PHOTODYNAMIC THERAPY OF COLON ADENOCARCINOMA WITH AMINOLEVULINIC ACID: A CLINICAL AND SPECTROSCOPIC STUDY

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Abstract: We present initial results of our clinical and spectroscopic study of treatment of oncological patients by photodynamic therapy (PDT). We have used aminolevulinic acid based photodynamic therapy (ALA-PDT) for the treatment of patients with colon adenocarcinoma. Aminolevulinic acid (ALA) is a precursor of light-sensitive protoporphyrin IX, formed in heme biosynthetic pathway. The whole procedure was performed by fibre laser system, whose light guides can be inserted in biopsy channel of endoscope. Before PDT, biopsies were taken and cryoslits were prepared. The slits were scanned by laser scanning confocal microscope for further characterization. Each patient had a positive response to therapy. In one case there was a total response and in other four cases more than forty percent of suspected area (adenocarcinoma polyps) was destroyed. From the results of our first five patients appears, that the treatment of intestinal colon adenocarcinoma with ALA-PDT could be successful.

Introduction

Colon cancer is the second most common cancer diagnosed in North America and Western Europe. It is a type of gastrointestinal carcinomas and has the best prognosis. The 5-year survival rate is approximately 50%. Survival rates may be improved by screening and removal of adenomatous polyps. Almost all colonic cancers are primary adenocarcinomas, which are a primary cause of mortality and morbidity.

Photodynamic therapy (PDT) is a suitable method for treatment of various type of cancer. It involves light-induced activation of administered photosensitiser in tissue to produce local necrosis [1]. Aminolevulinic acid (ALA) is an efficient substance used in PDT, which is not the photosensitiser as such, but it is a precursor of light-sensitive protoporphyrin IX (PpIX) in heme biosynthetic pathway. One of the main advantages of ALA-PDT is the metabolically short life of this photosensitiser (about 48 hours). Thus, the patient is at risk of sunburn for only a short period. PpIX is a light-sensitive compound typically activable by red light at a wavelength of 633 nm. Selective accumulation of PpIX in malignant tissue after administration of ALA has been reported [1, 2, 5]. Underlying reason is believed to be due to the altered activity of the heme biosynthetic pathway enzymes in such tissue. Excess PpIX production also occurs in normal tissues but at slower rate, because of a more efficient conversion to heme [2] Upon irradiation of PpIX energy of absorbed photons is partially spent on fluorescence and partially transferred to molecular oxygen through a metastable triplet state [3]. Oxygen under these conditions is transferred to an oxidation activity. Therefore, in the concurrent presence of photosensitizer and oxygen, light with a wavelength corresponding to photosensitizer absorption is able to destroy biological macromolecules. As the concentration of a photosensitizer and oxygen in cells reach certain critical level, these cells die [3]. Since the energy of absorbed photons is partially emitted in the form of fluorescence, it is possible to detect this radiation in order to determine the photosensitizer accumulation in a biological tissue and to monitor the dynamics of this irradiated tissue in the time domain.

Successful performance of PDT requires knowledge of spectral characteristics of used photosensitizer. PpIX has a typical absorption spectrum with major band around 400 nm (Soret band) and series of four less intensive Q-bands in the longer wavelength interval from 500 nm to 633 nm. Emission spectrum is characteristic with more intensive band around 633 nm and with less intensive band around 703 nm [1]. For ALA-PDT the most suitable wavelength for excitation is 633 nm (Q_1 band of absorption spectrum) because of the deeper penetration of red light in the tissue. Light of this wavelength with sufficient power (range 100mW – 1W) is capable to produce PDT effect. Interval for total dose in PDT is quite extensive from 30 J/cm^2 to 540 J/cm^2 [4]. It depends on type of tissue.

It was shown that PpIX is photodegraded by a photooxidation process and that its photoproduct – photoprotoporphyrin (PPp) has a characteristic emission band around 675 nm. Such process was observed both in solution and in cells incubated with ALA [2]. The formation of PPp is a photo-oxidation process and no photobleaching takes place in the absence of oxygen. In malignant tissue there is an excess of PpIX, so that even after photobleaching, sufficient amount of sensitizer remains for tumor eradication [6].

