ABSTRACT

Charles University
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Martina Půlkrábková
Supervisor: Mgr. Miloslav Macháček, Ph.D.
Title of diploma thesis:
Evaluation of photodynamic activity of tetrapyridoporphyrazine derivatives for treatment of tumorous diseases.

Cancerous diseases are one of the most common causes of death, especially in industrialized countries. Their more effective treatment requires development of new potential anti-tumor agents and therapeutic methods. Photodynamic therapy (PDT) is a clinically approved method for the treatment of both tumorous and some other diseases. Its main advantages are non-invasiveness and potential for high selectivity to target cells, which distinguishes it from current conventional anticancer treatments (chemotherapy, radiotherapy).

The effect of PDT is based on a combination of three effective components - molecular oxygen, light and photosensitiser (PS). These substances are inherently non-toxic, but they can form reactive oxygen species (ROS) if combined. Then, ROS significantly damages cellular structures in target cells, which can result in cell death. PS is a substance capable of absorbing light of suitable wavelength and transferring the received energy to surrounding molecules, to molecular oxygen in particular. In addition to the direct toxic effect on tumour cells, damage to the tumour vasculature and induction of the immune response are also involved in the PDT effect.

In this diploma thesis, the photodynamic activities of new potential PSs from the group of azaphtalocyanines and phthalocyanines (P39-1Zn-Me, ZIP252Zn-Me, ZIP280Zn, ZIP288-OHZn) are evaluated. Two substances, meant for clinical practice in PDT (Photosens®, methylene blue), were also studied. PSs evaluation was performed *in vitro* on the HeLa tumor cell line. The activity of these substances after exposure to the

activating radiation (phototoxicity) as well as their own toxicity without activation by light (dark toxicity) was determined together with TC_{50}/EC_{50} ratio. Cationic derivatives P39-1Zn-Me and ZIP252Zn-Me achieved the best results. They showed a relatively high toxicity after irradiation (up to 10^{-2} μM range) and low toxicity without irradiation (10^{2} μM range); P39-1Zn-Me did not show toxicity even after reaching the solubility limit in the cell culture medium.

Subcellular localization of new PSs after accumulation in cells was determined by fluorescence microscopy. All of the substances were primarily localized to the lysosomes. In the case of the most potent PS P39-1Zn-Me, morphological changes occurred in cells after photoactivation of this substance were also documented. This evaluation was performed using PS in concentrations corresponding to their EC_{15} and EC_{85}; cells which were not exposed to PS were used for control. The formation of ROS resulted in massive cell membrane damage with membrane blebbing, a change in the shape of the mitochondria, condensation of the nuclear chromatin and fragmentation of the nucleus, and reorganization of the cytoskeleton. Observed morphological changes indicated necrotic cell death, however some cells showed changes that could indicate apoptosis.

For PS P39-1Zn-Me, the time course of ROS creation was determined by DCF assay. Fluorescence signal generated in cells was evaluated, depending on the amount of ROS produced before, during and after light irradiation. From the results obtained, it is obvious that the ROS mass production occurs when cells containing PS are irradiated, whereas their production is minimal before and after the irradiation.