

The aim of the project is the investigation of the mechanism of cell death in cancer HeLa cells, after induction of reactive oxygen species by photodynamic therapy (PDT) sensitized by porphycenes. Photodynamic therapy is the method of treatment approved in anticancer applications. It requires two factors applied together: a substance called photosensitizer and the light of the proper wavelength. After photosensitizer administration, the treated spot is illuminated. Only in such conditions PDT action can occur. The reactive oxygen radicals are created to immediately react with the surrounding tissue, which leads to the cell death. It is crucial to use the substance which is not harmful in the dark. Such therapy is useful in the variety of medical conditions, from acne to the cancer.

In this project the group of compounds selected to be studied for their photosensitizing performance are porphycenes. They exhibit some structural similarity with porphyrins, which are commonly known in the world of PDT. What is the advantage of our compounds then? In fact the answer is simple. Human tissue has some permeability for the light, but not all colours of light can get equally deeply through our skin. The best is the red light and porphycenes can really make use of that feature. How? Their absorption in the red region of the spectrum is around ten times higher than for porphyrins, which can give more efficient treatment of the malignances located deeper under the skin.

The research to be done in this project will focus on the death mechanisms of the cancer cells from HeLa line. Does it seem boring to the chemists? Not necessarily. Cellular death pathways induced under conditions of oxidative stress, such as PDT, originate from the phenomena occurring on a much smaller scale than the cell. The first symptoms initiated on the molecular level lead to the inevitable consequences for the tissues, organs and whole body. Talking about mechanisms of cell death we distinguish three basic pathways: necrosis, apoptosis and autophagy. In the case of PDT they usually occur in parallel, however it is important to know that we can have some control over them, changing the intensity of capacity of the stress factors, such as the amount or produced radicals, concentration of photosensitizer or light dose. Why is it important? The apoptosis, which is sometimes called programmed suicide is the mildest possible way of cell destruction. It does not make any harm to the tissues in the surrounding environment, and the released organic material can be used by the organism again. At the same time, the cell that undergoes necrosis is falling apart in an uncontrolled way, which causes the inflammation that expands over the neighbouring tissues. Such situation can lead to the kidney failure and to the death of a patient.

On the basis of our preliminary studies we conclude that porphycenes can be efficient photosensitizers in PDT. They are killing the cells even two orders of magnitude more efficiently than a commonly available PDT drug. However, the mechanism of that process is not yet known and we believe it is worth investigation. In the presented project we want to investigate what are the molecular level changes induced in HeLa cells by PDT. The example of a difference between apoptotic and non-apoptotic cells is the expression of phosphatidylserine on the surface of their membrane. This phenomenon can be used by adjusting the proper dye, such as annexin V to the moieties that sticks out and observing the fluorescence by microscopy technique. This simple method will be a starting point of the research. After that, some other markers of apoptosis such as cytochrome c relocation from mitochondria to cytosol and expression of proapoptotic proteins will be determined. A more difficult task is to discriminate necrotic cells, but we will make an effort to do it by means of DNA chromatin visualization, since the characteristic feature of necrosis is the condensation of chromatin, which can be followed with the high resolution fluorescence microscopy. The final result of the project will be an optimized method of PDT treatment with the use of porphycene sensitizers.