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CYTOTOXIC ACTIVITY OF ε-AMINOCAPROYLAMINO ACIDS IN BREAST CANCER MCF-7 AND FIBROBLAST CELL LINES

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Abstract: The effect of H-EACA-L-Cys(S-Bzl)-OH, H-EACA-L-Leu-OH, H-EACA-L-Nle-OH and EACA on the viability of MCF-7 and fibroblast cells was examined. The antibacterial activity of these compounds was also tested. H-EACA-L-Leu-OH and H-EACA-L-Nle-OH showed cytotoxic activity against MCF-7 and fibroblast cell lines, particularly in the highest studied 20 mM concentration. None of the examined dipeptides showed antibacterial activity.

Keywords: ε-aminocaproylamino acids, cytotoxity, MCF-7, fibroblast cells, antibacterial activity

ε-Aminocaproic acid (EACA) and *trans*-aminomethylcyclohexanecarboxylic acid (AMCHA) are clinically used as the inhibitors of fibrinolytic activity. They block the lysine binding sites of plasminogen and plasmin. The effect of these ω-amino acids towards plasmin with respect to fibrinogen, other proteins and synthetic substrates is much weaker than towards fibrin because these compounds do not influence the enzyme active centre.

The ε-aminocaproyl derivatives of α-amino acids with a bulky, hydrophobic side chain significantly inhibited the fibrinolytic activity of plasmin (1). It can be the result of forming an antifibrinolytic active conformation (2). ε-Aminocaproyl-S-benzyl-L-cysteine (H-EACA-L-Cys-(S-Bzl)-OH) showed only antifibrinolytic activity but the ε-aminocaproyl derivatives of leucine (H-EACA-L-Leu-OH) and norleucine (H-EACA-L-Nle-OH) showed also certain levels of antifibrinogenolytic and antiamidolytic activities (1). Because these dipeptides can be potential antifibrinolytic agents, their effect on the fibrinolytic activity of euglobulin fraction, aggregation of platelet and some other aspects of hemostasis was also examined (3, 4). Plasmin, a key enzyme of fibrinolysis plays an important role in a variety of biological processes such as wound healing, tissue repair, cell migration and is also important in pathological phenomena such as inflammation, tumor cell growth and metastasis (5). Plasmin inhibitors, eg. N^{α}-acetyl-L-lysine methyl ester (6) and the derivative of dipeptide containing AMCHA – YO-2 (*trans*-aminomethylcyclohexanecarbonyl-(O-picolyl)-tyrosine octylamide) (7, 8) possess proapoptocic action. YO-2 exerts an apoptosis-inducing effect on various human cell cultures (9, 10), it also inhibits the growth of human tumors, decreases the amount of metastasis and it can be a potential antitumor agent (9). Antibacterial activity of some ω-aminoacyl derivatives of amino acids is well known (11, 12). ε-Aminocaproyl derivatives of L-histidine and L-lysine possessed antistaphylococcal activity but only ε-aminocaproyl-L-lysine (H-EACA-L-Lys-OH) was antifibrinolytic agent.

It seems to be interesting to check if EACA containing dipeptides which possessed antifbrinolytic activity can also show antitumor or antibacterial activity. In the present paper we examine the

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influence of H-EACA-L-Cys(S-Bzl)-OH, H-EACA-L-Leu-OH, H-EACA-L-Nle-OH and EACA on the viability of MCF-7 and fibroblast cells. Antibacterial activity of these compounds was also examined and compared with the activity of chloramphenicol.

EXPERIMENTAL

Reagents

The examined compounds were synthesized in the Department of Organic Chemistry of University of Białystok (1). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma. Dulbecco's minimal essential medium (DMEM) and fetal bovine serum (FBS) used in cell culture were products of Gibco (USA). Penicillin and streptomycin were obtained from Quality Biological Inc. (USA).

Fibroblast cultures

Normal human skin fibroblasts were maintained in DMEM supplemented with 10% FBS, 50 U/mL penicillin, 50 µg/mL streptomycin at 37°C in 5% CO_2 in an incubator. The cells were used between the 12th and 14th passages. The fibroblasts were subcultivated by trypsinization. Subconfluent cells from Costar Flasks were detached with 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) in calcium-free phosphate-buffered saline (PBS). Cells were counted in hemocytometers and cultured at 1×10^5 cells per well in 2 mL of growth medium.

MCF-7 cultures

Breast cancer MCF-7 cells were maintained in DMEM supplemented with 10% FBS, 50 µg/mL penicillin, 50 µg/mL streptomycin at 37°C in 5% CO_2 in an incubator. Cells were cultured in Costar flasks and subconfluent cells were detached with 0.05% trypsin, 0.02% EDTA in calcium-free phosphate-buffered saline, counted in hemocytometers and inculated at 5×10^5 cells per well of six-well plates (Nunc, Wiesbaden, Germany) in 2 mL of growth medium. Cells reached about 80% of confluence at day 3 after inoculation and in most cases such cells were used for the assays.

