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Effect of Peptide AEDG on Telomere Length and Mitotic Index of PHA-Stimulated Human Blood Lymphocytes

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We studied the effect of peptide AEDG on telomere length and mitotic index of PHA-stimulated blood lymphocytes from young (18-22 years, *N*=5) and middle-aged (49-54 years, *N*=6) men. In the younger age group, no significant changes in the mitotic index were detected, while in the middle-aged group, a decrease in this parameter was found in one case. The relative length of telomeric regions of metaphase chromosomes was evaluated by *in situ* fluorescence hybridization with DNA probes specific to telomeres. After incubation with peptide AEDG, significant changes in the relative telomere length were found in 7 of 11 individuals (3 cases in the younger age group and 4 cases in the middle age group). Significant increase in telomere length after exposure to peptide AEDG was revealed in 5 cases, including two individuals of the younger age group (by 41 and 55%) and three individuals of the middle age group (by 156, 18, and 76%). In one individual of the younger age group and in one of the middle-age group, a significant decrease in telomere length (by 37 and 15%, respectively) was found. A tendency to normalization of telomere lengths was noted: this parameter increased in individuals with initially lower telomere length relative to the group mean value and decreased in individuals with initially longer telomeres compared to the mean length in the group.

Key Words: *telomere length; peptide AEDG; human blood lymphocytes; young and middle age*

Telomeres located at the chromosome ends are dynamic nucleoprotein structures consisting of tandem 5'-TTAGGG-3' repeats and complexes of proteins (*e.g.* shelterin) with non-coding RNA (TERRA) [5,12,13]. Being located at the ends of chromosomes, telomeres protect them from nucleases, degradation [3,9,14], and terminal fusion [4]. In view of incomplete terminal replication determined by peculiarities of the biological mechanism of DNA polymerase action, cell divisions lead to gradual shortening of telomeres [10]. However, the mechanisms of telomere elongation also exist; they are associated with activity of telomerase and homologous recombination between telomere sequences (alternative chromosome elongation; ALT) [6]. Thus, telomere length is in a dynamic equilibrium regulated by the balance between elongation and shortening of telomeres.

Telomere length can change critically under the influence of negative exogenous and endogenous factors, which leads to premature cell aging, loss of viability, and apoptosis [11]. Some substances contribute telomere lengthening via activation of telomerase. For instance, addition of peptide AEDG to cultured human embryonic fibroblast induces expression of telomerase gene and activation of telomerase, which results in a 2.4-fold increase in telomere length [7].

Here we studied the effect of peptide AEDG on telomere length and mitotic activity of mitogen-stimulated blood lymphocytes from humans of different ages.

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MATERIALS AND METHODS

Peripheral blood lymphocytes were isolated from 5 young men (18-22 years) and 6 middle-aged men (49-54 years). Inclusion criteria were the absence of tobacco, alcohol, and drug dependence, symptoms of acute viral infection over 1 month before blood sampling, chronic diseases, as well as systematic drug therapy.

Lymphocytes were cultured using a standard semi-micromethod with phytohemagglutinin (PHA) stimulation [1]. At the 48th hour, peptide AEDG was added to the culture vials in a final concentration of 1000 ng/ml. No peptide was added to the control vial. At the 71st hour, 1% colchicine was added to the vials. The cell suspension was fixed with methanol and glacial acetic acid mixture (3:1), then dropped to wet slides cooled to 4°C over a steam bath.

The mitotic index of PHA-stimulated lymphocytes was calculated by the ratio of dividing cells to the total cell number in the preparation.

