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Detection of 1270 nm Emission from Singlet Oxygen due to Photodynamic Therapy in vitro and in vivo.

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Photodynamic therapy (PDT) is a cancer treatment modality which utilizes the cytotoxicity of the active singlet oxygen derived from irradiation of a tumor accumulated photosensitizer. As the oxygen in the singlet state radiates an emission of 1270nm wavelength when it decays to the triplet state, detection of the emission helps us to understand the mechanism of PDT or to evaluate photosensitizers. We detected the 1270nm emission from photosensitizers Photofrin and ATX-S10 in vitro and in vivo by means of high sensitive NIR detectors. We obtained the maximum amount of singlet oxygen at irradiation wavelength of 665~670nm from a HeLa tumor in a nude mouse which is injected with ATX-S10.

Key words: photodynamic therapy (PDT), Photofrin, ATX-S10, singlet oxygen, 1270nm emission

INTRODUCTION

Photodynamic therapy (PDT) is a modality of treatment for patients with malignant tumors. It is based on the photochemical reaction that occurs when the photosensitizer is exposed to the light. The photosensitizer administered intravenously accumulates in malignant tumors selectively at a higher level than the surrounding normal tissues. On exposure to the light of a specific wavelength, the photosensitizer is activated from its ground state S_0 to the excited state S_1. A part of the photosensitizer which exists in the S_1 state transfers to the triplet state T_1 by way of the intersystem crossing, and then the photosensitizer in the T_1 state transfers its energy to the triplet state oxygen ($^3O_2$), and as a result we have a generation of the active singlet oxygen ($^1O_2$). This is the main cause of the cytotoxic effect upon the target area which is encountered in PDT. The discrepancy in concentration of the photosensitizer makes it possible to destroy malignant tumors selectively with less damage to surrounding normal tissues.

PDT with photosensitizer Photofrin® has proved its efficacy in the treatment of patients worldwide. In Japan, PDT with Photofrin and an excimer dye laser (an excimer laser pumped dye laser, 630nm wavelength) as an excitation light source has been approved for treatments of superficial early state diseases such as lung cancer, esophagus cancer, stomach cancer, cervical cancer and its dysplasia. It has been supported by the governmental medical insurance since 1996.

Detection of the singlet oxygen in the course of PDT is important to understand and to investigate its mechanism. Near infrared emission of 1270nm wavelength is obtained when the oxygen decays from the singlet state to the triplet state and this detection helps us to investigate the optimal irradiation method (excitation wavelength, exposure time after the photosensitizer injection, fractionation of the exposure, etc), the photobleaching phenomenon of photosensitizers, the comparison of photosensitizers by the power of generating singlet oxygen, etc. J.G.Parker (1987) was the first to detect it from the tumor bearing mouse which was injected with 50mg/kg Photofrin II by the modulated argon-dye laser (an argon laser pumped dye laser, 630nm) irradiation[1]. For the synchronized detection with the laser irradiation, a Ge photodiode and a lock-in amplifier were used.

For the efficient detection of the 1270nm emission, we adopted the photon-counting method using a high sensitive single channel detector, photomultiplier tube (PMT) [2),3)] or a multichannel detector. This paper describes the efficient detection system which incorporates a photon-counting method and some results of the detection from water solutions of photosensitizers and from tumors in mice injected with photosensitizers.

MATERIALS AND METHODS

Samples of water solutions (concentration of 1, 10, 100

uM) of Photofrin (supplied from Wyeth Lederle Japan) or ATX-S10 (supplied from Photochemical Co Ltd, Okayama, Japan) and nude mice with HeLa tumors injected with these photosensitizers were irradiated with a second harmonic generated YAG laser (532nm) or a wavelength tunable optical parametric oscillator (OPO; Figure 1, Figure 2). HeLa cells of 1×10^{7} ml^{-1} were implanted intracutaneously into the right side of the back of the nude mouse. When the tumor diameter reached 6-8mm, the irradiation was performed. The wavelength of 630nm was used for irradiation of Photofrin-injected mice and the wavelength of 670nm was used for the irradiation of ATX-S10-injected mice. Photofrin with 25mg/Kg was injected intravenously into a nude mouse 24hours before irradiation and ATX-S10 was injected with 25mg/Kg intravenously 2hours before irradiation.

