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Research article

Effect of Epitalon on biomarkers of aging, life span and spontaneous tumor incidence in female Swiss-derived SHR mice

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Abstract

From the age of 3 months until their natural deaths, female outbred Swiss-derived SHR mice were subcutaneously injected on 5 consecutive days every month with 0.1 ml of normal saline (control) or with 1.0 μg/mouse (~30–40 μg/kg) of tetrapeptide Epitalon® (Ala-Glu-Asp-Gly) dissolved in 0.1 ml saline. There were 54 mice in each group. The results of this study show that treatment with Epitalon did not influence food consumption, body weight or mean life span of mice. However, it slowed down the age-related switching-off of estrous function and decreased the frequency of chromosome aberrations in bone marrow cells (by 17.1%, $P < 0.05$). It also increased by 13.3% the life span of the last 10% of the survivors ($P < 0.01$) and by 12.3% the maximum life span in comparison with the control group. We also found that treatment with Epitalon did not influence total spontaneous tumor incidence, but inhibited the development of leukemia (6.0-fold), as compared with the control group. The data obtained suggest a geroprotector activity of Epitalon and the safety of its long-term administration in mice.

Introduction

The search for new effective and safe means to prevent premature aging is one of the priorities in gerontology (Anisimov 2001; Butler et al. 2002; De Grey et al. 2002). During the last decade a number of reports have appeared on the role of the pineal gland in aging (Armstrong and Redman 1991; Reiter 1995; Reppert and Weaver 1995; Pierpaoli 1998; Reiter et al. 2002). A modulating effect of the pineal gland on the neuroendocrine and the immune system was shown to change during aging (Arendt 1995). Pinealectomized rats showed a reduced life span (Malm et al. 1956; Reiter et al. 1999), whereas the administration of the pineal hormone melatonin to rodents or syngeneic transplantation of pineal glands from young donors into the thymus or *in situ* of old mice prolonged the life span of the recipients (Pierpaoli and Regelson 1994; Lesnikov and Pierpaoli 1994; Anisimov et al. 2001b; Oxenkrug et al. 2001). Most investigators invoked melatonin as a primary mediator of the endocrine capabilities of the pineal gland. However, some of the effects of the pineal gland might have obviously resulted from pineal peptide secretion (Benson 1977; Bartsch et al. 1992; Yuwiler and Brammer 1993; Arendt 1995). Some crude peptide extracts or purified peptides isolated from pineal glands were shown to have antigonadotropic, metabolic and anti-

tumor activity (Anisimov et al. 1994; Bartsch et al. 1992; Lapin and Ebels 1979). One of the complex peptide bioregulators isolated from the pineal gland, Epithalamin®, was shown to slow down aging rate, prolong life span in fruit flies, mice and rats, and inhibit spontaneous and induced carcinogenesis in rodents (Anisimov et al. 1994; Khavinson et al. 2001c; Khavinson 2002).

Tetrapeptide Epitalon® (Ala-Glu-Asp-Gly, molecular weight 390.35 dalton) was designed on the basis of Epithalamin amino acid analysis and synthesized (Khavinson et al. 2000). The geroprotective activity of Epitalon was studied in three strains of *Drosophila melanogaster* (Khavinson et al. 2000; Mylnikov and Lyubimova 2000). Epitalon increased the life span of imagoes significantly by 11–16% when applied at unprecedentedly low concentrations – from 0.001×10^{-6} to 5×10^{-6} wt% of the culture medium. A recent study by us demonstrated a geroprotective effect of long-term Epitalon administration in female inbred CBA mice (Anisimov et al. 2001a). The bioregulator slowed down aging of the reproductive function, inhibited free radical processes, and decreased total spontaneous tumor incidence in female CBA mice (Anisimov et al. 2001a). Epitalon inhibited mammary carcinogenesis and metastasis in transgenic *HER-2/neu* mice (Anisimov et al. 2002b) and colon and small intestine carcinogenesis induced by 1,2-dimethylhydrazine in rats (Anisimov et al. 2002a). Administration of Epitalon to young (6–8 years old) and senescent (20–26 years old) female monkeys *Macaca mulatta* restored the evening level of melatonin and the circadian rhythm of cortisol in the blood serum of senescent monkeys (Khavinson et al. 2001a).

