EXPERIMENTAL BIOLOGY

Circadian Variations of Melatonin Concentration in Saliva and Blood Content of Immunocompetent Cells in Healthy Individuals

G. I. Litvinenko, A. V. Shurlygina, O. A. Malysheva, O. T. Kudaeva**, V. S. Shirinskii**, V. A. Kozlov**, and V. A. Trufakin***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 5, pp. 578-581, May, 2002
Original article submitted February 7, 2002

Salivary content of melatonin and parameters of the immune status were studied in young healthy individuals. Morning and evening levels of melatonin showed different correlations with some immunity parameters. It was assumed that the degree and type of correlation between the immune system and melatonin depend on the phase of circadian cycle, which should be considered during evaluation of immunoendocrine status.

Key Words: *immune status; melatonin; saliva; circadian cycle*

A wide spectrum of biological activities of pineal hormone melatonin attracted much recent attention. Melatonin participates in the regulation of the central and autonomic nervous systems, endocrine and immune systems, and their circadian rhythms. Antigen-dependent and antigen-independent effects of melatonin involve its binding with receptors on lymphoid cells and are mediated by opioids, thymic hormones, cytokines, and others [8]. However, the data on the mechanisms of immunomodulating effects of melatonin are contradictory. Thus, A. V. Shai *et al.* reported that melatonin can selectively activate Th2-dependent immune response [11]. On the contrary, S. Garsia-Maurino *et al.* [7] found that melatonin stimulated production of interleukin-2 by human Th1 lymphocytes, but did not stimulate production of interleukin-4 by Th2 lymphocytes. The effects of melatonin depend on the degree and way of cell activation before melatonin application, on the dose of melatonin and the time of its application [7]. The interrelations between endogenous melatonin and immune parameters are even less studied. Many authors point to immunomodulatory and oncostatic role of endogenous melatonin. However, some studies on animals with leukemias showed that pinealectomy increased survival of tumor-bearing mice, while injections of melatonin promoted progression of leukemia in PNX animals [6].

Circadian rhythm of melatonin production is characterized by significant differences between maximum and minimum hormone levels. Subpopulational composition of immunocompetent cells, their receptor system, and metabolic potential also undergo circadian variations [3,5]. Thus, both the content and activity of immunocompetent cells and blood melatonin concentration vary during the day. However, the correlations between daily fluctuations of these parameters and their interrelations remain unclear.

Studies in this field are important for better understanding of the neuroendocrine regulation of circadian organization of the immune system and for the de-

Institute of Clinical and Experimental Lymphology, *Institute of Physiology, **Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** A.V.Shurlygina@iph.ma.nsc.ru; anna_v_s@mail.ru. Shurlygina A. V.

0007-4888/02/1335-0500$27.00

velopment of rational scheme of immunomodulation with melatonin and its inductors.

The aim of the present study was examination of circadian variation of melatonin concentration in the saliva of healthy individuals and parallel evaluation of the content and functional properties of immunocompetent cells in the peripheral blood.

MATERIALS AND METHODS

Healthy men and women (n=16, 22-25 years, students of Novosibirsk State Medical Academy) were examined in February-May 2000. The blood and saliva for evaluation of the immune status and melatonin content were obtained at 9.00 and 21.00. The blood (20 ml) from the cubital vein was stabilized with heparin. Mononuclear cells were isolated in Ficoll-Verographin gradient, stained with FITC- and phycoerythrin-labeled monoclonal antibodies against CD3, CD4, CD8, CD16, CD20, CD25, and HLA-DR antigens (Medbiospectr and Sorbent), and counted by flow cytometry on a FACSCalibur cytometer (Becton Dickinson). Phagocytic activity of granulocytes and monocytes was also evaluated by flow cytometry. To this end the cells containing FITC-labeled latex particles were counted [4].

Blood smears were prepared on glass slides, fixed in 60% cold acetone, and used for measuring activities of succinate (SDH) and lactate (LDH) dehydrogenases, and NADP-diaphorase in lymphocytes. Enzyme activity was detected by quantitative cytochemistry using nitroblue tetrazolium (NBT test) [2]. The concentration of melatonin in the saliva was determined by immunoenzyme assay using Melatonin-ELISA-kit (ICN-Biomedicals) according to the protocol supplied with the kit.

The significance of differences between groups was estimated by Mann—Whitney nonparametric U test.

RESULTS

In most subjects circadian rhythm of salivary melatonin was characterized by high evening and low morning concentrations. All subjects can be divided into the following groups: individuals with high, medium, and low evening melatonin levels (80, 13-21, and 0-5 μg/ml, respectively), and with medium (10-20 μg/ml) and low (0-5.5 μg/ml) morning melatonin content. High melatonin concentrations (above 20 μg/ml) were never found in morning samples.