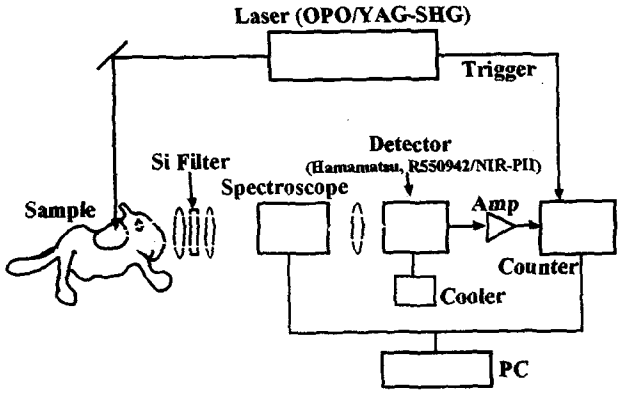

Figure 1. Detection system of 1270nm emission from the singlet oxygen.

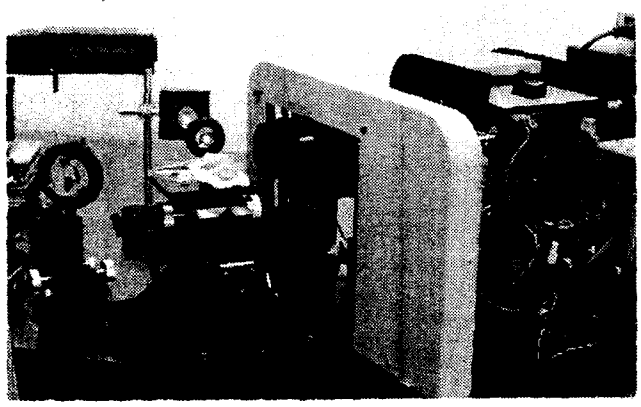

Figure 2. Setup of the experimental detection system.

The reflected or scattered light from the sample was guided to the detection system which was comprised with a spectroscope, a PMT (Hamamatsu Photonics KK, R5509-42) or a multichannel detector (Hamamatsu Photonics KK, NIR-PII) and a photon-counter. To separate the 1270nm emission from the fluorescence of the photosensitizer or the tissue, the variable time window for detection was adopted to the system.

RESULTS AND DISCUSSION

The laser beam with 532nm wavelength and 25mW power at 12Hz was irradiated to the water solution of Photofrin or ATX-S10, and the emission of 1270nm was detected by the system. As the emission was quenched by an addition of the singlet oxygen quencher NaN_3 to the solution, we confirmed that it was derived from the oxygen when it decays from the singlet state to the triplet state (Figure 3, Figure 4). The intensity of the emission

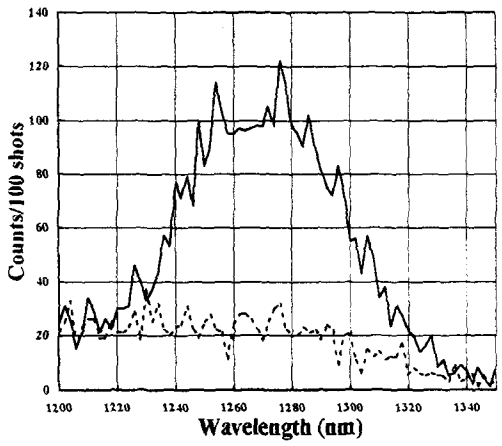

Figure 3. Emission of 1270nm wavelength from the water solution of Photofrin. **upper:** Photofrin (100uM). **lower:** Photofrin (100uM) +NaN_3 (1mM).

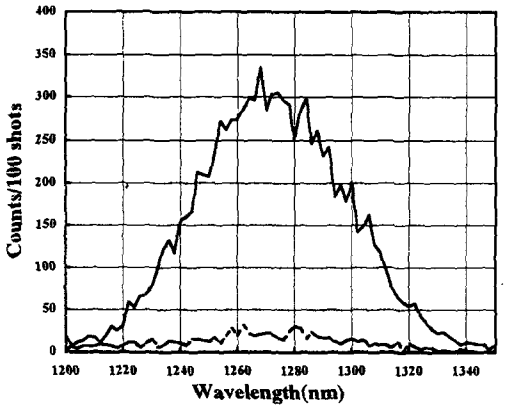

Figure 4. Emission of 1270nm wavelength from the water solution of ATX-S10. **upper:** ATX-S10 (100uM) **lower:** ATX-S10 (100uM) +NaN_3 (1mM)

increased as the concentration of the photosensitizer increased from 1, 10 to 100uM. Although the spectral shapes for 1 or 10uM solutions were not distinct due to the weak intensity of the emission, the shapes for 100uM were clearly marked as shown in Figure 3 and Figure 4.