This paper presents data on the effect of Epitalon on life span, estrous function, incidence of chromosome aberration in the bone marrow cells and spontaneous tumorigenesis in outbred Swiss-derived SHR mice.

Materials and methods

Animals

Female outbred Swiss-derived SHR 2-month-old mice (108 specimens) were purchased from the Rappolovo Animal Farm of the Russian Academy of Medical Sciences (St. Petersburg). The mice were kept in polypropylene cages ($30 \times 21 \times 9$ cm), 5 mice to a cage, at a temperature of 22 ± 2 °C. A regimen was followed of 12 hours of light and 12 hours of dark. The animals received sterilized standard laboratory feed (Anisimov et al. 2003) and tap water *ad libitum*. Mice were checked daily by animal care personnel and weekly by a veterinarian. The study was conducted in accordance with the regulations for ensuring the humane treatment of animals under the approval of the Committee on Animal Research of the N.N. Petrov Research Institute of Oncology.

Experiment

At the age of 3 months the mice were randomly divided into two groups, 54 animals in each, and they were individually marked. Mice of the control group were subcutaneously injected with 0.1 ml of 0.9% normal saline for 5 consecutive days every month, whereas the mice of the second group received subcutaneously 1.0 μg of Epitalon dissolved in 0.1 ml of saline. This treatment dosage and regimen were effective for the inhibition of spontaneous tumorigenesis in female CBA mice (Anisimov et al. 2001a). Epitalon was synthesised in St. Petersburg, Institute of Bioregulation and Gerontology, by E.I. Grigoriev and was 99.8% pure. Four intact female SHR mice were euthanized at the age of three months to evaluate the initial level of chromosome aberrations. Additionally four mice from each group were euthanized at the age of 12 months for a cytogenetic study of chromosome aberrations in bone marrow cells (see below). Once every 3 months, simultaneously with weighing, the amount of food consumed was measured. Thirty grams of food were given in each cage after cleaning and twenty-four hours thereafter the food that had not been consumed was collected from each cage and weighed. The mean amount of food (grams) consumed per mouse during this day was calculated for each group.

Once every three months, vaginal smears taken daily for two weeks from the animals were examined cytologically to estimate the phases of their estrous functions. In the same period, the rectal body temperatures of the mice were measured with an electronic thermometer, TPEM (KMIZ, Russia). Animals were observed until their natural death. The date of each death was recorded, and the mean life span, the age by which 90% of the animals died, and the maximum life span were estimated.

Cytogenetic study

Chromosomal aberrations in bone marrow cells were studied by a modified Ford's method, described by Rosenfeld et al. (2001). Mice were sacrificed by ether anaesthesia. Both femurs of each mouse were dissected and bone marrow cells were flushed gently with 0.56% KCl solution into a centrifuge tube. Cells were treated for 20 min with hypotonic solution and fixed with an ethanol : acetic acid mixture (3:1). Slides were stained with 4% acetoorseine: 20–30 well spread anaphases were analyzed for each animal and cells with chromosome breaks, acentric fragments, and other aberrations were evaluated at 1000× magnification under a light microscope (Leitz, Germany).

Pathomorphological examination

All animals that died, or were sacrificed when moribund, were autopsied and their skin and internal organs were examined. Neoplasias were classified according to the recommendations of the International Agency of Research on Cancer (IARC) as 'fatal' (i.e., those that directly caused the death of the animal) or 'incidental' (in cases where the animal died of a different cause) (Gart et al. 1986). All tumors, as well as tissues and organs with suspected tumors, were excised and fixed in 10% neutral formalin. After routine histological processing, tissues were embedded in paraffin. Thin, 5–7 μm histological sections were stained with hematoxylin-eosine and examined microscopically. The experimental group to which the mouse belonged was blinded. Tumors were classified according to IARC recommendations (Turusov and Mohr 1994).

