ONCOLOGY

Evaluation of Antitumor Activity of Peptide Extracts from Medicinal Plants on the Model of Transplanted Breast Cancer in CBRB-Rb(8.17)1Iem Mice

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We studied antitumor effects of peptide extracts from plants on slowly growing mammary adenocarcinoma in CBRB-Rb(8.17)1Iem mice used as a model of breast cancer in humans. The antitumor effect of a single injection of the test peptides was evaluated by the delay of the appearance and growth of palpable breast cancer in mice over 4 weeks. Peptides from *Hypericum perforatum* and a mixture of *Chelidonium majus L.*, *Inula helenium L.*, *Equisetum arvense L.*, and *Inonotus obliquus* exhibited maximum activity. Peptide extracts from *Frangula alnuc Mill.* and *Laurus nobilis L.* were less active. No antitumor effect of *Camelia sinesis Kuntze* was detected.

Key Words: *plant peptides; experimental model of human breast cancer; CBRB-Rb(8.17)1Iem mice; antitumor activity*

Breast cancer (BC) is one of the most prevalent causes of female mortality in countries with well-developed industry [2]. The search for new drugs, including those of plant origin, for the treatment of these patients is never ceasing. Testing of these drugs on mice is an important problem [3].

Peptides of animal origin, for example, angiogenesis inhibitors, attract now special interest [1]. In addition to peptides of animal origin, antitumor activity was detected for peptides extracted from plants, for example, cyclotides [8]. The interest to plant peptides with antitumor activity is increasing in recent years [5,7].

We studied antitumor activity of peptide extracts from natural medicinal plants on the model of BC in mice.

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MATERIALS AND METHODS

Peptides were extracted from plant preparations manufactured by the Zdorovye Company. Dry plants (10 g) were pulverized in a knife homogenizer. Acetic acid (1 M; 100 ml) was added to the resultant powder and thoroughly mixed. The homogenate was sonicated and heated at 100°C. The mixture was cooled and centrifuged. Acetone was added to the supernatant (2:5), the precipitate was separated by centrifugation and dried (the procedure was described in detail previously [4]). Dry precipitate was dissolved in 0.1 M acetic acid and centrifuged for 20 min at 40,000*g*. The resultant supernatant was lyophilized twice and dry residue was dissolved in water. The peptide material output was 50-100 mg.

The following peptide preparations of natural origin were obtained: peptide extracts from St. John

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wort (*Hypericum perforatum L.*), alder buckthorn (*Frangula alnuc Mill.*), green tea (*Camelia sinesis Kuntze*), Grecian laurel (*Laurus nobilis L.*), and a mixture of plants: celandine (*Chelidonium majus L.*), elecampane (*Inula helenium L.*), horsetail (*Equisetum arvense L.*), and tree fungus *Inonotus obliquus*. The preparations (0.5-2.0 mg) were put into glass tubes, 200 μl mixture of hydrochloric and propionic acids (50:50) was added, and the mixture was incubated at 140°C for 2.5 h in thermoblocks (Pierce). Analysis on an LC-3000 amino acid analyzer (Eppendorf/Biotronik) showed that the preparations contained 85-95% peptide material.

Antitumor activity was studied on male CBRB-Rb(8.17)1Iem (CBRM) mice aging 4.5±0.5 months, 5-7 animals per group. Recipients received 10^6 tumor cells of slowly growing spontaneous mammary adenocarcinoma obtained from syngeneic females as described previously [6]. On the next day after transplantation, experimental animals received single injection of plant peptide extracts (1 mg in 0.1 ml saline) to the site of transplantation. Controls received an equivalent volume of saline.

Anticancer activity of the preparations was evaluated by the dynamics of appearance and growth of transplanted palpable BC in mice over 4 weeks after transplantation. Three perpendicular sizes of the tumor were measured and its mean diameter was calculated from these values for each recipient [6]. The results were evaluated using Student's *t* test.

RESULTS

The anticancer effect of single injection of medicinal plant peptide extracts was tested on transplanted slowly growing spontaneous mammary adenocarcinoma of CBRB mice.

The dynamics of appearance of palpable BC is presented in Figure 1. The preparations differed by the degree and duration of antitumor activity, exhibiting short-term (week 1) or lasting (up to week 3 after tumor transplantation) effects.

