DOI 10.1007/s10517-014-2687-6

PHARMACOLOGY AND TOXICOLOGY

Effect of Melatonin on Cellular Composition of the Spleen and Parameters of Lipid Metabolism in Rats with Alimentary Obesity

V. A. Trufakin, A. V. Shurlygina*, M. I. Dushkin, M. V. Khrapova**, S. V. Michurina*, E. V. Mel'nikova*, N. G. Panteleeva*, and M. V. Tenditnik***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 158, No. 7, pp. 49-52, July, 2014
Original article submitted August 9, 2013

We studied the effects of melatonin on the status of immune organs and parameters of lipid metabolism in rats with alimentary obesity and parameters of lipid metabolism and immune status in Wistar rats kept on high-fat diet and receiving melatonin solution *per os*. Melatonin leveled the changes in blood and liver parameters of lipid metabolism, which was paralleled by normalization of cellular composition of immune organs. We conclude that melatonin can be a promising agent for the treatment of lipid metabolism and immune status disorders in alimentary obesity.

Key Words: *obesity; melatonin; lymphocytes; thymus; spleen*

Obesity is more prevalent in developed countries is one of the most urgent problems of modern society, because it is associated with various metabolic disorders such as type 2 diabetes mellitus, atherosclerosis, hypertension, and fatty liver disease. In this context, prevention of obesity and correction of lipid metabolism are essential in many clinical fields. The available data also strongly suggests that adipose tissue dysfunction is tightly linked with systemic inflammation. In particular, it is shown that shifts in the balance of pro- and anti-inflammatory cytokines towards the synthesis of TNF-α and IL-1 contribute to impairment of insulin receptor activity [7] and dyslipoproteinemia [12] playing an important role in the development of obesity. Increased blood levels of adipokines, proinflammatory cytokines, and leptin in obese individuals contribute to the development of a number of obesity-related co-morbidities, such as cardiovascular disease, diabetes, hypertension, arthritis, and stroke [10,11]. Inflammation and changes in the functions of T cells and macrophages indicate impaired immune regulation in obesity [14]. Melatonin (MT) was shown to be effective in the correction of lipid metabolism disorders: it reduces the amount of adipose tissue and body weight due to its antioxidant effect [13]. At the same time, MT is an effective immunomodulator that stimulates differentiation of T cells and their circadian activity and contributes to activation of type 1 T helpers [2,6]. However, MT effects on the immune system in obesity were never studied.

Here we studied the effects of MT on the status of immune organs and parameters lipid metabolism in rats with alimentary obesity.

Research Institute of Physiology and Fundamental Medicine, Siberian Division of Russian Academy of Medical Sciences; *Research Institute of Clinical and Experimental Lymphology, Siberian Division of Russian Academy of Medical Sciences; **Research Institute of Internal Medicine, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** anna_v_s@mail.ru. A. V. Shurlygina

MATERIALS AND METHODS

The study was carried out on female Wistar rats weighing 180-200 g at the beginning of the experiment (vivarium of the Research Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences). The animals were kept at 20-22°C and had free access to water and food. The experiments were carried out in the fall and winter. The rats were divided into 4 groups: group 1 rats (intact) were kept on standard vivarium diet; group 2 (high-fat diet) received animal fats (lard) without restriction in addition to standard vivarium diet over 3 months; group 3 (high-fat diet+placebo) received high-fat diet and 1.0 ml 0.9% NaCl *per os* every day after sunset on experimental days 90-104; group 4 (high-fat diet+MT) received melatonin (Sigma) solution in saline in a dose of 0.1 mg per 100 g body weight *per os* every day after sunset on experimental day 90-104. All experimental procedures were performed in accordance with International Rules of Animal Welfare of the Helsinki Declaration for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive of the European Communities (86/609/EC).