Statistics

Experimental results were statistically processed by the methods of variation statistics (Goubler 1978). The significance of discrepancies was defined according to Student's t-criterion, Fischer's exact method, χ^2-analysis, and the non-parametric criterion of Wilcoxon–Mann–Whitney (Goubler 1978). To estimate discrepancies in neoplasm incidence, an IARC method of combined contingency tables calculated individually for the fatal and incidental tumors (Gart et al. 1986). For survival analysis, Cox's method (Cox and Oakes 1996) was used. All reported test values for survival analyses are two-sided.

Survival models and estimations

The mathematical model used to describe survival is the Gompertz model with the survival function:

$$S(x) = \exp\left\{-\frac{\beta}{\alpha}\left[\exp(\alpha x) - 1\right]\right\}$$

where parameters α and β are associated with demographic aging and initial mortality rate, respectively. Parameters for the model were estimated from data using the maximum likelihood method implemented in the GAUSS statistical system (Gauss System 1994). Confidence intervals for the aging rate parameter estimates were calculated using log-likelihood functions (Cox and Oakes 1994).

Results

Age-related body weight dynamics

Mean values of body weight for mice at different ages in the control and treated with Epitalon groups are displayed in Table 1. The body weight of the mice in both groups increased with age, exceeding by 13 months the body weight of 3-month-old animals by 44.5% in the control group ($P < 0.001$), and by 48.5% in the group given Epitalon ($P < 0.01$). There were no differences in the body weight between groups at any period of observation.

Age-related dynamics of food consumption

Measurements showed that the amount of food consumed by the mice in the control (saline) group was practically stable from the age of 5 months to the age of 16 months, increasing slightly at the age of 18 months. Mice treated with Epitalon consumed more food from the 5th to the 16th months of their life than the control group (Table 2).

Age-related dynamics of estrous function in mice

The estrous function in the animals of both age groups was examined every three months, starting when the mice were three months old. The following parameters of estrous function were estimated: the length of the estrus, the relative rate of estrous cycle phases (in percent); and the relative number of short (< 5 days) and long (> 5 days) estrous cycles. The relative number of animals with regular cycles and irregular

Table 1. Body weight gain dynamics in female SHR mice treated with saline or Epitalon

Group	Body weight (g)							
	3 mo	5 mo	7 mo	9 mo	11 mo	13 mo	16 mo	18 mo
Saline	24.7 ± 0.29	27.8 ± 0.63	30.9 ± 0.85	32.4 ± 1.30	34.3 ± 1.24	35.7 ± 1.67	34.0 ± 1.73	32.4 ± 1.50
Epitalon	24.1 ± 0.41	28.1 ± 0.40	30.1 ± 0.74	33.2 ± 1.04	34.4 ± 1.08	35.8 ± 1.68	33.9 ± 1.67	30.7 ± 1.49

Table 2. Food consumption dynamics in female SHR mice treated with saline or Epitalin

Group	Daily food consumption (g/mouse)							
	3 mo	5 mo	7 mo	9 mo	11 mo	13 mo	16 mo	18 mo
Saline	4.6 ± 0.03	2.8 ± 0.05	3.3 ± 0.02	2.8 ± 0.03	3.1 ± 0.01	3.4 ± 0.03	3.8 ± 0.06	5.3 ± 0.15
Epitalon	4.3 ± 0.17	4.9 ± 0.15*	5.4 ± 0.09*	5.6 ± 0.13*	5.6 ± 0.22*	5.7 ± 0.24*	5.9 ± 0.32*	5.8 ± 0.47

The difference from the saline group is significant. * – $P < 0.001$ (Student's *t*-test).

cycles (persistent estrus and anestrus) were also calculated. Judging by the data presented in Table 3, the length of estrous cycle in the control female SHR mice increased with advancing age ($P < 0.05$; Student's *t*-test). Thus, no essential age-related alterations in the rate of estrous cycle phases were observed. However, the relative number of short estrous cycles decreased significantly with age (37.1% at the age of 3 months, 9.4% at the age of 12 months ($P < 0.05$; Fischer's exact test) and zero at the age of 15 months, whereas the number of long cycles rose (5.1% at the age of 6 months and 36% at the age of 15 months, $P < 0.05$; Fischer's exact test).