For evaluation of the relative length of telomeres of metaphase chromosomes, fluorescent *in situ* hybridization (FISH) with DNA probes specific to telomere sequences of human chromosomes (Telomere PNA Kit/Cy3; Dako) and locus in the short arm of chromosome 9 (CDKN2A; Abbott Molecular) as a reference probe was performed according to the recommendations of DNA probe manufacturers. After FISH, the metaphase plates were photographed (Fig. 1). The mean fluorescence intensity of the hybridization signal of telomere-specific DNA probe on chromosomes 1, 9, and 16 was determined using ImageJ 1.48 software. To neutralize the effect of chromosome condensation on the measurement result, the mean relative length of chromosome telomeres was calculated as the ratio of the mean fluorescence intensities of the hybridization signals of the telomer-specific DNA probes and the reference DNA probe. Fluorescence intensity of 7280 hybridization signals on 260 metaphase plates was measured.

Statistical analysis of the results was carried out using GraphPad Prism 6.01 software. To analyze changes in the mitotic index after exposure to peptide AEDG, χ^2 test with Yates correction for conjugacy tables was used. The changes in the relative telomere length after exposure to peptide AEDG were evaluated using Mann—Whitney test. The differences were significant at $p<0.05$.

RESULTS

The percentage of dividing cells in the control varied from 4.1 to 8.6% in the younger group and from 5.9 to 9.5% in the middle age group. After addition of peptide AEDG, this parameter did not significantly change in the younger age group, but decreased by 38% in one individual of the middle age group (Table 1).

The relative telomere length in PHA-stimulated lymphocytes in the control varied from 0.86 to 1.75 in young and from 0.63 to 1.64 in middle-aged individuals. The groups did not significantly differ by this parameter, although there was a tendency to decrease the relative telomere length in the middle age group (Fig. 2).

After incubation with peptide AEDG, significant changes in the relative telomere length were found in 7 of 11 individuals (3 cases in the younger age group and 4 cases in the middle age group). A significant increase in the relative telomere length was shown in two young individuals (by 41 and 55%) and in three middle-aged individuals (by 156, 18, and 76%, respectively) (Fig. 2). A significant decrease in the relative telomere length was found in one young (by 37%) and one middle-aged men (by 15%) (Fig. 2).

Thus, peptide AEDG added to the culture of PHA-stimulated lymphocytes induced changes in telomere length in 7 out of 11 individuals. Significant changes in telomere length were more typical of middle-aged than for younger individuals (4 and 3 cases, respectively). The increase in telomere length after exposure to peptide AEDG was by 2.5 times more frequent than its decrease (5 and 2 cases, respectively). Significant increase in the relative telomere length was revealed in two individuals of the younger age group and in three individuals of the middle age group. Significant decrease in the relative telomere length was revealed in one individual of the younger age group and in one middle-aged individual. The maximum increase in telomere length (by 156%) was found in one individual of the middle age group, who also showed a

TABLE 1. Mitotic Index (%) of PHA-Stimulated Lymphocytes Incubated in the Presence and Absence of peptide AEDG

Subject No.	No peptide	Peptide AEDG
1	8.6	10.3
2	6.7	5.8
3	4.1	3.7
4	6.1	6.2
5	8.3	10.2
6	9.5	10.6
7	7.7	6.5
8	7.6	4.7**
9	5.9	7.5
10	7.4	7.2
11	7.0	8.6

Note. **$p<0.01$ (Mann—Whitney test).

