BIOGERONTOLOGY

Effect of Vilon on Biological Age and Lifespan in Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 7, pp. 88-91, July, 2000
Original article submitted June 26, 2000

Subcutaneous administration of vilon (Lys-Glu) to female CBA mice starting from the 6th month of life increased physical activity and endurance, decreased body temperature, prolonged the lifespan, and prevented the development of spontaneous neoplasms. Vilon had no effect on age-related changes of estrous function and free radical processes. Long-term administration of vilon caused no unfavourable effects on animal development. The obtained results show safety of chronic vilon administration and allow to use this preparation for geroprotection and prophylaxis of age pathology.

Key words: *vilon; lifespan; reproduction; physical activity; CBA mice*

According to immunological theory of aging, age-dependent dysfunction of the immune system results in the loss of resistance against infections and increases the risk of autoimmune and oncological diseases [7, 8,10]. Attempts were undertaken to decelerate aging of the immune system in old animals by transplanting lymphocytes and thymus or administering immunomodulators and thymus preparations [3,7,12]. During the past 25 years the ability of a polypeptide preparation from the thymus, thymalin, to increase lifespan of mice and rats was demonstrated [1,2,4,5]. Some other peptide preparations from the thymus, in particular, thymosin-α, possess weak geroprotective and immunomodulating properties [3,6,12]. However, despite high effectivenes of thymalin its clinical application is limited due to deficit of raw material. Synthetic biologically active peptides of the thymus can be widely used in clinical practice. Vilon (Lys-Glu) was constructed on the basis of the analysis of amino acid sequence of thymalin and other thymus peptides and cytokines [9].

In the present study we examined the effects of vilon on the lifespan, physical and motor activity, reproductive function, body temperature, spontaneous tumor formation, and free radical processes in mice.

MATERIALS AND METHODS

Six-month-old female CBA mice (n=120) were divided into 2 groups (60 mice each). Experimental group was treated with 0.1 μg vilon subcutaneously for 5 days monthly. Control group was injected with physiological saline according to the same scheme. Estrous function was estimated by cytology of vaginal smears daily for 2 weeks each third month. In parallel, rectal temperature was measured with a TPEM-1 electronic thermometer (KMIZ, Russia) and motor activity was examined in an open field. The number of crossed squares, rearings, time of face, body and genital grooming were recorded. Muscle power and fatigue were estimated 1 year after the start of the experiment. To this end, the mice were suspended at the height of 75-80 cm clinging a wire with their fore-

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0007-4888/00/0007-687$25.00

paws. The time of hanging was recorded twice with a 20 min rest. The difference between two these values was considered as the measure of power recovery. The observation was conducted to natural death of the animals. The mean and maximum lifespans, and the age corresponding to 90% mortality were calculated. All dead or sacrificed asthenic animals were subjected to autopsy. All tumors were examined histologically. The results were processed statistically using Statgraphics program.

RESULTS

Eighteen- and 21-month-old mice treated with vilon had significantly ($p<0.01$) higher body weight. Regular (each 3 month) measurements of food consumption reveal no differences between the experimental and control groups. Therefore, the higher body weight in the experimental group was not due to the effect of vilon on food consumption.

Behavioral test showed that 6–9-month-old control mice were more active in the open field ($p<0.001$), but later (12 and 18 months) these differences became insignificant (Fig. 1). Muscular power, fatigue, and time recovery in 18-month-old mice showed considerable individual variability. However, the ratio between body weight and time of hanging was significantly lower in mice with high body weight (over 34 g) treated with vilon compared to control mice of similar weight, *i. e.* heavy mice receiving vilon showed higher hanging time during the first and second suspension compared to control mice of the same weight. Thus, long-term vilon administration increased muscle power and reduced fatigue.

The duration of estrous cycle in control mice increased with age. The relative number of short estrous

