Effects of cathepsin D inhibitor from *Vicia sativa* L. seed hulls on human skin fibroblasts and breast cancer cells (*in vitro* studies)

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Abstract

Cathepsin D is a lysosomal protease which plays an important role in cancer invasion and metastasis. There are known inhibitors of that enzyme, such as pepstatin and potato inhibitor. In this study, we examined effects of the cathepsin D inhibitor from *Vicia sativa* L. seed hulls on cell cultures of human skin fibroblasts and breast cancer cells. There is no effect of the D-cathepsin inhibitor from Vicia sativa L. seed hulls on the proliferative activity of either human skin fibroblasts or breast cancer cells, measured by the [3H] thymidine incorporation assay.

Key words: cathepsin D inhibitor, cathepsin D, fibroblasts, breast cancer cells, *Vicia sativa* L.

Introduction

Cathepsin D is ubiquitous lysosomal protease with important functions in protein catabolism. Three forms of the enzyme are known: 52 kDa procathepsin D, a 48-kDa intermediate form and stable cathepsin D with 34-kDa heavy and a 14-kDa light chain. Cathepsin D plays a key role in metastatic spread by promoting destruction of normal tissue architecture and in tumour growth by influencing of growth factors [1, 2]. There are known inhibitors of cathepsin D, such as pepstatin and potato inhibitor [3, 4].

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Material and methods

The specific objective of the studies was to examine the susceptibility of the cathepsin inhibitor D from seeds of *Vicia sativa* L. to the action of cathepsin D and its effect on DNA and cathepsin D activity in human skin fibroblasts and breast cancer cells MDA MB-231.

Peptide cathepsin D inhibitor was isolated from *Vicia sativa* L. seed hulls [5, 6]. Normal human skin fibroblasts were maintained in DMEM, supplemented with 10% FBS, 2 mmol glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin at 37°C in 5% CO_2 in an incubator. The cells were used between 12th and 14th passages. The fibroblasts were subcultivated by tripsinization. Breast cancer cells were maintained in DMEM, supplemented with 10% FBS, 2 mmol glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin at 37°C in 5% CO_2 in an incubator. The cells were cultured in Costar flasks and subconfluent cells were detached with 0.05% trypsin and 0,02% EDTA in calcium free PBS, plated at six-well plates (Nunc) in 2 ml of growth medium (DMEM without phenol red). The cells reached confluence at day 4 and, in most cases; such cells were used for the experiments.

In order to examine the effect of the studied inhibitor on cell proliferation, the fibroblasts were plated in 24-well tissue culture dishes at 1 x 10^5 cells/well and breast cancer cells at 1 x 10^6cells/well in 1 ml of growth medium. After 48h the cells were incubated at varying concentrations of the inhibitor (0.02, 0.2, 2.0 and 20 µmol/l) and 10 µl of 6.7 Ci/mM of [^{3}H] thymidine for 24 h at 37°C. The cells were rinsed three times with PBS, solubilized with 1 ml of 0.1 mol/l of sodium hydroxide, containing 1% SDS, scintillation fluid (3 ml) was added and radioactivity incorporation into DNA was measured in a scintillation counter. The activity of cathepsin D was determined, according to the used method [7]. Briefly, the monolayer was washed three times with 0.15 M NaCl. The cells were collected by scraping and suspended in 0.15 NaCl and centrifuged (2700 x g, 30 min) at 4°C. The cells were sonicated three times for 10 s at 0°C. The sam-

Table 1. Cathepsin D activity and protein concentration in supernatant and sediments of fibroblasts and breast cancer cells in presence of the inhibitor from *Vica sativa* L. seed hulls.

Cell inhibitor concentration	**Supernatant**		**Sediments**	
	Protein, mg/ml	Cathepsin D activity, Tyr, nmol/ml	Protein, mg/ml	Cathepsin D activity, Tyr, nmol/ml
Fibroblasts				
0.15 mol/l NaCl	4.64	2.4	0.098	24.8
0.02 ěmol/l	4.42	2.5	0.102	19.2
0.2 ěmol/l	4.47	2.4	0.102	19.2
2.0 ěmol/l	4.65	2.5	0.107	22.8
20 ěmol/l	4.69	2.4	0.118	24.8
MDA MB-231				
0.15 mol/l NaCl	4.65	5.3	0.103	80.0
0.02 ěmol/l	4.55	5.3	0.108	28.0
0.2 ěmol/l	4.58	5.3	0.108	28.8
2.0 ěmol/l	4.64	5.1	0.106	20.0
20 ěmol/l	4.61	5.2	0.104	12.8

ples were then centrifuged (16 000 x g, 30min) at 4°C. The sediment and the supernatant were used for protein determination by the Bradford method [8] and then by the cathepsin D activity assay.

Results and discussion

There is no antiproliferative activity of the cathepsin D inhibitor on human skin fibroblasts and breast cancer cells, measured by the [^{3}H] thymidine incorporation assay. D-cathepsin activity, measured in fibroblast supernatant, was 2.4 nmol Tyr/ml and, in breast cancer cells, it was 5.3 nmol Tyr/ml and there was no correlation between the activity and the inhibitor concentration Table 1. In the sediments of fibroblast homogenates, D-cathepsin activity in control without the inhibitor was 24.8 nmol/ml, while with the inhibitor, it ranged from 19.2 to 24.8 nmol/ml. In breast cancer cell sediments, the control activity was 80 nmol/ml and with the inhibitor activity, it was decreased to 12-28 nmol/ml, according to inhibitor concentration. The protein concentration in the supernatant fibroblast and breast cancer cells was 4.6 ng/ml and, in the sediments, it was 0.106 mg/ml.

There is no effect the D-cathepsin inhibitor from *Vicia sativa* L. seed hulls on the proliferative activity of human skin fibroblasts and breast cancer cells, measured by the [^{3}H] thymidine incorporation assay. The cathepsin D inhibitor from seeds of *Vicia sativa* L. does not penetrate into fibroblasts cells, but it penetrates inside breast cancer cells.

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