After completion of MT or saline administration, the rats were decapitated under light ether anesthesia. The thymus, spleen, mesenteric lymph nodes, and liver were isolated; the blood was collected from the decapitation wound. Lymphoid organs were weighed and the cell suspension prepared routinely. The number of cells in 1 ml of suspension was counted in a Goryaev's chamber and then the number of cells per organ was calculated. For estimation of lymphocyte subpopulations in lymphoid organs, the cell suspension was treated with FITC- or phycoerythrin-labeled monoclonal antibodies to lymphocyte surface antigens CD3, CD4, CD8, CD11b/c, CD45RA, and CD25 (BD Pharmingen). The samples were assayed using a FACSCalibur flow cytofluorometer (Becton Dickinson). Lactate (LDH) and succinate dehydrogenases (SDH) activities in lymphocytes were assayed cytochemically in blood smears using p-nitro violet tetrazolium after R. P. Nartsissov [4]. Serum and liver homogenate levels of total cholesterol (CH), HDL CH, and triglycerides were determined by enzymatic colorimetric method with Biocon kits.

The data were statistically processed using Statistica 6.0 software. The significance of intergroup differences was evaluated using non-parametric Mann–Whitney *U* test.

RESULTS

High-fat diet increased the body weight of rats, elevated serum and liver levels of triglycerides, total cholesterol, and total lipids, and reduced serum levels of HDL CH (Fig. 1; Tables 1 and 2). MT reduced body weight by 13.1% (Table 1) and blood triglyceride level by 45.5% (Fig. 1) and lowered liver content of total lipids by 32%, triglycerides by 42.6%, and total CH by 24%.

The parameters of the immune system did not differ significantly in the "high-fat diet" and "high-fat diet+placebo" groups, therefore, Tables 1, 2, and 3 show measures for the group "high-fat diet+placebo". High-fat diet reduced the spleen weight index calculated as organ weight (mg)/body weight (g) and elevated blood leukocytosis; MT normalized these parameters (Table 1).

High-fat diet increased the relative content of mature $CD8^+4^-$ and $CD4^+8^-$ and decreased the content of poorly differentiated $CD4^+8^+$ cells in the thymus. There were no significant differences between the group treated with MT and placebo group (Table

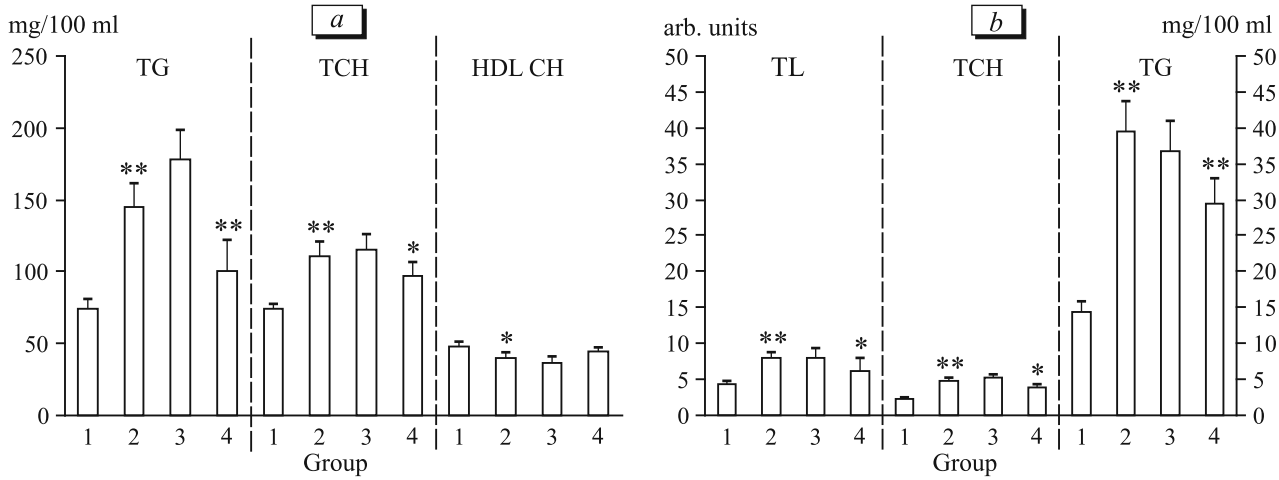

Fig. 1. Effects of MT on triglyceride (TG), total cholesterol (TC), and HDL CH content in serum (*a*) and liver (*b*) in rats maintained on high-fat diet. TL: total lipids (arb. units=optical density/g tissue weight). **p*<0.05, ***p*<0.01 in comparison with group 3.

