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Biological Activity of a Newly Synthesized Specific Inhibitor of Aminopeptidase A: A Preliminary Study

Vessela Petrova[1], Donka Tasheva[2], Ivan Iliev[1], Mashenka Dimitrova[1], Ivaylo Ivanov[3]*

[1] *Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria*
[3] *Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria*

*Corresponding author e-mail: mashadim@abv.bg

According to the recent studies, aminopeptidase A (APA) activity is very low in the tumor tissue of human mammary gland carcinoma, whereas the enzyme is well expressed in the normal breast tissue. In order to elucidate if this is a secondary phenomenon or part of the tumor phenotype of the cells, we determined (MTT test) the effect of the newly synthesized APA inhibitor α-glutamylhydroxamate (GH) on the proliferative activity and survival rate of three types of human cells: MCF-10A (epithelial cells of mammary gland), MCF-7 (luminal adenocarcinoma type A) and MDA-MB-231 (triple negative carcinoma). The results show that the inhibitor is not toxic to all three cell lines, but its application enhances the proliferation of normal MCF-10A cells. According to these results, the decrease of APA activity may increase the survival and division of normal cells (tumor phenotype).

Key words: aminopeptidase A, enzyme inhibitor, cultured cells, MTT test, mammary gland carcinoma

Introduction

Aminopeptidase A (APA, EC 3.4.11.7) is a plasma membrane-associated enzyme, catalyzing the hydrolysis of glutamic (Glu) or aspartic (Asp) acidfrom the N-terminal of natural peptides and synthetic substrates ($pH_{opt} \approx 7.6$) [7]. The enzyme is a part of the systemic and local renin-angiotensin systems (RAS), where it hydrolyzes angiotensin II to angiotensin III (AngII to AngIII) thus participating in the regulation of blood pressure [3]. APA has been also found in the invasive front of different types of tumors [e.g. 2, 8] where it participates in the opening of free spaces for tumor growth, together with matrix metallopeptidases. On the other hand, in AngII-mediated cancers (like breast cancer) the enzyme may act as a tumor-suppressor by minimizing the cell migration and angiogenesis, induced by AngII [1]. Biochemical studies of biopsies from patients with mammary gland carcinoma have shown a lower activity of APA in the tumor tissue in comparison to the adjacent normal tissue [4, 5]. We obtained similar results in Erlich's

model of breast carcinoma of mice and in a model system of three types of human mammary gland cells [unpublished results]. Since APA is a tumor suppressor in breast carcinoma, it would be valuable to test whether the inhibition of the enzyme may result in a promotion of tumor phenotype in normal breast epithelial cells, which is the aim of the present study.

Materials and Methods

Three types of human cells were used in the study: MCF-10A (immortalized epithelial cells of mammary gland), MCF-7 (luminal adenocarcinoma type A) and MDA-MB-231 (triple negative carcinoma). The tumor cells were cultured in DMEM high glucose medium with 10% FBS and 100 µg/ml penicillin/streptomycin in a humidified atmosphere with 5% CO_2 at 37ºC. The normal cells were grown in the same conditions but with the addition of 20 µg/ml hEGF, 0.5µg/ml hydrocortisone, 0.1µg/ml cholera toxin and 10 µg/ml insulin. Cell viability and proliferative activity were measured (MTT-test [6]) after the incubation of the cells with the APA inhibitor GH in the concentrations range from 0.001 to 10 mg/ml for 72 hours. Cells were also cultivated with the inhibitor on cover slips for 72 hours, stained with acridine orange /propidium iodide and studied under a fluorescent microscope.

Results and Discussion

AngII is well known to act not only as a vasoconstrictor but also as a stimulator of cell migration, invasiveness and angiogenesis in AngII-mediated carcinomas [1]. On the other hand, AngII is the main natural substrate of APA. Thus, the lower APA activity in AngII-mediated tumors is expected to favor the tumor growth. In the present study, we test the effect of a recently synthesized specific APA inhibitor GH on cell viability and proliferation potential in a model system of three types of human mammary gland cells: normal (MCF-10A), luminal breast cancer of low invasiveness (MCF-7) and triple negative (highly invasive) cancer of the mammary gland. According to our results, GH affects the cells growth in a concentration-dependent manner with IC_{50} in all the three cell lines being above 6 mg/ml (**Fig. 1** and **2**). Since these IC_{50} are high, we concluded