In the group of mice exposed to Epitalon the length of estrous cycles did not change with the age of the animals and decreased in comparison with the age-matched controls at the age of 15 months ($P < 0.05$). There was no significant age-related decrease in the number of short cycles, or an increase in the number of long cycles. The number of mice with regular cycles did not change significantly with age in both groups (Table 3).

Age-related dynamics of body temperature in mice

Data on body temperature alterations in the mice exposed to saline or Epitalon are presented in Table 4. The control mice and mice treated with Epitalon revealed a significant decrease in body temperature with age, both on the whole (irrespective of the estrous cycle phases) and in any of the phases. No cyclic alterations in rectal body temperature during the estrus cycle were observed in mice of the control group, but the temperature at diestrus was significantly higher than that in estrus in mice treated with Epitalon at the age of 15 months ($P < 0.05$). It should be noted that the average body temperature in the mice treated with Epitalon was not significantly different from the control mice during the entire period of observation (Table 4).

Chromosome aberrations in mouse bone marrow cells

The incidence of chromosome aberrations in bone marrow cells of 3-month-old female SHR was 2.1 ± 0.29%. At the age of 12 months this parameter increased to 8.2 ± 0.41% ($P < 0.001$; Wilcoxon–Mann–Whitney test) in the group injected with saline. In mice treated from the age of 3 months with Epitalon the incidence of chromosome aberrations at the age of 12 months was 6.8 ± 0.21 (– 17.1%; $P < 0.05$).

Survival and longevity of female SHR mice

Survival dynamics in the mice treated with either saline or DSIP are demonstrated in Table 5 and Figure 1. The survival dynamics were in general similar in all groups up to the age of 22 months. However, thereafter, the number of survivors was much higher in Epitalon-treated groups.

The last mouse in the control group died at the age of 739 days (24.3 months), whereas in the groups treated with Epitalon 12% of mice survived to this age, and the maximum life span was 830 days (27.3 months, + 12.3%). The mean life span of mice treated with Epitalon did not change as compared with controls. However, the life span in the last 10% of the

Table 3. Age-related dynamics of estrous functional parameters in SHR mice treated with saline or Epitalon

Age (mo)	No. of mice	Length of estrous cycle (days)	Rate of separate phases of estrous cylce (%)			Rate of estrous cylces (%)			Rate of mice with regular cycles (%)	Rate of mice with irregular cycles (%)
			E	D	P + M	< 5 d	5–7 d	> 7 d		
					Saline					
3	28	5.68 ± 0.19	42.4	54.7	2.9	31.7	55.0	13.3	88.0	12.0
6	22	5.38 ± 0.19	45.4	50.5	4.1	30.8	64.1	5.1	95.5	4.5
9	22	5.77 ± 0.30	34.2	62.4	3.4	25.6	59.0	15.4	95.5	4.5
12	22	6.25 ± 0.26	47.1	51.3	1.6	9.4**	81.2	9.4	95.5	4.5
15	17	6.84 ± 0.26*	34.2	65.1	0.7	0**	64.0	36.0**	88.2	11.8
					Epitalon					
3	50	5.87 ± 0.24	42.8	53.1	4.1	29.1	47.3	23.6	100	0
6	48	5.81 ± 0.23	46.5	48.9	4.6	16.2	75.7	8.1	91.4	8.6
9	36	5.33 ± 0.21	51.5	46.1	2.4	35.7	54.8	9.5	95.5	4.5
12	20	5.96 ± 0.34	48.9	49.2	1.9	22.2	59.3	18.5	94.7	5.3
15	15	5.87 ± 0.33[a]	43.3	54.4	2.3	10.7	78.6	10.7	85.7	14.3

Note: E = estrus; D = diestrus; P = proestrus; M = metaestrus.
Difference from the parameters at the age of 6 months in the same group: $^{*}P < 0.05$ (Student's *t*-test); $^{**}P < 0.05$ (Fischer's exact test).
Difference from the corresponding age in the control group: $^{a}P < 0.05$ (Student's *t*-test).

