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Effect of Tripeptides on Lymphoid and Stem Cells

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Tripeptides T-36 and, particularly, T-38 in concentrations of 0.1, 1, and 10 ng/ml inhibited proliferation of primary trypsinized embryonic mesenchymal stem cells, rat transplantable KF-1 fibroblasts, and human erythromyelosis K-562 cells. Inhibition of proliferation in embryonic and immortalized cells under the influence of tripeptides probably reflects antitumor activity of these substances. Tripeptides had no effect on lymphocyte survival and their adhesive, cytotoxic, and induced proliferative activities. T-36 did not modulate the proliferative properties of erythromyelosis K-562 cells. Tripeptides did not change engulfment activity and spontaneous and induced bactericidal activities of granulocytes. T-36 in a concentration of 0.1 ng/ml increased spontaneous proliferation of normal lymphocytes. These data suggest that tripeptides stimulate nontumor immune cells in adult people.

Key Words: *tripeptides; lymphoid cells; mesenchymal stem cells; proliferation*

The regulation of proliferation and differentiation of lymphoid cells and mesenchymal stem cells (MSC) is an urgent problem of immunology of aging [1,3,9,10]. Studying the properties of peptide geroprotectors (synthesized at the St. Petersburg Institute of Bioregulation and Gerontology), including tripeptides T-36 and T-38, showed that short peptides can be used to regulate proliferative activity of immune and stem cells from humans and animals [1,5,6,8]. However, little is known about the effects of both peptide bioregulators on various functions of lymphocytes and MSC that provide favorable microenvironmental conditions for these cells.

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Here we studied the effects of tripeptides T-36 and T-38 on stem and lymphoid cells.

MATERIALS AND METHODS

Experiments were performed on the following cell cultures: human peripheral blood leukocytes; transplantable cell culture of human erythromyelosis (K-562); transplantable culture of rat fibroblasts (KF-1); and primary trypsinized culture of rat embryonic-muscular MSC.

The cells were cultured using Hanks solution, trypsin, Versen (BioTestLaboratoriya), MTT (PanEco), Histopaque-1077, trypan blue, phytohemagglutinin (PHA), and DMSO (Sigma). The nutrient media for culturing of MSC contained DMEM/F12 medium, 10% FBS, 2 mM L-glutamine, and 0.1 mM Hepes sodium salt. The nutrient media for culturing of K-562

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cells contained RPMI 1640 medium, 10% FBS, 2 mM L-glutamine, 0.1 mM Hepes sodium salt, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml kanamycin, and 10 μg/ml fluconazole.

Primary trypsinized MSC from 14-day-old rat embryos were isolated by mechanical and enzymatic treatment. Trypsin was inhibited by adding FBS. The cells were washed, pipetted in the nutrient medium, and put in culture flasks (25 cm^2). These flasks were incubated in a CO_2 incubator at 37°C and 5% CO_2 and passaged (1:5) at 3-day intervals using a mixture of trypsin and Versen solution. The transplantable culture of rat KF-1 fibroblasts was similarly treated and maintained. The suspension culture of K-562 cells was maintained in penicillin flasks. The cells were passaged at 4-5-day intervals. They were resuspended (3×10^5 cells) in 5 ml nutrient medium. Proliferative activity of cultures was evaluated by cell counting in a Goryaev chamber.

Proliferative activity of lymphocytes was measured in the reaction of blast transformation (RBTL) in PHA. Functional activity of mouse bone marrow MSC was evaluated from fibroblast colony formation (CFU-F) in the CFU test with monolayer cultures [7].

Natural cytotoxic activity of lymphocytes was measured colorimetrically. This method is based on the ability of mitochondrial enzymes in livable cells to transform the MTT tetrazolium salt (added to the cell culture) into a yellow-colored crystalline MTT-formazan product [11]. K-562 cells served as a target. The cytotoxic index was calculated as follows:

$$CI=100-(\frac{OD_{TC,EC}-OD_{EC}}{OD_{TC}}\times100),$$

where $OD_{TC,EC}$ is optical density of the target cells incubated with the effector cells (lymphocytes); OD_{TC} is optical density of the target cells; and OD_{EC} is optical density of the effector cells.

Adhesion activity of human lymphocytes was evaluated from cytoskeleton-dependent interaction of CD2 molecules with the surface structure of sheep erythrocytes homologous to CD58 molecule [4].

Engulfment activity of neutrophils was evaluated from the ability of these cells to absorb heat-inactivated *St. aureus* cells. Bactericidal activity of neutrophils was measured in the NBT test. This test is based on the ability of activated phagocytes to utilize oxygen with the formation of high-reactivity free oxygen radicals and deposition of dark-blue diformazan granules in the cell [2].

T-36 (H-Glu-Asp-Pro-OH) and T-38 (H-Lys-Glu-Asp-OH) were used in final concentrations of 0.01, 0.1, 1, 10, 100, and 1000 ng/ml. These tripeptides were added to the culture media for the following periods: 18-20 h (studying the survival and cytotoxic activity of lymphocytes), 2 h (studying the adhesive properties); 72 h (RBTL); and 2 h (studying the engulfment and bactericidal activity of granulocytes). The test peptides were added to the culture of K-562 cells for 18-20 h. Tripeptides were added to the primary trypsinized culture of rat embryonic MSC and transplantable culture of rat fibroblasts for 2 days. These products were present in the culture for 14 days to study CFU-F.

The results were analyzed by nonparametric Mann–Whitney test.