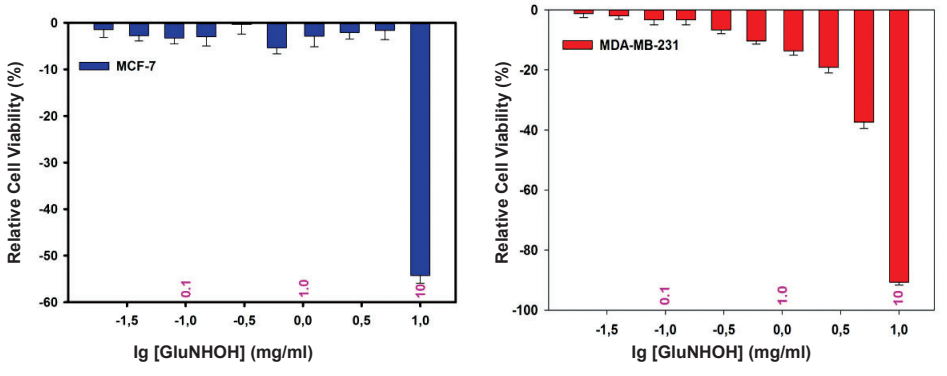

Fig. 1. Effect of the inhibitor GH on the tumor cell lines. Left – MCF-7 (IC_{50} = 9.0 mg/ml); Right – MDA-MB-231 (IC_{50} = 6.5 mg/ml).

that GH is not toxic to the three types of cells. Additionally, the inhibition of APA in the normal mammary gland epithelial cells (MCF-10A) led to an increase of cells viability by 30% (effective concentration EC_{50} = 0.150 mg/ml) in comparison to the non-treated control (**Fig. 2**). Furthermore, the number of mitotic figures under the fluorescent microscope was substantially higher than the control cells (**Fig. 3**).

Thus, it can be concluded that the suppression of APA activity leads to a considerable increase of the survival rate and proliferative capacity of the normal mammary gland epithelial cells, which corresponds to the tumor phenotype. According to our results, the inhibition of APA is part of the tumor phenotype of the cells.

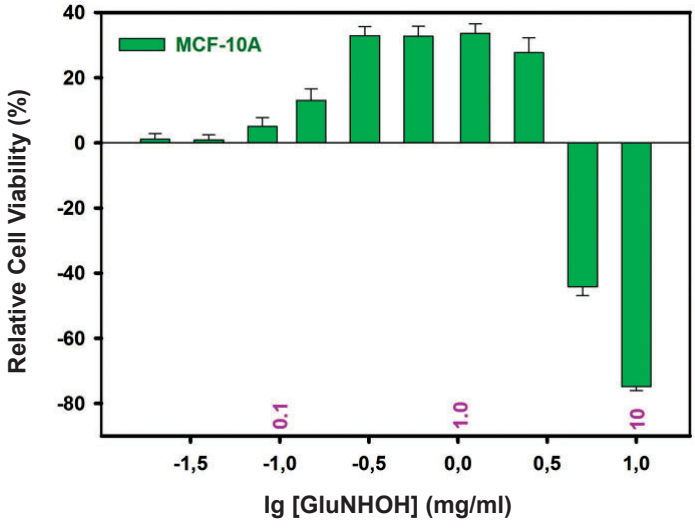

Fig. 2. Effect of GH on the normal cell line MCF-10A (IC50 = 6.0 mg/ml; EC50 = 0.15 mg/ml).

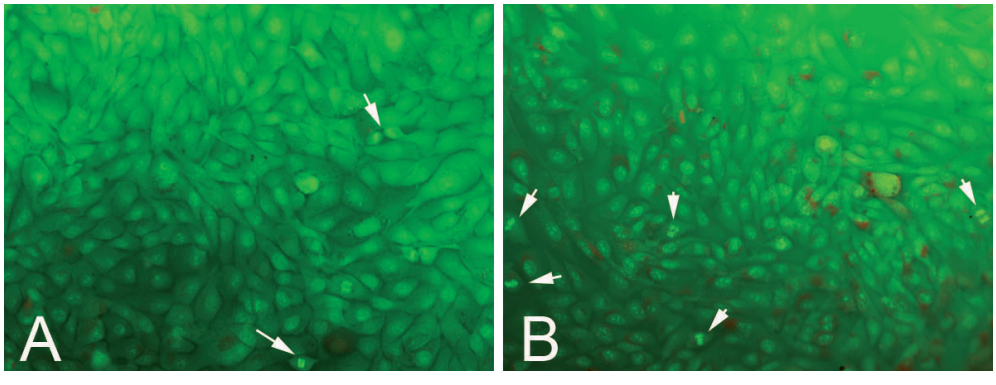

Fig. 3. Increased number of mitotic figures (arrows) in the cells, treated with GH (B) in comparison to the non-treated cells (A). 200X

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