ISSN 2079-0570, Advances in Gerontology, 2015, Vol. 5, No. 3, pp. 176–179. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © N.I. Chalisova, N.S. Linkova, A.N. Zhekalov, A.O. Orlova, G.A. Ryzhak, V.Kh. Khavinson, 2014, published in Uspekhi Gerontologii, 2014, Vol. 27, No. 4, pp. 699–703.

Short Peptides Stimulate Cell Regeneration in Skin during Aging

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Abstract—The search for new safe and efficient low-molecular weight substances stimulating skin regeneration is an important objective of geriatric cosmetology. The present study addressed the effects of the peptides LK and AEDG at concentrations of 0.05–2.00 ng/mL on the proliferation of organotypic tissue cultures of skin from young and old animals. The application of peptides enhanced fibroblast proliferation in skin cell cultures from young and old animals by 29–45%. The effect on aging skin was observed in a narrower concentration range; in addition, it was less pronounced than the effect on skin cell cultures obtained from young animals. These data reveal the potential of LK and AEDG as components of cosmetic products that restore the structure of aging skin.

Keywords: short peptides, cell proliferation, organotypic culture, skin, aging
DOI: 10.1134/S2079057015030054

Skin serves as a protective function and a border zone between the external environment and the interior of an organism. Consequently, skin is affected by various physical, mechanical, and chemical damaging factors that promote its aging. Age-related skin involution is directly associated with dysfunction of dermal fibroblasts. Age-related fibroblast degeneration (rigidity increase, hypertrophy, and decrease of functional activity) leads to disruption between the contact of cells and collagen of the extracellular matrix; therefore, the viscoelastic properties of skin collagen deteriorate [8]. Two types of skin aging are currently known, photoaging and chronoaging, of which the latter is associated with fibroblast hypotrophy related to a decrease in hyaluronic acid content in the skin at 35–40 years of age [8, 12]. Moreover, microcirculation in the derma of the epidermis may be impaired. Photoaging is accompanied by thickening of the corneal layer of the skin. Decline of functional activity of the fibroblasts manifested as impairment of collagen synthesis and extracellular matrix remodeling is the common mechanism underlying internal and external aging of the skin [12–14]. Therefore, the search for substances capable of restoring the functional activity of skin fibroblasts is a relevant task for modern cosmetology and molecular medicine. Short peptides are promising geroprotectors for many tissues, including skin.

Short peptides are capable of regulating tissue repair processes by stimulating cell proliferation and cell–cell interactions. *L*-carnosine (*L*-AH) is among the most thoroughly characterized regulatory peptides involved in controlling cell metabolism in various tissues. Carnosine is involved in melatonin synthesis and improves the functional activity of neuroimmunoendocrine cells affected in age-related pathology [7]. The peptide epitalon (AEDG) presents another example of a short peptide stimulating tissues of the neuroimmunoendocrine system and regulating melatonin synthesis by pinealocytes. The peptides thymogen (*L*-GW), vilon (KE) [4, 10], and bestim (*D*-G-L-W) [1] are used as immunomodulators. Moreover, it was shown that the role of proteinogenic amino acids is not limited to functioning as plastic material for the production of peptides and proteins, but rather involves multidirectional regulation of cell renewal (mediated by proliferation and apoptosis) [11, 15]. Opposite actions of two amino acids on a certain tissue were assumed to be the most efficient approach for maintaining the balance between proliferation and apoptosis, the two fundamental cellular processes, and thus promote tissue regeneration. Skin is composed of both ectodermal and mesodermal tissue. Lysine was previously found to stimulate cell proliferation in cultured tissue of young rat skin, while it suppressed cell proliferation in skin explants from old rats, and leucine had no effect on proliferation in both cases [3, 5]. The peptide *LK* was synthesized with allowance for the distinctive effects of leucine and lysine.

The aim of this study was to characterize and compare the effects of LK and AEDG on cell proliferation in organotypic tissue cultures of skin from young and old rats.

MATERIALS AND METHODS

Organotypic cultivation of tissue fragments and analysis of the growth zone of explants is the most suitable and convenient method for rapid quantitative assessment of the effects of biologically active substances, because the change in cell numbers reflects the stimulation or inhibition of cell proliferation and can therefore serve as a criterion for initial general evaluation of biological activity of substances [3, 5, 9].