Cell viability assay

The assay was performed according to the method of Carmichael using MTT (13). Confluent cells, cultured for 48 h with various concentrations of studied compounds in 6-well plates, were washed three times with PBS and then incubated for 4 h in 1 mL of MTT solution (0.5 mg/mL of PBS) at 37°C in 5% CO_2 in an incubator. The medium was removed and 1 mL of 0.1 M HCl in absolute isopropanol was added to attached cells. Absorbance of

Table 1. Cytotoxic activity of dipeptides, expressed as percentage of the survivability MCF-7 mammal tumor cells.

Concentration of compound (mM)	EACA	H-EACA-Nle-OH	H-EACA-LeuOH	H-EACA-Cys(S-Bzl)-OH
0	100	100	100	100
0.02	98 ± 0.63	98 ± 0.63	97 ± 0.63	98 ± 0.63
0.2	98 ± 0.63	95 ± 1.09	97 ± 0.63	98 ± 0.63
2	98 ± 0.63	70 ± 1.67	80 ± 1.06	98 ± 0.63
20	95 ± 1.09	0	0	80 ± 1.09

Table 2. Cytotoxic activity of dipeptides, expressed as percentage of the survivability of fibroblast cell lines.

Concentration of compound (mM)	EACA	H-EACA-Nle-OH	H-EACA-LeuOH	H-EACA-Cys(S-Bzl)-OH
0	100	100	100	100
0.02	100	100	95 ± 1.09	100
0.2	100	85 ± 1.72	80 ± 1.09	100
2	100	80 ± 1.09	50 ± 1.26	100
20	95 ± 1.09	15 ± 1.66	5 ± 0.63	90 ± 0.89

Table 3. Minimum inhibitory concentrations (μg/mL) of ε-aminocaproylamino acids and EACA (chloramphenicol was used as a standard compound).

Microorganism	Compound				
	Chloramphenicol (*D-threo-*)	EACA	H-EACA-Nle-OH	H-EAC-Leu-OH	H-EACA-Cys(S-Bzl)-OH
Staphylococcus aureus ATTC 6538	0.5	> 200	> 200	> 200	> 200
Micrococcus luteus ATCC 9391	0.5	> 200	> 200	> 200	> 200
Bacillus subtilis ATCC 6633	0.5	> 200	> 200	> 200	> 200
Pseudomonas aeruginosa ATCC 27853	> 200	> 200	> 200	> 200	> 200
Escherichia coli ATCC 29922	1.0	> 200	> 200	> 200	> 200

converted dye in living cells was measured at a wavelength of 570 nm. Cell viability of breast cancer MCF-7 and fibroblast cells cultured in the presence of examined compounds was calculated as a percent of control cells ± SD. The results are given in Tables 1 and 2.

Antibacterial assay

The assay was performed according to the literature method (14). The mother solution of the compound in ethanol, of the concentration of 10 mg/mL was twice diluted by the Mueller-Hinton broth. Two more dilutions of the solutions with the broth were performed in order to obtain working solutions of the concentrations from 512 μg/mL to 0.5 μg/mL. Fifty μL of each working solution was placed in the plate cavities for microcoagulation. To each portion of the solution 50 μL of a suspension of microorganisms in the broth, containing 2×10^5 of cells in 1 μL was added. The obtained mixtures were incubated for 18 h at 37°C. After 18 h, it was established whether the growth of microorganisms had been halted in the colonies. The results are given in Table 3.

RESULTS AND DISCUSSION

The two examined dipeptides: H-EACA-L-Leu-OH and H-EACA-L-Nle-OH show antitumor properties against MCF-7 cells, particularly in the largest studied 20 mM concentration (Table 1). Both compounds also show the cytotoxic activities against cells of fibroblasts (Table 2). In the case of H-EACA-L-Leu-OH, this effect was observed also at lower 2mM concentration, in which this compound reduces the survivability of fibroblast cells by 50%. In the case of EACA and H-EACA-L-Cys(S-Bzl)-OH, the cytotoxity in the both tests practically was not observed. Only at the maximum 20 mM concentration, the last dipeptide showed a weak cytotoxic effect against MCF-7 breast cells. According to the results obtained, it is possible to suggest that the compounds with specific antifibrinolytic activity (EACA and H-EACA-L-Cys(S-Bzl)-OH) do not show cytotoxic activity against MCF-7 and fibroblast cells (Table 1, 2).

Antitumor activity was observed only in the case of the antifibrinolytic dipeptides which are also the weak inhibitors of proteolytic and amidolytic activities of plasmin (1). These compounds influence not only the lysine binding sites but also the active centre of the enzyme. The relationship between antitumor activity and plasmin inhibition was observed earlier in the case of YO compounds (8, 15) but the correlation was not clear. Poor plasmin inhibitors did not effect apoptosis induction, but not every potent active centre directed inhibitor of this enzyme showed proapoptocic activity. Additionally, the apoptosis induction was not observed in the case of classic plasmin inhibitors such as diisopropylfluorophosphates or leupeptin (15). The problem of the possible relationship

between an inhibition of plasmin and anticancer activity needs further investigation. The examined antifibrinolytics did not showed antibacterial activity (Table 3) and probably there is no relathionship between these activities. Described earlier (12) antistaphylococcal activity of antifibrinolytic dipeptide: H-EACA-L-Lys-OH is probably rather connected with the fact that some of lysine derivatives (16) can show antibacterial properties.

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