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Pineamin Increases Melatonin Synthesis in Pineal Gland of Elderly People

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Abstract—The effect of a pineal gland polypeptide complex, Pineamin, on urine excretion of 6-sulfatoxymelatonin (6-SOMT) was investigated by immunoenzyme assay in 55 elderly patients with decreased melatonin formation. Pineamin at a dose of 100 mg increased the level of 6-SOMT excretion in overnight urine by 1.9 times in comparison with the respective indicator before the treatment. A similar effect was previously obtained upon the administration of Epitalamin, which also facilitated the restoration of melatonin synthesis in human and animal pineal glands affected by aging. Hence, Pineamin and Epitalamin exhibit a one-directional stimulating property with respect to the melatonin-forming function of the pineal gland in elderly people.

Keywords: Pineamin, 6-sulfatoxymelatonin, pineal gland, aging
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INTRODUCTION

The pineal gland is one of the main organs of the endocrine system. The rate of aging of the endocrine system is related to a significant extent to involution of the pineal gland, which is manifested by distortion of its melatonin-forming function [2, 13, 23]. Information is available worldwide on the application of melatonin drugs for the treatment of various age-related conditions and as a prophylactic of accelerated aging of an organism. The pharmaceutical melatonin preparation Melaxen (Unipharm, United States) is approved for use in the Russian Federation. Despite successful clinical testing, a number of side effects and risk factors associated with this hormonal preparation must not be ruled out [17, 24]. For example, Melaxen can cause the aggravation of certain psychiatric conditions, the initiation of autoimmune processes and allergic reactions, and the development of tumors (lymphomas and leukemia); it is not used in breast-feeding women, and it cannot be combined with certain drugs (preparations affecting functions of cytochrome P450 and microsomal oxidases) [17, 18]. Thus, the search for novel approaches to increase the synthesis of endogenous melatonin appears to be important.

In 1974 V.G. Morozov and V.Kh. Khavinson isolated a complex of polypeptides with a molecular mass of approximately 10 kDa from the cattle pineal gland by extraction with acetic acid, which was later approved under the name Epitalamin as a therapeutic preparation (reg. no. 90/250/6, Order of the Ministry of Health of the Soviet Union no. 250, June 19, 1990). According to the instruction provided with the preparation, it is mainly used to treat climacteric myocardial dystrophy in women. Epitalamin was also successfully used in clinical practice during the postoperative rehabilitation period in oncology patients, in patients with various traumas and myocardial ischemia, in neurology, ophthalmology, stomatology, endocrinology, and as a geroprotector [2, 5–7, 9, 12, 19–22]. The main effects of Epitalamin are associated with normalization of indicators of cellular and humoral immunity and the blood levels of cortisol, insulin, serum thymic factor, melatonin, and other hormones. Epitalamin normalizes indicators of functional activity of cardiovascular and antioxidant systems, as well as those of homeostasis [7–9, 19, 22]. It has been established that the administration of Epitalamin facilitated a reliable decrease of mortality rate in elderly people [7, 8, 22].

Many of the mentioned effects of Epitalamin are due to its ability to restore the nighttime peak of melatonin secretion in blood, which is reduced in people over the age of 60 [9]. It was shown for a group of 40 elderly people that injections of Epitalamin (five intramuscular injections of 10 mg with two-day intervals) increased the overnight peak of melatonin secretion by 2.45 times in comparison with a placebo group

Furthermore, increased physical and psychomotor productivity, restoration of the circadian rhythm of the cardiovascular and vegetative nervous system, and normalization of the hormonal status was observed in the process of Epitalamine administration to elderly people [9].

These data are in good agreement with the results from research on the effect of Epitalamin on the melatonin-forming function in animals. It was shown in the work of O.V. Korkushko and coauthors (2007) that a blood concentration of melatonin at 21 h (corresponds to the nighttime peak of melatonin synthesis in humans) increased by 1.79 times in aging monkeys with a reduced melatonin level when Epitalamin was administered [5]. In another study, a five-day administration of Epitalamin (2.5 mg/kg of body weight) to young and older rats at 10 a.m. resulted in an increased nighttime melatonin concentration in the blood by 55 and 49%, respectively [1, 7]. However, in 2006, the pharmacological production of Epitalamin was canceled for a number of technological reasons.

