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Fund “Nauka” Project № 17010 Resume – Competition-Based Session 2017:

“New RGD/polyamines – synthesis and study for antitumor activity”

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Malignant neoplasms are a major public health problem and a leading cause for death worldwide.

As far as cancer chemotherapy is concerned, one of the main problems is that the drugs are not selective for cancer cells. The resulting damage of normal cells leads to severe adverse drug reactions, which limits the dose of antitumor drugs to the highest tolerated dose. In addition, some of the chemotherapeutics are not soluble and this makes very difficult their delivery to the cell.

In recent years, a new approach to drug design has been used – combining two or more pharmacological effects into one ligand. The bivalent ligand retains the power of effects of individual drug molecules (synergistic effect, lower doses and fewer side effects) but at the same time, some limitations of the individual molecules are overcome.

Among a whole range of short-chain peptides, RGD analogs are an attractive possibility for a selectively delivery of cytostatics to cancer cells. They have a strong affinity and selectivity for integrins overexpressed on the cell membrane of some tumor and angiogenic endothelial cells. Linear and cyclic peptides containing the RGD (Arg-Gly-Asp) amino acid sequence are used as delivery agents for low molecular weight drugs, peptides and proteins to endothelial and tumor cells.

Aim:

Synthesis of new hybrid structures – RGD/polyamines that act as specific carriers for therapeutic agents.

Tasks:

1. Synthesis of new hybrid peptide structures:
2. In vitro study of the potential cytotoxic effect of the synthesized peptide conjugates.
3. Investigation of the potential of newly-synthesized structures as carriers for therapeutic agents.

Expected results:

- New, not described in the literature, analogs of RGD-peptides and their conjugates with more pronounced cytotoxic properties will be synthesized.

- The results obtained might be a basis for the development a new class of dosage forms.
- The scientific research will contribute to the academic development of the project team.
- The participation of students in the project will increase their interest in the novelties in the field of experimental medicine.

Achieved results:

As a result of the project activities, two new RGD peptide mimetics were synthesized with the participation of arginine non-protein amino acid analogues Agb (2-amino-4-guanidino-butyric acid) and Agp (amino-3-guanidino-propanoic acid), as well as the native RGD and its canavanine analogue CavGD.

Eight new RGD peptide conjugates were synthesized with the participation of arginine non-protein amino acid analogues – Agb, Agp, canavanine (Cav) and two diamines – 1,2-ethylenediamine (1,2-EDA) и 1,4-diaminobutane (1,4-DAB). A standard solid phase peptide synthesis protocol was used. Peptide mimetics and analogs are obtained with high purity and good yield.

For the design of the target peptide mimetics – RGD analogs and conjugates, the following modifications were made in the structure of the native RGD:

- modification in position 1: the arginine residue was replaced by its structural analogues – the non-protein amino acids Agb, Agp and Cav;
- modification in position 3: modification of the C-terminus of Asp with diamines – 1,2-EDA and 1,4-DAB.

After completion of the synthesis and purification of the peptides by gel filtration, the degree of chemical purity of the obtained compounds was determined by high performance liquid chromatography and capillary electrophoresis. Mass spectrometric analysis confirmed the pre-calculated molecular weights of the synthesized peptides.

A biological evaluation of the two new RGD analogs, modified at position 1 with Agb and Arg, was performed. The cytotoxic effect of AgbGD and AgpGD mimetics and the parent RGD peptide was determined on six tumor cell lines by MTT assay. The results showed that the peptide mimetic, AgbGD, had an increased cytotoxic effect on HT-29, MDA-MB-231 and MCF-7 cells compared to the parent RGD peptide. Compared to the RGD peptide, both analogs showed an increased inhibitory effect on HepG2 cells. AgbGD showed the highest cytotoxic effect on HT-29 cells. AgpGD had an increased inhibitory effect compared to the parent RGD peptide on PC-3 cells. A-549 cells were not sensitive to AgbGD, AgpGD and RGD. Shortening the length of the side chain of the amino acid Arg altered the activity of analogs, increasing their cytotoxicity. The effect of the tested peptides was concentration-dependent and showed specificity for the cell lines. On the MCF-10 reference non-cancer line,

the peptide analogs, as well as the RGD peptide, did not have a pronounced cytotoxicity. This is a good indicator of the lack of toxicity of the substances.

Peptides are very attractive for the discovery and development of drugs with cytotoxic effects due to their low cost, good oral bioavailability and the ability to perform relatively easy studies on the relationship between molecular structure and activity. On the other hand, the use of natural polyamines and their analogues is a promising strategy for cancer treatment. The preparation of conjugates from these two groups of natural substances would lead to potentiation of their antitumor effect. RGD peptides can improve the delivery of polyamines to tumor cells.

The results of the project contribute to the discovery and development of new molecules with cytotoxic effects.

The project contributes to the development of one of the priority scientific fields in MU - Varna: direction “Oncology”.

The project is directly related to the topic of the doctoral dissertation of Momchil Konstantinov Lambev: “New RGD/ polyamines – synthesis and study for antitumor activity.” The results are included in this dissertation.