Comparison of photodynamic therapy with phthalocyanine and photofrin in human colorectal carcinoma

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Received August 10, 2007

Photodynamic therapy (PDT) has been developed in recent years as a new modality for the treatment of various neoplastic and non-neoplastic lesions. Although the method of combining light with photosensitizers for treatment has been around for a century, further understanding has been evolved over the past decades. The method is based on the phenomenon involving the combination of photosensitizer and light. Neither drug nor light alone are effective as therapeutic agents. The antitumour effects result from direct cell damage, destruction of tumor vasculature and activation of a nonspecific immune response. The more accepted use of PDT is still restricted for ophthalmology, dermatology and treatment of some stages of esophageal, lung and urinary bladder cancer. In our experiments, the effect of phototherapy with disulfonated hydroxyaluminium phthalocyanine (Al(OH)S2Pc) and photofrin (control group) on the growth of human colorectal carcinoma on nude mice was studied. We chose colorectal carcinoma, because the Czech population has the highest incidence and it is still increasing. We try to offer a new possibility of treatment for patients with this severe disease.

Key words: phthalocyanine, colorectal carcinoma, photodynamic therapy

PDT is a two-stage procedure that requires administration of photosensitizer, followed by illumination of the tumor with light of the proper wavelength. A third component of PDT is molecular oxygen, which in photodynamic reaction generates singlet oxygen and reactive oxygen species. [1,2,3] A photosensitizer can be applied topically or can be administered systemically. The ideal photosensitizer should be a stable, pure compound of known composition, with the ability to target tissues with a high degree of selectivity, rapid clearance and minimal or absent skin photosensitivity. [4,5,6]

Light sources are mostly lasers with wavelength between 600 and 900 nm. [7,8,9] Tissue penetration is affected by several processes, but most important is wavelength of light. Longer wavelengths penetrate deeper into tissue, but the limitation of light penetration into tissue is about 8 mm. After a period necessary for efficient accumulation of photosensitizer in the tumor, the requisite area is irradiated.

Tumor damage is a result of energy transfer from a photosensitizer that has been excited by light of a specific wave length. In the presence of molecular oxygen, the light illumination of the photosensitizer can lead to two kinds of reactions. It can directly react with substrate to produce radical substrate forms and after interaction with oxygen it generates peroxides and other oxygenated products (type I reaction). The result of the second reaction is singlet oxygen, which is transferred from the ground state by an excited photosensitizer (type II reaction). Both reactions can occur simultaneously and they lead to destruction of biological macromolecules, such as membrane lipids and proteins. [1,2,3]. Histology after PDT shows appearance of cells with nonpycnotic nuclei, indicating the existence of nontumorous cells and irregularly located capilaries penetrating the tissue indicated tumor remission [8].

The main disadvantages of PDT are limited tissue penetration and photosensitivity. Maximum tissue penetration is about 8 mm, so PDT can be used only for small lesions, but it can be used repeatedly. Photosensitivity is the main problem

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of the first generation of photosensitizers. Among other discussed problems belong angiogenic affect, which can be responsible for regrowth of the tumor and pronounced inflammatory response in the tissues surrounding the tumor.

Colorectal cancer is one of the most common cancers in both men and women in the Czech Republic. Risk factors including age, a diet rich in fat and cholesterol (typical Czech cuisine), smoking and alcohol, inflammatory bowel disease and genetic predisposition, including hereditary polyposis and nonpolyposis syndromes are well known. Progress has been made in understanding the molecular basis of colorectal cancer predisposition and progression. The lifetime risk for Czech citizens is 5.9 percent and it is still on the increase. Incidence in Czech men is the highest in Europe. The number of cases of colorectal carcinoma in men has redoubled in the last 20 years. [13] Prognosis relates to the stage of the disease at the time of diagnosis and to initial treatment. If detected early, colorectal cancer is curable by surgery. Adjuvant (or neoadjuvant) chemotherapy can prolong survival in disease in patients with advanced disease. Radiotherapy is used in cases of rectal cancer.

Unfortunately there is still a large group of patients with advanced disease, which is indicated only for palliative therapy, i.e. diverting stoma, stent placement or local laser therapy. Efforts are underway to develop better screening strategies and novel therapies to improve patient survival rates. Despite all efforts, colorectal cancer remains the leading cause of death from cancer in the Czech Republic.

In our study, we decided to demonstrate the efficacy of PDT with phthalocyanine for treatment of colorectal carcinoma in an experiment on nude mice. Phthalocyanines have been the most extensively studied among other "second generations" photosensitizers. [3,6,10,14] They absorb minimally in the wavelength region 400-600nm, so that the potentiation of the skin sensitivity to sunlight is negligible in contrast to that of porphyrins. The aim of our experimental work is to offer a new possibility of therapy of colorectal carcinoma.

Materials and methods

Disulfonated hydroxyaluminium phthalocyanine (Al(OH)S2Pc) was synthesized in the Research Institute of Organic Synthesis, Pardubice, Czech Republic. The dye was used as a solution of sodium salt in 0, 9% (w/v) saline solution. Photofrin was obtained from Axcan Scandipharm Inc. The dose applied was 2.5 mg of Al(OH)S2Pc per kilogram of body weight. The dose of photofrin was also 2.5 mg per kg of body weight.

