | 0                             | 3                                  | 0                 |
| II               | 40.0          | 0                             | 3                                  | 0                 |
| III              | 80.0          | 1                             | 2                                  | 33.3              |
| Control          | -             | 0                             | 3                                  | 0                 |

As a result, the study found that after single subcutaneous administration to animals of the experimental groups, changes in behavior and activity did not occur. Also, no signs of intoxication and animal death were observed except for group III – 1 were killed. Thus, LD<sub>50</sub> for GKP-305 in the acute toxicity phase determined 40.0 mg/kg animal weight. According to the acute toxicity study, the dynamics of body weight of animals of all groups were studied to evaluate the toxic effects of potential drugs on mammals (table 2).

**Table 2.** Dynamics of body weight of animals

| Group of animals | Days of observation / Body weight, g |               |               |
|------------------|--------------------------------------|---------------|---------------|
|                  | 1                                    | 7             | 14            |
| I                | 292.6±8.7                            | 302.8±13.6*   | 348.5±15.6**  |
| II               | 287.3±11.3                           | 303.5±10.4    | 347.4±11.5    |
| III              | 291.8±12.5                           | 311.4±12.2*** | 424.8±13.6*** |
| Control          | 286.5±9.7                            | 305.9±10.5    | 365.3±12.7*** |

Note: \* (P<0.05); \*\* (P<0.01); \*\*\* (P<0.001).

We determined that the animals in groups I and II on the 7th day of observation did not differ from the control, and on the 14th day slightly lagged in body weight by 18.0 and 17.0 g, respectively. In animals of group III, on the contrary, body weight on the 7th day of observation was higher than the control by 6.0; and on day 14, 59.0 g (P<0.05), respectively.

On the 14th day of observation, all animals had a neat wool blanket, unchanged mucous membranes of natural openings. Subcutaneous lymph nodes are normal in size and to the touch. The study of the morpho-biochemical parameters of the blood of the tunicates with long-term administration of the GKP-305 are shown in Tables 3 and 4.

Most plasma proteins are synthesized by the liver. Note the increase in total protein in the III experimental group after drug use in comparison with I, II and intact animal group by 24.6 (P<0.05); 12.9 and 15.9% due to globulins by 41.2 (P<0.05); 29.6 (P<0.05); and 30.1% (P<0.05), respectively. There was also a sharp decrease in protein ratio in the experimental group III by 40.0% (P<0.05) than in animals I and 30.8% (P<0.05) in group II and control.

AST in the first experimental group for long-term administration of GKP-305 decreased by 27.7% and was 2.0 times (P<0.05) below control, whereas this indicator in the animals of group III exceeded the intact tussles by 3.0 times (P<0.05). Aspartate aminotransferase catalyzes the formation of glutamic acid from 2-oxoglutarate by the transfer of an amino group. AST in maximum concentration is found in the liver, heart and skeletal muscles. Increasing the serum AST concentration in group III animals is, in our opinion, associated with dysfunctional changes in the liver.

After the application of the test preparation in the troughs of group III there was an increase of alkaline phosphatase by 34.8%, which is by 19.7 (P<0.05); 12.4 and 14.6% higher than in I, II and controls, respectively. Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monoesters in an alkaline medium. The enzyme is present in almost all tissues of the body, especially in cell membranes, and is found in especially high concentrations in the placenta, intestinal epithelium, renal tubules, osteoblasts and liver. Increase in alkaline phosphatase content in group III may be associated with hepatobiliary disorders – drug-induced hepatotoxicity.