Experiments were carried out in organotypic cultures with 800 skin explants from young and old (aged 3 and 24 months, respectively) male Wistar rats. Dissected fragments of lower abdominal skin were separated into pieces 1 mm^3 in size, which were then placed into Petri dishes with collagen-coated bottoms. The cultivation medium consisted of 35% Eagle medium, 35% Hank's solution, and 25% fetal calf serum; it was supplemented with glucose (0.6%), insulin (0.5 U/mL), and gentamicin (100 U/mL). LK and AEDG were added to the Petri dishes with the experimental explants in 3 mL of medium to the final concentrations of 0.001, 0.01, 0.05, 0.1, 1, 2, and 10 ng/mL. The amino acids lysine and leucine were added to skin tissue cultures at concentrations of 0.001, 0.01, 0.05, 0.1, 1, 2, and 10 ng/mL; the effective concentration was 0.05 ng/mL. When the cultures were treated with leucine and lysine simultaneously, both amino acids were used at subthreshold concentrations of 0.01 ng/mL. Petri dishes with control explants contained nutrient medium only (3 mL), i.e., experimental and control explants developed in the same volume of culture medium. The tissue cultures were divided into six groups: 1—control, 2—LK peptide, 3—AEDG peptide, 4—the amino acid lysine, 5—the amino acid leucine, and 6—a combination of amino acids leucine and lysine.

The petri dishes were placed in an incubator at 37°C; the cells were cultivated for 3 days and subsequently analyzed using phase contrast microscopy. The area index (AI) was calculated as the ratio of the total explant area (including the zone of expulsed cells) to the area of the central zone of the explant and expressed in arbitrary units. A microteleattachment for a microscope (series 10, MTN-13) manufactured by Al'fa Telekom (Russia) was used to visualize the explants. PhotoM 1.2 software was used to calculate the explant AI. The number of explants used for the control and each of the experimental conditions was 17–20. AI values were expressed in %, with a control AI value of 100%.

The Shapiro–Wilk test was used to analyze the distribution of the values. Statistical homogeneity of multiple samples was tested using a nonparametric ANOVA procedure (Kruskal–Wallis test). If statistically significant heterogeneity of multiple samples was detected by ANOVA, further pairwise comparison for the detection of non-homogeneous groups was performed using the Mann–Whitney *U*-test. The threshold level of significance (no difference) was assumed to be 0.05.

RESULTS AND DISCUSSION

The spreading of explants on a collagen substrate and the expulsion of proliferating and migrating cells that formed a growth zone along the edge of the explant occurred during the first day of cultivation. The major structural elements of the peripheral zone of the explants were represented by the peripheral growth zone and a discontinuous capsule formed by one or two layers of fibroblasts. The presence of large gaps in the capsule enabled migration of part of the cells outside the explants; these cells subsequently proliferated, forming the growth zone. Epithelial cells and fibroblasts constituted the growth zone of cultured rat skin fragments (Fig. 1). These cells formed the peripheral growth zone of explants; the size of this zone was measured to determine the AI. The AI value of the experimental explants measured after 3 days of cultivation exceeded that of the control explants in the case of stimulation of growth zone development; in other cases, the explant AI was the same as or lower than the control value.

Investigation of the effect of amino acids (at an effective concentration of 0.05 ng/mL) on skin tissue culture showed that lysine increased the AI significantly (by 26%), while leucine reduced the AI by 12% ($p < 0.05$). Treatment of the cultured cells by a mixture of leucine and lysine in subthreshold concentrations (0.01 ng/mL each) resulted in an increase in AI exceeding the stimulatory effect of the individual stimulatory amino acid by 2–4%. Ttreatment of the cultured cells by a mixture of these two amino acids resulted in growth zone stimulation and an 2% higher increase in AI (by 28%) ($p < 0.05$) than that induced by lysine alone.

