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Circadian Variations in Immune Values and Serum Melatonin in Asthmatics

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Circadian variations in the immune status and serum melatonin were studied in asthmatics during exacerbation stage before and after treatment. Deviations from the normal (donor) immunogram values at 9.00 and 21.00 and decreased correlations between immune values and blood melatonin level were detected. The correlations of immune values between each other and with melatonin over the circadian cycle increased after treatment.

Key Words: *circadian rhythm; immune status; melatonin; asthma*

Circadian rhythms of the immune system maintain optimal level of its functioning and are disturbed in immunopathological processes [7]. Pineal gland hormone melatonin playing an important role in the regulation of immunity functions by stimulating T cell differentiation is the leading synchronizer of circadian rhythms in mammals and humans [10]. However, the relationships between circadian fluctuations of melatonin and immune values in health and diseases associated with immune disorders remain unclear. In order to clear out these relationships, rational protocols of immunomodulation by melatonin and/or its inductors have to be developed.

Normally the relationships between endogenous melatonin levels and T and B cell subpopulations and functional activity of blood phagocytes vary during different phases of the circadian and annual cycles [3]. We studied these relationships in immune disorders.

MATERIALS AND METHODS

Twenty-nine patients with atopic and mixed forms of asthma running a medium severe and severe course and 24 donors were examined in 2003-2005 at Clinical Immunopathology Department of Institute of Immunology. The mean age of patients was 42.60 ± 2.51 years, disease duration 15.11 ± 2.14 years, mean age of donors 25.20 ± 1.34 years. The patients were observed during the exacerbation phase. All patients received topical glucocorticosteroids, β_2-receptor agonists, methylxanthines, and mucolytic therapy.

Examinations were carried out twice a day (at 9.00 and 21.00). Serum concentration of endogenous melatonin was measured by enzyme immunoassay using Melatonin—Elisa—Kit (ICN Biomedicals, Inc.) according to manufacturer's protocol.

Leukocytes for evaluation of the immune status were routinely isolated from venous blood. Activity

of phagocytic cells was evaluated by incubating the leukocyte suspension with FITC-labeled 0.1% latex charged with human immunoglobulin (Biopreparat Company). Leukocyte surface markers were evaluated using FITC- and phycoerythrin-labeled monoclonal antibodies to CD3, CD4, CD8, CD20, CD16, and HLA-DR (MedBioSpectr, Sorbent). Positive cells in studies of phagocytosis and leukocyte surface phenotype were detected on a FACS Calibur immunocytometer (Beckton Dickinson). The level of HLA-DR expression was estimated as the ratio of the percentage of monocytes with high expression of HLA-DR to the percentage of HLA-DR expressing monocytes, immunoregulatory index (CD4$^+$/CD8$^+$ lymphocytes). Serum content of circulating immune complexes was determined by high-speed nephelometry on an immunochemical analyzer (ICS, Beckman) as described previously [2]. Immunohistochemical studies of venous blood dehydrogenases (SDH, lactate dehydrogenase (LDH), and NADP-diaphorase (NADP-D)) were carried out [4].

The results were statistically processed using Statistica 5.0 software. The significance of differences between the groups was evaluated using Mann—Whitney's test, Kruskal—Wallic test (ANOVA) at 95% significance level. Analysis of correlations was carried out using Spearman correlation coefficient.

RESULTS

The immune values in asthmatics differed from the normal (donors): the levels of HLA-DR molecule expression on monocytes and the content of active phagocytic granulocytes in the morning and evening hours decreased, the percentage of HLA-DR$^+$ monocytes increased at 9.00, and count of T cells (CD3$^+$) and the immunoregulatory index decreased at 21.00 (Table 1).

Analysis of correlations showed that morning level of serum melatonin in asthmatics was in negative correlation with the morning blood levels of CD4$^+$ and CD20$^+$ lymphocytes before therapy (Table 2). A weak correlation was found between activities of lymphocyte energy metabolism enzymes: positive correlation was observed only for evening SDH and LDH activities. Cytochemical characteristics of lymphocytes correlated with only few immunity parameters (CD20$^+$- and CD8$^+$-lymphocyte percentage and CD4$^+$/CD8$^+$ ratio) (Table 2). Correlations between the immunological parameters were detected only in the morning (Table 3).

After therapy, the general status and function of external respiration improved, the morning levels of CD4$^+$ and CD8$^+$ lymphocytes and the phagocytic activity of monocytes increased, and the level of circulating immune complexes decreased in all patients. Granulocyte phagocytic activity at 9.00 and 21.00 increased. The percentage of CD3$^+$ and CD4$^+$ lymphocytes at 21.00 increased, while the level of CD20$^+$ cells during the same hour decreased (Table 1). Hence, the treatment led to positive changes in the clinical immunological parameters.

Analysis of correlations revealed significant shifts in the relationships between the studied parameters after treatment. The morning level of serum

TABLE 1. Immune Status Parameters of Donors and Asthmatics before and after Therapy ($M\pm m$)