An Epitalamin analog—Pineamin—was developed in 2011–2015 by the Gerofarm group of companies. Similarly to Epitalamin, Pineamine comprises a complex of polypeptides isolated from the cattle pineal gland. It follows from the description of the Pineamin that this preparation optimizes epiphysis-hypothalamus interactions, normalizing the functions of the hypophysis front lobe and balance of gonadotropic hormones. The multicenter, double-blind, randomized, placebo-controlled clinical study of therapeutic efficacy and safety of the Pineamin preparation proved the efficiency of its application for the treatment of climacteric syndrome in women [10].

It can be suggested that Pineamin achieves its effect on the melatonin-forming function of the pineal gland via the same mechanism as Epitalamin, because both preparations have a similar polypeptide composition and both regulate the reproductive system in women.

The objective of this study was to study the effect of Pineamin on the synthesis of melatonin in the pineal gland of elderly people.

MATERIALS AND METHODS

The study was conducted at the St. Petersburg Institute of Bioregulation and Gerontology. A group of 75 elderly patients (60–74 years old, 45 women and 30 men) were examined. All patients provided written consent for participation in the study. Based on the results of clinical, laboratory, and instrumental examination, all of the participants were classified as practically healthy in accordance with their age. No diseases associated with cardiovascular, respiratory, nervous, or the endocrine system in an acute state were revealed. During the study they all received standard meals and were free to follow their daily routines at the usual level of physical activity. The study was conducted in the winter–spring period (December–April) of 2016.

During the initial examination, it was established that 20 patients (control group) had secretion level of 6-sulfatoxymelatonin (6-SOMT) that was normal for their age [3, 11]; hence, they were not treated. The rest of the patients (55 participants) had reduced secretion of 6-SOMT. The age-adjusted level of overnight secretion of 6-SOMT for people of 60–74 years of age varies in the range 380–800 ng/h [3]. Patients with reduced melatonin-forming function of the pineal gland were divided into two groups: the first ($n = 20$) received a placebo, and the second ($n = 35$) received Pineamin. The patients in the placebo group were treated with 0.9% sodium chloride solution (2 mL daily, intramuscular injections for 10 days); the patients in the second group were treated with Pineamin (10 mg in 2 mL of 0.9% sodium chloride solution, daily intramuscular injection for 10 days).

In order to evaluate the melatonin-forming function of the pineal gland, the secretion of 6-SOMT in urine was determined in patients according to the standard technique [15] prior to treatment and 5 days after completion of the treatment. The unified data protocol used in the study was fulfilled during the clinical determination of the 6-SOMT excretion in humans [4]. Urine samples were collected in the period from 11:00 p.m. to 7:00 a.m. All patients were required to avoid any light exposure during sample collection. After measurement of the total overnight urine volume, three 1-mL aliquots were placed into tubes, which were then frozen and stored at a temperature of –20°C. Information about the patient was entered into the protocol on the determination of 6-SOMT excretion, which was performed on an immunoenzyme analyzer (BioTek Instruments, model ELx808) with a 6-Sulfatoxymelatonin ELISA Kit (Buhlmann Laboratories AG, Switzerland) that included all materials required for the determination of 6-SOMT in human urine. The method for immunoenzyme assay of 6-SOMT in urine allows determination of the 6-SOMT concentration in the urine but does not consider such an important parameter as urine volume. The amount of urine excreted overnight in patients depends on various factors (amount of liquid consumed during the day, kidney functional state, individual peculiarities of the organism, and other) and can vary significantly (in our case in the range 150–550 mL). In order to take into account this factor, we calculated the 6-SOMT concentration in the urine according to the following equation: $A = KV/t$, where A is the concentration of 6-SOMT in urine over 1 h, ng/h; K is the concentration of 6-SOMT in the urine over 8 h, ng/mL; V is the volume of urine (mL) collected during 8 h (in the period from 11:00 p.m. to 7:00 a.m., $t = 8$). Hence, in our study we calculated the excretion of 6-SOMTin ng/h; the same approach was used in other works [3, 16].

