Effect of Epithalamin on the Rhythm of Immune and Endocrine Systems Functioning in Patients with Chronic Coronary Disease

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In contrast to young people, changes in the rhythms of parameters manifested in elderly and senile chronic coronary patients in inversion (decreased T cell count in the peripheral blood, values of lymphocyte blastogenesis test with phytohemagglutinin, and phagocytic index in the fall), monotony (B cell count in the peripheral blood), decreased amplitude of serum hydrocortisone, and desynchronization. The levels of thymic serum factor and IgG were higher in the fall than in the spring, but the seasonal difference leveled after 2.5 years of observation. On the other hand, the rhythms of changes in the thymic serum factor and hydrocortisone were retained in patients with chronic coronary disease after 6 courses of epithalamin by the optimal protocol (period of observation 30 months) and blood T cell count increased in the fall. Improvement of the rhythms of the parameters was associated with a benign clinical course of the disease.

Key Words: *epithalamin; thymic serum factor; immune system; rhythm; chronic coronary disease*

The circannual rhythm of the functioning of the immune system is essential for adaptation to periodically changing environmental conditions, *e.g.* variations in illumination and temperature [8,11,12]. Some rhythm parameters change with aging, which is paralleled by the formation of age-associated pathological processes, when the diseases present by that time can cause secondary changes in the rhythms of the functioning of the immune system [1,3,11]. Chronic coronary disease is one of such conditions. Immune factors play an important role in the development of chronic coronary disease, which, in turn, is associated with acceleration of age-specific immune dysfunctions [2,4]. Chronic coronary disease is also associated with age-specific changes in the concentrations of glucocorticoids, essential for the functioning of the thymus, immune and cardiovascular systems [2,15].

The pineal gland, in which indole and peptide substances are synthesized, is the main synchronizer of the circadian program of many functions of the body, including functions of the immune and endocrine systems [7,9,13]. Experimental and clinical studies demonstrated positive effects of epithalamin, a pineal peptide, on the age-associated changes in the functions of the thymus, immune system, and adrenal cortex [2,9]. The drug restored circannual rhythms of thymic hormone and corticosterone levels in old animals [5].

We studied the possibility of using epithalamin for the correction of circannual rhythms of the function of the thymus, the peripheral component of the immune system, and the adrenal cortex in elderly and senile patients with chronic coronary disease.

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MATERIALS AND METHODS

Fifty-four chronic coronary patients (aged 60-84 years) were examined at clinical department of Institute of Gerontology. Chronic coronary disease (clinical forms: functional classes I-II stable effort angina and atherosclerotic cardiosclerosis) was diagnosed after general clinical examinations (Rose questionnaire and ECG) and exercise tests (bicycle ergometry) using WHO criteria [2].

Patients of the experimental group (n=27) received epithalamin: 9 patients in the spring and 18 in the fall. Discordance of body functions and exacerbations of many diseases are particularly pronounced during these seasons [3,8]. Patients of the control group (n=27) received no preparation. Epithalamin was injected by our optimal protocol based on experimental data indicating that the sensitivity of the pineal gland, thymus, and immune system to the drug increased with age [2,5,6]. A course consisted of 5 injections at 3-day intervals (single dose 10 mg, total dose per course 50 mg, intervals between the courses 6 months). The patients were observed over 30 months (2.5 years) and received 6 courses [2]. Control group was observed during the same period. Chronic coronary patients were examined in the spring and fall before and 7 days after the first and sixth courses of epithalamin. In parallel with them, controls were examined (12 in the spring and 15 in the fall). Blood for analysis was collected from the ulnar vein at 8.00-9.00 at natural light.

The endocrine function of the thymus was evaluated by the titer of thymic serum factor (TSF). The count of B cells in the peripheral blood was evaluated by the immunofluorescent method with monoclonal antibodies to CD3, T cell count was evaluated by rosette formation with sheep erythrocytes. The results were comparable [10]. Functional activity of T cells was studied in the lymphocyte blastogenesis test (LBGT) with phytohemagglutinin (PHA). The percent of intact and typical blast forms was evaluated in morphological preparations stained by the method of Romanowskii—Giemsa under a microscope [2]. Neutrophil phagocytic activity was evaluated in the whole blood. The smears were stained by the method of Romanowskii—Giemsa. The percent of neutrophils phagocytosing *E. coli* were counted (phagocytic index) under a light microscope. Serum IgG concentration was evaluated by radial diffusion in agar [2]. Serum hydrocortisone was measured by radioimmunoassay using commercial kits (Beloris).