Parameter	Time of analysis	Donors	Patients before therapy	Patients after therapy
HLA-DR$^+$ monocytes, %	09:00	84.10±2.57	89.00±0.85*	89.70±1.41
CD8$^+$ lymphocytes, %	09:00	24.6±1.8	22.89±2.13	26.55±1.78$^+$
HLA-DR expression on monocytes	09:00	0.56±0.03	0.44±0.01*	0.45±0.02*
Circulating immune complexes	09:00	—	23.22±1.21	19.33±1.03$^+$
CD4$^+$ lymphocytes, %	09:00	35.80±1.26	35.80±1.73	41.40±1.75*$^+$
Active phagocytic granulocytes, %	09:00	76.90±2.23	60.00±1.96*	68.00±3.38$^+$
Active phagocytic monocytes, %	09:00	56.30±2.67	56.30±3.42	59.00±2.66$^+$
CD3$^+$ lymphocytes, %	21:00	66.80±1.64	57.8±1.9*	70.20±2.69$^+$
CD4$^+$/CD8$^+$	21:00	1.51±0.07	1.46±0.30*	2.32±0.82
HLA-DR expression on monocytes	21:00	0.62±0.03	0.46±0.01*	0.43±0.02*
Active phagocytic monocytes, %	21:00	61.2±1.5	50.80±1.11*^{o}	56.60±3.55
Active phagocytic granulocytes, %	21:00	73.90±0.98	55.00±4.49	67.3±2.8*
CD20$^+$ lymphocytes, %	21:00	13.60±0.75	13.90±1.38	9.80±1.31*$^+$
CD4$^+$ lymphocytes, %	21:00	37.30±1.25	32.40±2.69	43.00±3.05$^+$

Note. $p<0.05$ compared to: *donors, $^+$patients before therapy, ^{o}values at 9.00.

TABLE 2. Results of Analysis of Correlations of Immune Values with Serum Melatonin and Lymphocyte Dehydrogenase Activities in Asthmatics

Parameter		Coefficient of correlation
1	2	
Before therapy		
Melatonin (9.00)	CD4$^+$ (9.00)	-0.66
Melatonin (9.00)	CD20$^+$ (9.00)	-0.85
SDH (21.00)	LDH (21.00)	+0.73
LDH (9.00)	IRI (9.00)	-0.71
LDH (9.00)	CD20$^+$ (9.00)	-0.77
SDH (9.00)	CD8$^+$ (21:00)	+0.89
SDH (9.00)	IRI (21.00)	-0.89
After therapy		
Melatonin (9.00)	LDH (9.00)	-0.82
Melatonin (9.00)	LDH (21.00)	-0.82
Melatonin (9.00)	CD16$^+$ (9.00)	+0.81
Melatonin (9.00)	GP (21.00)	-0.90
SDH (9.00)	NADP-D (9.00)	+0.78
SDH (9.00)	NADP-D (21.00)	+0.89
LDH (9.00)	LDH (21.00)	+0.96
NADP-D (9.00)	NADP-D (21.00)	+0.89
SDH (9.00)	CD8$^+$ (21:00)	-0.90
SDH (9.00)	IRI (21.00)	+0.90
LDH (9.00)	CD3$^+$ (21:00)	-0.90
NADP-D (9.00)	CD8$^+$ (21:00)	-0.90
NADP-D (9.00)	IRI (21.00)	+0.90
LDH (21.00)	CD3$^+$ (21:00)	-0.90
NADP-D (21.00)	LIM (21.00)	-0.90
LDH (9.00)	HLA-DR$^+$ monocytes (9.00)	+0.74
SDH (21.00)	CD3$^+$ (9.00)	-0.84
SDH (21.00)	CD8$^+$ (9.00)	-0.84

Note. Here and in Table 3: GP: granulocyte phagocytosis; LIM: lymphocyte count/mm^3 blood; IRI: immunoregulatory index; APG: active phagocytic granulocytes; APM: active phagocytic monocytes.

melatonin strictly correlated with morning and evening LDH activities in blood lymphocytes, morning percentage of CD16$^+$ lymphocytes, and evening phagocytic activity of granulocytes. The number of correlations between lymphocyte dehydrogenase activities increased, as well as between the parameters of enzyme spectrum of lymphocytes and immune values (Table 2). Correlations between the studied immune values in the evening appeared after the treatment (Table 3).

Hence, the positive changes in clinical and immunological parameters during treatment of patients with asthma were associated with rearrangement of the circadian organization of the endocrine—immune relationships. The increase in the number of correlations between immune values and blood melatonin level probably attests to increasing immunoregulatory role of this hormone, one of the main synchronizers of mammalian and human circadian system. The increase in the total number of correlations between immunity parameters and metabolic characteristics of lymphocytes presumably reflects better coordination between the circadian dynamics of these parameters due to increased synchronizing role of melatonin.

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TABLE 3. Results of Analysis of Correlations between Immune Parameters in Asthmatics

Parameter		Coefficient of correlation
1	2	
Before therapy		
LIM (9.00)	CD4$^+$ (9.00)	0.65
LIM (9.00)	CD8$^+$ (9.00)	0.68
CD3$^+$ (9.00)	CD4$^+$ (9.00)	0.86
CD3$^+$ (9.00)	CD8$^+$ (9.00)	0.76
CD4$^+$ (9.00)	CD20$^+$ (9.00)	0.67
CD3$^+$ (9.00)	APG % (9.00)	0.80
CD3$^+$ (9.00)	HLA-DR$^+$ monocyte % (9.00)	0.73
LIM (9.00)	CD3$^+$ (9.00)	0.70
After therapy		
CD4$^+$ (9.00)	IRI (9.00)	0.95
CD3$^+$ (9.00)	CD8$^+$ (09:00)	0.80
LIM (21.00)	IRI (21.00)	-0.94
CD3$^+$ (21:00)	APM % (21.00)	-0.60
CD4$^+$ (21:00)	LIM (21.00)	-0.81
CD4$^+$ (21:00)	IRI (21.00)	0.90
CD8$^+$ (21:00)	LIM (21.00)	0.84
LIM (21.00)	CD4$^+$ (21.00)	0.81
LIM (21.00)	CD8$^+$ (21.00)	0.84
CD8$^+$ (21.00)	IRI (21.00)	-0.90

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