The synthetic peptide LK had a stimulatory effect on the growth of cultured skin explants from young rats when applied at 0.05, 0.1, 1, and 2 ng/mL, with the most pronounced stimulation (45%, $p < 0.05$) observed at 0.1 ng/mL (Fig. 2a). The stimulatory effect of AEDG on skin explants from young animals was similar, albeit less pronounced: the AI increased by 34% relative to the control value at the most efficient concentration of 0.1 ng/mL (Fig. 2a).

LK and AEDG exerted stimulatory effects on cells of cultured skin from old rats, although the effect was not as pronounced and the range of stimulatory concentrations was not as broad as in the case of young animals. LK used at 1 and 2 ng/mL induced an AI increase of 29 and 31%, respectively, as compared to the control ($p < 0.05$) (Fig. 2b). AEDG evoked a 30% increase in the AI relative to the control when applied at a concentration of 1 or 2 ng/mL ($p < 0.05$).

The treatment of cultured tissue with LK at a concentration of 0.1 ng/mL resulted in an increase in skin explant AI by 45%; i.e., the difference between this

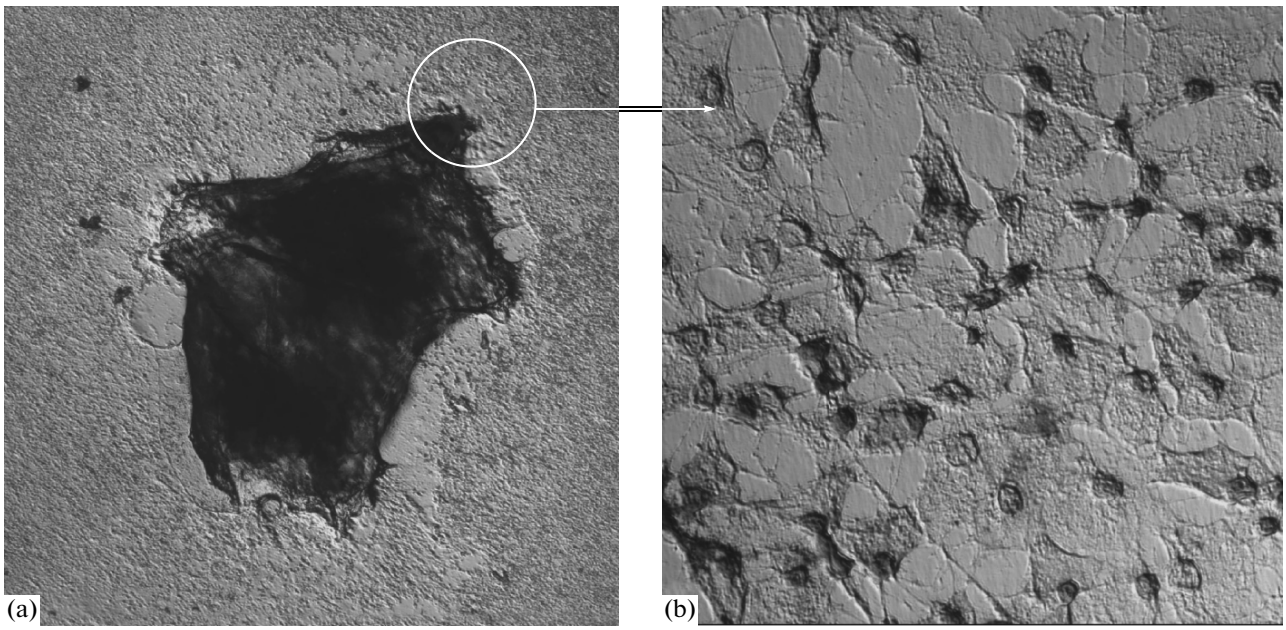

Fig. 1. In vivo phase-contrast microscopy of skin explant from young rat after 3 days of cultivation. (a) ×50 magnification, (b) ×400.