Table 4. Body temperature dynamics in SHR mice treated with saline or Epitalon

Age (mo)	Number of mice	Total cycle (without phase sub-division)	Mean body temperature (°C)		
			Estrus	Diestrus	Metaestrus + proestrus
			Saline		
7	22	39.95 ± 0.14	39.80 ± 0.30	40.00 ± 0.20	39.40 ± 0.10
12	22	38.83 ± 0.18[a]	38.73 ± 0.30[b]	38.90 ± 0.24[a]	–
15	17	38.73 ± 0.17[a]	38.68 ± 0.30[b]	38.75 ± 0.20[a]	–
17	12	38.05 ± 0.24[a]	37.40	38.10 ± 0.30[a]	–
19	10	37.70 ± 0.11[a]	37.60 ± 0.10[a]	37.70 ± 0.14[a]	–
			Epitalon		
7	23	40.68 ± 0.16	40.60 ± 0.20	40.40 ± 0.40	40.96 ± 0.20
12	20	38.90 ± 0.22[a]	39.07 ± 0.30[a]	38.90 ± 0.30[a]	–
15	15	39.07 ± 0.17[a]	38.20 ± 0.10[a]	39.10 ± 0.20[b,c]	
17	12	38.28 ± 0.21[a]	38.75 ± 0.30[a]	38.10 ± 0.30[a]	
19	10	37.32 ± 0.14[a]	37.10	37.40 ± 0.15[a]	–

Difference from the age of 7 months in the same group is significant: $^{a}P < 0.01$; $^{b}P < 0.05$ (Student's *t*-test).
Difference with the parameter at phase estrus of the same group and age: $^{c}P < 0.05$ (Student's *t*-test).

Table 5. Survival distribution of female SHR mice treated with saline or Epitalon

Group	No. of survivors at the age of:														
	3 mo	4 mo	6 mo	8 mo	10 mo	12 mo	14 mo	16 mo	18 mo	20 mo	22 mo	24 mo	26 mo	27 mo	28 mo
Saline	50	50	38	36	36	34	33	28	20	16	10	2	0	0	0
Epitalon	50	49	39	36	34	33	32	25	17	14	11	7	3	3	0

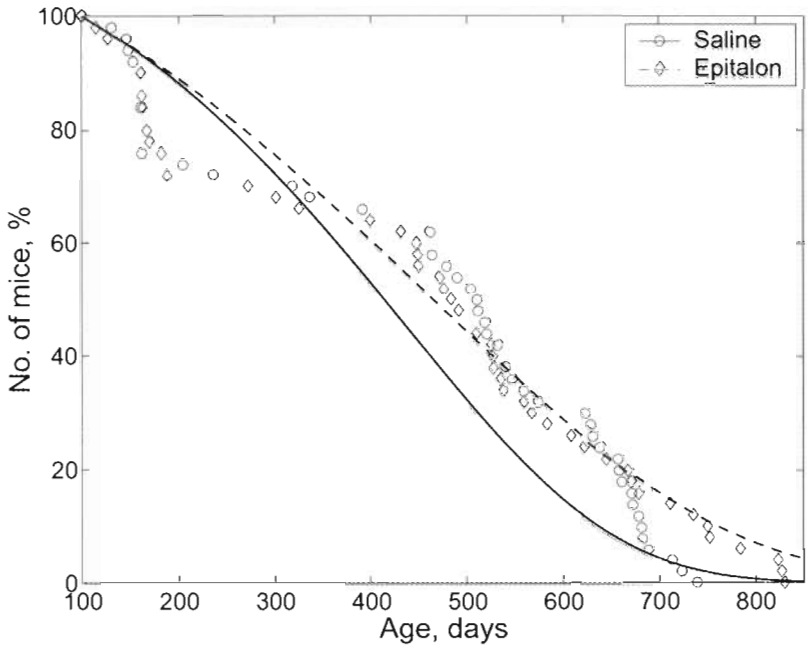

Figure 1. Effect of Epitalon on the survival curves of female SHR mice. *Y*-axis: number of mice as percentage of total.