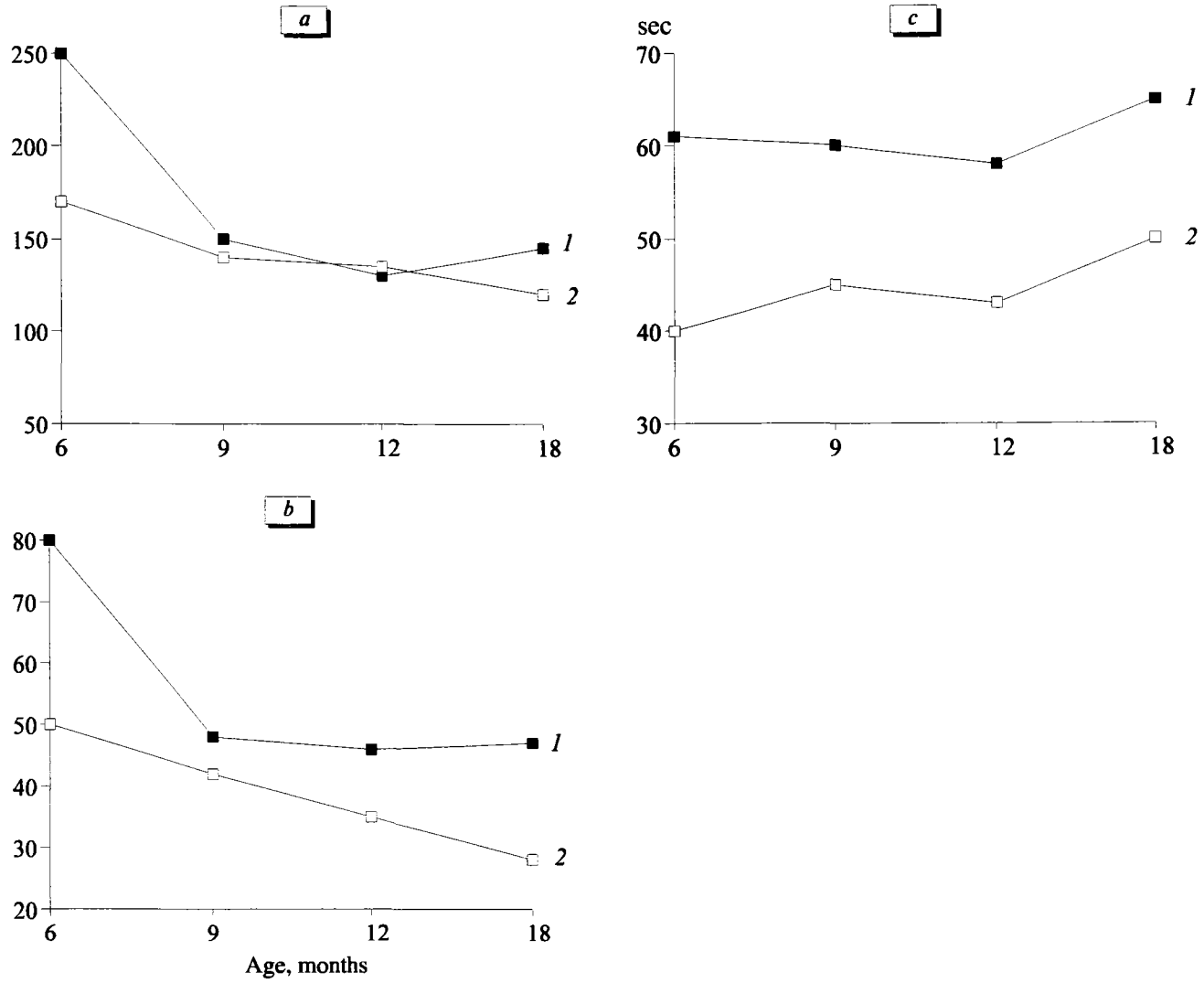

Fig. 1. Age dynamics of motor activity in open field estimated by the number of crossed squares (*a*), rearings (*b*), and time of grooming (*c*) in control (*1*) and vilon-treated mice (*2*).

TABLE 1. Effect of Vilon on Age Dynamics of Mouse Body Temperature ($M \pm m$)

Phase of estrous cycle	Age, months							
	9		12		15		18	
	control	experiment	control	experiment	control	experiment	control	experiment
Estrus	35.8±1.6	37.300±0.116^{+}	37.7±0.2	36.940±0.136^{*+++}	34.960±2.469	36.940±0.153^{++}	37.110±0.167	37.030±0.085
Diestrus	37.480±0.112	37.330±0.096^{+++}	37.73±0.08	36.960±0.122^{++}	37.370±0.183	35.990±0.107^{++}	37.180±0.111	36.430±0.212^{++}
Metaestrus and proestrus	37.400±0.207	36.920±0.319	37.310±0.234	36.610±0.229*	37.260±0.219	36.240±0.146^{++++}	37.120±0.136	36.920±0.384^{*+++}
Mean	37.420±0.062	37.270±0.076	37.670±0.092***	36.900±0.085***	37.370±0.120	36.000±0.079^{++}	37.130±0.077	36.650±0.144^{++}

Note. $^{*}p<0.001$, $^{**}p<0.01$, $^{***}p<0.05$ compared to 12-month-old mice; $^{+}p<0.001$, $^{++}p<0.01$, $^{+++}p<0.05$ compared to age-matched controls.

cycles decreased and the number of long cycles increased. In contrast to young mice, older mice showed anestrus. Prolonged vilon administration had no effect on the age dynamics of estrous function.

