BIOGERONTOLOGY

Peptide Geroprotector from the Pituitary Gland Inhibits Rapid Aging of Elderly People: Results of 15-Year Follow-Up

O. V. Korkushko, V. Kh. Khavinson*, V. B. Shatilo, and I. A. Antonyk-Sheglova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 3, pp. 343-347, March, 2011
Original article submitted January 13, 2010

The paper presents the results of randomized comparative study of the efficiency of peptide geroprotector from the pituitary gland in elderly patients with rapidly aging cardiovascular system. Over three years 39 coronary patients received, in addition to basic therapy, regular courses of epithalamin (peptide drug), while 40 coronary patients (control group) received basic therapy alone. Long-term treatment with epithalamin (6 courses over 3 years) decelerated aging of the cardiovascular system, prevented age-associated impairment of physical endurance, normalized circadian rhythm of melatonin production and carbohydrate and lipid metabolism. A significantly lower mortality in the group of patients treated with epithalamin in parallel with basic therapy also indicated a geroprotective effect of the peptide preparation from the pineal gland.

Key Words: *rapid aging; pineal gland; epithalamin; peptides; correction*

Reduction of the melatonin-producing function of the pineal gland is a characteristic manifestations of physiological aging [1,2,12,13,15]. Disorders in melatonin production are augmented in accelerated aging and age-associated diseases (coronary disease, arterial hypertension, Alzheimer's disease, and non-insulin-dependent diabetes mellitus) [8,11-14].

Reduction of melatonin production with aging is assumed to be caused by functional, but not structural changes in the pineal gland [1,10,15] and hence, there is a possibility of restoring normal secretion of the hormone in elderly and senile patients. The stimulatory effects of pineal hormones on melatonin production have been proven in rats [6], old primates [1,7], and elderly humans [2]. In addition, numerous experimental studies have shown that peptide preparations fromn the pineal gland inhibit age-associated changes in the mechanisms of neuroendocrine regulation and reproductive function, correct disorders of carbohydrate and lipid metabolism, improve the immune and antioxidant status, and exhibit clear-cut oncostatic and geroprotective effects [1,4,7-9]. On the other hand, the efficiency of long-term use of peptide preparations from the pineal gland in rapidly aging elderly patients is a very interesting problem.

We studied the efficiency of long-term epithalamin treatment of elderly coronary patients with rapid aging and evaluated delayed results in these patients in the course of subsequent 12-year follow-up.

Institute of Gerontology, the National Academy of Medical Sciences of Ukraine, Kiev, Ukraine; *St. Petersburg Institute of Bioregulation and Gerontology, North-Western Division of the Russian Academy of Medical Sciences, Russia. ***Address for correspondence:*** miayy@yandex.ru. V. Kh. Khavinson

0007-4888/11/1513-0366

MATERIALS AND METHODS

All patients signed informed consent for participation in the study. The protocol of the study was confirmed by local ethical committee of Institute of Gerontology the National Academy of Medical Sciences of Ukraine.

A total of 79 elderly patients (60-69 years) took part in the study. The criteria for selection were as follows: 1) coronary disease with stable effort angina, functional class II; 2) rapid aging of the cardiovascular system (CVS); and 3) low plasma melatonin level (<40 ng/liter) at 3.00 at night.

The patients selected in 1992 were divided at random into 2 basically similar groups (by age, number of men and women, CVS functional age (FA), exercise tolerance, disease severity, *etc.*). Patients of both groups received in 1992-2007 similar basic therapy for the underlying disease (coronary disease): acetylsalicylic acid (100-125 mg daily), angiotensin converting enzyme inhibitors, low dose β-adrenoblockers, and, if necessary, nitrates.

In addition to basic therapy, group 1 patients (N=39) received epithalamin (6 courses over 3 years, from 1992 to 1995) [4]. The protocol of peptide treatment was as follows: 10 mg in 2 ml saline intramuscularly, every 3 days, 5 injections per course, with 6-month intervals between the courses. The drug was injected in the morning, because previous experimental and clinical studies showed that the morning injections of epithalamin led to the maximum increase of melatonin production at night [3,6]. Epithalamin dose per course was 50 mg, total dose 300 mg.

Group 2 patients (N=40) received basic therapy for coronary disease in 1992-2007.

The treatment efficiency was evaluated in both groups by shifts in the following parameters: CVS FA, degree of CVS aging, threshold exercise power, total cholesterol and LDL cholesterol, glucose concentration after overnight fasting and 2 h after standard oral glucose tolerance test (OGTT), and plasma melatonin concentration. Lethal outcomes in 1992-2007 were recorded and their causes were diagnosed.

Plasma melatonin concentration was radioimmunoassayed (DPC) at different time of the day (03.00, 09.00, 15.00, and 21.00).