All peptide extracts except green tea extract delayed the appearance of the tumor during week 1: palpable tumors were detected in 33% treated mice *vs.* 60% in the control.

During week 2 after transplantation, tumor appearance was delayed only in mice receiving *Hypericum perforatum L.* extract and extract from a mixture of plants: palpable tumors were detected in 67% treated mice *vs.* 100% controls.

Hence, single injection of green tea extract had virtually no effect on tumor appearance. *Frangula alnuc Mill.* and *Laurus nobilis L.* peptide extracts provided short-term inhibition of tumor appearance.

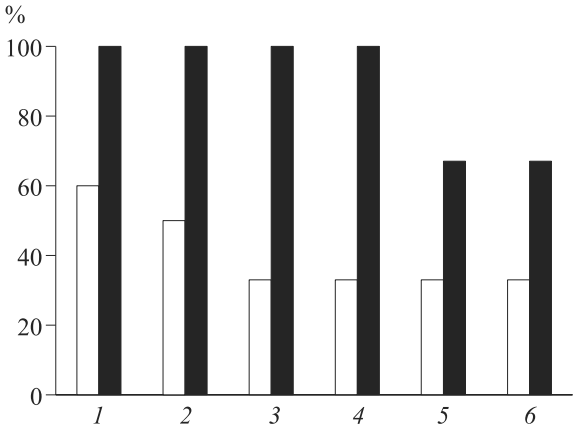

Fig. 1. Delay of tumor appearance over 1 (light bars) or 2 weeks (dark bars) in CBRB mice after single treatment with plant peptide extracts. *1*) control group; *2*) peptide extract from *Camelia sinesis Kuntze*; *3*) peptide extract from *Frangula alnuc Mill.*; *4*) peptide extract from *Laurus nobilis L.*; *5*) peptide extract from *Hypericum perforatum L.*; *6*) peptide extract from a mixture of medicinal plants and *Inonotus obliquus*.

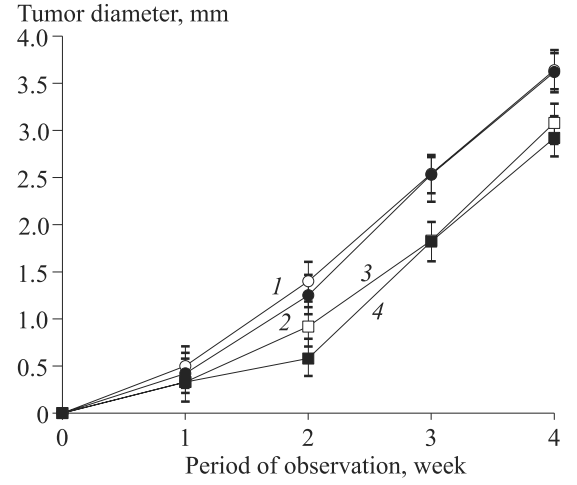

Fig. 2. Tumor growth inhibition in CBRB mice after single treatment with peptide extracts. *1*) control group; *2*) peptide extract from *Camelia sinesis Kuntze*; *3*) peptide extract from *Hypericum perforatum L.*; *4*) peptide extract from a mixture of medicinal plants and *Inonotus obliquus*.

The maximum delay of tumor appearance was observed in mice injected with peptide extracts from a mixture of plants and *Hypericum perforatum L.*

Tumor growth in mice injected with green tea peptides did not differ from that in the control (Fig. 2). A significant inhibition of tumor growth by 20 and 25% during week 4 after transplantation was observed only in animals treated with peptides from *Hypericum perforatum L.* and a mixture of plants, respectively. *Frangula alnuc Mill.* and *Laurus nobilis L.* peptide extracts negligibly inhibited tumor growth.

Hence, peptide extracts from *Hypericum perforatum L.* and a mixture of medicinal plants exhibited a pronounced antitumor activity in CBRB mice with BC. *Frangula alnuc Mill.* and *Laurus nobilis L.* peptide extracts exhibited short-term effects on the dynamics of appearance and growth of palpable BC. Green tea peptide extract had no antitumor activity.

The results indicate that peptide preparations from *Hypericum perforatum L.*, *Chelidonium majus L.*, *Inula helenium L.*, *Equisetum arvense L.*, and *Inonotus obliquus* are perspective objects for further studies.

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