In this study we present application of ALA-PDT in the area of human colon together with related spectroscopic study.

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Subjects and Methods

Five patients (three male, average age of 55 years) with superficial intestinal adenocarcinoma polyps were treated. They were given 60 mg/kg ALA dissolved in fruit juice to drink. After the drug administration, patients were asked to wear sun-glasses and stay in the dark room in order to avoid exposure to sunlight. Four hours later endoscopic treatment was performed. For detection of accumulated PpIX in tissue, fibre spectrometer LESA-01-BIOSPEC (JSC BioSpec) with He:Ne laser operating at 632.8 nm with power of 2 mW was used (Fig. 1).

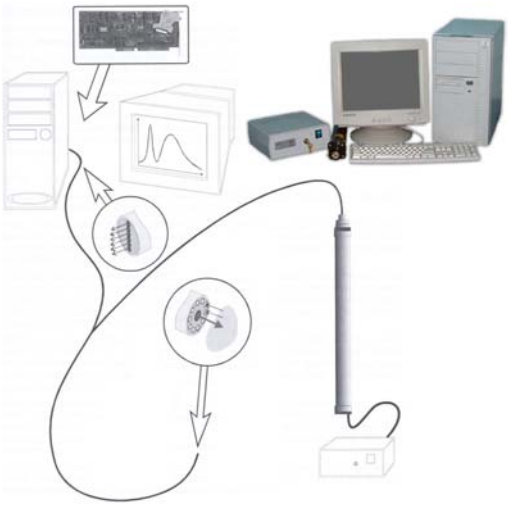

Figure 1: Scheme and photography of LESA-01-BIOSPEC

The instrument has a special output end of catheter (Fig. 2) with central light-guide, delivering the light to the specimen and ambient light-guides, collecting reflected and emitted light from the specimen.

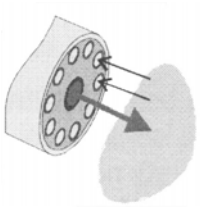

Figure 2: Scheme of catheter output end with central and ambient light-guides

Before PDT treatment emission spectra from both health tissue and suspected area of the same patient were compared. After the fluorescence detection, biopsy was taken and cryoslits were prepared. They were scanned by laser scanning confocal microscope LSM 510 Meta (Zeiss) with C-Apochromat 40x/1.2 water immersion objective. The samples were excited by 458 nm Ar:ion and 633 nm He:Ne laser lines (Lasos Lasertechnik), respectively (Fig. 3).

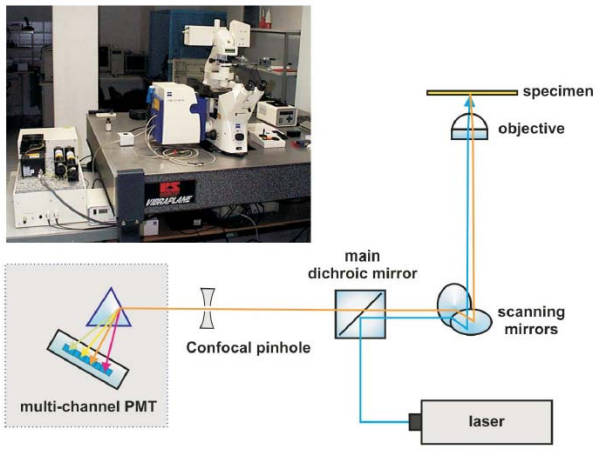

Figure 3: Scheme and photography of LSM

For PDT procedure diode laser system LPht-630/675-01-BIOSPEC (JSC Biospec) was used. The light power was 400 mW, delivered via diffusing fibre for 120 sec., giving the total dose of 48 J/cm^2. The fluorescent spectra of the treated tissue were measured after the PDT treatment as well as one week later during the endoscopic control.

Results

In vivo fluorescence measurements

Five patients suffering from intestinal cancer, namely superficial adenocarcinoma polyps were involved in the present study. An example of superficial intestinal adenocarcinoma polyps as seen during endoscopy is shown in Figure 4.