Table 6. Parameters of life span in female SHR mice treated with saline or Epitalon

Parameters	Saline	Epitalon
Number of mice	50	50
Mean life span, days (M ± S.E.)	456 ± 29	455 ± 31
Median	512	487
Mean life span of last 10% of survivors, days	709 ± 10.8	803 ± 15.0* (+ 13.3%)
Maximum life span, days	739	830 (+ 12.3%)

Differences from saline is significant: $^*P < 0.01$ (Student's *t*-test).

mice increased for the duration of Epitalon treatment by 3.1 months (+ 13.3%, $P < 0.01$; Student's *t*-test) (Table 6).

Spontaneous tumor development in female SHR mice

The total tumor incidence in the control female mice was 36%. Mammary carcinomas and leukemias developed most frequently, corresponding to the oncological characteristics of female SHR mice (Anisimov et al. 1989). The treatment with Epitalon failed to influence the total or malignant tumor incidence in comparison with that of the control group. However, the incidence of leukemias during the treatment with Epitalon decreased 6-fold ($P < 0.01$; Fischer's exact test). There was no significant difference in the incidence of any other tumors between the group of mice treated with the peptide and saline (Table 7). The treatment with Epitalon significantly shifted to right the total tumor yield curve as compared with the control group (Figure 2).

Mathematical model and estimations of survival of tumor-free and tumor-bearing mice

A mathematical analysis of the survival data of the mice from the control and melatonin- treated groups has been done separately for three different contexts: (1) for all animals in each group (total cases); (2) for fatal tumor-bearing mice, and (3) for fatal tumor-free mice. We composed the groups of animals without consideration of possible effects caused by dependence between these groups. The Gompertz model shows a slowdown (by 29.0.4%) of the population aging rate (calculated as α in the Gompertz equation) and a corresponding increase in MRTD under the influence of Epitalon. The mortality rate in the group of fatal tumor-free mice treated with Epithalon was decreased by 32.5% as compared with the controls ($P < 0.05$, Table 8).

Table 7. Incidence, localization and type of tumors in female SHR mice treated and not treated with Epitalon

Parameters		Saline	Epitalon
Number of mice		50	50
Number of tumor-bearing mice		18 (36%)	16 (32%)
Number of malignant tumor-bearing mice		15 (30%)	16 (32%)
Total number of tumors		25	22
Total number of malignant tumors		18	20
Number of tumors per tumor-bearing mice		1.38	1.38
Mean life span of fatal-tumor bearing mice, days		549 ± 29	551 ± 21
Mean life span of fatal tumor-free animals, days		416 ± 38	410 ± 43
Localization and type of tumors:			
Mammary gland:	adenocarcinoma	12 (10)[a]	17 (13)[b]
	Nos. of metastases	7	6
Leukemia		6	1*
Lung:	adenoma	1	0
	Adenocarcinoma	0	2
Utery:	polyp	1	1
Ovary:	cyst	5	1

[a]Two mice had two mammary tumors each.
[b]Four mice had two mammary tumors each.
Differences from saline is significant: $^*P < 0.05$ (Fischer's exact test).

