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The role of melatonin, IL-6, and IL-10 in the immunopathogenesis and clinical course of tuberculosis

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Conflict of interest: none

BACKGROUND. The present day dictates a new relevance of tuberculosis, which consists in the increase in the number of patients with resistant forms of tuberculosis and the decrease in treatment adherence due to the long duration and toxicity of antimycobacterial therapy. Treatment regimens should be adjusted to include additional non-microbial agents that can simulate the immune system's activity in combating mycobacteria and synergistically enhance the activity of anti-tuberculosis drugs themselves.
MATERIALS AND METHODS. The determination of melatonin levels and cytokine levels (interleukin-6 and -10) in blood plasma was performed during the first 2 weeks of antimycobacterial treatment. The study included 30 patients with new cases of pulmonary tuberculosis with preserved sensitivity and 30 patients with multidrug resistance.
RESULTS AND DISCUSSION. It was found that the level of melatonin in the blood plasma in the study groups was significantly lower compared to the control group. The average value of melatonin was 33.2±1.2 pg/ml in group 1 and 29.4±2.3 pg/ml in group 2 and in the control group – 46.2±0.8 pg/ml ($p=0.035$).
It was proven that the content of interleukin-6 in the studied groups of patients significantly exceeded the practically healthy individuals indicator: in group 1, the level of interleukin-6 was 11.04 times higher, in group 2 – 13.9 times ($p<0.001$).
The concentration of interleukin-10 in the blood plasma of tuberculosis patients is significantly increased: in group 1, the level of interleukin-10 was 2.2 times higher, in group 2 – 1.7 times ($p<0.001$).
The linear correlation coefficient showed that in tuberculosis patients, there is almost no correlation between the level of interleukin-6 and melatonin ($r=0.14$), and there is a positive, close relationship between the level of interleukin-10 and melatonin ($r=0.73$).

KEY WORDS: tuberculosis, melatonin, interleukins, resistant, intoxication.

Роль мелатоніну, ІЛ-6 та ІЛ-10 в імунопатогенезі й клінічному перебігу туберкульозу

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ОБҐРУНТУВАННЯ. Сьогодення диктує нову актуальність туберкульозу, яка полягає в прирості кількості хворих з резистентними формами хвороби та зниженні прихильності до лікування через довготривалість і токсичність антимікобактеріальної терапії. Схеми лікування мають бути скореговані так, щоби включати додаткові немікробні агенти, які можуть моделювати активність імунної системи в боротьбі з мікобактеріями та синергійно посилювати активність власне протитуберкульозних препаратів.
МАТЕРІАЛИ ТА МЕТОДИ. Рівні мелатоніну та цитокінів (зокрема, інтерлейкіну-6 і -10) у плазмі крові визначали впродовж перших 2 тижнів антимікобактеріального лікування. У дослідження залучено 30 пацієнтів з новими випадками туберкульозу легень зі збереженою чутливістю та 30 пацієнтів із множинною лікарською стійкістю.

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РЕЗУЛЬТАТИ ТА ЇХ ОБГОВОРЕННЯ. Встановлено, що рівні мелатоніну в плазмі крові в досліджуваних групах були значно нижчими порівняно з групою контролю. Середнє значення мелатоніну становило 33,2±1,2 пг/мл у групі 1 та 29,4±2,3 пг/мл у групі 2, у контрольній групі – 46,2±0,8 пг/мл (p=0,035).
Доведено, що вміст інтерлейкіну-6 у досліджуваних групах пацієнтів вірогідно перевищував показник практично здорових осіб: у групі 1 рівень інтерлейкіну-6 був вищим в 11,04 раза, в групі 2 – у 13,9 раза (p<0,001). Концентрація інтерлейкіну-10 у плазмі крові хворих на туберкульоз достовірно підвищується: в групі 1 рівень інтерлейкіну-10 був вищим у 2,2 раза, в групі 2 – в 1,7 раза (p<0,001 відповідно).
Коефіцієнт лінійної кореляції показав, що у хворих на туберкульоз між рівнем інтерлейкіну-6 і мелатоніном майже відсутній кореляційний зв'язок (r=0,14), але між рівнем інтерлейкіну-10 і мелатоніном наявний позитивний тісний зв'язок (r=0,73).

