Melatonin effect on the thymus cell composition in mice with functional pinealectomy

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More and more data is accumulating about the close connection between the pineal gland and the immune system. Prolonged continuous lighting contributes to the development of light-induced functional pinealectomy, as a result of which melatonin (MT) synthesis in the pineal gland is reduced/blocked. It was found that the character of the 24-hour rhythms of the number of B- and T- cells, T-helper cells and T-suppressors/killers, the expression of CD45, CD5, CD3 and CD4 surface molecules on rat lymphocytes depends on the light regime and changes when animals are kept in conditions of constant light or constant darkness (Pelegri C. et al., 2003). Situations with functional pinealectomy or MT production inhibition lead to immunosuppression, to a decrease in adaptive cellular and humoral immune response. The use of this hormone helps to restore the immune system functions. However, the peculiarities of MT effect on the thymus cellular composition of animals under the conditions of functional pinealectomy remain not fully investigated.

T-cells originate from hematopoietic stem cells, which are found in the bone marrow. The precursors of T-lymphocytes in the thymus do not self-renew, so they must be constantly imported from the blood into the thymus to maintain T-cell production. T-lymphocytes complete most of their development in the thymus. The intra-thymus pathway of development eventually leads to the formation of naive T-cells, which then migrate from the thymus and populate the periphery. T-cells occupy a special place in the immune system and serve as the main population in the development of cellular immune response. A common marker for all varieties of T-lymphocytes is the TCR-CD3 molecular complex, which is carried by all mature T-lymphocytes.

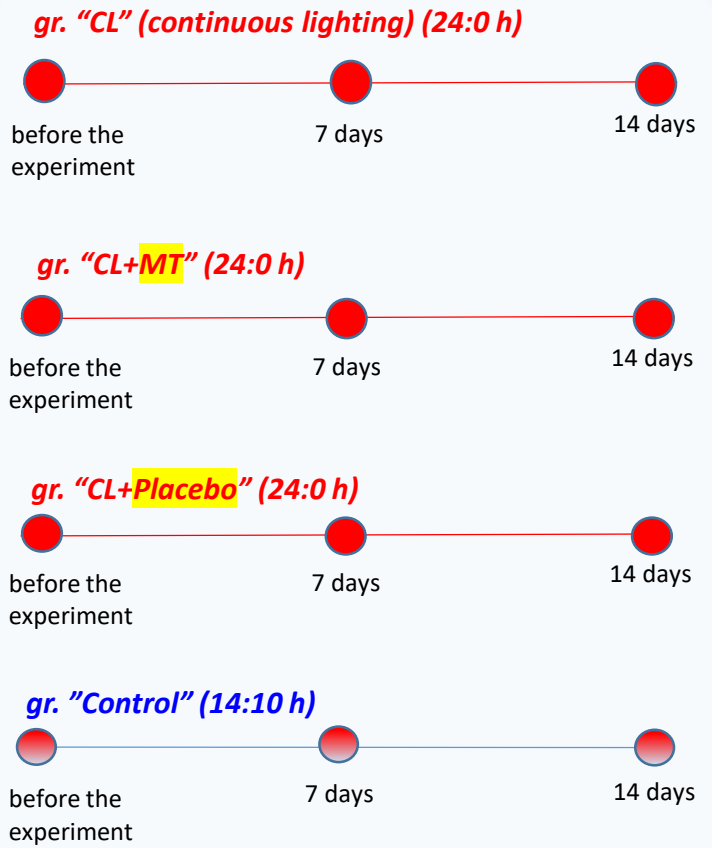
The aim of the study was to determine MT effect on the content of young $CD3^{low}$ and mature $CD3^{hi}$ forms of T–lymphocytes in the thymus in C57Bl/6 mice male with a model of functional pinealectomy (FP).

Material and Methods. 4 groups were formed for the experiment:

- **«CL»** – animals with a model of functional pinealectomy (FP), which were kept under constant lighting for 14 days (CL, **light 24:dark 0 hours**);
- **«CL+MT»** - animals with FP, who were intragastrically treated with melatonin (MT, 0.664 g/kg of body weight) in 200 ml of distilled water daily against the background of constant lighting;
- **«CL+Placebo»** - animals with FP, intragastrically receiving 200 ml of water daily;
- **«Control»** - intact C57Bl/6 mice kept under standard lighting mode (**light 14:dark 10 hours**).