In 9–15-month-old control mice, rectal temperature markedly increased during diestrus (compared to estrus) (Table 1) due to functioning of the corpus luteum. No increase in rectal temperature during diestrus was observed in 18-month-old mice and the temperature was constant throughout all phases. No age-related changes in the average or phase-dependent body temperature were observed in control mice. Vilon-treated mice showed no cyclic estrus- or age-dependent changes in rectal temperature. Notably, the average body temperature during diestrus in 12-18-month-old experimental mice was lower than in controls.

No differences in the survival rate were noted up to 21 months, after this age vilon significantly decreased mortality (Fig. 2). In experimental group, the number of mice survived to 22 and 23 months was 1.24- and 2.57-fold higher than in the control ($p<0.01$). Thus, the survival rate curve in vilon-treated mice was shifted to the right compared to that for control mice. The mean life span in both groups did not differ significantly (685.0±9.2 and 694.0±12.5 days). However, vilon increased lifetime in 10% of the oldest animals (737.0±1.1 and 761.0±7.7 days in control and experimental groups, respectively, $p<0.05$) and maximum lifespan by about 2 months (740 and 792 days, respectively) indicating deceleration of aging during the second half of life.

In the control group, the incidence of spontaneous tumors reached 30%. Lung adenomas and breast tumors developed most frequently (20 and 8% cases, respectively). Vilon inhibited spontaneous carcinogenesis reducing the incidence of all tumors and multiple tumors 1.5- and 1.8-fold, respectively, and increased lifespan of animals with tumors (more than by 1 month). It 2-fold decreased the incidence of lung adenomas ($p<0.05$) and tended to decrease the occurrence of breast adenocarcinomas.

Thus, long-term vilon administration was associated with an increase in the lifespan and prevention of spontaneous neoplasms in female CBA mice. Vilon had a positive effect on animal physical activity and endurance. This was accompanied by an increase in body weight and decrease of body temperature, which is important for prolongation of life, because decreased motor activity and body temperature decelerate metabolic processes, thus increasing the lifespan [12].

It should be noted that geroprotective and antineoplastic effect of vilon were not associated with its effects on body weight and food consumption. Low-calorie diet and reduction of body weight significantly decelerate aging, inhibit spontaneous tumorigene-

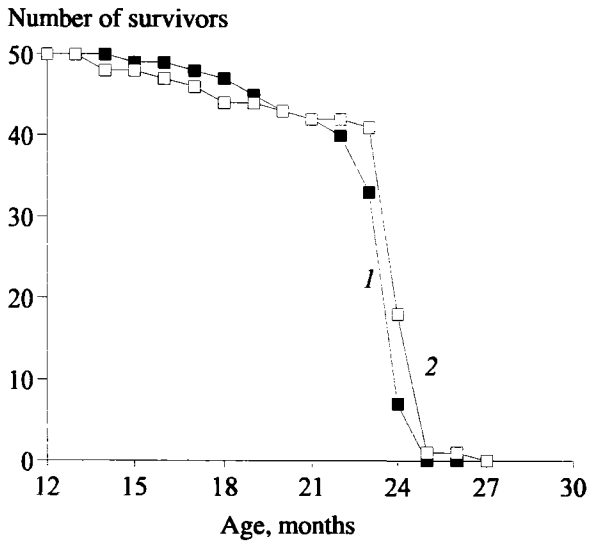

Fig. 2. Effect of vilon on lifespan in mice.

sis in experimental rodents [3,12], and decelerate aging of the immune system [12]. It can be assumed that immunomodulatory properties of vilon [9] play a key role in its geroprotective and antineoplastic effects.

Our findings agree with previous data on the safety of long-term administration of peptide thymus preparations from the thymus and their geroprotective and antineoplastic properties [1,2,4,5], which allows to recommend vilon as a preparation for geroprotection and prophylaxis of age-dependent pathology.

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