PHARMACOLOGY AND TOXICOLOGY

Cytostatic Activity of Peptide Extracts of Medicinal Plants on Transformed A549, H1299, and HeLa Cells

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Biological activity of peptide extracts of medicinal plants was studied on transformed non-small-cell lung carcinoma A549 cells, lung cancer H1299 cells, and cervical cancer HeLa cells at various cell densities. Cell survival and proliferation were evaluated 72 h after treatment with extracts in concentrations of 0.05, 0.25, and 0.5 μg/μl. The cytostatic effect was produced by peptide extracts of *Camelia sinesis Kuntze*, *Inonotus obliquus,* and a mixture *Inula helenium L., Chelidonium majus L., Equisetum arvense L.*, and *Inonotus obliquus.* Peptide extracts of *Hypericum perforatum L.* and *Laurus nobilis L.* in the same concentrations had no effects on proliferative activity and growth of tumor cells.

Key Words: *plant peptides; A549, H1299 and HeLa cells; cytostatic effect*

The search for new antitumor drugs exhibiting low toxicity against normal cells is now in progress. Among them are peptides of plant origin [5,6,7,11]. It is known that cyclotides (astins, viscotoxins, *etc.*), peptides initially isolated from plants belonging to some genera of the *Rubiaceae* and *Violaceae* families, exhibit antitumor activity [5,9,10]. We previously isolated peptide extracts from some plants and demonstrated *in vivo* antitimor activity of extract from *Hypericum perforatum L.* (PE-Hp) and extract from plant mixture *Chelidonium majus L., Inula helenium L., Equisetum arvense L.* and *Inonotus obliquus* (PE-PM) on the models of mouse breast cancer [3].

Here we evaluated biological activity of a panel of peptide extracts from plant raw material on various strains of human cancer cells.

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MATERIALS AND METHODS

Peptide extracts were prepared from plants approved by Pharmacopeia of the Russian Federation as drug plants: a plant mixture *Inula helenium L., Chelidonium majus L., Equisetum arvense L.*, and *Inonotus obliquus* (PE-PM); and from individual plants *Camelia sinesis Kuntze* (PE-Cs), *Inonotus obliquus* (PE-Io), *Hypericum perforatum L.* (PE-Hp), and *Laurus nobilis L.* (PE-Ln) (Zdorov'e company). Peptide extracts were prepared as described previously [3,4] and quantitatively characterized by methods of amino acid and carbohydrate analysis [1,2]. According to quantitative amino acid analysis, extracts primarily consisted from peptides (85-95%) and contained less than 5-10% carbohydrates.

The antitumor effects of the analyzed plant extracts were evaluated on A549 and H1299(p53-/-) human non-small-cell lung cancer cell strains and Hela human cervical cancer cells. The cells were incubated in nutrient mixtures: RPMI-1640 (Pan-Eko) for A549 and H1299 cells and DMEM (Pan-

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Eko) for HeLa cells. The cells were seeded into 96-well plates with various densities designated as high (100% confluence 24 h after seeding), medium (50-70%), and low (20-50%) density. Peptide extracts disolved in nutrient medium in concentrations of 0.05, 0.25, 0.5 μg/μl (in triplicates for each concentration) were added 12 h after seeding.

Activity of extracts was detected using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)-assay, a method of quantitative eva-