Thymus samples were taken to study its cellular composition using a flow cytofluorimeter. A cell suspension was prepared from the thymus by soft crushing in a glass homogenizer. Then fixation was carried out in ice 70% ethanol. Immunophenotyping of thymic $CD3^{hi}$ and $CD3^{low}$ T-lymphocyte subpopulations was performed by staining with CD3-APC antibodies (BioLegend Inc., USA). 3 µl of monoclonal antibody in the presence of 1% albumin was added to 50 µl of each sample. The samples were incubated at room temperature in the dark for 30 min and then washed twice with PBS. The analysis of obtained thymocytes was carried out using a CytoFlexS-100 flow cytofluorimeter (Beckman Coulter, Inc, USA). The $CD3^{low}/CD3^{hi}$ ratio was calculated.

Statistical processing was performed using the software package "Statistica12". Median values, values of the first and third quartiles, and the arithmetic mean were determined. The reliability of the differences between compared values was determined using Mann-Whitney test. The differences were considered statistically significant: at $p<0.05$ - *, at $p<0.01$ - **.



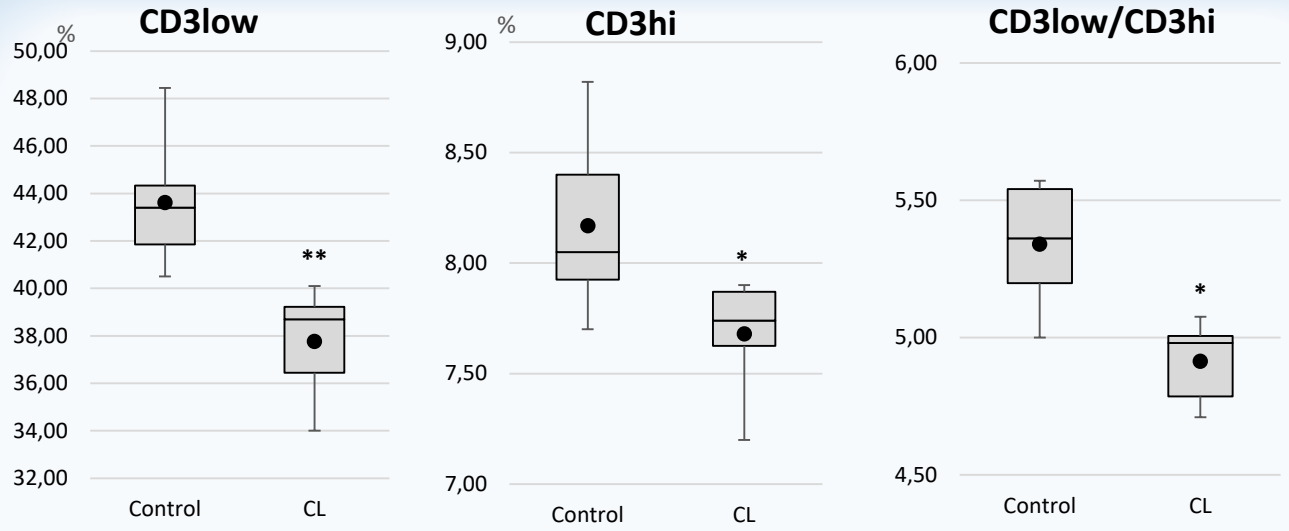


Figure 1. The relative amount of CD3^{low} and CD3^{hi} in the thymus and the ratio of CD3^{low}/CD3^{hi}.

