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A new method for monitoring the efficiency of photodynamic therapy with HpD in real-time. First result "in vitro"

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Abstract

We have investigated the feasibility of using optical spectroscopy as a method for monitoring the efficiency of Photodynamic Therapy in real time.

1. Introduction

Photodynamic Therapy (PDT) has proved effective in the treatment of patients with localised malignant tumours [1]. In this treatment a dye, Hematoporphyrin Derivative (HpD), is first administered intravenously and is preferentially retained by the neoplastic cells. When the HpD is excited by UV radiation it fluoresces, so this fluorescence can be used for tumour detection and localization using flexible fiber optic endoscopes. Besides of this property, when the detected tumour is irradiated with red light, the HpD is thereby photoactived, generating highly reactive oxygen intermediates, mainly singlet molecular oxygen, which cause cell death.

The effectiveness of PDT rests on the retention of the HpD in the neoplastic tissue, on its photoactivation with red light and on the amount of singlet oxygen in the medium. Other authors had indirectly verified the efficiency of PDT measuring the oxygen tension with transcutaneous eletrodes [2, 3] and using chemiluminescent materials to detect the oxygen production [4]. In this work we report an optical method to verify directly the HpD energy transfer to form singlet oxygen.

The aim of this work was to detect the fluorescence intensity alternatively during *UV*, and *UV* plus red radiation, and then associate its decrease with the energy transfer process to form singlet oxygen. Therefore this method can be used to verify the efficiency of PDT during the treatment.

2. Theory

When HpD is excited with *UV* radiation an intense visible fluorescence occurs. In this process the excited electrons from fundamental level populate a higher level of energy, and then decay emitting light. The tissue with retained HpD can be excited simultaneously with another wavelength from another absorption process beginning from the same initial level involved in the fluorescence. This resonant excitation can be the one used in the energy transfer process to singlet oxygen – which causes cell necrosis. In this case, a part of the available population at fundamental level of HpD will be transfered to the excited triplet level, as it is represented in Fig. 1. The second excitation process decreases the population at the fundamental level available to the fluorescence process, decreasing its intensity.

Consequently, when monitoring the fluorescence, excited by UV radiation and emitted by neoplastic tissue with HpD, there is a competitive excitation in the red region and we can expect a fluorescence intensity decrease. This shows that energy was available for singlet oxygen formation, and during its progressive formation, a non linear decrease in the fluorescence intensity can be observed. This decrease is connected with the efficiency of the PDT because it shows that singlet oxygen is formed, and it can be used as a new method to monitor the PDT efficiency *in vivo* and in real time.

3. Material and methods

In order to verify this assumption we performed an *in vitro* experiment with a rotating cell containing a diluted solution of HpD in saline. An argon ion laser (I) provided the *UV* wavelength ($\lambda = 364nm$) and a Argon-Dye laser (II) system provided the red wavelength ($\lambda = 619nm$). Both laser beams entered the cell with the same power, travelling the same path and focal point. The signal was detected at 90° by a double spectrometer with a photomultiplier and then amplified by a Lock-in. Figure 2 shows the experimental arrangement.

The HpD was obtained from Hp.2HCl Sigma Chemical; the rotating cell from Hellma. The argon laser (I) is Spectra Physics, mod. 2045E; the argon laser (II) is a Spectra Physics mod. 2025, 4W multiline pumping a Dye laser Coherent Radiation mod. 490 using Rhodamine 6G. It was used a Dou-

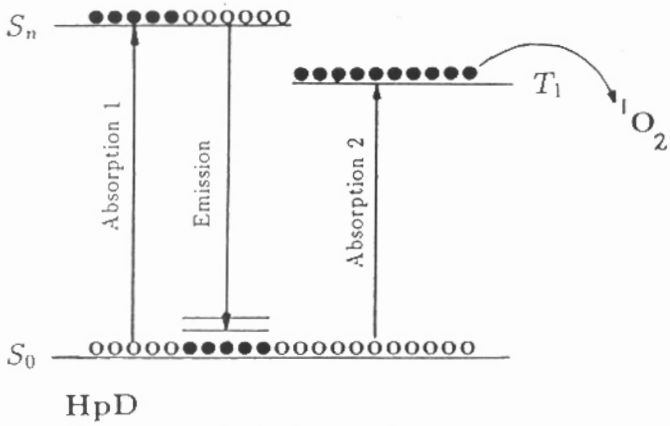

Fig. 1 – Energy diagram.