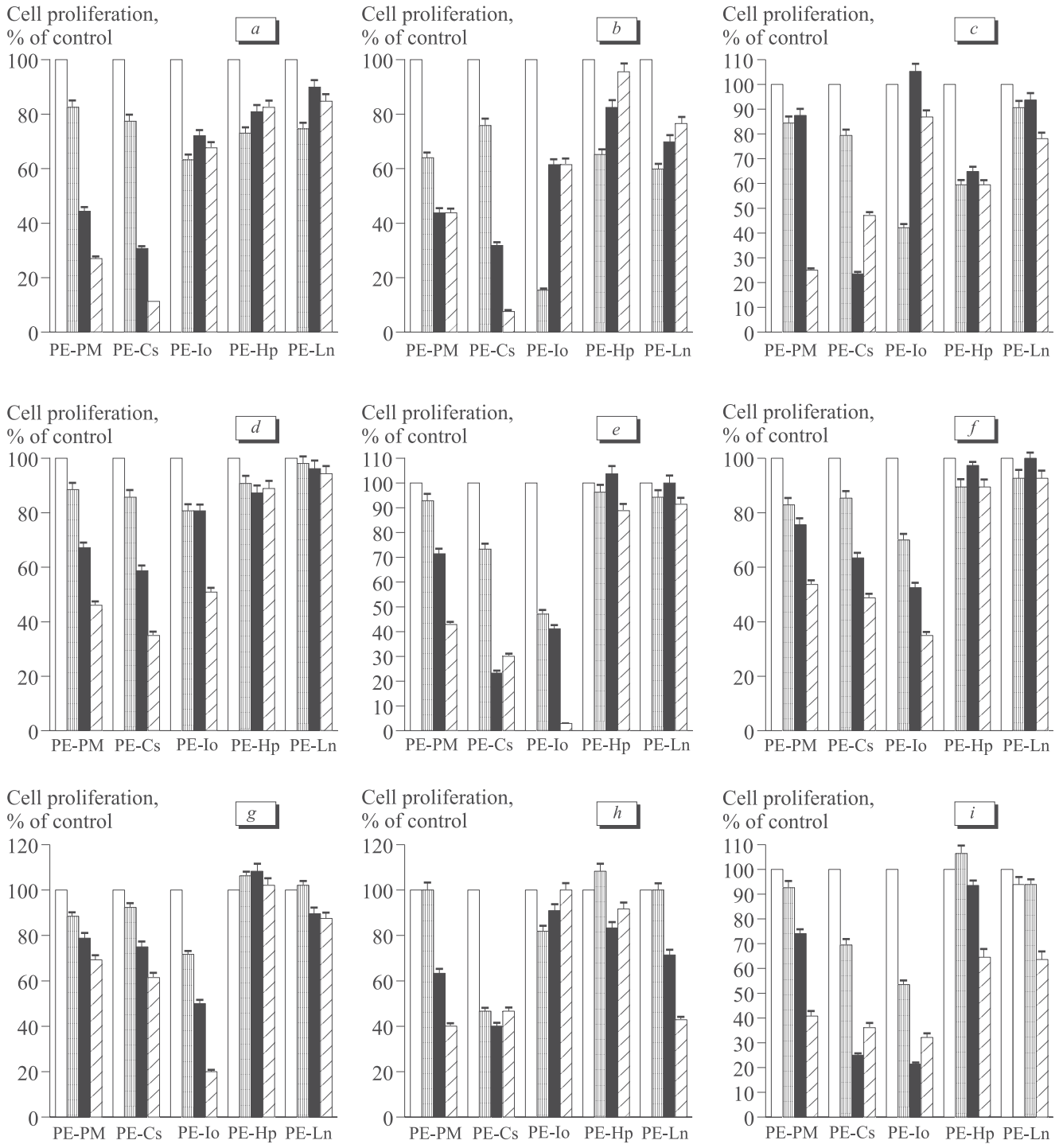

Fig. 1. Effect of peptide extracts on proliferation of A549, H1299, and HeLa cells after 72 h (MTT assay). *a*) high density of A549 cells, *b*) high density of H1299 cells, *c*) high density of HeLa cells, *d*) medium density of A549 cells, *e*) medium density of H1299 cells, *f*) medium density of HeLa cells, *g*) low density of A549 cells, *h*) low density of H1299 cells, *i*) low density of HeLa cells. Light bars: control; vertical shading: peptide concentration 0.05 μg/μl; dark bars: 0.25 μg/μl; horizontal shading: 0.5 μg/μl

luation of cell survival during incubation in a medium containing a cytotoxic agent [8]. Cell survival in the control and after treatment with the plant extracts was determined after 72-h incubation. The medium in the plate was replaced with a medium with MTT (0.5 mg/ml, 100 μl per well). The cells were incubated in a CO_2 incubator for 1 h and then the medium was removed, the cells were lysed in 100 μl 0.04 M HCl in isopropanole. Optical density was measured at λ=590 nm using a Bio-Rad Benchmark Plus spectrophotometer.

For morphological analysis, the wells with cells were examined under an Olympus BX40 and photographed with Olympus C-3030 camera.