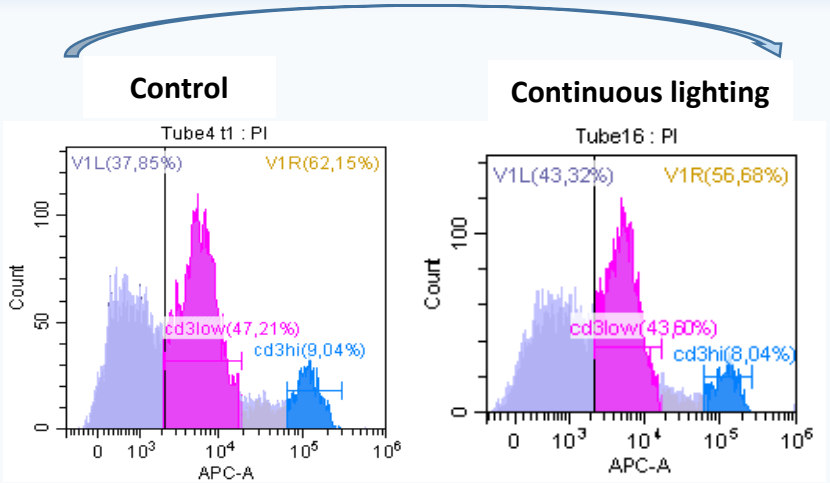


Figure. 2. Histograms of flow cytometry of CD3^{low} and CD3^{hi} distribution of T-lymphocytes in C57Bl/6 mice' thymus in groups: "Control" and "CL" (animals with the FP model). On the ordinate axis: the number of cells, on the abscissa axis: pink color - CD3^{low} fluorescence intensity, blue color - CD3^{hi}.

Prolonged continuous lighting for 14 days (mice with the FP model) led to a significant decrease in the relative number of CD3^{low} and CD3^{hi} T-lymphocytes in the thymus and a decrease in the ratio of CD3^{low}/CD3^{hi}.

In this study, no development of accidental thymus involution was recorded in C57Bl/6 mice exposed to CL. Perhaps this is due to the high level of proliferation in the G2/M phases of the cell cycle for a pool of large stroma cells consisting of thymus epithelial cells, endothelial, mesenchymal, dendritic cells, macrophages. At the same time, activation of apoptosis and a decrease in the proliferative activity of young and dividing thymocytes were noted (work in print).

Such rearrangements can be explained by the stressful effects of prolonged continuous lighting (Kinsella S. and Dudakov J.A., 2020; Hofmann T. et al., 2020) and a violation of the daily biorhythms of proliferation and migration of immune system cells under altered light conditions (Pivonello C. et al., 2022).

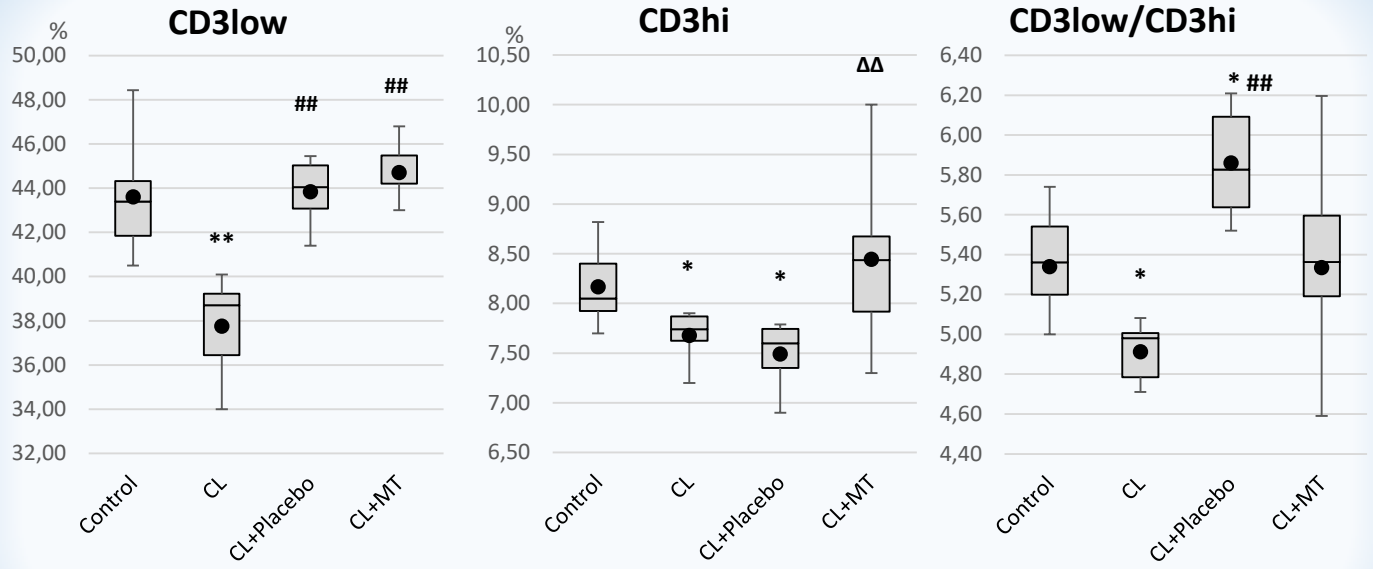


Figure 3. The relative amount of CD3^{low} and CD3^{hi} in the thymus and the ratio of CD3^{low}/CD3^{hi}.