Threshold exercise power (in W) was evaluated by bicycle ergometry using common diagnostic criteria [3].

Cardiovascular system FA was evaluated by the method developed at out Institute using mathematical formulas based on the threshold exercise power and hemodynamics at the peak of threshold exercise [3]. The degree of CVS aging was calculated as the difference between FA and actual age (AA).

Oral glucose tolerance test with 75 g glucose was carried out as recommended by the WHO. Plasma glucose concentrations after overnight fasting and 2 h after OGTT were measured by the glucose oxidase method. Serum concentrations of total cholesterol and LDL cholesterol were measured by the common biochemical methods.

The data were processed by the parametrical method using t test for evaluation of the significance of differences between the groups and significance of changes in the parameters in each group during treatment. The results were also statistically processed by plotting the life-span tables and survival curves and by Kaplan–Meier evaluations. The significance of differences was evaluated by the Breslow (generalized Wilcoxon) and Log Rank (Mantel–Cox) tests.

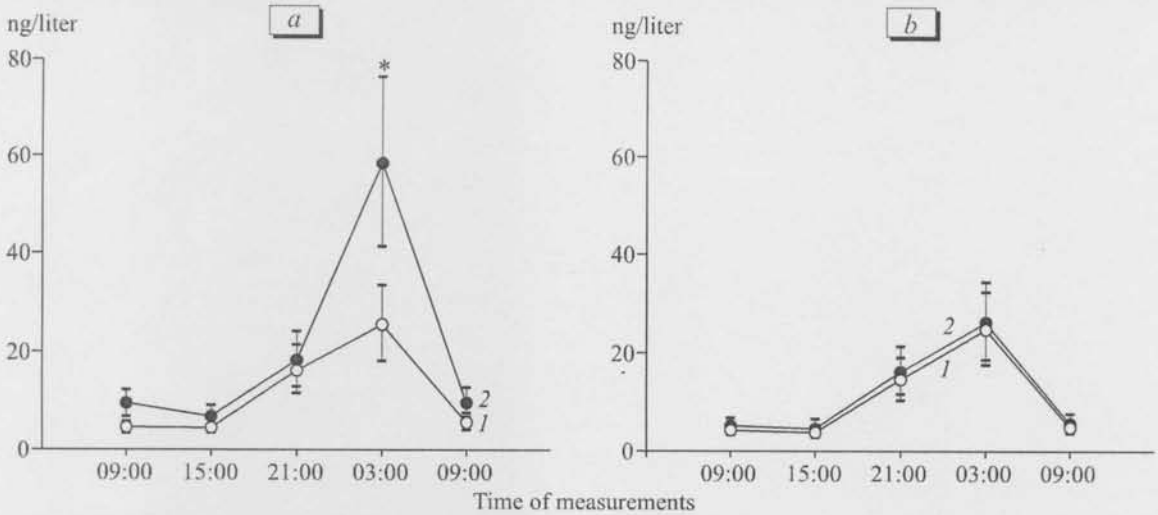

Fig. 1. Circadian rhythm of plasma melatonin concentrations in elderly patients with rapidly aging CVS before and after epithalamin treatment. *a*) basic therapy+epithalamin: *1*) before; *2*) after treatment; *b*) basic therapy: *1*) before; *2*) after treatment. *p<0.05 compared to the parameter before injection.

TABLE 1. The FA and Degree of CVS Aging in Elderly Patients during 3-Year Epithalamin Treatment and Subsequent Long-Term Follow-Up

Parameter	Period of study, year	Group 1 (basic therapy+epithalamin)	Group 2 (basic therapy)
AA, years	1992	64.5±0.9	65.1±1.1
	1995	67.6±1.0*	68.3±1.0*
	2005	76.4±1.2*	77.8±1.1*
CVS FA, years	1992	89.1±1.7	86.2±1.8
	1995	88.7±1.8	93.9±1.9+
	2005	96.2±2.0*	102.2±2.1+
Degree of CVS aging (FA-AA), years	1992	+24.6±1.3	+21.1±1.5
	1995	+21.0±1.7*	+25.6±1.9+
	2005	+19.8±1.9*	+24.4±1.7+

Note. $p<0.05$ compared to the corresponding parameter *in 1992, +in group 1.

RESULTS

Initial studies in both groups of elderly coronary patients revealed a significant reduction of the nocturnal melatonin level in the plasma. That fact indicated functional insufficiency of the pineal gland. Peptide (epithalamin) treatment of group 1 patients led to a significant (2-fold) increase in the nocturnal melatonin level in the plasma and recovery of the circadian rhythm of the melatonin-producing function of the pineal gland (Fig. 1). These favorable shifts were observed after the very first course and persisted over the entire period of epithalamin treatment. No changes in the circadian rhythm of plasma melatonin concentrations were found in group 2 patients.

