BIOGERONTOLOGY

Effects of Livagen Peptide on Chromatin Activation in Lymphocytes from Old People

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 10, pp. 451-455, October, 2002
Original article submitted September 20, 2002

We studied the effects of the synthetic peptide Livagen on activity of ribosomal genes, denaturation parameters of heterochromatin, polymorphism of structural C-heterochromatin, and variability of facultative heterochromatin in lymphocytes from old people. Livagen induced activation of ribosomal genes, decondensation of pericentromeric structural heterochromatin, and release of genes repressed due to age-related condensation of euchromatic regions in chromosomes. Our results indicate that Livagen causes de-heterochromatinization (activation) of chromatin, which is realized via modification of heterochromatin and heterochromatinized regions in chromosomes from old people.

Key Words: *peptide; Livagen; aging; chromatin activation; heterochromatinization*

Aging is accompanied by abnormal changes in genetic processes, progressive dysfunction, and development of age-related diseases, which contributes to death of the organism. Changes in homeostasis and age-related loss of functional activity in cells result from alterations in chromatin domains that act as functional units of the hereditary structure. In old people chromosomes undergo progressive heterochromatinization (condensation of euchromatin and heterochromatin regions), which promotes inactivation of genes with normal functional activity [10]. Previous studies showed that hormones and some physical and chemical agents induce de-heterochromatinization (decondensation) of chromosomes in cells from old people. These data indicate that directed treatment holds promise for the therapy of patients with age-related diseases.

Peptide bioregulators are of considerable interest in this respect. These substances decrease the risk of accelerated aging and development of age-related diseases and, therefore, are widely used in geriatrics [9]. One of these preparations is synthetic tetrapeptide Livagen. Direct chemical synthesis of Livagen was performed after statistical analysis of the amino acid composition of a complex preparation isolated from the liver [9] and stimulating protein synthesis in old animals [1].

Here we evaluated whether Livagen affects chromatin domains in lymphocytes from old people. The ability of Livagen to stimulate de-heterochromatinization of total heterochromatin, pericentromeric structural heterochromatin, and facultative heterochromatin (condensed euchromatin) and activate synthesis of ribosomal genes by de-heterochromatinization of nucleolar organizer regions (NOR) in acrocentric chromosomes was studied on cultured peripheral blood lymphocytes from old people.

MATERIALS AND METHODS

Experiments were performed on chromosomes of lymphocytes obtained from 23 conventionally healthy do-

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0007-4888/02/1344-0389$27.00

nors (76-80 years). We studied intact and Livagen-treated cultures of lymphocytes from each donor. The procedure allowed us to compare experimental and control results for each volunteer. We studied 1040 metaphases in 15 intact and 15 Livagen-induced lymphocyte cultures. Differential scanning microcalorimetry (DSM) was performed on 4 intact and 8 Livagen-induced cell cultures. Livagen was added in a concentration of 0.005 μg/ml producing no mutagenic effect [8].

Activity of ribosomal genes was evaluated by the incidence of cells with associations of acrocentric chromosomes, number of NOR in acrocentrics, and intensity of staining with Ag [3]. The significance of differences between the count of argentophilic NOR and their presence in satellite associations of acrocentric chromosomes in intact and Livagen-treated cultures was determined by comparing 2 binomial populations.

Denaturation parameters of total heterochromatin were estimated by the method of DSM [11]. The method is based on different thermostability of chromatin fractions. The sensitivity of DSM was 10^{-7} cal/min. The measurements were performed at 20-150°C and heating rate of 35°C/h. The volume of a measuring cell was 0.3 ml.

Polymorphism of structural C-heterochromatin was determined as described elsewhere [14]. The comparative analysis of C-stained chromosomes in intact and Livagen-treated cultures was performed using the standard system of classification. The size of C-segments in chromosomes 1, 9, and 16 was compared with that of the short arm in chromosome 16. The results were divided into 5 categories (*a*, *b*, *c*, *d*, and *e*). The formula of Zaks was used to calculate χ^2.

The variability of facultative heterochromatin was determined by the incidence of sister chromatid exchanges (SCE) in intact and Livagen-treated lymphocytes. The cells were incubated with 5-bromodeoxyuridine in a final concentration of 7.7 μg/ml over 2 cycles of replication. Differential staining of sister chromatids was performed without fluorochromes [2]. The mean level of SCE per metaphase was calculated. The differences between intact and Livagen-treated cultures were estimated by Student's *t* test.