RESULTS

The survival of cultured lymphocytes after addition of T-36 and T-38 in various concentrations (except for the highest concentration) did not differ from the control. Lymphocyte survival was significantly reduced (94.8%, $p<0.05$) under the influence of T-38 in a concentration of 1000 ng/ml. Tripeptides had no effect on adhesive and cytotoxic activities of lymphocytes. They did not modulate the engulfment and bactericidal properties of granulocytes. The test products did not affect the ability of mouse bone marrow cells to form fibroblast colonies.

PHA-induced proliferative activity of lymphocytes remained unchanged after treatment with T-38. However, addition of T-36 in a concentration of 1.0 ng/ml was followed by the increase in spontaneous RBTL from 2.3 to 10.0% ($p<0.05$).

T-36 had no effect on proliferation of rat transplantable fibroblasts and primary trypsinized rat embryonic MSC at low density of inoculation (3×10^3 cells/cm^2). At a greater density of cells (5×10^3 cells/cm^2), T-36 in concentrations of 0.1, 1, and 10 ng/ml significantly decreased proliferative activity of MSC (Table 1).

T-38 was more potent that T-36 in inhibiting proliferation of embryonic MSC. T-38 in concentrations of 0.1, 1, and 10 ng/ml decreased the proliferative activity of MSC at high density of cell inoculation (5×10^3 cells/cm^2). Moreover, T-38 in concentrations of 0.1 and 1 ng/ml had an antiproliferative effect at low density of MSC inoculation (Table 2). T-38 inhibited the proliferation of rat transplantable fibroblasts at low density (0.1, 1, and 10 ng/ml) and high density of cell inoculation (1 and 10 ng/ml; Table 3). T-38 in concentrations of 0.1, 100, and 1000 ng/ml inhibited the proliferation of cultured human erythromyelosis K-562 cells (Table 4). T-36 did not modulate the proliferative activity of cultured fibroblasts and human erythromyelosis cells.

TABLE 1. Effect of T-36 on Proliferation in the Primary Trypsinized Culture of Rat Embryonic MSC (%, *M*±*m*)

Initial number of cells	T-36 concentration, ng/ml					
	0.01	0.1	1	10	100	1000
3×10^3/sm^2	98.2±4.5	97.0±2.9	96.5±6.6	91.0±9.8	96.2±10.3	96.8±9.7
	(88-120)	(88-106)	(77-124)	(49-120)	(53-132)	(61-141)
15×10^3/sm^2	96.5±3.2	92.0±2.4*	91.3±2.3*	88.5±2.4*	91.2±5.7	91.5±4.7
	(86-109)	(83-98)	(82-98)	(78-96)	(66-106)	(75-106)

Note. Here and in Tables 2-4: range of variations. *$p<0.05$ compared to the control (100%).

TABLE 2. Effect of T-38 on Proliferation in the Primary Trypsinized Culture of Rat Embryonic MSC (%, *M*±)

Initial number of cells	T-38 concentration, ng/ml					
	0.01	0.1	1	10	100	1000
3×10^3/cm^2	94.0±6.2	85.5±4.1*	86.3±3.4*	78.5±4.5*	89.5±9.8	83.0±10.3
	(75-120)	(66-95)	(71-96)	(65-95)	(52-120)	(40-109)
15×10^3/cm^2	98.5±2.8	97.3±2.5	91.0±2.7*	80.7±8.3*	87.2±9.4	90.78±8.9
	(89-110)	(87-106)	(81-99)	(40-99)	(43-110)	(53-120)

TABLE 3. Effect of T-38 on Proliferation in the Transplantable Culture of Rat Fibroblasts (%, *M*±)

Initial number of cells	T-38 concentration, ng/ml					
	0.01	0.1	1	10	100	1000
3×10^3/cm^2	99.8±2.3	86.5±3.4*	91.8±1.8*	89.3±2.3*	105.8±6.3	98.3±2.6
	(89-106)	(75-98)	(87-99)	(84-98)	(88-138)	(89-109)
15×10^3/cm^2	106.8±7.4	106.3±7.1	94.3±1.9*	95.3±2.2*	106.8±7.6	90.8±4.3
	(86-443)	(95-145)	(89-103)	(88-103)	(91-145)	(94-125)

TABLE 4. Effect of T-38 on Proliferation in the Culture of Human Erythromyelosis K-562 Cells (%, *M*±)

Parameter	T-38 concentration, ng/ml					
	0.01	0.1	1	10	100	1000
Cell number	101.8±4.5 (72-130)	91.7±4.0* (65-114)	90.5±5.3 (56-129)	95±4.8 (62-123)	91.3±3.5* (69-114)	92.5±3.5* (70-112)

Hence, T-36 and T-38 dose-dependently inhibit proliferation of cultured rat embryonic MSC, transplantable immortalized rat fibroblasts, and immortalized human erythromyelosis K-562 cells. These agents do not suppress the formation of fibroblast colonies and RBTL. Moreover, T -36 in the lowest concentration (0.1 ng/ml) increases spontaneous proliferative activity of normal lymphocytes. The data suggest that

tripeptides stimulate nontumor immune cells in adult people. This suggests that embryonic and immortalized cells can serve as a main target for the antiproliferative effect of tripeptides. The inhibition of proliferation in embryonic and immortalized cells under the influence of tripeptides probably reflects antitumor activity of these substances. Tripeptides have no effect on survival and adhesion, cytotoxic, and PHA-induced proliferative activities of lymphocytes. They do not change the engulfment activity and spontaneous or induced bactericidal action of granulocytes.

Our results indicate that tripeptides have various specific effects on the cell. They decrease proliferative activity of immortalized and embryonic cells, but stimulate proliferation of human leukocytes. The data allow us to classify these tripeptides as regulators of proliferation. They have a positive effect on normal cells, but inhibit embryonic and immortalized cells. Therefore, these tripeptides hold much promise for immunotherapy of tumor diseases.

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