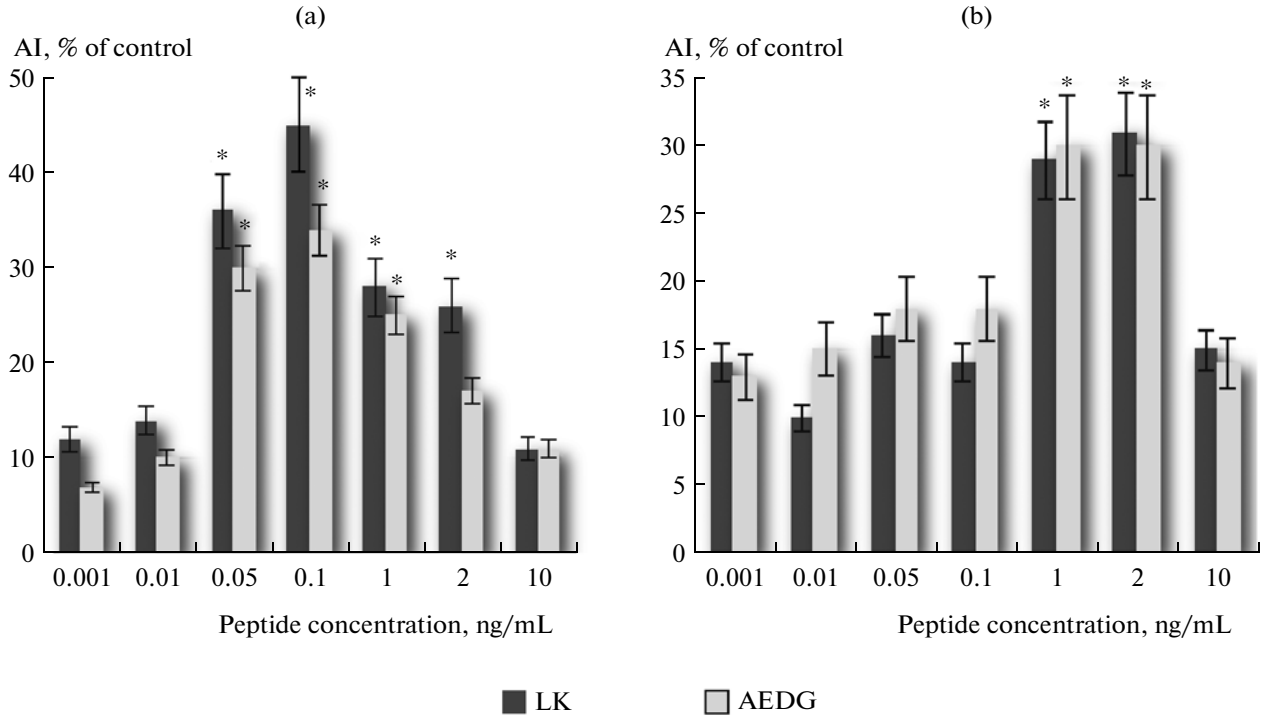

Fig. 2. Effect of short peptides on AI of growth of skin explants from young (a) and old (b) animals. *$p < 0.05$ compared with control (0-line).

value and the increase observed upon the application of an amino acid mixture was 17% ($p < 0.05$). This demonstrates the important biological role of the peptide bond. Regulation by amino acids may have been characteristic of the early period of evolution of living organisms (prokaryotes): e.g., the regulatory effects of amino acids on the reproduction of various bacterial species were reported. The emergence of the peptide

bond enabled more efficient and specific regulation of cell function as compared to regulation by the amino acids composing the peptides.

Thus, LK and AEDG stimulated cell proliferation in animal skin when applied in a wide range of concentrations. The concentration range associated with the effect on skin tissue of young rats was broader than in the case of skin tissue from old rats; in addition, the concentrations required to stimulate skin cells from older animals were higher: 1–2 ng/mL. The stimulatory effect of the dipeptide was somewhat more pronounced than that of the tetrapeptide. It was shown earlier that the polypeptide complexes epithalamin and timalin improve the functional state of the skin and its appearance in elderly people [2]. The data demonstrate enhancement of the functional activity of fibroblasts in aging skin via the short peptides LK and AEDG, and this can lay the foundations for the use of short peptides in cosmetology to correct age-related changes in skin [2]. LK and AEDG are capable of penetrating cell membranes [6] and can therefore probably be used as components in creams to maintain the functional activity of skin cells in middle-aged women and and be introduced into deeper layers of the dermis by electrophoresis as part of machine cosmetology treatments for elderly women.

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Translated by S. Semenova

SPELL: 1. explants