**Table 3.** Biochemical indices of blood murchak during long-term administration of GKP-305 (n = 10)

| Indicators                | Group of animals |            |            |              |            |              |            |              |
|---------------------------|------------------|------------|------------|--------------|------------|--------------|------------|--------------|
|                           | I                |            | II         |              | III        |              | Control    |              |
|                           | Before           | After      | Before     | After        | Before     | After        | Before     | After        |
| Total protein, g/L        | 54.2±3.4         | 51.3±3.8   | 53.9±4.2   | 59.2±4.3     | 55.2±3.6   | 68.1±4.3*    | 55.0±3.4   | 57.2±2.9*    |
| Albumin, g/L              | 33.1±2.6         | 31.5±3.6   | 33.0±2.9   | 33.5±3.4     | 32.9±3.2   | 32.0±4.1     | 33.2±3.5   | 32.0±3.9     |
| Globulins, g/L            | 21.1±1.7         | 21.3±2.1   | 20.9±2.5   | 25.5±2.6     | 22.3±2.8   | 36.2±3.6**   | 21.8±2.5   | 25.3±2.8*    |
| Protein ratio, U          | 1.6±0.21         | 1.5±0.32   | 1.6±0.12   | 1.3±0.32     | 1.5±0.23   | 0.9±0.16     | 1.5±0.12   | 1.3±0.34     |
| Urea, mmol/L              | 4.0±0.43         | 4.25±0.78  | 3.5±0.36   | 4.7±0.67     | 4.1±0.36   | 3.9±0.64     | 3.7±0.55   | 4.8±0.76     |
| Blood urea nitrogen, mg%  | 7.1±0.78         | 8.1±1.1    | 7.0±0.81   | 8.95±1.3     | 6.8±0.78   | 7.5±1.1      | 6.5±0.89   | 9.2±0.77     |
| Creatinine, µmol/L        | 66.5±6.4         | 92.3±7.4*  | 62.3±5.6   | 129.2±10.3** | 60.1±5.9   | 143.8±12.6** | 56.1±6.2   | 118.1±9.9*** |
| AST, U/L                  | 89.2±8.9         | 64.5±6.4*  | 82.4±9.6   | 12.3±11.2*   | 79.2±7.5   | 388.2±21.9** | 69.1±5.7   | 130.3±11.5** |
| ALT, U/L                  | 80.3±6.8         | 49.5±5.9** | 78.9±7.6   | 80.5±8.3**   | 73.5±7.4   | 72.2±6.8*    | 63.5±6.4   | 67.1±7.2     |
| AST/ALT, U                | 1.1±0.21         | 1.2±0.34   | 1.0±0.19   | 1.5±0.32     | 1.1±0.12   | 5.4±0.67***  | 1.1±0.12   | 1.9±0.14***  |
| Alkaline phosphatase, U/L | 200.3±13.6       | 244.3±16.1 | 234.4±11.8 | 266.4±13.7   | 198.3±12.8 | 304.2±19.4** | 193.1±12.7 | 259.9±15.7** |
| Total bilirubin, µmol/L   | 3.6±0.54         | 3.6±0.45   | 3.5±0.53   | 14.7±1.9***  | 3.4±0.64   | 12.8±1.3***  | 3.4±0.54** | 12.1±1.1     |
| Glucose, mmol/L           | 5.8±0.45         | 7.6±0.63   | 6.5±0.72   | 12.9±1.3***  | 7.0±0.97   | 10.1±1.3     | 7.2±0.88   | 13.6±1.6**   |
| Ca, mmol/L                | 3.7±0.57         | 2.25±0.31  | 3.0±0.42   | 2.55±0.32    | 3.3±0.48   | 2.3±0.32     | 3.1±0.43   | 2.7±0.31     |
| P, mmol/L                 | 4.2±0.44**       | 1.75±0.67  | 3.8±0.59   | 2.7±0.33     | 3.9±0.21   | 2.9±0.42     | 3.7±0.37   | 2.4±0.19     |
| Ca/P, U                   | 0.9±0.13         | 1.3±0.21   | 0.8±0.12   | 0.9±0.14     | 0.8±0.16   | 0.8±0.14     | 0.8±0.22   | 1.1±0.11     |
| Cholesterol, mmol/L       | 1.6±0.21         | 1.45±0.32  | 1.5±0.34   | 1.15±0.43    | 1.4±0.32   | 1.4±0.25     | 1.5±0.18   | 1.0±0.12     |

Note: \* (P<0.05); \*\* (P<0.01); \*\*\* (P<0.001).