As we mentioned, the CVS FA of elderly patients before epithalamin treatment was significantly higher in comparison with the AA – by 10 years and more, which indicated rapid aging of the CVS. After 3 years the CVS FA in patients treated with epithalamin virtually did not differ from the initial values (Table 1), and hence, the degree of CVS aging (difference between FA and AA) reduced by 3.6±1.7 years ($p<0.05$). In the control group, receiving no epithalamin, FA increased over 3 years, and the degree of CVS aging increased by 4.5±2.2 years ($p<0.05$) over the same period. This indicated rapid aging of the CVS.

The CVS FA was repeatedly evaluated in both groups after 10 years. In 2005 the degree of CVS aging in the patients treated with epithalamin in 1992-1995 was significantly less than in those who had received no epithalamin. Those results obviously attested to a geroprotective effect of the pineal peptide preparation in patients with rapidly aging CVS.

The favorable shifts in physical endurance, resulting from courses of epithalamin treatment, administered for a long period, are worthy of note (Fig. 2). The threshold exercise power increased by 21% in 58% patients after the first course of epithalamin, *vs.* just 7% examined subjects in the group receiving no epithalamin. Physical endurance remained high over 3 years of epithalamin treatment, while in the control group it reduced significantly.

Long-term therapy with the peptide geroprotector led to favorable shifts in carbohydrate and lipid metabolism in elderly patients. Before epithalamin treatment 56% patients had disorders of glucose tolerance (glucose level 2 h after OGTT above 7.8 mmol/

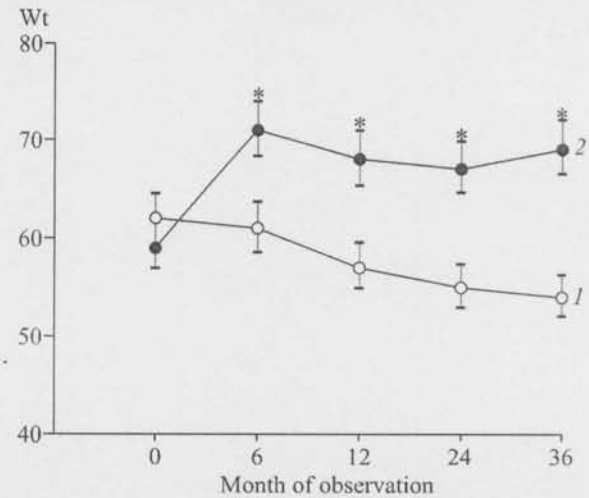

Fig. 2. Time course of physical endurance in elderly patients with rapidly aging CVS. Here and in Fig. 3: *1*) basic therapy; *2*) basic therapy+epithalamin. *$p<0.05$ compared to basic therapy.

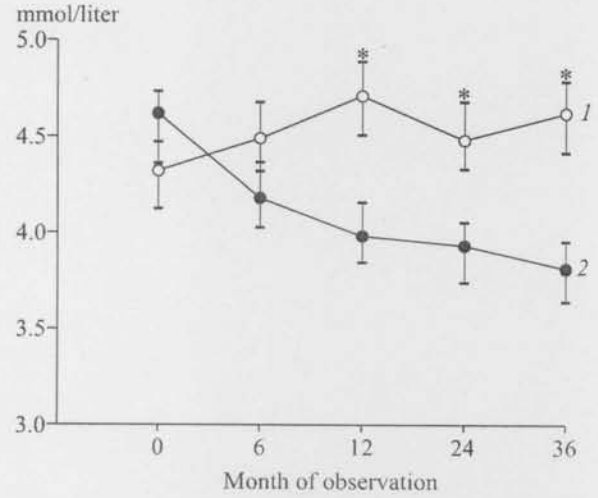

Fig. 3. Time course of serum LDL cholesterol concentrations in patients with rapidly aging CVS.