RESULTS

Differential Ag-staining showed that human ribosomal genes involved in protein synthesis are localized in secondary centromeres of chromosomes (chromatid satellite threads). The presence of two such threads determines association of acrocentric chromosomes.

Positive staining with silver (Ag binding) during metaphase was typical of NOR, whose genes functioned during interphase. The intensity of staining correlates with activity of synthetic processes [13]. Thus, association of satellite threads is proportional to the intensity of Ag-staining, which, in turn, depends on activity of ribosomal genes. The absence of satellite threads reflects inactivation of ribosomal genes [15].

Livagen markedly increased the number of Ag^+-NOR in individual acrocentrics and associates (Fig. 1). After treatment with Livagen the mean number of Ag^+-NOR in associated acrocentrics markedly surpassed that in intact cultures ($p<0.001$, Fig. 1, *a*). Livagen improved associative activity of acrocentric chromosomes. In lymphocytes treated with Livagen the incidence of cells with associations of acrocentrics was much higher than in intact cultures ($p<0.001$, Fig. 1, *b*). It should be emphasized that Livagen evenly increased the number of DD, DG, and GG associations.

Our results are consistent with published data that hormones and growth factors induce decondensation

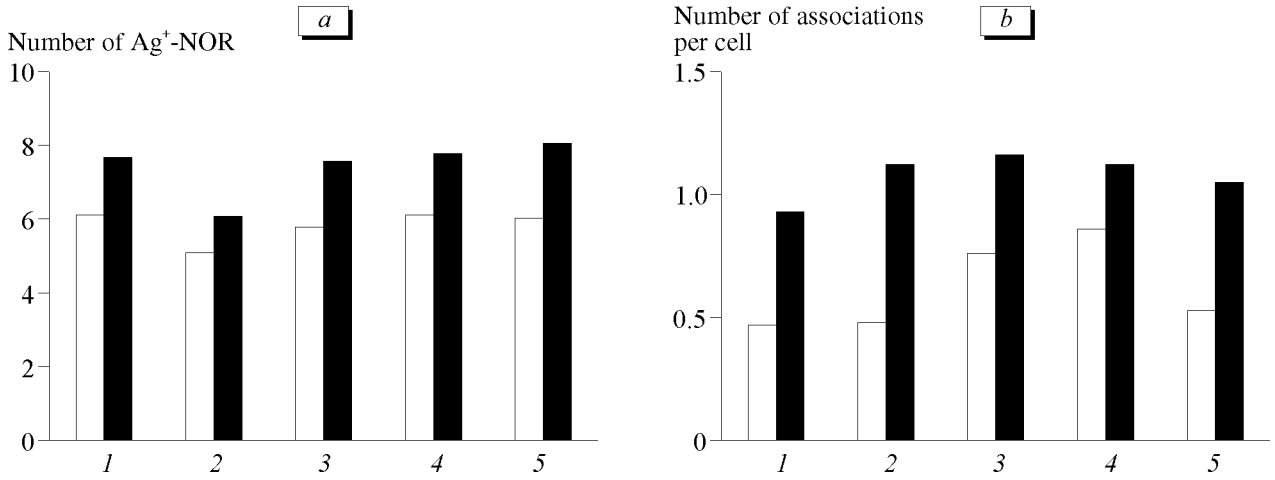

Fig. 1. Number of Ag-positive nucleolar organizer regions (NOR) in individual and associated acrocentrics (*a*) and incidence of cells with associations of acrocentrics in lymphocyte cultures (*b*). Light bars: control (intact lymphocytes). Dark bars: Livagen. *1-5*: cultures from various patients.