**Table 4.** Morphological indices of guinea pig blood with prolonged administration of GKP-305 (n = 10)

| Indicators                        | Group of animals |              |             |            |             |             |            |             |
|-----------------------------------|------------------|--------------|-------------|------------|-------------|-------------|------------|-------------|
|                                   | I                |              | II          |            | III         |             | Control    |             |
|                                   | Before           | After        | Before      | After      | Before      | After       | Before     | After       |
| Hemoglobin, g/L                   | 108.5± 9.5       | 130.5±11.4   | 111.3±8.9   | 113.3±13.1 | 118.5±10.9  | 112.0±9.8   | 119.8±12.6 | 92.4±8.9    |
| Hematocrit, %                     | 37.5±5.6         | 41.2±4.1     | 35.3±3.6    | 40.3±4.7   | 36.5±3.7    | 39.3±5.2    | 35.9±4.9   | 34.3±3.9    |
| Erythrocytes, 10 <sup>12</sup> /L | 4.1±0.2          | 5.1±0.6      | 4.7±0.7     | 4.8±0.5    | 4.1±0.4     | 4.6±0.3     | 3.9±0.2    | 4.1±0.3     |
| ESR, mm/hour                      | 1.0±0.1          | 1.1±0.1      | 0.9±0.1     | 1.0±0.1    | 1.0±0.1     | 1.0±0.1     | 1.0±0.1    | 1.0±0.1     |
| Platelets, 10 <sup>9</sup> /L     | 285.3±14.4       | 347.2±23.11* | 273.5±15.8  | 274.5±16.8 | 223.2±13.9  | 263.1±14.7  | 266.5±14.9 | 276.2±15.2* |
| Leukocytes, 10 <sup>9</sup> /L    | 6.5±0.2***       | 3.3±0.16     | 4.5±0.34*** | 1.8±0.22   | 4.3±0.35*** | 1.9±0.17    | 4.1±0.5*** | 2.0±0.14    |
| Leucocyte formula, %              |                  |              |             |            |             |             |            |             |
| Basophils                         | 0                | 0            | 0           | 0          | 0           | 0           | 0          | 0           |
| Eosinophils                       | 1.0±0.1          | 1.0±0.1      | 1.0±0.1     | 1.0±0.1    | 1.0±0.1     | 1.0±0.1     | 1.0±0.1    | 2.0±0.1     |
| Neutrophils                       |                  |              |             |            |             |             |            |             |
| Myelocytes                        | 0                | 0            | 0           | 0          | 0           | 0           | 0          | 0           |
| Young cell                        | 0                | 0            | 0           | 0          | 0           | 0           | 0          | 0           |
| Band cell                         | 2.0±0.12         | 1.5±0.1      | 2.0±0.21    | 1.5±0.23   | 1.0±0.1     | 3.0±0.25*** | 2.0±0.11   | 2.0±0.14    |
| Granulocytes                      | 38.5±5.6         | 28.3±2.5     | 35.5±4.7    | 25.4±5.7   | 41.0±7.4    | 30.5±4.6    | 35.1±3.6   | 41.2±5.7    |
| Lymphocytes                       | 53.5±6.9         | 66.5±7.8     | 56.0±5.8    | 71.5±10.3  | 50.8±8.9    | 65.0±9.7    | 54.0±8.8   | 46.2±7.8    |
| Monocytes                         | 5.2±0.45***      | 2.7±0.42     | 5.5±0.37*** | 0.6±0.32   | 6.2±0.53*** | 0.5±0.12    | 7.9±0.42   | 8.6±1.2***  |

Note: \* (P<0.05); \*\* (P<0.01); \*\*\* (P<0.001).

Urea is synthesized in the liver as a by-product of the amino acid deamination reaction. Its elimination in the urine is the main route of excretion of nitrogen. The reduced concentration of urea in the plasma of the troughs of group III is a consequence of the decrease in protein catabolism and weak dehydration. This is lower than group I, group II and intact animals by 8.2; 17.0

and 18.8% ( $P<0.05$ ), respectively. Blood plasma creatinine determines the glomerular filtration rate, an integral indicator of renal excretory function. We noted an increase in creatinine in animals in the experimental group III compared to tadpoles I, II and intact animals by 35.8 ( $P<0.05$ ); 10.2 and 17.9% ( $P<0.05$ ), respectively. An increase in this indicator indicates the presence of an inflammatory reaction in the body of group III tentacles. Blood biochemical parameters in animals of the first and second experimental groups are within the physiological norm. Analyzing the morphological indices of murch blood with prolonged administration of GKP-305, we note an increase in the number of rod-core neutrophils in group III animals compared to I, II and intact murchak by 2.0 ( $P<0.05$ ); 2.0 ( $P<0.05$ ) and 1.5-fold ( $P<0.05$ ) against a decrease of 5.4 ( $P<0.05$ ) monocytes; 1.2 and 17.2 ( $P<0.05$ ) times, respectively. The mass coefficients of the internal organs of the tentacles for long-term administration of the GKP-305 is presented in Table 5.