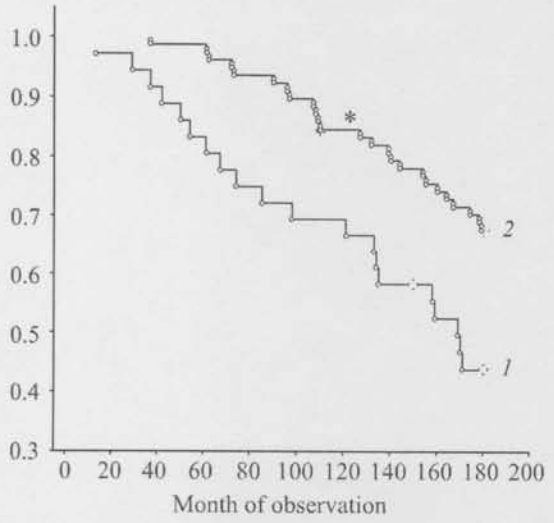

Fig. 4. Cumulative survival curves of elderly coronary patients with rapidly aging CVS treated by different protocols. *1*) basic therapy; *2*) basic therapy+epithalamin. *$p<0.05$ compared to the parameters in the basic therapy group throughout the entire period of observation.

liter), while after course 6 the number of patients with abnormal glucose tolerance decreased to 24%. The incidence of this disorder of carbohydrate metabolism virtually did not change in the control group (48 and 42% cases, respectively).

In addition to normalization of glucose tolerance in the course of long-term epithalamin treatment, favorable shifts in the lipid spectra of the blood were recorded. The levels of total cholesterol and LDL cholesterol gradually decreased (Fig. 3). By contrast, in the control group the levels of total cholesterol and LDL cholesterol increased significantly over 3 years.

After 3 years of peptide therapy, the two groups of patients receiving only basic therapy were followed up in 1995-2007. By the end of this period, 16 of 40 patients in the control group (40%) and 26 of 39 patients treated with epithalamin (66.7%) were living. The survival tables and Kaplan–Meier survival curves (Fig. 4) indicated a statistically significant reduction of the lethal outcome risk in the group treated with the peptide geroprotector in comparison with the control group.

In the epithalamin group, myocardial infarction and stroke caused death in 6 of 13 patients (46.2%) *vs.* 20 of 24 (83.3%) in the control group. Hence, long-term treatment with the pineal peptide preparation significantly reduced cardiovascular mortality. These data confirm favorable effect of epithalamin on the delayed results of treatment of elderly coronary patients with rapidly aging CVS.

Hence, 15-year follow-up of two groups of elderly patients with rapidly aging CVS showed that long-term (for 3 years) treatment with pineal peptide geroprotector inhibited CVS aging, prevented age-associated reduction of physical endurance, and normalized circadian rhythm of melatonin production and carbohydrate and lipid metabolism. One more evidence of the geroprotective effect of pineal peptide preparation is a significant reduction of mortality in the group receiving epithalamin in parallel with basic therapy.

REFERENCES

1. N. D. Goncharova, V. Kh. Khavinson, and B. A. Lapin, *The Pineal Gland and Age-Associated Diseases (Mechanisms and Correction)* [in Russian], St. Petersburg (2007).
2. O. V. Korkushko, V. Kh. Khavinson, and V. B. Shatilo, *The Pineal Gland. Approaches to Correction during Aging* [in Russian], St. Petersburg (2006).
3. O. V. Korkushko, V. Kh. Khavinson, G. M. Butenko, and V. B. Shatilo, *Pineal and Thymic Peptide Preparations for Prevention of Rapid Aging* [in Russian], St. Petersburg (2002).
4. V. Kh. Khavinson and V. N. Anisimov, *Peptide Bioregulators and Aging* [in Russian], St. Petersburg (2003).
5. V. Kh. Khavinson and V. V. Malinin, *Byull. Eksp. Biol. Med.*, **133**, No. 1, 4-10 (2002).
6. V. N. Anisimov, L. A. Bondarenko, and V. Kh. Khavinson, *Ann. N. Y. Acad. Sci.*, **673**, 53-57 (1992).
7. V. N. Anisimov, S. V. Mylnikov, and V. Kh. Khavinson, *Mech. Ageing Dev.*, **103**, No. 2, 123-132 (1998).
8. L. Girotti, M. Lago, O. Ianovsky, *et al.*, *J. Pineal Res.*, **29**, No. 3, 138-142 (2000).
9. N. D. Goncharova, A. A. Vengerin, V. Kh. Khavinson, and B. A. Lapin, *Exp. Gerontol.*, **40**, Nos. 1-2, 51-57 (2005).
10. L. H. Greenberg and B. Weiss, *Science*, **201**, 61-63 (1978).
11. M. Jonas, D. Garfinkel, N. Zisapel, *et al.*, *Blood Press.*, **12**, No. 1, 19-24 (2003).
12. M. Karasek, *Exp. Gerontol.*, **39**, Nos. 11-12, 1723-1729 (2004).
13. F. Magri, S. Sarra, W. Cinchetti, *et al.*, *Pineal Res.*, **36**, No. 4, 256-261 (2004).
14. D. J. Skene and D. F. Swaab, *Exp. Gerontol.*, **38**, Nos. 1-2, 199-206 (2003).
15. Y. Touitou, *Ibid.*, **36**, No. 7, 1083-1100 (2001).