TABLE 1. Effects of High-Fat Diet and MT on Body Weight, Weight Parameters of the Immune System, and Blood Leukocytosis in Rats (*M*±*SE*)

Parameter	Group		
	intact	high-fat diet+placebo	high-fat diet+MT
Rat weight, g	242.40±11.23	328.40±9.89**	285.33±7.84*+
Spleen weight index, mg/g	2.62±0.14	1.96±0.07**	2.47±0.08++
Number of leukocytes in blood, 10^6/ml	1.64±0.42	2.99±0.31*	1.68±0.40

Note. Here and in Table 2: *p<0.05, **p<0.01 in comparison with intact group; +p<0.05, ++p<0.01 in comparison with group "high-fat diet+placebo".

2). The diet increased the relative content of B cells ($CD45RA^+$) in rat spleen, reduced the relative content of T cells ($CD3^+$), antigen-presenting $CD11b/c^+$ cells (monocytes/macrophages and dendritic cells, T-helper cells ($CD4^+8^-$), and activated lymphocytes ($CD25^+$) in the organ. In rats kept on high-fat diet, content of activated lymphocytes in the mesenteric lymph nodes ($CD25^+$) was reduced. MT normalized this parameter, which was accompanied by an increase in T helper cells ($CD4^+8^-$) in comparison with the control (Table 2).

High-fat diet increase SDH activity in blood lymphocytes and reduced the LDH/SDH ratio activity. In rats treated with MT, SDH activity in blood lymphocytes did not change, but a strong tendency to increase in LDH activity was detected (Table 3). Thus, MT restored the LDH/SDH ratio almost to normal, which may indicate normalization of energy metabolism and functional activity of lymphocytes [1,3].

Thus, impaired blood and liver lipid profile typical of the increased consumption of dietary fat was associated with body weight increase. It is now recognized that obesity is accompanied by chronic subacute inflammation underlying the majority of pathologies associated with lipid disorders [11]. Chronic inflammation is often associated with immune system dysfunction, imbalance in immune cell populations, and cytokine production. We revealed shifts in the ratio of mature and immature thymocyte subpopulations towards more differentiated cells in rats kept on high-fat diet. This was probably associated with the level of leptin and proinflammatory cytokines, in particular TNF-α [10], in obesity because these factors regu-

TABLE 2. Effects of High-Fat Diet and MT on Cell Populations (%) in Rat Thymus, Spleen, and Lymph Nodes (*M*±*SE*)

Parameter	Group		
	intact	high-fat diet+placebo	high-fat diet+MT
Thymus			
$CD8^+4^-$	4.44±0.23	8.20±0.49*	6.82±0.56**
$CD4^+8^+$	81.56±1.13	71.40±0.63*	71.47±1.13**
$CD4^+8^-$	10.98±0.70	16.18±0.78*	17.47±0.73**
Spleen			
$CD45RA^+$	35.70±1.25	41.98±1.80*	38.98±1.69++
$CD3^+$	45.10±1.51	37.88±2.91**	49.35±1.85
$CD11b/c^+$	22.26±0.22	16.92±0.33**	19.62±1.17
$CD4^+8^-$	27.64±0.82	22.54±1.08**	27.12±0.81+
$CD25^+$	13.20±0.68	10.42±0.39**	11.87±0.47
Lymph node			
$CD4^+8^-$	43.76±0.84	42.62±1.53	51.45±0.23**++
$CD25^+$	9.90±0.61	6.96±0.57*	11.28±0.37++