Outbred athymic nude CD-1 mice of 18 – 22 g body weight at the time of transplantation (AnLab ltd., Charles River, Czech Republic) were kept in an air laminar flow box under aseptic conditions with bedding sterilized by irradiation and fed by irradiated diet and having access to autoclaved water.

A human colorectal carcinoma (HCT-116 tumor line) was purchased from European bank of tumors ECACC (European Collection of Cell Cultures, CAMR, Salisbary, Wiltshire, United Kingdom). This tumor line was multiplied in a tissue culture and was administered subcutaneously with 1 x 10^7 tumor cells together with Matrigel onto the abdominal right flank. The testing started after reaching a tumor volume of 0.3-0.4 cm^3.

Photodynamic therapy. Mice were divided into 4 groups of at least 8 animals per group, each with a grown tumor. The first group received disulfonated hydroxyaluminium phthalocyanine (dose 2.5 mg/kg), the second group received photofrin (dose 2.5 mg/kg) and the other two groups were control ones. Treated groups received IV injections into the tail vein of phthalocyanine and photofrin. Al(OH)S2Pc reaches high concentration in the tumor after a very short time, so ten minutes after application, mice were subjected to Thiopental narcosis and the tumor area was irradiated from a distance of 1 cm for 8 minutes. The first group with disulfonated hydroxyaluminium phthalocyanine and the second group with photofrin were irradiated with xenon lamp with filter (600-700 nm) with total impact energy 120 J/cm2. The optimal wavelength is 675 nm for disulfonated hydroxyaluminium phthalocyanine and 632 nm for photofrin, but we wanted to use the same conditions for both photosensitizers, because longer wavelength has deeper penetration into tissue. The third group was only irradiated (no administration of photosensitizer) and the last one did not receive any photosensitizer or irradiation. The mice were kept and the tumor volumes were measured in three day interval for 30 days. Tumor volumes and visible surface necrotic area were measured by caliper by the following procedure: tumor volume $V = (l \times w^2)/2$ and necrotic area as $S = l \times w$, where l is the length and w is the width. First signs of necrosis were found in groups with photosensitizers 24– 48 hours after illumination. In successful cases, the result of PDT was a scar covered by epithelial tissue. All mice were observed regularly because there is a risk of regrowth of the tumor, which was observed in both groups treated with photosensitizers. In control groups no surface necroses were observed and rapid spread of tumor was visibly confirmed and malignant epithelial cells were confirmed histologically. All mice were sacrificed 30 days after PDT unless they had died previously.

Histology. The resulting tumor remission was histologically confirmed by disappearance of tumor cells. All mice from each treated group were sacrificed after 30 days and their tumor areas in 10% formaldehyde were left for histology. There were no differences in histological examination of the tumor area after successful PDT between phthalocyanine and photofrin. One mouse from each group was autopsized, with histological examinations of the liver, kidneys, small intestine, lungs and muscle tissue done to exclude deterioration by photosensitizer and/or laser irradiation. No signs of any deterioration were registered in all groups.

Results

In our study, we compare the efficacy of PDT with disulfonated hydroxyaluminium phthalocyanine and

photofrin on tumor remission after a single cycle of therapy. PDT with Al(OH)S2Pc and photofrin were successful in the majority of cases. Both treated groups of mice received the same dose of photosensitizer and were irradiated with light of the same wavelength so there was the same depth of light penetration into tumor. Administration of phthalocyanine had an 87% success rate after a single dose. Regrowth of tumor was registered in 1 mouse (13%). Administration of photofrin had a 62% success rate. Regrowth of tumor was found in 3 cases (38%). Al(OH)S2Pc has a 25% higher efficacy in PDT of colorectal carcinoma in comparison to photofrin. The resulting tumor remission was histologically confirmed. Among other advantages of Al(OH)S2Pc belong: better absorption spectra (and deeper penetration of light), minimal photosensitivity and photofrin is still a mixture of various mostly unidentified compounds. No remission and no side effects of illumination were registered in control groups.

Discussion

There is still an increasing interest and research effort focusing on developing new photosensitizers, [8,10,11] exploring PDT mechanisms at molecular and tissue levels, enhancing PDT efficacy, and evaluating potential clinical indications. Photodynamic therapy is an established modality for the treatment of neoplastic and non-neoplastic tumors and other accessible lesions. [2,6,12] In our study, we proved efficacy of PDT with phthalocyanine for the treatment of colorectal carcinoma in experiments on nude mice. We try to offer a new possibility of treatment for patients with colorectal carcinoma in early stages or for palliative treatment for patients with late and advanced carcinoma. Efficacy of PDT is mostly limited by penetration of light into tissue (5-8 mm), so PDT can be used only for small lesions or must be used repeatedly. PDT cannot be a curative procedure for large and disseminated tumors, but it can improve the quality of patient's life and prolong survival. The main advantages of PDT are: no need of anesthesia, any blood loss and minimal postoperative pain. [5,9] The success of PDT in clinical practice is based on the confirmation that the efficacy of PDT alone or with combination of other standard clinical methods is higher than methods used today.

This work was supported by grant GACR 203/06/1038, The League against Cancer, Prague and by Scientific Purpose MSM 0021620808.

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