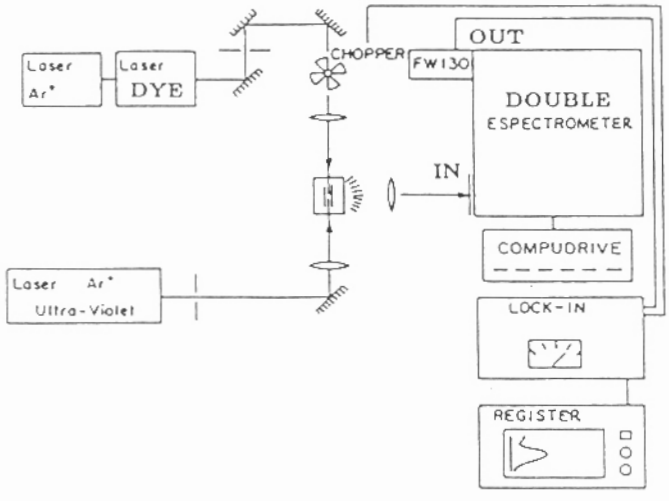

Fig. 2 – Experimental arrangement.

ble Spectrometer Spex mod. 1401, slit 0.2mm; a photomultiplier RCA mod. 124A; a Stanford Research Chopper System mod. SR540; a Princeton Applied Research Lock-in mod. 124A and a Hewlett Packard Chart Recorder mod. 7100BM/17505A.

4. Results and discussion

By fixing the spectrometer and recording data in the wavelength of maximum fluorescence intensity, excited by *UV*, we observed a non linear decrease when having the *UV* plus red wavelengths inciding in the sample. We need to have in mind that the oxygen decay is not a fast phenomenon since the transition to the triplet fundamental state is forbidden, so a certain amount of singlet oxygen still exists in the beginning of the interval without red excitation, causing a delay in the fluorescence intensity to return to the initial level, which can be verified by the spectra shape of Fig. 3.

We have also made a qualitative analogy with an experiment that shows a non linear decrease of oxygen tension during PDT [3], reinforcing our assumptions. In this paper it is observed a decrease

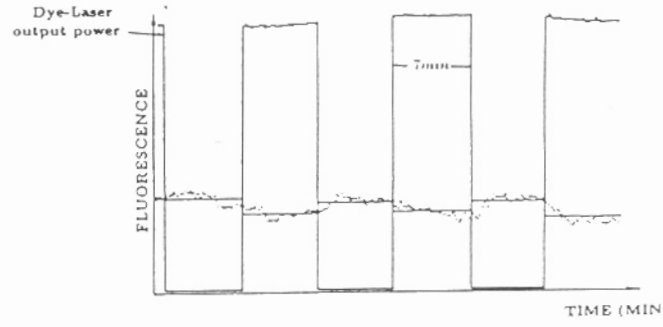

Fig. 3 – Fluorescence intensity in time, at $\lambda = 614.5$ nm.

of oxygen pressure during the red irradiation. This is attributed to the singlet oxygen consumption that will react with the medium. When the red irradiation stops, the vascularization will provide new amount of oxygen, that can be consumed if a new red irradiation will be done. In analogy, in Fig. 3 we can observe the average fluorescence intensity in each interval, as "steps", representing the energy transfer, and consequently the singlet oxygen formation.

Our system is not vascularized, so it will use only the oxygen molecules existing in the solution. So, in our experiments the singlet oxygen formation occurs, and a certain amount of it still exists in the next excitation interval and then the fluorescence intensity will return at a slower rate to its average level. So, the cyclic pattern of our measurements agrees with the results of the reference [3].

5. Conclusion

These results show that the monitoring of the fluorescence intensity excited by *UV* radiation simultaneously with the PDT treatment (irradiation with red wavelength) can be used as a method to verify the efficiency of the singlet oxygen formation.

Acknowledgements

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