PHOTOACOUSTIC SPECTROSCOPY APPLIED TO THE STUDY OF PROTOPORPHYRIN IX INDUCED IN MICE

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RESUMEN

El desarrollo de la Terapia Fotodinámica la cual es una técnica prometedora en el tratamiento de diferentes tumores ha conducido a investigar un gran número de materiales como sensibilizadores. Los sensibilizadores endógenos que pueden ser inducidos por otras sustancias son algunos de los mas interesantes debido a sus ventajas respecto a los efectos colaterales. Se estudia por espectroscopia fotoacústica la inducción de Protoporfirina IX en ratones con el uso del ácido aminolevulínico ALA.

ABSTRACT

The increasing development of the Photodynamic Therapy (PDT) as a promising technique in the treatment of different tumours has lead to investigate a great number of compounds as photosensitizers. The endogenous photosensitizers which could be induced by other substances are one of the most interesting due to their advantages in respect with the side effects. A study of the aminolevulinic acid (ALA) induced Protoporphyrin IX (PpIX) in mice by Photoacoustic spectroscopy is presented.

INTRODUCTION

Aminolevulinic acid (ALA) is used to induce protoporphyrin IX (PpIX) accumulation, it is one of the photosensitizers most powerful and specific for Photodynamoic Therapy (PDT), this is accumulated in high concentrations in cancerous cells and in low concentrations in normal cells [1]. It is important to measure the distribution of the PpIX in tissues and to study the products of its photobleaching in order to find possible collateral effects and to optimize the PDT. Among the not conventional techniques, that allow us to accomplish these studies, The Photothermal Techniques (PT) stand out because of allow us to obtain information about the samples that present inherent difficulties to the high dispersion of the light, and of structures that vary with the depth (for example: semiconductors, mineral, vegetable and animal tissue) [2].

Objectives: To determine the PpIX in situ accumulation in skin from mice exposed to different ALA concentrations, to obtain the optical absorption spectrum of PpIX in situ, to calculate the degradation time of this molecule when irradiated at its maximum optical absorption wavelength and to obtain the optical absorption coefficient as a function of the wavelength radiation.

MATERIALS AND METHODS

Five groups of seven CD1 female mice were used, four of which were exposed by intraperitoneal route to different ALA concentrations: 40, 80, 160, 320 mg/Kg, they were sacrificed to 0.5, 0.75 and 1 h after their exposition respectively. One group was treated with physiological saline solution and after one hour it was sacrificed. Mouse skin biopses(1cm2) of the abdominal zone were obtained, these were stored to – 20°C until their analysis. In order to obtain their optical absorption spectra by Photoacoustic Spectroscopy the samples were defrosted, shaved and dried. From the optical absorption spectra will be determined the optical absorption coefficient as a function of the wavelength. Also, from the difference between the external and internal face absorption spectra, it will be identified the zone of the epithelium with greater concentration of PpIX. To determine the degradation time of PpIX, it will be performed an in vitro study of this molecule which will be irradiated in its maximum absorption wavelength and by monitoring the evolution of the photoacoustic signal it will possible to obtain this time. The optical absorption spectra were obtained in the range of 300-700 nm by the use of a PA spectrometer. The experimental set-up consists of a 1000 W Xenon lamp (Oriel), a variable frequency mechanical chopper set at 17 Hz, a monochromator, an air filled

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brass cell with a condenser microphone and a lock-in amplifier. The sample compartment is a cilindrical chamber. The PA signal obtained is detected by a lock-in amplifier (SR-850), interfaced to a personal computer, which simultaneously displays the wavelength-dependent signal amplitude and phase.

RESULTS AND CONCLUSIONS

The optical absorption spectra from the skin samples, external and interna face, indicated that the PpIX is mainly accumulated in the dermis. From absorption spectra also were found the optical absorption coefficient as a function of the wavelength. See Graphic 1. The determination of this optical coefficient is very important in PDT in order to determine the ligth penetration in the skin.

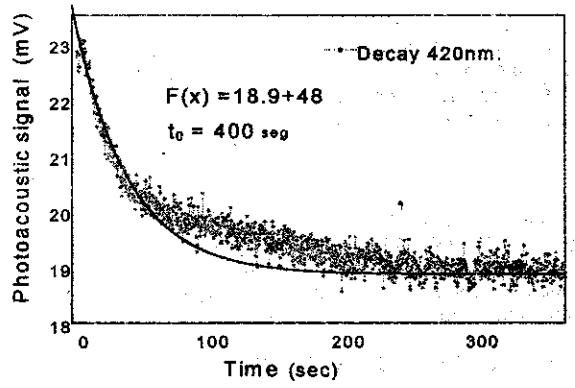
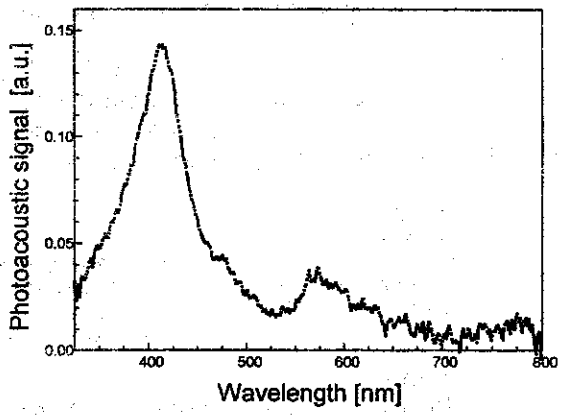


Figure 1. Photobleaching of the PpIX diluted in

Considering that the photobleaching process could be approximate to an exponential decay the PpIX degradation time was found around of 510 sec of exposition at the maximum absorption wavelength, what will allow us to have a criterion for the radiation time used in PDT. The graphic obtained for the photobleching and the fitted exponential decay are presented in Graphic 1.

The photoacoustic absorption spectra of the PpIX in acid chloride 25 N is presented in Graphic 2. It is clearly seen a Soret band peak at 417 nm and secondary peaks at 565 nm, and 620 nm .



Graphic 2. Photoacoustic spectra of PpIX.

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