**Table 5.** Mass coefficients of the internal organs of the tentacles for prolonged administration of the GKP-305, g, M  $\pm$  m

| Indicators  | Group of animals |                  |                  |                  |                  |                  |                  |                 |
|-------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-----------------|
|             | I                |                  | II               |                  | III              |                  | Control          |                 |
|             | Body weight      | Mass ratio       | IOM              | Mass ratio       | IOM              | Mass ratio       | IOM              | Mass ratio      |
| Body weight | 348.5 $\pm$ 15.6 | -                | 347.4 $\pm$ 11.5 | -                | 347.4 $\pm$ 11.5 | -                | 347.4 $\pm$ 11.5 | -               |
| Heart       | 1.45 $\pm$ 0.12  | 0.42 $\pm$ 0.02  | 1.5 $\pm$ 0.22   | 0.43 $\pm$ 0.045 | 1.7 $\pm$ 0.15   | 0.41 $\pm$ 0.02  | 1.5 $\pm$ 0.32   | 0.41 $\pm$ 0.07 |
| Lungs       | 4.1 $\pm$ 0.31   | 1.15 $\pm$ 0.03  | 4.1 $\pm$ 0.37   | 1.18 $\pm$ 0.025 | 4.6 $\pm$ 0.32   | 1.08 $\pm$ 0.05  | 4.1 $\pm$ 0.43   | 1.12 $\pm$ 0.07 |
| Liver       | 18.2 $\pm$ 2.11  | 5.22 $\pm$ 0.38  | 18.4 $\pm$ 3.12  | 5.32 $\pm$ 0.72  | 22.2 $\pm$ 3.56  | 5.22 $\pm$ 0.67  | 17.8 $\pm$ 2.87  | 4.87 $\pm$ 0.62 |
| Spleen      | 0.7 $\pm$ 0.10   | 0.20 $\pm$ 0.015 | 0.6 $\pm$ 0.17   | 0.17 $\pm$ 0.04  | 1.2 $\pm$ 0.12** | 0.28 $\pm$ 0.02* | 0.6 $\pm$ 0.22   | 0.16 $\pm$ 0.05 |
| The kidneys | 3.1 $\pm$ 0.17   | 0.89 $\pm$ 0.02  | 3.3 $\pm$ 0.55   | 0.94 $\pm$ 0.13  | 3.5 $\pm$ 0.44   | 0.82 $\pm$ 0.07  | 2.9 $\pm$ 0.55   | 0.79 $\pm$ 0.12 |

Note: \* ( $P<0.05$ ); \*\* ( $P<0.01$ ); \*\*\* ( $P<0.001$ ). IOM - The mass of the internal organ

We determined an increase in all indicators of internal organs in animals of the third experimental group in comparison with troughs I, II group and control, and the mass ratio of the spleen was higher by 28.6 ( $P<0.05$ ); 39.3 ( $P<0.05$ ) and 42.9% ( $P<0.05$ ), respectively. The analysis of this indicator in toxicological studies makes it possible to identify the target organ of the toxicant, to identify signs of endocrine-related effects. The unique properties of 1,2,4-triazole make this heterocycle very attractive for research (Bihdan et al., 2019; Hunchak et al., 2020). Studies have shown the high reactivity and low toxicity of 1,2,4-triazole derivatives and the prospect of their use as potential biologically active compounds. Interesting results have also been found regarding the use of some 1,2,4-triazoles derivatives as effective plant growth regulators (Zazharskyi et al., 2019; Palchykov et al., 2020).

We have determined the safety of the use of the GKP-305 on tadpoles up to 4 times the introduction. The biochemical parameters and mass coefficients of the internal organs in animals were within the physiological control of the intact group.

The preparation GKP-305 can be used as a tuberculocide with a level of safety up to 4 times the dose.

## Conflicts of interest

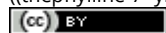
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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## Citation:

Gotsulya, A.S., Zazharskyi, V.V., Davydenko, P.O., Zazharskaya, N.M., Kulishenko, O.M., Panasenko, O.I., Gutty, B.V., Pryima, O.B., Mazur, I.Y., Pritsak, V.V., Drachuk, U.R., Sobolta, A.G., Riy, M.B. (2020). Features of experimental modeling of tuberculosis in guinea pig with the participation of *N*-(2-(5-((theophylline-7'-yl)methyl)-4-R-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide. *Ukrainian Journal of Ecology*, 10(4), 191–194.



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