In the group with high evening melatonin concentrations the mean phagocytic activity of granulocytes

TABLE 1. Immune Parameters and Dehydrogenase Activity in Blood Lymphocytes (Number of Formazan Granules per Cell) in Individuals with Different Evening and Morning Concentration of Melatonin in Saliva

Parameters	Melatonin concentration, μg/ml saliva				
	evening			morning	
	0-5	13-21	80	0-5.5	14-20
Lymphocyte subpopulations, %					
CD3	65.9	64.25	68.75	67.46	58.83^{+}
CD4	35.65	36.88	38.25	36.85	58.83
CD8	26	22.75	22	26.1	18.5^{+}
CD16	12.7	12	5.75*	9.65	20.33^{+}
CD20	13.6	12	12.25	13.12	12.67^{+}
Immunoregulatory index (CD4/CD8)	1.43	1.73	1.75*	1.47	1.88^{+}
Number of monocytes expressing HLA-DR, %	86.13	85.13	73.75*	81.45	93.67^{+}
Total number of cells expressing HLA-DR, %	0.61	0.59	0.69	0.64	0.56
Phagocytosis, % cells					
monocytes	58.9	58.875	62.25	59.27	59.5
granulocytes	78.05	75.75	71.25*	76.69	76.33
Mean melatonin concentration in saliva, μg/ml	2.5	13.1	40.5	9.99	9.6
SDH	13.8±0.3	15.0±1.5	13.1±1.5	14.2±2.1	12.9±2.6
LDH	14.9±0.5	12.4±2.4	16.8±0.05	15.5±2.4	10.9±4.6^{+}
NADP-diaphorase	14.1±0.3	13.6±0.8	15.3±0.01	14.3±1.5	12.9±2.1

Note. p<0.05 *compared to groups with minimum and medium evening melatonin level and $^{+}$group with minimum morning melatonin level.

and the content of HLA-DR$^+$-monocytes and CD16$^+$-lymphocytes were decreased, while CD4/CD8 ratio was higher than in individuals with low and medium melatonin concentrations (Table 1). In the group with medium morning melatonin concentrations the percentage of HLA-DR$^+$-monocytes and CD16$^+$-lymphocytes, and CD4/CD8 ratio were increased, but the content of CD3$^+$ and CD8$^+$ cells and LDH activity in lymphocytes were decreased compared to the group with low morning melatonin levels (Table 1).

High parameters of the immune system in individuals with high morning melatonin concentrations agree with the data on the stimulatory effect of this hormone on Th1 cells producing antiinflammatory cytokines and on macrophages producing interleukin-1 [8]. Low LDH activity in lymphocytes also reflects the stimulatory effect of high morning melatonin concentrations on functional activity of these cells. This is associated with a shift in activity of redox enzymes toward Krebs cycle enzymes SDH and NADP-diaphorase, *i. e.* to predominance of more effective aerobic processes.

High evening melatonin level was associated with decreased parameters of the immune system (functional activity of granulocytes and lymphocytes and the number of natural killers in the peripheral blood). This contradicts previous data on immunostimulatory effect of evening melatonin administration [1]. It is possible that the effects of exo- and endogenous melatonin are different. Moreover, a negative feedback between cytokines and melatonin probably exists: melatonin increases interferon-γ and interleukin-2 production by immunocompetent cells, while interferon-γ decreases pineal secretion of melatonin via a feedback mechanism [12]. Activity of Th1 lymphocytes and production of antiinflammatory cytokines, in particular interferon-γ, is enhanced in the evening and night [9]. It is possible that the combination of low evening melatonin concentrations in and high immune parameters is determined by inhibition of melatonin production by enhanced secretion of antiinflamatory cytokines. The type and level of feedback regulation between immune and neuroendocrine systems can also undergo circadian changes. Moreover, endogenous melatonin acts as an immunomodulator in the circadian cycle: it inhibits hyperactivation of T-cells and macrophages in the evening and night (at the peak of their activity) and stimulates them in the morning (minimum activity).

Our data confirm that melatonin participates in the coordination and synchronization of the content and activity of immunocompetent cells under physiological conditions. At the same time, impaired melatonin production and reception is probably a pathologic component of many diseases associated with immune disorders [10]. Thus, the level and type of interrelations between the immune system and pineal gland depend on the phase of circadian cycle, which should be considered during evaluation of the immunoendocrine status and application of melatonin and its inductors for immunocorrection.

REFERENCES

1. O. A. Malysheva and V. S. Shirinskii, *Int. J. Immunorehabilit.*, No. 9, 80-92 (1998).
2. R. P. Nartsissov, *Arkh. Anat.*, No. 5, 55-91 (1969).
3. V. A. Trufakin, A. V. Shurlygina, T. I. Dergacheva, and G. I. Litvinenko, *Byull. Exp. Biol. Med.*, **199**, No. 2, 181-183 (1995).
4. V. S. Shirinskii, N. M. Starostina, S. M. Petrova, *et al., Byull. SO RAMN*, Nos. 3-4, 52-55 (1999).
5. A. V. Shurlygina, I. G. Kovshik, L. V. Verbitskaya, and V. A. Trufakin, *Ibid.*, No. 5, 129-133.
6. A. Conti, N. Haran-Ghera, and G. J. M. Maestroni, *Med. Oncol. Tumor Pharmacother.*, No. 9, 87-92 (1992).
7. S. Garcia-Maurino, M. G. Gonzalez-Haba, J. R. Calvo, *et al., J. Immunol.*, **159**, No. 2, 574-581 (1997).
8. G. J. M. Maestroni and A. Conti, *Neuroimmunomodulation*, **3**, No. 6, 325-332 (1996).
9. S. Palm, E. Postler, H. Hinrichsen, *et al., Chronobiol. Int.*, **13**, No. 6, 423-434 (1996).
10. P. Penev and P. C. Zee*, Ann. Neurol.*, No. 42, 545-553 (1997).
11. A. V. Shaji, S. K. Kulkarni, and J. N. Agrewala, *Clin. Exp. Immunol.*, **111**, No. 1, 181-185 (1998).
12. B. Withyachumnarnkul, K. O. Nonaka, and A. M. Attia, *Role of Melatonin and Pineal Peptides in Neuroimmunomodulation*, Eds. F. Fraschini and R. Reiter, London (1991), pp. 79-90.