The data were statistically processed using Student's t test.

RESULTS

The titers of TSF, counts of T and B cells, and IgG level in the blood of healthy young people were higher in the fall than in the spring, the levels of IgM and IgA less depended on the season [4,12]. The values of LBGT and neutrophil phagocytic activity also increased in the fall [8,11].

The seasonal difference between the initial TSF titer and IgG level was retained in chronic coronary patients (Table 1). Blood count and proliferative potential of T cells and the phagocytic index were higher in the spring than in the fall, while B cell counts in the peripheral blood were the same in the spring and fall.

The stimulatory effect of epithalamin on the blood TSF titer in chronic coronary patients was observed during the entire period of the study (Table 1). After the 6th course, the parameter was significantly higher in the fall than in the spring. Before the 6th course, blood T-cell count increased in the fall, while LBGT decreased. Epithalamin treatment did not change blood counts of B cells, while IgG concentration increased significantly after the 6th course in the spring. After 2.5 years of treatment the phagocytic index decreased significantly in the spring and fall, and the difference between these values leveled.

No appreciable seasonal differences in TSF titers of chronic coronary patients receiving no epithalamin were detected after 2.5 years of observation (6.0±1.8 in the spring *vs.* 4.0±0.9 in the fall); peripheral blood counts of T cells also did not differ (55.0±4.3% in the spring and 62.3±1.8% in the fall). The count of B cells and the phagocytic index did not change from the initial values. Serum concentration of IgG was 9.0±0.2 and 11.7±2.5 g/liter in the spring and fall, respectively, the value in the fall being significantly lower than at the beginning of observation, while the value in the spring was lower than in patients treated with epithalamin.

Serum concentration of hydrocortisone in young healthy subjects was higher in the fall than in the spring [12]. A trend to fall elevation of hydrocortisone concentration was observed in chronic coronary patients at the beginning of the study; this trend was retained during the entire period of epithalamin treatment (Table 1). In controls examined simultaneously with patients after the first course of epithalamin, hydrocortisone concentration in the spring (659.0±93.7 nmol/liter) was higher than in the fall (513.0±51.2 nmol/liter), this parameter being significantly higher in the control group in comparison with patients treated with epithalamin.

TABLE 1. Functional State of the Thymus, Immune System, and Adrenal Cortex in Chronic Coronary Patients during Different Seasons and Their Changes after Epithalamin Treatment (*M*±*m*)

Parameter	Before treatment	After course 1	Before course 6	After course 6
Spring				
TSF, log_2	2.9±0.6	5.1±0.9*	7.0±0.5*	6.5±0.3*
T cells, %	60.8±4.1	50.8±5.4	47.3±8.6	56.6±6.9
T cells, 10^9/liter	0.99±0.11	0.94±0.09	0.85±0.25	1.09±0.27
LBGT, %	38.5±3.6	38.3±7.3	29.2±14.2	32.8±8.2
B cells, %	16.0±1.8	15.3±1.3	14.8±3.2	13.6±2.4
B cells, 10^9/liter	0.24±0.03	0.32±0.05	0.24±0.06	0.24±0.04
IgG, g/liter	9.1±0.3	8.9±0.5	18.1±1.7*	16.2±1.5*
Phagocytic index, %	83.3±4.5	76.9±2.1	41.00±9.04*	49.0±8.4*
Hydrocortisone, nmol/liter	334.4±44.8	364.2±62.0	—	318.5±55.8
Fall				
TSF, log_2	4.5±0.3$^+$	5.4±0.3*	6.6±0.7*	8.2±0.4$^+$
T cells, %	50.5±3.7$^+$	54.9±2.9	66.3±2.9$^+$	57.0±5.0
T cells, 10^9/liter	0.81±0.07	0.83±0.08	2.25±0.60$^+$	1.0±0.3
LBGT, %	27.2±2.9**	29.4±2.9	23.5±6.2	28.7±13.0
B cells, %	18.2±2.1	16.1±0.7	14.3±2.1	19.3±9.2
B cells, 10^9/liter	0.25±0.03	0.26±0.03	0.50±0.15	0.35±0.19
IgG, g/liter	17±1$^+$	16.4±1.0$^+$	14.1±3.6	17±6
Phagocytic index, %	69.8±4.2$^+$	65.1±3.3$^+$	33.9±5.9*	39.8±8.5*
Hydrocortisone, nmol/liter	433.6±73.2	425.4±70.0	—	393.3±54.9

Note. $p<0.05$ compared to value *before treatment, $^+$in the spring.

