ABSTRACT

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Tumor diseases are slowly but surely becoming the most widespread disease in the world. Malignant conditions affect a wide range of people who inherited a predisposition to the disease genetically, or were influenced by environmental factors. However, expensive therapy is a problem not only in developed countries, but especially in third world countries. Financial aspect and availability are only part of the problem. Early diagnosis, prevention before the onset of this type of disease and effective therapy are the key elements to succesfull treatment.

Due to the high prevalence and mortality associated with malignant diseases, which are increasing every year, great efforts are devoted to the prevention of these diseases and the correct and timely diagnosis associated with a healthy lifestyle. Great attention is also paid to the research and development of new and effective anti-tumor drugs and therapeutic approaches. One such modern and promising method is photodynamic therapy (PDT).

It‘s a selective, minimally invasive, clinically approved therapeutic method associated with a minimal occurrence of negative side effects compared to methods such as chemotherapy, radiotherapy, surgery or their combinations. PDT is becoming an increasingly utilized and recognized alternative in the therapy of malignant or non-malignant conditions, thanks to the less expensive treatment and better availability of therapy. The principle of the method is the local or system administration of a separately inactive compound, referred to as a photosensitizer (PS), followed by the irradiation of the tumor with light of a suitable

wavelength and intensity. After irradiation and activation of PS together with the presence of molecular oxygen, as the last main component, a photochemical reaction occurs with the formation of highly reactive oxygen species. These species lead to a state of oxidative stress, which causes damage and subsequently death of tumor cells, damage to the tumor vasculature, and even formation of an immune reaction in the body.

The aim of this work is to evaluate the photodynamic activity, efficiency and mechanism of action of novel phthalocyanine derivatives, photosenzitizers from the group referred to as subphthalocyanines (SubPc). The studied derivates are axially substitued with benzocrown or tyrosine methyl ester moieties. The experiments were carried out *in vitro* on two cell lines: HeLa (human cervical carcinoma) and SK-MEL-28 (human melanoma). Cytotoxicity after light irradiation with a suitable wavelength and intrinsic toxicity of the compounds were investigated. Furthermore, the subcellular localization assessed by fluorescence microscopy, determination of the type of cell death using Annexin V and the monitoring of the cellular uptake were evaluated as well.

Based on obtained results, very promising photodynamic activity of the four investigated SubPcs (3I-SubPc-CE, 3EtS-SubPc-CE, 3EtS-SubPc-Tyr-FB and 3EtS-SubPc-Tyr-Boc) was found after irradiation on both cell lines. 3EtS-SubPc-CE (EC_{50} = 2.3 ± 0.7 nM) and 3I-SubPc-CE (EC_{50} = 37.8 ± 7.6 nM) tested on SK-MEL-28 cell line proved to be the most effective even in comparison with clinically used PS. Studied compounds were localized in lysosomes and adiposomes. After irradiation, the cell lines underwent apoptotic cell death, which was manifested by the increase in luminescence signal and the delay of the increase in fluorescence signal. As part of the cellular uptake, 3EtS-SubPc-CE was found to be taken up in the highes amount by the cells.

Key words: photodynamic therapy, photosensitizer, subphthalocyanines, cytotoxicity, cell death, Annexin V, subcellular localization, BCA