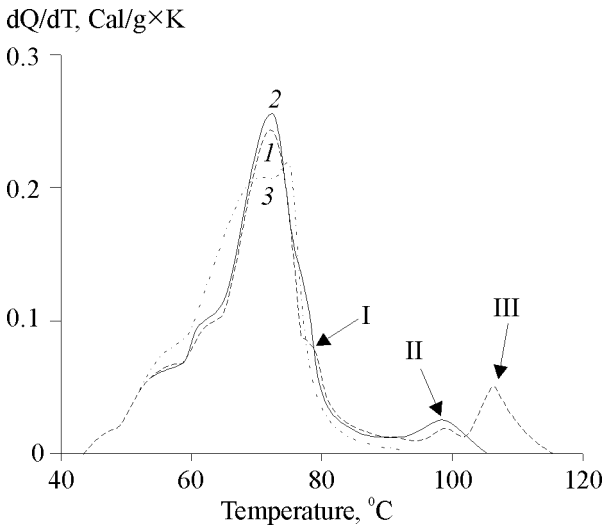

Fig. 2. Calorimetric curves for chromatin denaturation in intact (*1*), Livagen-treated (*2*), and phytohemagglutinin-stimulated and Livagen-treated lymphocytes from old people (*3*). Areas of endotherm: I (80±1°C), II (98.0±1.5°C), and III (107.0±1.5°C).

of chromosomes and increase transcriptional activity of NOR [5]. The count and size of Ag^+-NOR and the incidence of associations of acrocentric chromosomes increased in cultured lymphocytes treated with Livagen. These data indicate that Livagen causes de-heterochromatinization of satellite threads and activation of ribosomal genes in lymphocytes from old people.

Cell membranes, cytoplasmic structures, and nuclear proteins undergo denaturation during heating to 40-70°C. Denaturation of chromatin is observed at a higher temperature and proceeds in 3 stages. The curve for denaturation of lymphocytes in intact cultures included 3 peaks of heat absorption (endotherms) and 4 shoulders (Fig. 2). Areas at a low-temperature part of peak 1 characterized by denaturation temperatures (T_d) of 46±1, 55±1, and 63±1°C correspond to denaturation of membranes, nuclear matrix, and cytoplasmic structures, respectively. The most pronounced endotherm at 71.0±0.5°C and endotherms I, II, and III (Fig. 2) reflect denaturation of nucleosomes in a 10-nm filament-fibril with densely positioned adjacent nucleosomes, 30-nm solenoid, and supercoiled loop fixed to the nuclear matrix, respectively.

Livagen affected parameters of denaturation in lymphocytes. We observed a shift of heat absorption peak II and disappearance of endotherm III (Fig. 2). After treatment of phytohemagglutinin-stimulated cells with Livagen, the calorimetric curve was characterized by an increase in the intensity of heat absorption during thermal denaturation at 53-67°C. Our results indicate that Livagen stimulates uncoiling of a 10-nm dense filament in the chromatin of phytohemagglutinin-stimulated lymphocytes. These changes result in the formation of fibrils with separated nucleosomes that undergo 3-stage denaturation at T_d of 56, 70, and 75°C. An increase in the intensity of heat absorption at 53-67°C is probably associated with intensive denaturation of linker regions in nucleosomal DNA weakly protected by histone H1. Therefore, Livagen promotes

TABLE 1. Heteromorphism of C-Segments in Chromosomes 1, 9, and 16 of Livagen-Treated Human Lymphocytes

Chromosome, No.	Variants of C-segments	ν_i	μ_i	ν_i/n	$\nu_i+\mu_i/n+m$	χ^2
1	*a*	17	17	0.0867	0.0872	36.53; $p<0.001$
	b	55	61	0.2806	0.2974	
	c	68	79	0.3469	0.3769	
	d	52	37	0.2653	0.2282	
	e	4	0	0.0204	0.0103	
9	*a*	28	36	0.1451	0.1702	9.91; $p<0.05$
	b	75	85	0.3886	0.4255	
	c	75	58	0.3886	0.3537	
	d	15	4	0.0777	0.0505	
	e	0	0	0	0	
16	*a*	66	76	0.3492	0.3727	1.01; $p>0.05$
	b	103	99	0.545	0.5302	
	c	20	17	0.1058	0.0971	
	d	0	0	0	0	
	e	0	0	0	0	

Note. ν_i, number of variants *a*, *b*, *c*, *d*, or *e* in intact cells; μ_i, number of variants *a*, *b*, *c*, *d*, or *e* after treatment with Livagen; *n*, number of all variants of C-segments in intact cells; *m*, number of all variants of C-segments after treatment with Livagen.

TABLE 2. Effect of Livagen on the Incidence of SCE in Lymphocytes from Old People ($M \pm m$, n=30)

Donor	Intact cells		Livagen-treated cells	
	abs.	per cell	abs.	per cell
1	195	6.50±0.47	286	9.50±0.56*
2	175	5.80±0.44	249	8.30±0.53**
3	165	5.50±0.43	299	10.00±0.58*
4	171	5.70±0.44	245	8.20±0.52*
5	180	6.00±0.45	296	9.90±0.57*
Total	886	5.9±0.2	1375	9.2±0.2*

Note. *p<0.001 and **p<0.01 compared to intact cells.

decondensation of chromosomes and loosening of chromatin in lymphocytes from old people.