The emission of 1270nm was also detected in the irradiation of HeLa tumors of nude mice which were injected with Photofrin or with ATX-S10 (Figure 5). It was not detected from tumors without the injection of Photofrin or ATX-S10, so we confirmed that it was derived from the singlet oxygen.

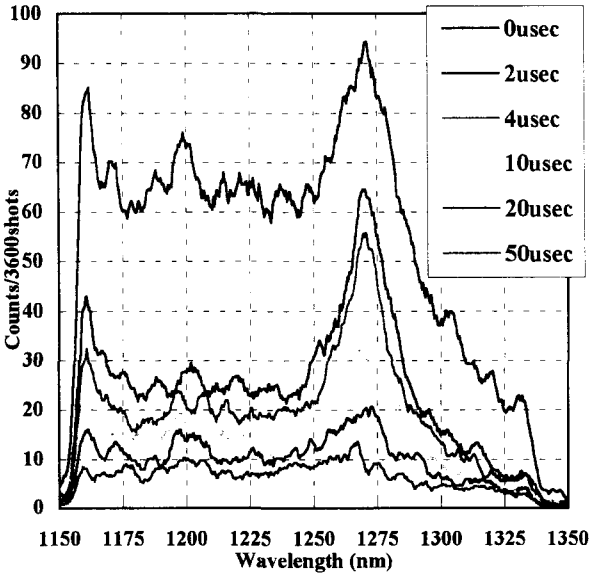

Figure 5. Emission of 1270nm wavelength from the HeLa tumor of the nude mouse injected with ATX-S10. The laser beam with 670nm wavelength and 30mW power at 20Hz was irradiated to the tumor. Results for delay time of 0, 2, 4, 10, 20 and 50usec (from top) are shown. Gate width: 5usec. Accumulation time: 3min.

We measured the 1270nm emission intensity when the excitation wavelength of the irradiation tuned to 645, 650, 655, 660, 665, 670, 675, 680, 685nm for the HeLa tumor of the mouse injected with ATX-S10. Irradiation powers were kept constant to 30uW (20Hz) for these wavelength. A transmission band pass filter of 1270nm wavelength was used in stead of the spectroscope for the efficient measurement. We obtained the maximum intensity around 665~670nm (Figure 6).

By means of the NIR-sensitive photon-counting method we obtained the optical singlet oxygen signal (1270nm emission) from photosensitizers directly. We tested two photosensitizers, Photofrin and ATX-S10. Photofrin is amphiphilic and is used for cancer treatment clinically worldwide and ATX-S10 is hydrophilic and is under development as a second generation photosensitizer in Japan. It is expected to be cleared from the patient's body in a few days, so the demerit of the present PDT with Photofrin which keeps patients in a dark room in a month after a PDT treatment to get rid of the photosensitivity will be overcome by ATX-S10. We confirmed ATX-S10 generated the singlet oxygen with comparable amount with Photofrin in vitro and in vivo experiment with our high sensitive NIR detection system. Our experiment showed ATX-S10 generated the maximum amount of singlet oxygen with 665~670nm excitation wavelength (Figure 5). The irradiation wavelength for the PDT with ATX-S10 willl be discussed considering this result.

Detection of the singlet oxygen generated in the course of PDT is of great interest for the understanding of the mechanism of PDT and our detection system was very useful for this study.

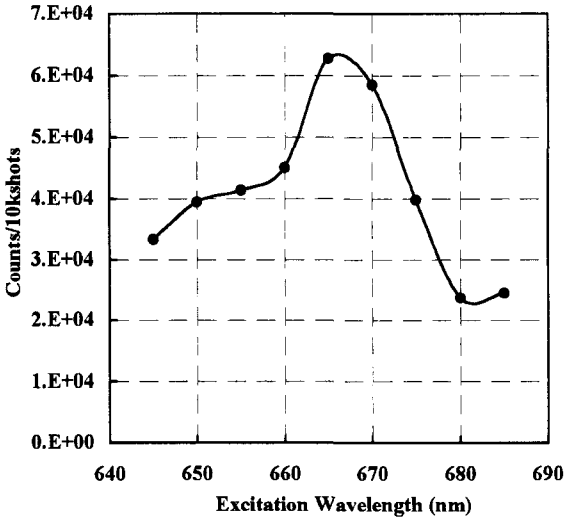

Figure 6. Excitation wavelength vs the 1270nm emission intensity from a HeLa tumor of a nude mouse injected with ATX S10 . Laser irradiation: 30uW, 20Hz.

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