RESULTS

Experiments on A549 cells seeded with high density revealed cytostatic effects of extracts from plant mixture (less than 30% viable cells) and *Camelia sinesis Kuntze* (10% viable cells); the concentration of peptides was 0.5 μg/μl (Fig. 1, *a*). *Inonotus obliquus* extract also inhibited cell growth in culture. At medium and low seeding densities, the inhibiting effects were produced by PE-PM (Fig. 1, *d*, *g*), PE-Cs (Fig. 1, *d*, *g*), and PE-Io (Fig. 1, *d*, *g*) at different concentrations of peptides. Peptide extracts from *Hypericum perforatum L.* (PE-Hp) and *Laurus nobilis L.* (PE-Ln) had no effects on A549 cells (Fig. 1, *a*, *d*, *g*) and even slightly stimulated cell growth at low cell seeding density (Fig. 1, *g*). Morphological analysis of A549 cells revealed the maximum cytostatic effect of PE-PM (Fig. 2, *d*) and PE-Cs (Fig. 2, *g*) at peptide concentration of 0.5 μg/μl and high seeding density.

All peptide extracts except PE-Hp produced a cytostatic effect on H1299 cell at high cell density (Fig. 1, *b*). Under conditions of medium cell den-

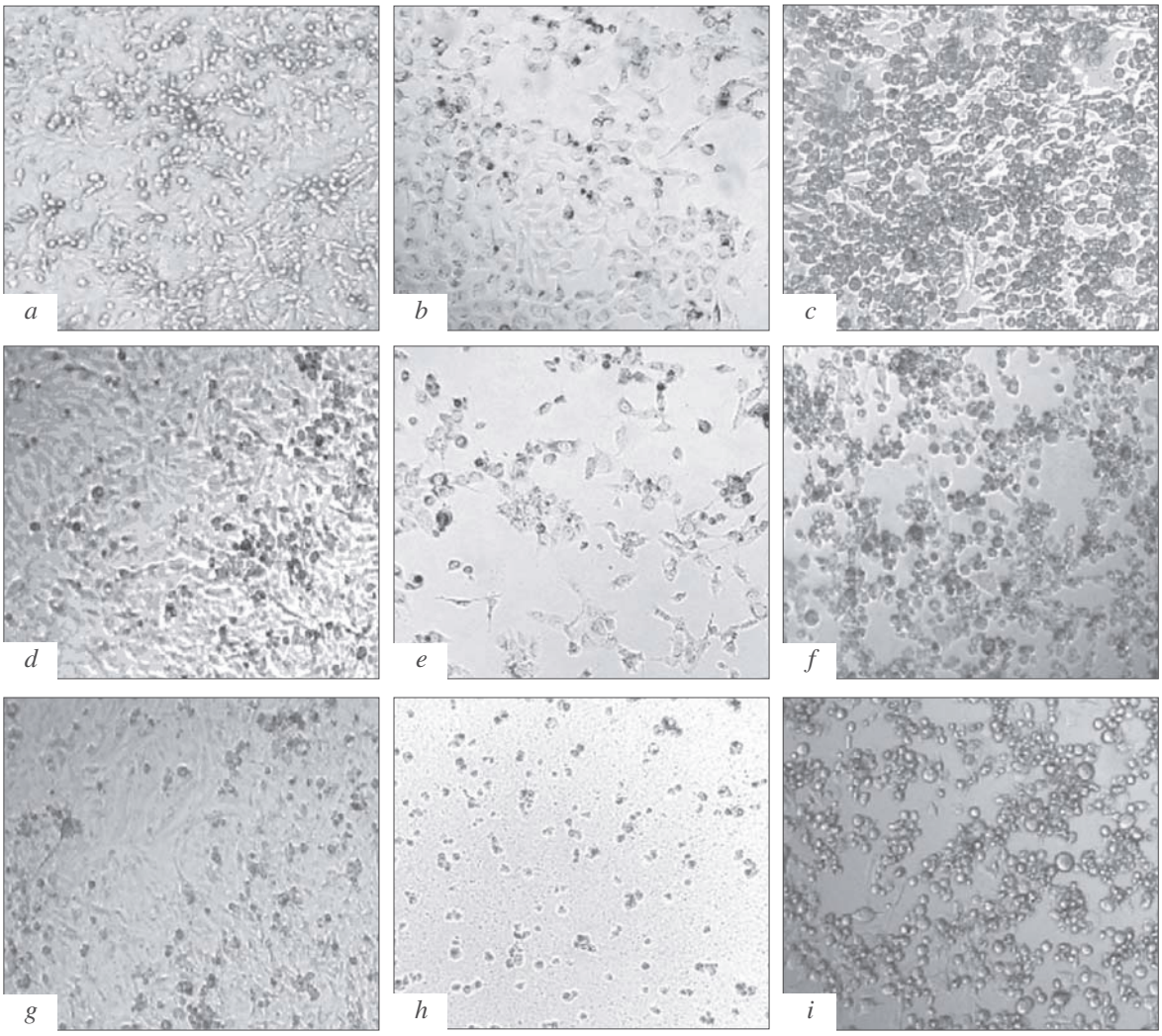

Fig. 2. A549, H1299, and HeLa cells at high density. Peptide concentration 0.5 μg/μl. *a*) control, A549; *b*) control, H1299; *c*) control, HeLa; *d*) plant mixture, A549; *e*) plant mixture, H1299; *f*) plant mixture, HeLa; *g*) *Camelia sinesis Kuntze,* A549; *h*) *Camelia sinesis Kuntze*, H1299; *i*) *Camelia sinesis Kuntze*, HeLa.

sity, the maximum effects were produced by PE-Io (3% viable cells, Fig. 1, *e*), PE-Cs (30%, Fig. 1, *e*), and PE-PM (45%, Fig. 1, *e*) at peptide concentration of 0.5 μg/μl. However, at low seeding density PE-PM in this concentration had no effect on cell growth (Fig. 1, *h*). PE-Hp in concentrations of 0.25 and 0.05 μg/μl produced a stimulating effect on H1299 cells grown at medium and low densities, respectively (Fig. 1, *e*, *h*). PE-Ln had no appreciable effect on the growth of H1299 cells. Morphological analysis of H1299 cells showed that PE-PM (Fig. 2, *e*) and PE-Cs (Fig. 2, *h*) produced maximum cytostatic effect at high cell seeding density compared to the control (Fig. 2, *b*).