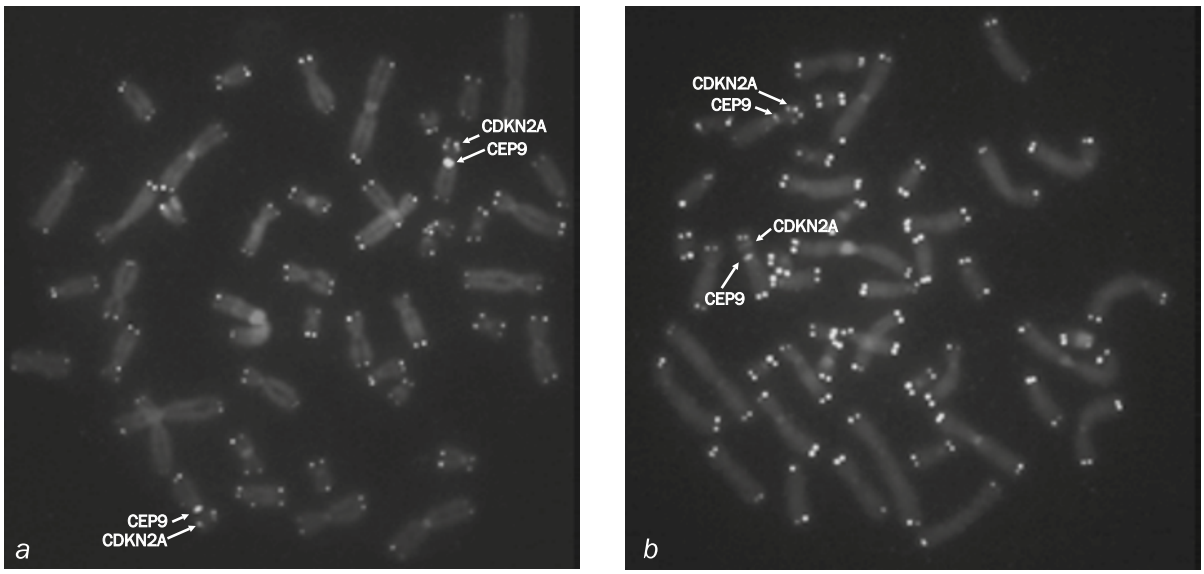

Fig. 1. Metaphase plates from PHA-stimulated lymphocytes cultured in the absence (*a*) and presence of peptide AEDG (*b*) after fluorescence *in situ* hybridization with telomere-specific reference (CDKN2A) and centromere-specific (CEP9) DNA probes.

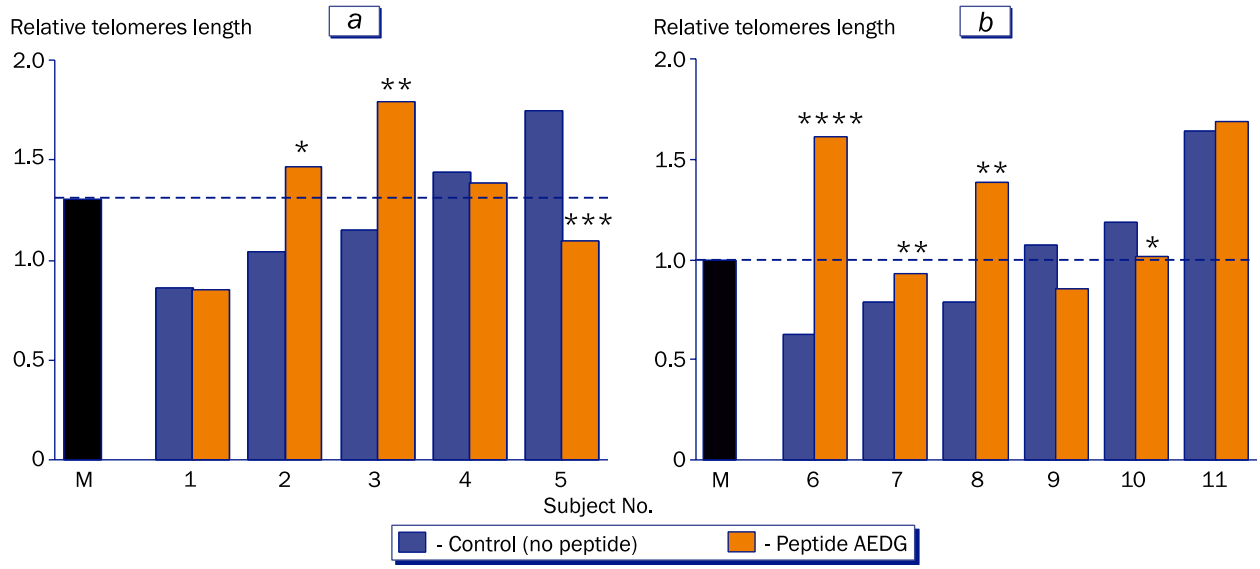

Fig. 2. Relative length of telomeres of PHA-stimulated lymphocytes in the control and after addition of peptide AEDG in young (*a*) and middle-aged (*b*) individuals. **p*<0.05, ***p*<0.01, ****p*<0.001 in comparison with the control (Mann—Whitney test). M bar and dashed line show the mean relative length of telomeres in the absence of peptide AEDG for this age group.