Hence, in contrast to young healthy people, the circannual fluctuations of immune values changed in elderly patients with chronic coronary disease, which manifested in inversion (T cell count in the peripheral blood, LBGT with PHA, phagocytic index), monotony (B cell count in the peripheral blood), and desynchronization. Though the rhythm of changes in TSF and IgG levels was retained at the beginning of observation, after 2.5 years it became monotonous in the control group. On the other hand, long treatment with epithalamin maintained SF titer and led to the appearance of a fall elevation of T cell count in the peripheral blood. The drug did not cause changes in the rhythm of T cell proliferative activity, B cell count in the peripheral blood, and phagocytic index, but essentially modified the phagocytic index and IgG concentrations during different seasons. The drug maintained the fall elevation of serum hydrocortisone level during the entire period of observation, while in the control group of patients the rhythm of fluctuations of this hormone became inverted.

A possible mechanism of the synchronizing effect of epithalamin on the functions of the immune and endocrine systems in chronic coronary disease is linked with seasonal stimulation of the pineal melatonin-producing function. According to our data, the concentration of melatonin in the blood of elderly people in fall/winter after a course of epithalamin treatment significantly surpassed the initial level (36.2±2.6 and 24.30±2.32 pmol/liter, respectively) [4,5]. Since thymic epithelial cells carry high-affinity receptors for melatonin and the hormone stimulates the expression of thymic hormone precursor in them [14,15], it cannot be excluded that recovery of the impaired rhythm of the pineal melatonin-producing function and the endocrine function of the thymus are closely related. Changes in the functioning of the thymus and peripheral component of the immune system are, in turn, synchronized [4,5,8,12]. The TSF titer and T cell count in the peripheral blood increase in the fall in young people, while in chronic coronary patients the unchanged fall peak of TSF is paralleled by inversion of the blood T cell count. This indicates intrasystemic desynchronization of these parameters and can be due to changed cyclic pattern of the metabolic activity of immunocompetent cells and of expression of receptors for thymic hormones on them [8]. On the other hand, melatonin and epithalamin modulate the synthesis of some cytokines, redox processes, gene expression [7,9,13]

and thus seem to promote the recovery of phase quantitative ratios between thymic hormones and blood T cells in the patients. Presumably, this mechanism is essential for improvement of the intersystems phase relationships: functions of the thymus and adrenal cortex. For example, melatonin modulates the expression of glucocorticoid receptors on thymic cells [14], while season-dependent secretion of TSF by the thymic stroma under the effect of glucocorticoids *in vitro* is restored in old animals after long-term epithalamin treatment [4].

Thymic hormones enhance the proliferative potential of T cells [2,9,11], but the LBGT values do not change after epithalamin treatment. The results confirm that less intense proliferation of lymphocytes in response to mitogens is one of the most pronounced and stable signs of age-specific changes in the immune system [1]. At the end of the period of observation, the seasonal difference in IgG concentrations was leveled in patients treated with epithalamin and control patients, the values in the latter group decreasing in comparison with the initial level. As a decrease in the blood concentration of IgG determines a more severe protracted course of pathological processes, its increase under the effect of epithalamin is regarded as a positive result. The pattern of fluctuations in the phagocytic index remains shifted after epithalamin treatment, but its significant reduction under the effect of the drug is essential for the course of chronic coronary disease, as activation of phagocytic cells augments the disease course [1,2].

Rhythmic functioning of the immune and endocrine systems in chronic coronary patients treated with epithalamin was associated with a benign clinical course of the underlying disease. During 3-year period of observations, positive shifts in common clinical symptoms were detected in 62% patients in the epithalamin group *vs.* only 25% in the control group [2].

Hence, our results confirm the role of the pineal peptide factors in the mechanisms of changes in the circannual rhythm of the immune and endocrine systems functioning in chronic coronary disease and can be used for correction of disordered rhythms of these systems by the optimal protocols. Chronobiological regularities of the immune and endocrine systems functioning should be taken into consideration in the diagnosis, development of prognostic criteria, and identification of risk factors for age-specific diseases. Drug effects on the functions of the immune and endocrine systems should be evaluated with consideration for the season.

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