Livagen-treated cells more rarely contained large C-segments (*d* and *e*) compared to intact cells. However, this peptide increased the incidence of small variants (*a* and *b*). We studied heteromorphism of C-segments in chromosomes 1, 9, and 16 of intact and Livagen-treated lymphocytes. The incidence of large and small C-segments in individual chromosomes underwent different changes. After treatment with Livagen, heteromorphism was typical of chromosomes 1 and 9. Table 1 shows that the degree of heteromorphism (*i.e.*, decrease in the size of large segments) varied in these chromosomes. The distribution of C-segments in heterochromatin of chromosome 16 remained unchanged and did not differ from that in intact cells (Table 1). Our results are consistent with published data that chemical substances decrease the size of C-heterochromatin in chromosomes 1 and 9, but do not affect structural C-heterochromatin in chromosome 16 [12]. However, some *in vitro* models of aging and diseases demonstrated variability of the absolute and relative size of C-heterochromatin in chromosome 16 [7].

Our results indicate that Livagen decreases the size of C-segments in chromosomes 1 and 9 of lymphocytes from old people. Therefore, the peptide causes decondensation of structural chromatin.

The incidence of SCE in cultured lymphocytes treated with Livagen was higher than in intact cultures (Table 2). We evaluated the average number of SCE per cell for various groups of chromosomes. Livagen increased the number of SCE practically in all groups of chromosomes. The exception was group F that included chromosomes 19 and 20 with a small size and central centromere. In this group the effect of Livagen was insignificant (p>0.05).

According to modern notions, exchange processes do not proceed in heterochromatic and heterochromatinized regions of chromosomes [4,6]. In our experiments Livagen increased the incidence of SCE, which reflects decondensation of euchromatin regions in chromosomes heterochromatinized during aging. Therefore, Livagen promotes the release of genes repressed during the development of age-related pathological changes in chromatin.

Our results indicate that Livagen activates chromatin in lymphocytes from old people by modifying heterochromatic and heterochromatinized regions in chromosomes.

REFERENCES

1. S. I. Anisimov, K. R. Bokheler, V. Kh. Khavinson, and V. R. Anisimov, *Byull. Eksp. Biol. Med*., **133**, No. 3, 340-347 (2002).
2. M. M. Antoshchina and N. A. Poryadkova, *Tsitol. Gen*., **12**, 349-352 (1978).
3. T. A. Lezhava, *Chromosomes in Extreme Old Age* [in Russian], Tbilisi (1991).
4. I. B. Lobov and O. I. Podgornaya, *Tsitologiya*, **41**, 562-573 (1999).
5. N. Mamaev and S. Mamaeva, *Ibid*., **34**, 3-24 (1992).
6. A. A. Podgornaya-Bel'govskaya, *Heterochromatic Regions in Chromosomes: Aging and Functions* [in Russian], Moscow (1986).
7. O. A. Podugol'nikova and V. G. Solonichenko, *Tsitol. Gen*., **28**, 85-88 (1994).
8. S. V. Rozenfel'd, E. F. Togo, V. S. Mikhaev, *et al*., *Byull. Eksp. Biol. Med*., **133**, No. 3, 320-322 (2002).
9. V. Kh. Khavinson and V. G. Morozov, *Peptides of the Epiphysis and Thymus in Regulation of Aging* [in Russian], St. Petersburg (2001).
10. T. A. Lezhava, *Biogerontology*, **2**, 253-260 (2001).
11. T. Lezhava, J. Monaselidze, Z. Chanchalashvili, *et al*., *Bull. Acad. Sci. Georgia*, **19**, 334-337 (1993).
12. G. Ott, T. Haaf, and M. Schmid, *Chromosome Res*., **6**, 495-499 (1998).
13. S. Stitou, R. Diaz de la Guardia, R. Jimenes, *et al*., *Ibid*., **8**, 305-311 (2000).
14. A. T. Sumner, *Exp. Cell Res*., **75**, 304-306 (1972).
15. D. Trere, *Micron*, **31**, 127-131 (2000).