significant decrease in the mitotic index. A tendency to normalization of telomere lengths was noted: this parameter increased in individuals with initially lower telomere length relative to the group mean value and decreased in individuals with initially longer telomeres compared to the mean length in the group.

Peptide AEDG did not affect mitotic activity of PHA-stimulated blood lymphocytes in young and middle-aged individuals, but was shown to stimulate proliferation of human fetal fibroblasts [8]. It can be hypothesized that fetal fibroblasts have greater biological reserve of mitotic activity than blood lymphocytes of young and middle-aged individuals. Moreover, peptide AEDG is tissue-specific [2] and its activity or the absence of activity depends on the type of cells used in the experiment.

Peptide AEDG modulates the relative telomere length of blood lymphocytes of young and middle-aged individuals. The stimulating effect of peptide AEDG in relation to the relative length of the telomeres in blood lymphocytes was revealed in individuals with initially reduced telomere length. These results are consistent with previous data on the stimulating effect of peptide AEDG on activity of the telomerase gene

and the corresponding enzyme in fetal fibroblasts [8]. It can be hypothesized that peptide AEDG regulates the activity of the telomerase gene, the function of the corresponding enzyme and telomere length in different types of human cells.

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REFERENCES

1. Kuznetsova TV, Loginova YuA, Chiryaeva OG, Pendina AA, Efimova OA, Fedorova ID, Baranov VS. Cytogenetic techniques. Medical Laboratory Technologies. Karpishchenko AI, ed. Moscow, 2013. Vol. 2. P. 623-650. Russian.
2. Anisimov VN, Khavinson VKh. Peptide bioregulation of aging: results and prospects. Biogerontology. 2010;11(2):139-149.
3. Azzalin CM, Lingner J. Molecular biology: damage control. Nature. 2007;448:1001-1002.
4. de Lange T. Protection of mammalian telomeres. Oncogene. 2002;21(4):532-540.
5. Déjardin J, Kingston RE. Purification of proteins associated with specific genomic Loci. Cell. 2009;136(1):175-186.
6. Dunham MA, Neumann AA, Fasching CL, Reddel RR. Telomere maintenance by recombination in human cells. Nat. Genet. 2000;26(4):447-450.
7. Khavinson VKh, Bondarev IE, Butyugov AA. Epithalon peptide induces telomerase activity and telomere elongation in human somatic cells. Bull. Exp. Biol. Med. 2003;135(6):590-592.
8. Khavinson VKh, Bondarev IE, Butyugov AA, Smirnova TD. Peptide promotes overcoming of the division limit in human somatic cell. Bull. Exp. Biol. Med. 2004;137(5):503-506.
9. Lundblad V. DNA ends: maintenance of chromosome termini versus repair of double strand breaks. Mut. Res. 2000;451(1-2):227-240.
10. Olovnikov AM. A theory of marginotomy: the incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J. Theor. Biol. 1973;41(1):181-190.
11. O'Sullivan RJ, Karlseder J. Telomeres: protecting chromosomes against genome instability. Nat. Rev. Mol. Cell Biol. 2010;11(3):171-181.
12. Ozturk S, Sozen B, Demir N. Telomere length and telomerase activity during oocyte maturation and early embryo development in mammalian species. Mol. Hum. Reprod. 2013;20(1):15-30.
13. Schoeftner S, Blasco MA. Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. Nat. Cell Biol. 2008;10(2):228-236.
14. Zhao Y, Hoshiyama H, Shay JW, Wright WE. Quantitative telomeric overhang determination using a double-strand specific nuclease. Nucleic Acids Res. 2007;36(3). e14.