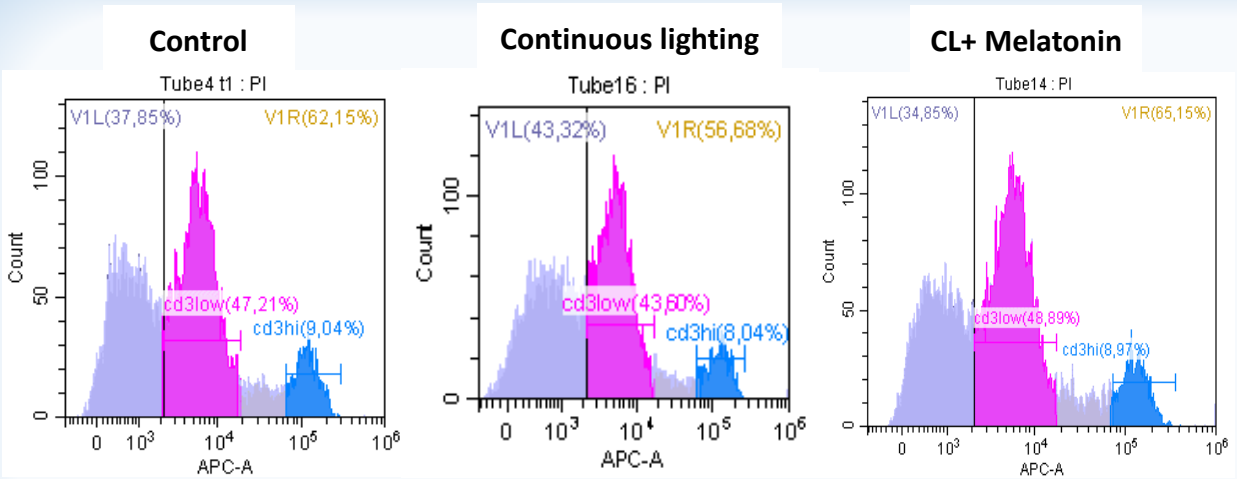


Figure. 4. Histograms of flow cytometry of CD3^{low} and CD3^{hi} distribution of T-lymphocytes in C57Bl/6 mice' thymus in groups: "Control", "CL" (animals with the FP model), "CL+MT" (melatonin administration on the background of CL). On the ordinate axis: the number of cells, on the abscissa axis: pink color - CD3^{low} fluorescence intensity, blue color - CD3^{hi}.

The melatonin administration to animals with the FP model restores the percentage of CD3^{low} and CD3^{hi} T-cells in the thymus, as well as the CD3^{low}/CD3^{hi} ratio to the values of the control group.

Our data are consistent with the results of many researchers. MT treatment increases the proliferative activity of T-lymphocytes (Chen F. et al., 2016; Ren W. et al., 2017; Hofmann T. et al., 2020), reduces the production of proinflammatory cytokines (Hofmann T. et al., 2020); reduces the level of thymocyte apoptosis increased after pinealectomy (Seema R. and Chandana H., 2013), restores the numerical density of thymocytes and cytokine expression in pinealectomized animals (Susko I. et al., 2017; Luo J. et al., 2020).

The melatonin effect on the biology of T-cells is associated with membrane and nuclear binding sites, as well as with receptor-independent pathways, including possible cytoplasmic and mitochondrial binding sites (W. Ren et al., 2017).

So, excessive light exposure at night due to light pollution or shift work leads to so-called "immune aging" (H.A. El-Bakry et al., 2018). The protective effects of melatonin may be a consequence of its combined antioxidant and immunomodulatory effects. Melatonin modulates oxidative damage, causing an antioxidant response, which contributes to the normalization of the thymus cellular composition. The protective effects of melatonin may also be the result of its anti-apoptotic activity and counteraction to excessive corticosterone production (V. Brazão, et al., 2020).

Conclusion: Melatonin in this model situation (model of light-induced functional pinealectomy) helps to compensate for the effects of continuous lighting, starting the process of cell proliferation in the thymus from the earliest stages. This indicates that the melatonin modifier molecule has immunotropic properties and can be used to correct the consequences of functional pinealectomy.

Thank you for your attention

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