Table 1. Excretion of 6-sulfatoxymelatonin (6-SOMT) by elderly people

Group	6-SOMT excretion, hg/h		
	age-adjusted norm 380–800	before treatment	after treatment
Control, $n = 20$	552 ± 37	–	–
Placebo, $n = 20$	–	258 ± 29*	273 ± 32*
Pineamin, $n = 35$	–	246 ± 30*	467 ± 35**

Here and in Tables 2 and 3: * $p < 0.05$ in comparison with control group; ** $p < 0.05$ in comparison with the respective indicator in placebo group.

Table 2. Excretion of 6-sulfatoxymelatonin (6-SOMT) by elderly women

Group	6-SOMT excretion, hg/h		
	age-adjusted norm 380–800	before treatment	after treatment
Control, $n = 13$	562 ± 42	–	–
Placebo, $n = 11$	–	255 ± 27*	282 ± 30*
Pineamin, $n = 21$	–	250 ± 34*	473 ± 35**

Table 3. Excretion of 6-sulfatoxymelatonin (6-SOMT) by elderly men

Group	6-SOMT excretion, hg/h		
	age-adjusted norm 380–800	before treatment	after treatment
Control, $n = 7$	542 ± 32	–	–
Placebo, $n = 9$	–	261 ± 31*	264 ± 34*
Pineamin, $n = 14$	–	242 ± 26*	461 ± 35**

The dynamics of the psycho-emotional and physical state of the patients was evaluated by the following tests: the test for the diagnostic of the state of wellbeing, activity, and mood and the Spilberg–Hanin test.

The obtained data was processed with the Statistica 7.0 software package. Parametric and nonparametric statistical methods were applied to analyze the results by the Student's t-test and Mann–Whitney U-criterion. The statistical significance of the results was assessed with the cut-off level of $p < 0.05$.

RESULTS AND DISCUSSION

The level of 6-SOMT excretion in urine was initially lower than normal for this age in the patients from the placebo group, and it did not change significantly following injections of physiological solution (Table 1). The 6-SOMT excretion increased by 1.9 times in patients after Pineamin injection in comparison with its level before treatment (see Table 1). The data on gender differences presented in Tables 2 and 3 indicate that the level of 6-SOMT excretion is the same for elderly men and women.

The survey revealed a positive trend in the psycho-emotional and general physical state of 92% of the patients treated with Pineamin, while the positive dynamic of wellbeing was revealed only in 26% of patients from the placebo group. It is important to note that no side effects were observed, either during the entire course of treatment or 20–30 days after treatment with Pineamin. Korkushko and coauthors in (2006) reported a 2.45-fold increase in the melatonin concentration in the blood in elderly people with reduced melatonin-forming function of pineal gland at 3:00 a.m. (from 24 ± 5 to 59 ± 13 ng/mL) after the administration of Epiltalamin [9], which was in agreement with the data obtained for aging animals [1, 5, 7].

It can be suggested that the mechanism of Pineamin effect on the melatonin-forming function of the pineal gland could be similar to that of Epitalamin. It was established that Epitalamin contained the AEDG peptide (epitalon), which produced the same biological effects as Pineamin but at a lower concentration. In particular, among other things, Epitalon facilitated normalization of the melatonin-forming function of the pineal gland [7, 13].

The effect of epitalon on melatonin synthesis and factors participating in this process—arylalkylamine N-acetyltransferase (AANAT) and transcriptional factor pCREB—were investigated with the use of pinealocyte culture [14]. It was established that epitalon was capable of stimulating the synthesis of AANAT and

pCREB and increasing the melatonin content in the cell culture. It seems likely that Pineamin, like Epitalamin, contains the AEDG peptide that regulates the synthesis of molecules stimulating production of melatonin in the pineal gland. However, this suggestion required further study.

Hence, Epitalamin and Pineamin exhibit similar stimulating effects with regard to the melatonin-forming function of the pineal gland in elderly people. Based on the results of conducted study, Pineamin can be recommended as a therapeutic preparation to restore the level of melatonin secretion in elderly people.

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