Summary of doctoral dissertation on:

„Evaluation of the usefulness of unconventional methods of generation and detection of $^1O_2$ in medical diagnostics"

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Introduction

Photodynamic therapy (PDT) is a cancer treatment that uses photo-generated reactive oxygen species (ROS) to damage target cells. To generate ROS, the PDT treatment method uses photosensitizers (PS), which are excited by light of a specific wavelength and power that does not damage healthy tissue. The main ROS generated is singlet oxygen ($^1O_2$), which reacts with cellular molecules, eventually causing tissue damage and cell death. One diagnostic method that uses photosensitizing dyes is photodynamic diagnostics, which allows you to see neoplastic changes that cannot be seen with conventional methods. It enables more accurate diagnostics of tumors and various types of neoplastic changes. Research on PDT is diagnostic in the form of PS delivery imaging and measurement of the amount of singlet oxygen produced in the tissue and the amount of $^1O_2$ molecules required to kill a single cell.

Material and method

The research used biological material in the form of tissue sections taken from 30 patients of the Provincial Clinical Hospital No. Frederic Chopin in Rzeszów with head and neck tumors. Tests using human tissues were approved under Resolution No. 143/B/2018 of the Bioethics Committee of the Regional Medical Chamber in Rzeszów of November 22, 2018. The studies were performed on freshly dissected normal and neoplastic thyroid tissue samples in a total of 30 healthy control tissue samples and 30 neoplastic tissue samples. The volume of the examined tissues was approx. 4×4×4 mm (6.4×10^{-5} L) for normal and cancerous laryngeal tissue and approx. 10×10×15 mm (1.5×10^{-3} L) for normal and malignant thyroid tissue. The concentration of the photosensitizer in the form of Rose Bengal used in the research did not exceed 5.8×10^{-4} M in the laryngeal tissue and 1.0×10^{-4} M in the thyroid tissue. The samples were illuminated with a MGL-III-532 nm / 300 mW semiconductor laser (CNI Lasers, China) with a wavelength of 532 nm and a visible light source in the form of a 400W halogen lamp, irradiation time ranging from 15 to 40 minutes. Studies have been carried out to achieve tumor cell killing using a photosensitizer, oxygen and laser light. T_1 was measured in the superficial and deep layers of tissues after a series of experimental steps in the form of irradiation to identify potential differences in T_1. An in vitro study of the penetration of the laryngeal photosensitizer was performed and an analysis related to the evaluation of the use of PDT in this type of cancer was performed. In cooperation with the Clinical Department of Pathomorphology of the Clinical Provincial Hospital No. 1 in Rzeszów, histopathological examinations of the tissues used in the research were performed. Histological image analysis was performed using a Leica DM1000 LED microscope (LEICA Microsystems, Wetzlar, Germany).

Results

Histological examination of the neoplastic tissues showed damage to the cells of the neoplastic cells by PDT after delivery of the PS directly to the tissue. Histological images showed visible tumor epithelium with an image of cellular picnosis. The microscopic analysis also showed that after RB was delivered to the tissue surface and irradiation was performed at a depth greater than 2 mm, the tissue was not stained by RB and that the cells at this depth were not damaged in any way. The measured times T_1 and T_2 showed effectiveness in the assessment of oxygen consumption during the photodynamic action by extending them. The applied research methods made it possible to evaluate the effectiveness of the therapy through the possibility of assessing the irradiation time needed to consume the oxygen present in the tissue structure. The T_2 mapping sequence enabled the characterization of laryngeal tissues and distinguished tumor tissue from normal tissue. The effect in the form of cell damage after PDT application was recorded only in those layers of the tissue where the photosensitizer directly interacted with its structure. Targeted delivery of the photosensitizer, directly to the tissue, made it possible to obtain the best therapeutic effect.

Conclusions

The presented study showed that PDT is an effective primary or alternative treatment for cancer of the larynx and thyroid gland. Photogenerated singlet oxygen, which is the main factor responsible for cell necrosis during photodynamic cancer therapy, led to damage of cancer cells, as confirmed by histopathological analysis. Research has shown the potential of photodynamic therapy and magnetic resonance imaging to solve the problem of treatment when the tumor occurs in surgically complex sites, such as when tumors are immediately adjacent to organs such as the larynx and thyroid gland. Direct measurement of T_1 relaxation time and T_2 mapping by MRI made it possible to monitor oxygen consumption due to photodynamic action and allowed for the differentiation of normal from cancerous tissue. The conducted research made it possible to define the limits of the application of PDT in the structure of tissues and proved to be a useful diagnostic tool.