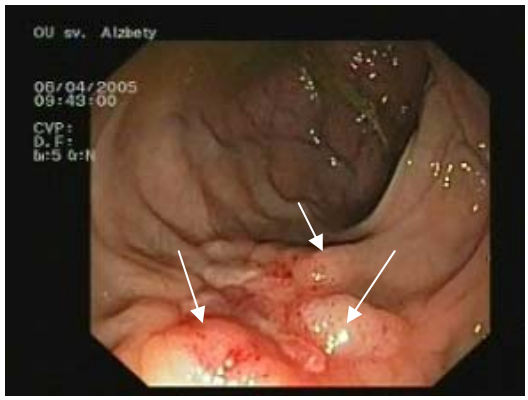

Figure 4: Superficial intestinal adenocarcinoma polyps (white arrows)

The fluorescence measurements performed during endoscopy with 632.8 nm excitation light 4 hours after ALA administration showed an increase of fluorescence intensity at 675 nm in suspected tissue as compared to healthy area (Figure 5).

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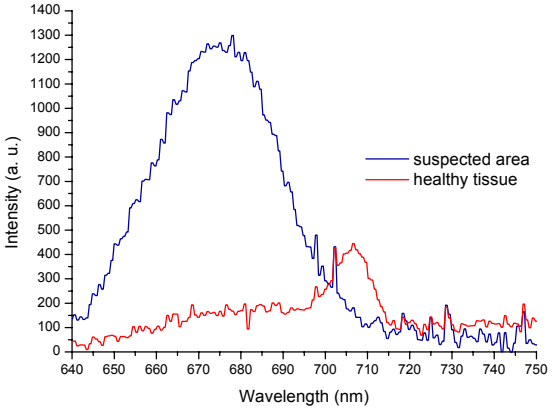

Figure 5: Fluorescence spectra from suspected area (blue) and healthy tissue (red).

Fluorescence emission spectra from suspected area were characterized by an intensive emission band around 675 nm, whereas for healthy tissue fluorescence maximum around 705 nm was observed in the region 640 nm - 750 nm. At the time of endoscopic control - one week after PDT fluorescence emission spectra from treated area were characterized by emission maximum around 705 nm. The same maximum was found in untreated tissue (Figure 6).

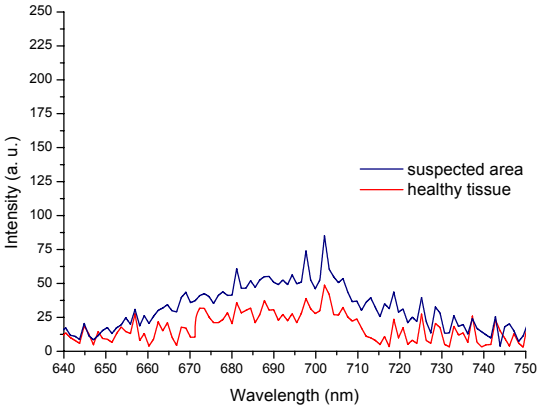

Figure 6: Fluorescence spectra from suspected area (blue curve) and health tissue (red curve) one week after the PDT.

Fluorescence microscopy

An example of fluorescence images of cryoslits from suspected area and healthy tissue is shown in Figure 7. Spectral images of cryoslits from suspected area clearly showed areas of increased red fluorescence (Figure 8). In corresponding fluorescence spectra maxima around 633 nm and 675 nm were seen after the excitation with 458 and 633 nm light, respectively. An emission band around 705 nm was also detected. In this case a typical emission band of PpIX (633 nm) could be seen, which was not possible in measurements of in vivo tissue fluorescence, because of the excitation at 632.8 nm. Fluorescence was found to be more intensive in suspected area than in healthy tissue. An example of the measurement performed with cryoslit from healthy tissue is shown in Figure 9.