TABLE 3. Effects of High-Fat Diet and MT on Dehydrogenase Activity in Peripheral Blood Lymphocytes of Rats (Formazan Granules per Lymphocyte; *M*±*SE*)

Dehydrogenase	Group		
	intact	high-fat diet +placebo	high-fat diet+MT
LDH	13.23±1.10	12.83±0.98	18.90±2.48
SDH	7.06±0.57	10.25±0.96*	11.76±1.88**

Note. *$p<0.05$, ** $p<0.01$ in comparison with intact group.

late maturation and apoptosis of T cells in the thymus [5,9]. The rats kept on a high-fat diet also showed changes in the cellular composition in secondary immune organs, the spleen and mesenteric lymph nodes. In this case, the percentage of B cells increased in the spleen with simultaneous decrease in the percentage of T cells, antigen presenting ($CD11b/c^+$), and activated splenocytes ($CD25^+$). The percentage of activated $CD25^+$ lymphocytes was also reduced in lymph nodes, where T cells prevailed. Thus, it can be concluded that high-fat diet impaired the balance between the B- and T-cell compartments of the immunity system. MT leveled the changes in lipid metabolism in the blood and liver, which was consistent with the data on its possible use as a hypolipidemic agent and as an effective protector of liver steatosis [8]. Furthermore, our study showed that MT normalized some parameters of cellular composition of immune organs, which can be regarded as restoration of the immunoregulatory mechanisms. Thus, MT producing potent antioxidant, hypolipidemic, immunomodulatory, and chronotropic effect is a promising agent for the treatment of lipid disorders and impaired immune status in alimentary obesity.

REFERENCES

1. T. I. Dergacheva, N. V. Yuklyaeva, A. V. Shurlygina, *et al*., *Byull. Sib. Otd. Ross. Akad. Med. Nauk*, No. 2, 21-26 (1999).
2. G. I. Litvinenko, A. V. Shurlygina, L. V. Verbitskaya, *et al*., *Bull. Exp. Biol. Med*., **140**, No. 2, 213-216 (2005).
3. G. I. Litvinenko, A. V. Shurlygina, and V. A. Trufakin, *Bull. Exp. Biol. Med*., **131**, No. 5, 490-492 (2001).
4. A. V. Merkur'yeva, G. L. Bilich, and R. P. Nartsissov, *Biochemical and Cytochemical Methods for Measuring Enzyme Activity and Enzyme-Substrate Systems of Different Cellular Localization. Methodical Recommendations* [in Russian], Moscow; Yoshkar-Ola (1982).
5. J. G. Baseta and O. Stutman, *J. Immunol*., **165**, No. 10, 5621-5630 (2000).
6. A. Carrillo-Vico, A. García-Pergañeda, L. Naji, *et al*., *Cell. Mol. Life. Sci*., **60**, No. 10, 2272-2278 (2003).
7. P. Dandona, A. Aljada, A. Chaudhuri, *et al*., *Circulation*, **111**, No. 11, 1448-1454 (2005).
8. A. Hattori, K. Mawatari, S. Tsuzuki, *et al*., *Obesity (Silver Spring)*, **18**, No. 1, 48-54 (2010).
9. R. W. Hick, A. L. Gruver, M. S. Ventevogel, *et al*., *J. Immunol*., **177**, No. 1, 169-176 (2006).
10. G. S. Hotamisligil, N. S. Shargill, and B. M. Spiegelman, *Science*, **259**, 87-91 (1993).
11. G. S. Hotamisligil, *Nature*, **444**, 860-867 (2006).
12. W. Khovidhunkit, M. S. Kim, R. A. Memon, *et al*., *Lipid Res*., **45**, No. 7, 1169-1196 (2004).
13. V. Srinivasan, Y. Ohta, J. Espino, *et al*., *Recent Pat. Endocr. Metab. Immune Drug Discov*., **7**, No. 1, 11-25 (2013).
14. M. D. Trottier, A. Naaz, Y. Li, and P. J. Fraker, *Proc. Natl Acad. Sci. USA*, **109**, No. 20, 7622-7629 (2012).