КЛЮЧОВІ СЛОВА: туберкульоз, мелатонін, інтерлейкіни, резистентний, інтоксикація.

Introduction

Worldwide, the treatment of tuberculosis (TB), namely drug-sensitive, has achieved significant success, and, accordingly, control over its spread has been achieved. However, today's reality highlights a renewed relevance of TB, associated with an increase in the number of patients with drug-resistant forms of the disease and a decline in treatment adherence due to the prolonged duration and toxicity of antitubercular therapy (ATT).

In our opinion, TB treatment regimens should be adjusted so that additional non-microbial agents that can simulate the activity of the immune system in the fight against mycobacteria and synergistically enhance the activity of ATT itself are included in pathogenetic treatment regimens. The literature shows that adjunctive therapeutic drugs [2, 6, 7, 13] in combination with antimycobacterial drugs can enhance the overall effect of ATT through a directly directed response to the immune system or pathogen.

Pathological processes such as specific inflammation, fibrosis, and oxidative stress underlie the pathogenesis of TB. Thus, additional pathogenetic therapy may be useful in the clinical management of TB patients.

Melatonin is one of the potent anti-inflammatory, antifibrotic and antioxidant agents and reducing the expression of inducible nitric oxide synthase and blocking the overexpression of inflammatory cytokines. Melatonin also prevents the increase in the level of interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α), and interferon-γ at the site of inflammation.

Melatonin plays an important role not only in the regulation of respiratory chain complexes I and IV but also in preventing mutation and deletion of mitochondrial DNA. This effect of melatonin is the result of a direct interaction between melatonin and the protein (fig.).

Pulmonary TB is characterised by a specific inflammatory response involving alveolar granulocyte colony-stimulating factor, α-chemokines, and pulmonary neutrophilosis. It has been shown that the immune system directs neutrophils and chemokine production, which regulate the accumulation of Th1 cells [5, 9, 12]. However, melatonin has the property of attenuating pulmonary neutrophil infiltration and reducing the levels of the cytokines TNF-α, IL-1β, and IL-6 [11, 13].

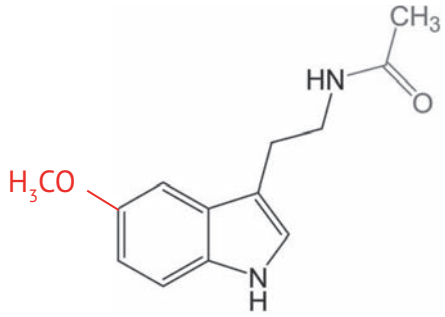

Fig. Molecular structure of melatonin

Melatonin demonstrates significant antimicrobial properties. In order to evaluate the susceptibility of *Mycobacterium tuberculosis* (MBT) to melatonin, strains resistant to rifampicin, streptomycin, and isoniazid were incubated with the compound. The findings revealed that melatonin exerts an inhibitory effect against multidrug-resistant TB (MDR-TB) [10]. These data offer strong evidence supporting the therapeutic potential of melatonin in the management of pulmonary TB.

At present, numerous experimentally modeled studies have investigated the effects of melatonin on MBT; however, data on plasma melatonin levels in patients with TB and its therapeutic efficacy remain unavailable.

The aim of this study was to determine the concentration of melatonin and the levels of selected cytokines in the blood plasma of patients with pulmonary TB.

Materials and methods

A randomized case-control clinical study included 30 patients with newly diagnosed drug-sensitive pulmonary TB (group 1), 30 patients with MDR-TB (group 2), and 10 practically healthy individuals – PHI (control group).

The patients were distributed by sex: males accounted for 76.5 %, and females – 23.5 %. The mean age of the patients was 38.9±5.2 years, ranging from 23 to 65 years. The majority of examined patients were male – 68.2 %, while females comprised 31.8 %. Objective examination methods revealed that heart rate, blood pressure, respiratory rate, and body mass

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index in the studied patients varied depending on the extent of the pathological process in the lungs.

Inclusion criteria for the study: patients with active TB confirmed by X-ray, positive molecular genetic analysis of sputum and microscopic analysis for AFB, HIV-negative.