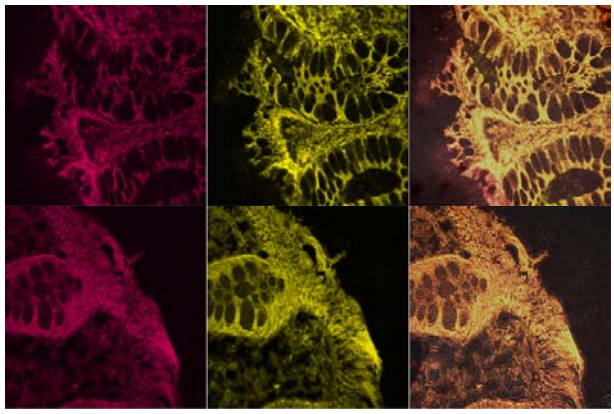

Figure 7: Fluorescence images of tissue cryoslits. Suspected area of tissue (upper), healthy area (lower). Emission from 560 nm to 710 nm (left), emission from 500 nm to 550 nm (middle), both together (right).

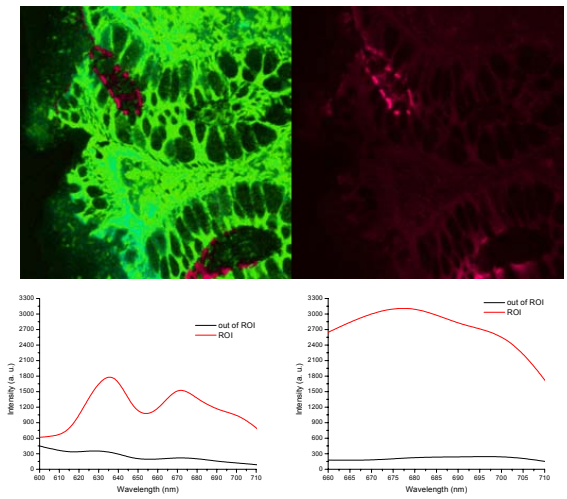

Figure 8: Spectroscopic images of cryoslits from suspected area of tissue and corresponding fluorescence spectra.

Excitation 458 nm (left), and 633 nm (right). The fluorescence spectra corresponding to the areas with (red curve) or without (black curve) visible red fluorescence (ROI, out of ROI) are shown below the images.

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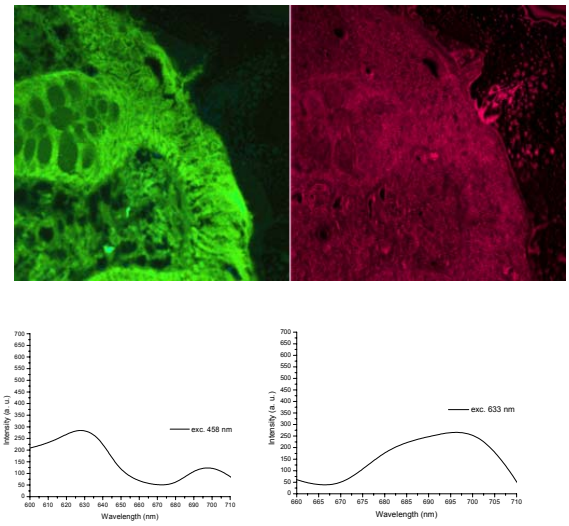

Figure 9: Spectroscopic images of a cryoslit prepared from healthy tissue after excitation by 458 nm (left) and 633 nm (right), respectively. Corresponding fluorescence spectra are shown below the images.

PDT

PDT performed 4 hours after ALA administration with red laser giving the total light dose of 48 J/cm^2 resulted in the apparent reduction of adenocarcinoma polyps. Each patient had a positive response to therapy. In one case there was a total response and in other four cases more than forty percent of suspected area was destroyed. All patients tolerated the ALA dose and the therapy without striking problems, however in two patients side effects, namely sunburn skin photosensitivity, were observed.

