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Photodynamic effect of Radachlorin on nerve and glial cells

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Summary

Background: Radachlorin, a chlorine-derived photosensitizer, is used currently in photodynamic therapy (PDT) of skin cancer. In this work we studied Radachlorin-PDT effect on peripheral nerve and glial cells that are damaged along with tumor tissue.

Methods: We used simple model objects — a crayfish stretch receptor that consists of a single sensory neuron surrounded by glial cells and crayfish nerve cord consisting of nerve fibers and ganglia. Radachlorin absorption and emission spectra were registered using spectrophotometer and spectrofluorimeter. Radachlorin accumulation and intracellular localization were studied using the fluorescence microscope. Necrotic and apoptotic cells were visualized using propidium iodide and Hoechst 33342. Neuronal activity was registered using standard electrophysiological methods.

Results: Radachlorin absorption spectrum in the physiological van Harreveld saline (pH 7.3) contained maximums at 420 and 654 nm. Its fluorescence band 620–700 nm had a maximum at 664 nm. In the crayfish stretch receptor Radachlorin localized predominantly to the glial envelope and penetrated slightly into the neuron body and axon. Radachlorin rapidly accumulated in the crayfish nerve cord tissue within 30 min. Its elimination in the dye-free solution occurred slower: 11% loss for 2 h. Radachlorin-PDT inactivated the neuron and induced necrosis of neurons and glial cells and glial apoptosis at concentrations as low as 10^{-10}–10^{-9} M.

Conclusions: Radachlorin rapidly accumulates in the nervous tissue, mainly in glial cells, and demonstrates very high photodynamic efficacy that characterize it as a promising photosensitizer.

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Introduction

Photodynamic therapy (PDT) is based on photoinduced generation of strongly cytotoxic singlet oxygen, following oxidative stress and death of stained cells under light exposure in the presence of oxygen. It is currently used in oncology [1—3]. Derivatives of hematoporphyrin (Photofrin II, Photoheme, Hpd), benzoporphin (vereporphin), chlorins (mTHPC or Foscan), and 5-aminoleveulinic acid (ALA) are the most popular photosensitizers for PDT. However no one of them satisfy all demands for an ideal photosensitizer [4]. Some very efficient photosensitizers are not used in clinics or have only limited applications because of one or two unacceptable parameters. For example, hematoporphyrin derivatives have relatively weak light absorbance in the red spectral region. Aluminum phthalocyanine Photosens retains in the organism for more than two months that imparts too long skin and eye photosensitivity.

Chlorine derivatives have excellent spectral and photochemical parameters. Radachlorin, the mixture of the sodium salts of chlorine e6, chlorine p6 and purpurin 18 (Fig. 1), is a promising photosensitizer. Radachlorin-PDT is adopted for skin cancer treatment in Russia and Korea and showed promising results for treatment of the cervical, gastrointestinal, head and neck, lung and bladder tumors [5—14]. However, in all cancer cases PDT damages not only tumor cells but also nearby peripheral nerve elements and glial cells. It is the most important in the case of treatment of brain tumors, when photodynamic injury of neighboring normal neurons and glial cells can induce unacceptable side effects and neurological disorders. The cellular and molecular mechanisms of photodynamic damage to neurons and glial cells are difficult to study in the brain because of its complexity and numerous intercellular interactions. Biochemical study cannot identify processes that occur separately in different cell types — neurons or glial cells.

The suitable simple object for simultaneous study of interacting neurons and glial cells is the crayfish stretch receptor that consists of a single mechanoreceptor neuron surrounded by a multilayer glial envelope [15]. The photodynamic effects of diverse hematoporphyrin, phthalocyanine,

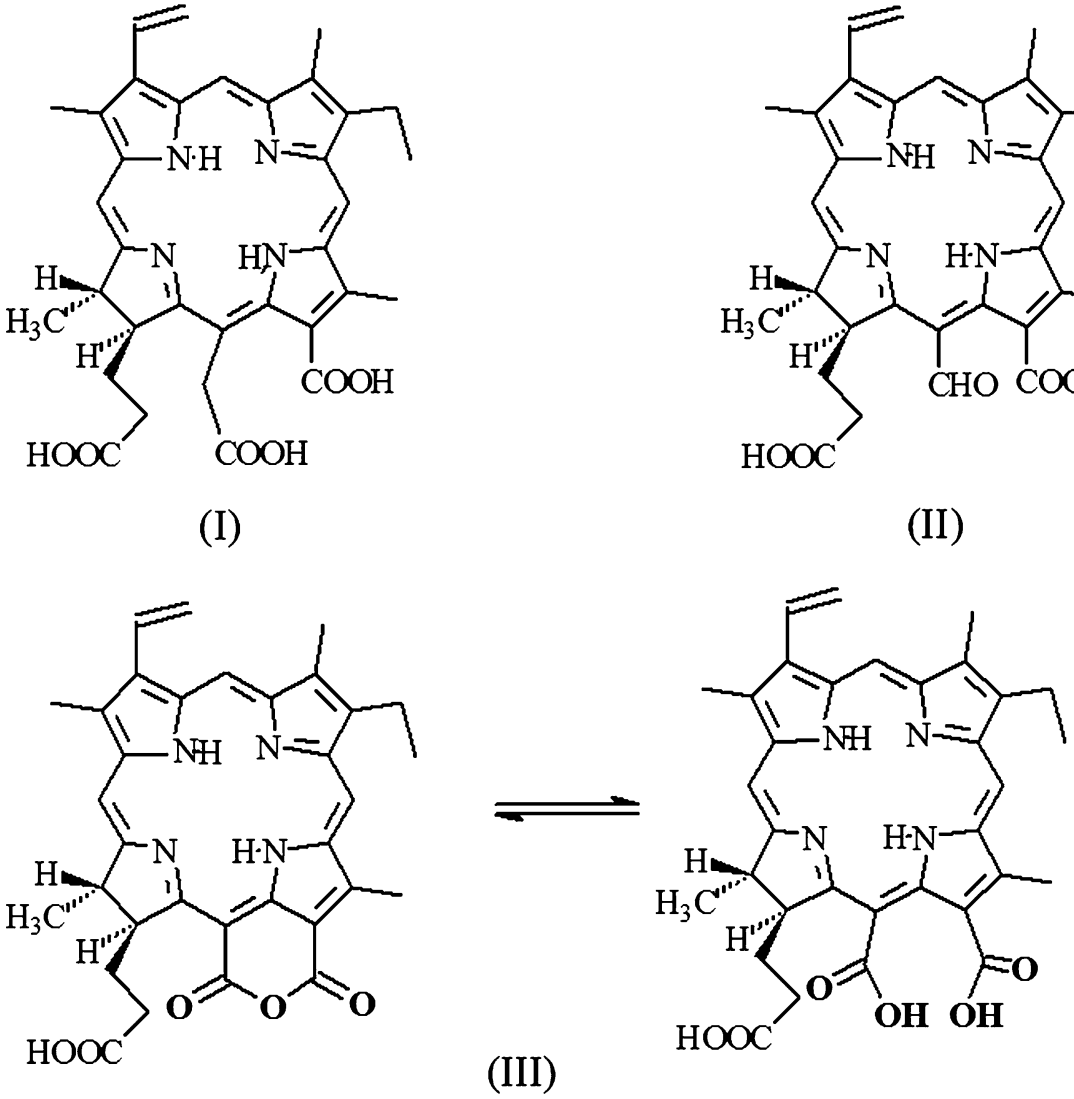

Figure 1 Radachlorin components: chlorine e6 (80%), chlorine p6 (15%), purpurin 18 (5%).