The determination of plasma melatonin levels in the examined patients of groups 1 and 2 was performed during the first 2 weeks of antimycobacterial treatment. Whole blood samples were collected into anticoagulant-containing tubes (for plasma preparation) between 08:00 and 09:00. The blood samples were centrifuged at 1000 rpm for 30 minutes to obtain plasma. Plasma samples were transferred into tubes covered with aluminum foil and stored at -30 °C until melatonin analysis. The study was conducted at the Educational and Scientific Laboratory of Bukovinian State Medical University. Plasma melatonin concentrations were expressed in picograms per milliliter (pg/mL), with reference values for daytime levels ranging from 3.8 to 80.4 pg/mL.

Statistical analysis was performed using quantitative and qualitative research tests with the SPSS Statistics 23.0 software package, applying paired sample t-tests and Chi-square tests. Differences were considered statistically significant at a confidence level exceeding 95 % and $p<0.05$. To assess differences between groups, Student's t-test and the Mann – Whitney U test were used. The results were presented as mean standard deviation and median (minimum – maximum). Spearman's correlation test was applied to determine relationships between variables. A probability level of $p<0.05$ was considered statistically significant.

Results and discussion

The general condition of the patients and the severity of the intoxication syndrome were assessed using a proprietary scoring scale developed by the authors. This scale incorporated the severity of clinical symptoms, body weight loss, radiological characteristics of the TB process, and haematological parameters (table 1).

Among the examined patients in group 1, 53.4 % demonstrated a moderate general condition and a mild to moderate intoxication syndrome, whereas in group 2, a severe general condition and pronounced intoxication syndrome predominated in 62.8 % of cases.

In patients with pulmonary TB, the erythrocyte sedimentation rate, C-reactive protein level, leukocyte count, and plasma melatonin concentration were assessed (table 2).

According to the results of our study, plasma melatonin levels in both study groups 1 and 2 were significantly lower compared to the control group. The mean melatonin concentration in group 1 was 33.2±1.2 pg/mL, and in group 2 – 29.4±2.3 pg/mL, while in the control group it was 46.2±0.8 pg/mL. The Mann – Whitney U test revealed a statistically significant difference with $p=0.035$.

The mean plasma melatonin concentration in the control group was 1.39 times higher compared to group 1 and 1.57 times higher compared to group 2.

In study groups 1 and 2, the erythrocyte sedimentation rate, C-reactive protein level, and leukocyte count were significantly elevated compared to the control group, with statistically significant differences ($p=0.001$, table 2).

Melatonin mediates seasonal changes in the immune system. It has been proven that melatonin plays an organisational role in changing the physiological state of the body on a seasonal basis [1, 4, 8, 10].

That is why the seasonal development of the TB process may be associated with seasonal changes in the immune system caused by annual fluctuations in melatonin levels. In an oxidative environment, MDR-TB develops quite well in macrophages [3, 10, 12]. In addition, oxidative stress caused by infection leads to damage to host tissues.

The endotoxins released by MDR-TB during decay cause dysfunction of internal organs, and above all, cause acute damage to the lung tissue. Specific mycobacterial lung damage releases proinflammatory mediators, protease, reactive oxygen and nitrogen species, which leads to alveolar-capillary

Table 1. Assessment of the general condition of patients and the severity of intoxication syndrome

Points	Group 1, n (%)	Group 2, n (%)
1-3	5 (12.3)	3 (10.4)
4-6	22 (53.4)	9 (26.8)
7-10	14 (34.1)	20 (62.8)

Table 2. Values of individual blood parameters and melatonin levels in blood plasma of the study groups and the control group

Parameters	Group 1 (n=30)	Group 2 (n=30)	PHI (n=10)	p
Melatonin (pg/ml)	33.2±1.2	29.4±2.3	46.2±0.8	0.035**
Leukocytes (10^9/l)	12±1.5	14±0.7	6.2±0.2	<0.001*
Erythrocyte sedimentation rate (mm/h)	16.8±2.4	25.5±3.09	7.2±0.5	<0.001**
C-reactive protein (mg/l)	22.4	28.3	3.21±0.4	<0.001**

Notes: * Student's criteria; ** Mann – Whitney U test.

