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Morphological changes in experimental tuberculosis resulting from treatment with quercetin and polyvinylpyrrolidone

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ABSTRACT

Research objective: Morphological study of tissue necrosis stages in experimental organ-preserving tuberculosis pharmacotherapy using Quercetin and Polyvinylpyrrolidone (QP).

Background and methods: 32 laboratory mice of C57BL/6JLacSto strain were used in the experiment. The animals were divided into five groups, six to seven mice in each: group 1- Mycobacterium tuberculosis (MBT) uninfected mice; group 2- MBT infected mice; group 3- MBT infected and treated with antituberculosis preparation (ATP); group 4- MBT infected and QP treated; group 5- MBT infected and treated with ATP and QP. The mice were infected through caudal vein injection with MTB H37Rv strain. The preparation QP, which belongs to the capillary-stabilizing-remedy group, was used for the research. The ATP were izoniazid and streptomycin.

Results: QP produced a strict delineation of caseous necrosis from the unaffected parts of the connective tissue with fibrosis in the center and a large number of Langerhans cells, which was not observed in the control groups without QP. The combination of QP and ATP had more pronounced effects. In MBT-infected mice, where QP was not used, unlike the group where QP was used, adipose dystrophy of hepatocytes was observed. Thus, the hepatoprotective effect of QP against TB can be suggested.

Conclusion: QP produces a clear delineation of caseous necrosis from an uninfected tissue by connective-tissue formation, and by forming fibrotic tissue in the center of epithelioid cells that prevents further TB dissemination by enhancing TB pharmacotherapy.

Introduction

In recent years in Ukraine, as in many countries, we observed an unstable epidemiological situation connected with tuberculosis (TB) [1], which became a silent social threat to humanity [2]. Therefore, in April 1993, the World Health Organization (WHO) recognized the fact that TB is a global threat [3]. In addition, TB is the leading disease among all

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infectious diseases and causes of death worldwide. In 2013, according to the estimations conducted by the WHO, 9.0 million people were infected with TB, and of those 1.5 million died [1].

The results of treatment of patients suffering from destructive pulmonary TB with bacterio excretion in spite of high possibilities of antibacterial therapy remain insufficient [4–6]. Antimycobacterial therapy regimes recommended by the WHO allow achieving negative smear in 67–96.3% of patients suffering from TB [7–9]. One of the factors that increases the treatment efficiency of patients with pulmonary TB is the development of new treatment technologies [4,10,11]. According to most authors, the main modern method of treatment of TB is connected with the integrated approach to the use of antibacterial drugs [12–15]; their use now is urgently needed. Thus, the problem of treatment of patients with destructive pulmonary TB is very topical and difficult.

One reason for the ineffective treatment of TB patients is the insufficient modern view concerning pathomorphism and pathophysiological interacting processes of the macroorganism with *Mycobacterium tuberculosis* (MBT), which dictates the need for the implementation of an integrated effect method into the medical process not only to suppress MBT, but also to preserve lung tissue from further destruction.

Organ preservation from the consequences of TB influences a patient's future life. With the progression of the tuberculous process and myocardial hypoxia, which occurs as a result of the destruction of lung tissue, cardiopulmonary insufficiency develops, from which the patient dies. The restoration of respiratory function in the case of pulmonary TB is an important criterion for clinical treatment, and for the medical and social rehabilitation of patients with respiratory diseases. The aforementioned effects may be obtained by applying an effective treatment.

In each form of TB, immunopathological inflammation caused by the violation of microcirculation and trophic factors of lung tissue occurs, which may be complicated by its melting, secreting of cheesy masses through the bronchi, and the formation of cavities (i.e., the process transition in the destructive form). This worsens the course of TB, resulting in the subsequent loss of a part of or even the whole organ, unless an adequate, effective type of therapy is applied. According to some authors, there is a necessity for a new effective integrated treatment in the therapeutic conditions [16–18]. According to the literature, treatment schedules primarily aim at the destruction of MBT. We have not seen works connected with the application of therapy of organ preservation by limiting the spread of the pathological process, preventing lung-tissue melting and the rapid decline of intoxication syndrome with the restoration of the immune-system functioning. We focused our attention on quercetin with polyvinylpyrrolidone (QP). QP is a chemical compound that is used in clinical practice for the treatment of patients suffering from myocardial infarction. However, in the literature, there is no information on the use of QP in the case of TB. QP is a chemical compound that belongs to a group of capillary-stabilizing drugs and antioxidants (bioflavonoids). The main effect of QP in the treatment of myocardial infarction is the separation of necrosis tissue from healthy areas. Thus, the spreading of necrosis to healthy areas is halted and localization of the pathological process in the affected organ is observed, which leads to its preservation. The attempt to find out the morphological changes that would confirm the separation of caseous necrosis convinced us to initiate the study on the effectiveness of QP against TB in mice, as a property of the drug consists not only of the effect on "sterile" necrosis, but perhaps also on inflammation in the infectious disease process.

Materials and methods

Thirty-two laboratory mice of C57BL/6J strain were used in the experiment. The experimental study of QP was conducted according to the literature [19], which provided an example of research of pharmacological compounds in the case of TB in mice.

The mice (17 males and 15 females) were all young (males: 3 months, 12 ± 5 days; females: 3 months, 26 ± 5 days). The weight of the female mice varied from 18.17 g to 19.83 g (18.83 ± 0.52 g), whereas that of the male mice varied from 19.35 g to 20.47 g (19.8 ± 0.38 g). None of the animals have been used in any previous research. All animals were kept under the same housing and feeding conditions.

The mice were infected via caudal-vein injection with MBT H37Rv strain [19]—0.5 mL of isotonic solution (according to group), which corresponds to 2.0×10^7 microbial bodies of the laboratory strain of MBT $H_{37}Rv$.

The animals were divided into five groups, with six to seven mice in each group: (a) Group 1, MBT-uninfected mice; (b) Group 2, MBT-infected mice; (c) Group 3, MBT infected and treated with anti-TB preparation (ATP); (d) Group 4, MBT infected and treated with QP; and (e) Group 5, MBT infected and treated with ATP and QP. The ATP included isoniazid and streptomycin.

Recalculation of the doses of the drugs used (QP, isoniazid, and streptomycin) for the mice was carried out using the conversion factor of doses between a person and a mouse, which is generally accepted in conducting research [20]. Thus, the drug doses for the mice contained the following: isoniazid (10%, 5 mL), 45 mg/kg; streptomycin (1 g), 90 mg/kg; and QP (0.5 g), 45 mg/kg of the body weight of a mouse. The medicines used in the experiment on the mice were applied as follows: isoniazid and streptomycin, intramuscularly once a day; and QP, intraperitoneally according to a schedule (on the 5th day after the introduction of the infection every 2 h, and then every 12 h; on the 6th day and 7th day two times a day every 12 h).

Based on the literature [19], treatment was performed on the 5th day after the animals had been infected and after pathomorphological (macroscopic and microscopic) confirmation of the presence of the TB process in the mice.

Mouse removal was conducted using chloroform overdose on the 11th day after the introduction of MBT [21].

The lungs, spleens, and livers of the control and experimental groups of animals were used for the morphological examination.

The degree of lesions on the internal organs of the mice and comparisons depending on the character of the given treatment were evaluated macroscopically [22] by the sum of points. The data on index lesions are shown in Table 1.