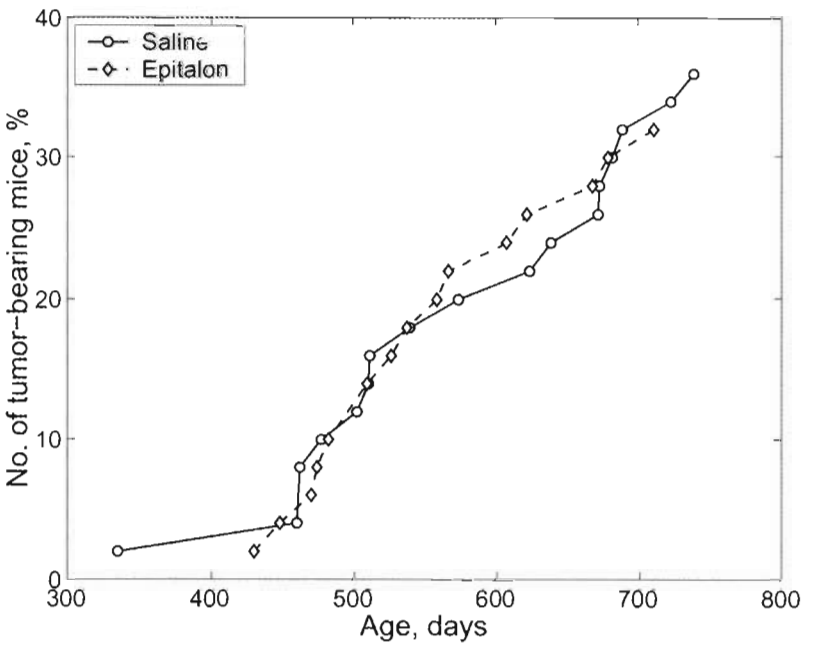

Figure 2. Effect of Epitalon on total tumor yield curve in female SHR mice. *Y*-axis: number of tumor-bearing mice as percentage of total.

Table 8. Parameters of life span in female SHR mice treated with saline or Epitalon

Group	Total no. of cases	Fatal tumor-bearing mice	Fatal tumor-free mice
Number of mice			
Saline	50	15	35
Epitalon	50	16	34
Mean life span (days)			
Saline	456 ± 29	549 ± 29	416 ± 38
Epitalon	455 ± 31	551 ± 21	410 ± 43
Mean life span of the last 10% of survivors (days)			
Saline	709 ± 10	731 ± 8	692 ± 7
Epitalon	803 ± 15*	695 ± 16	827 ± 2**
Aging rate $\alpha \times 10^3$ *(days*$^{-1}$*)*			
Saline	4.55 (4.43; 4.86)	9.15 (9.02; 10.4)	2.80 (2.74; 3.20)
Epitalon	3.23 (3.15; 3.46)#	11.8 (11.3; 11.35)#	1.89 (1.82; 2.16)#
MRDT (days)			
Saline	152.41	75.71	247.71
Epitalon	214.69#	58.91#	367.1#

Note: Mean life spans are given as mean ± standard error; 95% confidence limits are given in parentheses; MRDT = mortality rate doubling time.
Difference from controls is significant: $^{a}P < 0.05$ (Fischer's exact test); $^{*}P < 0.05$; $^{**}P < 0.001$ (Student's *t*-test); $^{\#}P$ 0.05 (Cox's method).

Discussion

The results of our study show that the long-term administration of Epitalon slows down demographic aging rate, increases survival and maximum life span and decreases the development of spontaneous leukemias in female SHR mice.

Treatment with Epitalon increased the food consumption in comparison to the controls between the 5th and 16th months of their life, but the body weight was similar in both the control and Epitalon-treated group. Administration of Epitalon to female CBA mice failed to influence the body weight, and increased food consumption at the age of 12 months (Anisimov et al. 2001a). No significant differences in the age-related dynamics of body temperature between both groups were observed in SHR mice. In CBA mice a slight decrease in the body temperature under the influence of Epitalon has been observed (Anisimov et al. 2001a). Thus, it could be suggested that increased food consumption induced by Epitalon was not followed by an increase in the basal metabolic rate, because both the body weight and the body temperature were not different from the controls.