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Table 3. Concentration of some pro- and anti-inflammatory cytokines in the blood plasma of patients with pulmonary TB (M±m)

Parameters	PHI (n=10)	Group 1 (n=30)	Group 2 (n=30)
IL-6 (pg/ml)	1.691±0.016	17.86±13.21 (p<0.001)	24.15±12.63 (p<0.001, p_1<0.01)
IL-10 (pg/ml)	1.74±0.131	3.9±0.81 (p<0.05)	2.83±0.81 (p<0.001, p_1>0.001)

Notes: p – the degree of probability of indicators in relation to the PHI; p_1 – the degree of probability of the intergroup difference in indicators (between groups 1 and 2).

damage with the development of pulmonary edema, impaired gas exchange, increased permeability and altered lung mechanics. Endotoxins stimulate the excessive production of proinflammatory cytokines, such as TNF-α, IL-6 and nitric oxide. At the same time, IL-10, an anti-inflammatory cytokine, is induced to limit the specific inflammatory response in the lungs.

We analyzed the indicators of individual cytokines in the blood serum of patients with NTB and MDR-TB and their correlation with melatonin. Prior to treatment, patients with susceptible and resistant TB showed a significant increase in the level of proinflammatory cytokine (IL-6) in the blood serum against a decrease in the production of anti-inflammatory cytokine IL-10, which characterises the active phase of a specific inflammatory process (table 3).

We found that the content of IL-6 in the studied groups of patients significantly exceeded the index of PHI: in group 1, the level of IL-6 was 11.04 times higher, in group 2 – 13.9 times higher (p<0.001). An increase in IL-6 concentration is one of the first markers of an inflammatory response that stimulates the synthesis of C-reactive protein and acute phase proteins. It has been proven that an increase in IL-6 concentration usually correlates with the stage and intensity of inflammatory process infiltration. The analysis of the intergroup dynamics of IL-6 levels showed that in group 2 it increased by 1.7 times compared to group 1 (p1<0.01).

The concentration of IL-10 in the blood plasma of TB patients significantly increased: in group 1, the level of IL-10 was 2.2 times higher, in group 2 – 1.7 times higher (p<0.001, respectively). IL-10 is an inhibitor of activated macrophages and dendritic cells, and an increase in concentration indicates suppression of cellular immunity and the onset of chronicity of a specific inflammatory process. It was found that IL-10 in group 1 was 1.2 times higher than in group 2.

A correlation analysis was performed between the studied cytokines and melatonin. The presence and closeness of a linear relationship between the two signs were determined using the linear correlation coefficient (Pearson correlation coefficient) r, which is calculated by the following formula.

$$r_{x,y} = \frac{\sum_{i=1}^{n}(y_i - \bar{y})\cdot(x_i - \bar{x})}{\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2 \cdot \sum_{i=1}^{n}(x_i - \bar{x})^2}}$$

It has been shown that in TB patients there is almost no correlation between IL-6 and melatonin levels (r=0.14), but there is a positive, close relationship between IL-10 and melatonin levels (r=0.73).

The role of melatonin in TB is obvious but not fully understood. To prevent the chronicity of the inflammatory process, the development of resistance, and mortality from TB, specific treatments are needed, as well as the inclusion of new immune system stimulating drugs such as melatonin in the treatment programme. Determining the plasma concentration of melatonin in TB patients is not a routine test in diagnostic laboratories. However, we believe that determining the level of melatonin in the blood plasma of TB patients can contribute to a better course of the specific process and further treatment of patients.

Thus, the immunomodulatory and antioxidant effects of melatonin indicate the possibility of its use as an additional pathogenetic therapy for pulmonary TB and its resistant forms.

Conclusions

1. It was found that the levels of melatonin in blood plasma in the study groups were significantly lower than in the control group. The average value of melatonin was 33.2±1.2 pg/ml in group 1 and 29.4±2.3 pg/ml in group 2, in the control group – 46.2±0.8 pg/ml (p=0.035).
2. It was proved that the content of IL-6 in the studied groups of patients significantly exceeded the PHI index: in group 1, the level of IL-6 was 11.04 times higher, in group 2 – 13.9 times higher (p<0.001). The concentration of IL-10 in the blood plasma of TB patients significantly increased: in group 1, the level of IL-10 was 2.2 times higher, in group 2 – 1.7 times higher (p<0.001, respectively).
3. The linear correlation coefficient showed that in TB patients there was almost no correlation between IL-6 and melatonin (r=0.14), but there was a positive, close relationship between IL-10 and melatonin (r=0.73).

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