The most pronounced effect on HeLa cells seeded with high density was produced by PE-PM (25% viable cells at peptide concentration 0.5 μg/μl) and PE-Cs (25% viable cells at peptide concentration 0.25 μg/μl, Fig. 1, *c*). Pe-Hp and PE-Ln had practically no effect on cell growth (Fig. 1, *c, f, i*). At low cell density, the cytostatic effect was produced by PE-PM (40% viable cells at peptide concentration 0.5 μg/μl), PE-Io (20% viable cells at peptide concentration 0.25 μg/μl), and PE-Cs (25% viable cells at peptide concentration 0.25 μg/μl, Fig. 1, *i*). Cytomorphological analysis of HeLa cells also revealed the cytostatic effect of PE-PM and PE-Cs (Fig. 2, *f, i*).

Our previous experiments on mouse breast cancer model demonstrated antitumor activity of extracts from *Hypericum perforatum L.* and from plant mixture, but extract from *Camelia sinesis Kuntze* exhibited no antitumor activity [3]. The results obtained on cell strains show that extract from *Camelia sinesis Kuntze* produced a pronounced cytostatic effect, while the preparation from *Hypericum perforatum L.* had no effect on cell growth. It can be concluded that the preparation from *Hypericum perforatum L.* probably exhibits an immunomodulating activity *in vivo*, but has no effect on proliferation of transformed cell strains *in vitro*.

Thus, we demonstrated the cytostatic effect of peptide extracts from plant mixture (*Chelidonium majus L., Inula helenium L., Equisetum arvense L.* and *Inonotus obliquus*) and extract from *Camelia sinesis Kuntze* on the growth of A549, H1299, and HeLa transformed cell strains. The peptide extracts from *Inonotus obliquus* exhibits the most pronounced cytostatic effect at medium density of H1299 cells, which can be explained by the absence of the expression of p53 in these cells. Extracts from *Laurus nobilis L.* and *Hypericum perforatum L.* were ineffective on these tumor cell cultures.

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