The administration of Epitalon was followed by a slowing down of the age-related disturbances in estrous function in female SHR mice. These observations are in agreement with data obtained with Epitalon in other strains of mice – CBA and FVB/N (Anisimov et al. 2001a, 2002b). It is worth nothing that long-term administration of melatonin or pineal peptide preparation Epithalamin was also followed by a slowdown of age-related switching-off of reproductive function in SHR, C3H/Sn and CBA mice and rats (Anisimov et al. 1989, 1998, 2001a, b; Meredith et al. 2000).

The aging process predisposes cells to accumulate mutations, some of which are necessary for initiation of tumor growth in target tissues (Vijg 2000; Bodyak et al. 2002). The incidence of chromosome aberrations increases with age in different strains of mice (Crowley and Curtis 1963; Sato et al. 1995). Previously we found age-related increases in chromosome aberrations in bone marrow cells and in primary spermatocytes in male SHR mice (Rosenfeld et al. 2001). In this study we observed a significant increase in the frequency of chromosome aberrations in the bone marrow cells in 12-month-old female SHR mice compared to 3-month-old specimens. Long-term treatment with Epitalon significantly decreased the age-associated increase in chromosome aberrations in female SHR mice. It was shown earlier that Epitalon

also inhibited the incidence of chromosome aberration in one-year-old senescence-accelerated mice (Rosenfeld et al. 2002).

The long-term administration of Epitalon failed to influence total spontaneous tumor incidence in female SHR mice, but it significantly inhibited the development of leukemias ($P < 0.001$) (Table 7). Treatment with Epitalon inhibited the growth of transplanted sarcoma M-1 (Khavinson et al. 2001b), decreased spontaneous tumor incidence in female CBA mice (mainly lung adenomas) (Anisimov et al. 2001a) and in *HER-2/neu* transgenic mice (Anisimov et al. 2002b). It also inhibited colon carcinogenesis induced by 1,2-dimethylhydtrazine in rats (Anisimov et al. 2002a). It possible to suggest that the capacity of Epitalon to prevent the development of spontaneous leukemias in female SHR mice can be related to its antioxidative activity. It has been shown that Epitalon inhibits free radical processes in *D. melanogaster* and CBA mice (Anisimov et al. 2001a; Khavinson and Mylnikov 2000).

Our observation of the positive effect of Epitalon on the life span of SHR mice is in agreement with observations of similar activities of Epithalamin in SHR and C3H/Sn mice and rats (Anisimov et al. 1989, 1994) and of Epitalon in female CBA mice (Anisimov et al. 2001a). The effective concentration of Epitalon was 1000–5000 times less than that of Epithalamin. In experimens with two strains of *D. melanogaster*, Epithalon treatment was followed by an increase in their mean life span (Khavinson and Mylnikov 2000). It is noteworthy that effective concentrations of the tetrapeptide were 1000 times less than of Epithalamin and 16,000–80 $\times$ 10^6 times less than that of melatonin (Khavinson and Mylnikov 2000). Epithalon increased catalase activity and decreased the level of conjugated hydroperoxides in fruit flies (Mylnikov and Lyubimova 2000). The results of this investigation agree with data obtained in previous observations on the safety of long-term administration of peptide preparations isolated from the pineal gland on their geroprotective and anti-tumour effects (Anisimov et al. 1994; Khavinson et al. 2001c; Khavinson 2002). Thus, the results obtained confirm a geroprotective potential of the peptide preparation Epithalon (Ala-Glu-Asp-Gly).

In the heart of CBA mice, the expression of 15,247 transcripts from the cDNA library was studied by the microarray technique (Anisimov S et al. 2002). The analysis of the results of hybridizing the cDNA clone containing microarrays with the heart samples of the control and Epitalon-exposed mice revealed intensified expression of 194 clones and reduced expression of 48 clones. The analysis identified the multiple genes involved in cell division (14 genes), cell signaling/communication (14), cell structure/motility (6), cell/organism defense (13), gene/protein expression (17), metabolism (11), and genes encoded by mitochondrial DNA (5). These subgroups may include gene products that can explain some of the physiological effects described above, and form a molecular basis for the geroprotective effects of these peptides.

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