GENETICS

Effect of Epithalon on the Incidence of Chromosome Aberrations in Senescence-Accelerated Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 3, pp. 320-322, March, 2002
Original article submitted October 22, 2001

The incidence of chromosome aberrations in bone marrow cells of 12-month-old SAMP-1 female mice characterized by accelerated aging was 1.8 times higher than in wild-type SAMR-1 females and 2.2 times higher than in SHR females of the same age. Treatment with Epithalon (Ala-Glu-Asp-Gly) starting from the age of 2 months decreased the incidence of chromosome aberrations in SAMP-1, SAMR-1, and SHR mice by 20%, 30.1%, and 17.9%, respectively, compared to age-matched controls ($p<0.05$). Treatment with melatonin (given with drinking water in a dose of 20 mg/liter in night hours) had no effect on the incidence of chromosome aberrations in SHR mice. These data indicate antimutagenic effect of Epithalon, which probably underlies the geroprotective effect of this peptide.

Key Words: *epithalone; melatonin; chromosome aberrations; SAM mice*

Age-realted increase in the incidence of somatic mutations can result from accumulation of non-repaired DNA damage and underlie the development of age-related pathology, including malignant tumors [3,15]. The dynamics of accumulation of these mutations with age differs in different mouse strains, which can determine differences in the life span, rate of aging, and the incidence of spontaneous tumors between different strains [3,14,15]. For example, SAMP mice are characterized by shorter life span (≤14 months) and exhibit signs of rapid aging compared to control SAMR mice [1,2,11-14]. The search for new means preventing premature aging and development of age-related diseases is a primary task of modern gerontology [4]. The most promising geroprotector is a complex epiphyseal peptide preparation Epithalamin and Epithalon tetrapeptide (Ala-Glu-Asp-Gly) constructed on its base. These agents prolong animal life span and decrease the incidence of tumors in mice and rats [4-7,10].

The aim of our study was to elucidate the effect of long-term Epithalon treatment on the incidence of chromosome aberrations (CA) in female SAMP, SAMR mice, and long-living SHR mice used in experimental oncology. Melatonin, an epiphyseal indole hormone, was used as the reference drug in SHR mice.

MATERIALS AND METHODS

Experiments were carried out on SAMP-1 and SAMR-1 female mice (kindly provided by Prof. A. A. Boldyrev, Moscow State University). These strains were bred at Department of Carcinogenesis and Oncogerontology of N. N. Petrov Institute of Oncology, Ministry of Health of the Russian Federation. SHR mice were from Rappolovo Breeding Center of the Russian Academy of Medical Sciences. The animals were kept in

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0007-4888/02/1333-0274$27.00

plastic cages with standard day-night regimen on standard fodder and free access to water. Starting from the age of 2 months the mice were monthly subcutaneously injected with Epithalon (1 μg/mouse, 5 times a week) or with 0.1 ml 0.9 NaCl according to the same protocol (controls). Epithalon was synthesized by E. I. Grigoryev, St. Petersburg Institute of Bioregulation and Gerontology [10]. In experiments with SHR mice, special groups received (monthly) 5 times a week melatonin (Sigma) with drinking water in concentrations of 2 or 20 mg/ml during night hours 18.00-21.00. The number of CA in bone marrow cells in mice of all groups was analyzed at the age of 12 months. Material for cytogenetic analysis was fixed and treated as described previously [9]. The animals were sacrificed by cervical dislocation, both tibias were isolated, and epiphyses removed. The bones were treated in hypotonic (0.56%) KCl and fixed in ethanol:glacial acetic acid mixture (3:1). Crushed preparations were stained with 4% acetoorsein. Bridges and fragments in cells during anaphase were considered as CA.

The data were statistically processed by methods for small samples; the significance of differences between experimental variants was evaluated using Student's *t* test.

TABLE 1. Effects of Epithalon and Melatonin on the Incidence of CA in Bone Marrow Cells of 12 Month-Old SHR, SAMP-1, and SAMR-1 Female Mice ($M \pm m$, n=4)

Mouse strain; experimental series	CA incidence, %	Δ, %
SHR		
intact	8.2±0.41	
0.9% NaCl	8.4±0.38	
melatonin, mg/liter		
2	7.5±0.25	-8,5[x]
20	7.2±0.42	-12.2[x]
epithalone	6.9±0.45*	-17.9*
SAMR-1		
0.9% NaCl	10.3±0.11[+]	
epithalone	7.2±0.08*	-30.1
SAMP-1		
0.9% NaCl	18.7±0.51[o]	
epithalone	15.0±0.28*	-19.8

Note. *p<0.05 compared to the control (0.9% NaCl); [+]p<0.01 compare to control SHR mice; [o]p<0.001 compared to control SHR and SAMR-1 mice, [x]compared to intact animals.

RESULTS

The incidence of CA in rapidly aging SAMP-1 mice was 1.8–fold higher than in SAMR-1 mice (p<0.001, Table 1), while in this latter strain this parameter 23% surpassed the corresponding value in age-matched SHR mice (p<0.01). Long-term Epithalon treatment significantly reduced the incidence of CA in mice of all three studied strains in comparison with age-matched controls. The most pronounced effect was observed in SAMR-1 mice (Table 1). Interestingly, the incidence of CA in Epithalon-treated SAMR-1 mice decreased even below the corresponding level in control long-living SHR mice of the same age (p<0.01). Melatonin had virtually no effect on the incidence of CA in SHR mice.

SAMP mice develop normally until the age of 4 months. This age is characterized by drastic accumulation of reactive oxygen species, appearance of aging signs, including rapid accumulation of CA (compared to SAMR mice) [1,2,11-13]. Long-term treatment with Epithalon prolonged the life span of *D. melanogaster* and CBA mice, suppressed free radical processes, and inhibited development of spontaneous tumors in mice [5,10]. It can be hypothesized that the antimutagenic effects of Epithalon play an important role in the realization of its geroprotective and antitumor effects. In our experiments melatonin had no appreciable effect on the incidence of CA in SHR mice. It was previously shown that the geroprotective effect of melatonin was associated with increased incidence of lymphomas and adenocarcinomas of the lungs in CBA mice. The antioxidant effect of melatonin is less pronounced than that of epithalone [8].

Hence, our experiments showed that 12-month-old senescence-accelerated SAMP-1 mice are characterized by higher incidence of CA in bone marrow cells compared to normally aging SAMR-1 mice and long-living SHR mice of the same age. Long-term treatment with Epithalon markedly decreased the incidence of CA in SAMP-1, SAMR-1, and SHR mice.

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