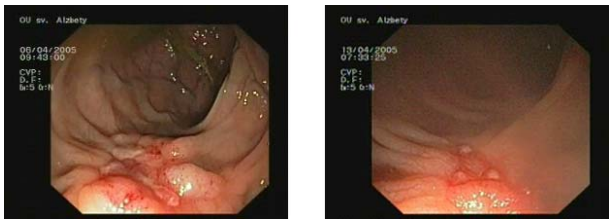

Figure 10: Endoscopic images of suspected area before (left) and one week after PDT (right)

Discussion

In this study ALA-PDT as a procedure for treatment of superficial colon adenocarcinoma polyps was presented. For determination of higher production and accumulation of PpIX in suspected area of tissue fluorescence diagnostics and fluorescence microscopy were used.

In vivo fluorescence diagnostics was performed by excitation at 632.8 nm, what is identical wavelength with Q_1 absorption band and also with emission band of PpIX (Figure 12).

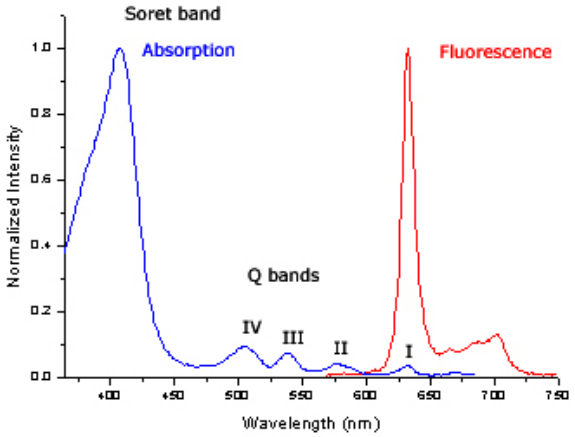

Figure 12: Absorption (blue) and emission (red) spectrum of PpIX .

In this case we supposed that in emission spectra we could detect emission maximum about 705 nm. But fluorescence emission spectra from suspected area were characterized by strong band around 675 nm, which is typical for PPp. Phototransformation of PpIX to PPp is a part of photodegrading process of PpIX and starts immediately after exposure of PpIX by light. In fluorescence spectrum this transformation is characterized by decreasing of fluorescence intensity at 633 nm and increasing at 675 nm [7]. In health tissue this peak was not observed. There was only peak about 705 nm, typical for tissue autofluorescence due to endogenous porphyrins.

Beginning of phototransformation was probably caused by light sources of endoscope during the guidance to suspected area. In fluorescence spectra of cryoslits from suspected area we could see emission band 633 nm that was more intensive as that at 675 nm. So this fact indicates that there remains sufficient amount of sensitizer for tumor eradication. Fluorescence from cryoslits of health tissue was characterized by typical porphyrin dual-band (633 nm – 705 nm) in case of 458 nm excitation and 705 nm in case of 633 nm excitation. Fluorescence was found to be more intensive in suspected area than in healthy tissue, what proved the presence of higher quantity of accumulated PpIX.

Initial results of our study suggest that PDT using endogenous photosensitization with ALA is indeed feasible mode for the treatment of adenocarcinoma of the colon. The results also indicate that tumor necrosis can be accomplished without significant gross damage to surrounding normal mucosa. Another benefit is that one treatment using ALA does not preclude additional treatments. There are a number of issues that require resolution before PDT can be used routinely. Among these are the timing of treatment, light dosage, depth of tissue destruction. Toxic reaction to ALA per se is low and involves three areas. Sunburn is a hazard if the patient is not kept in subdued light for approximately 48 hours. Relatively mild elevations in liver function test results may also occur, but these are transient. Finally,

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mild nausea and occasional vomiting may occur shortly after ingestion.

By changes in fluorescent spectra of PpIX in suspected area and in health tissue has been demonstrated that this photosensitizer is also suitable agent for fluorescence diagnostics of the precancerous tissue. On the other hand, spectrally resolved fluorescence microscopy is possible to reveals more details about accumulation and production of PpIX in tissue what can help to bring new improvements to ALA-PDT procedure.

Conclusions

We have demonstrated the initial results of our PDT and related spectroscopic study. It still running and one from ours aims is performed at least thirty PDT procedures in this study. From these starting results of the first five patients appears, that the treatment of intestinal adenocarcinoma with ALA PDT could be successful. These results constitute an important background for future study of concentration PpIX in tissue and also therapy flow.

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