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Quantification of Protoporphyrin IX concentration in murine pigmented melanoma by ex vivo, in situ and in vitro measurements

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PpIX is an endogenous porphyrin whose accumulation in target cells can reach sono- and photosensitizing concentrations by the exogenous application of ALA. Since a key component to trigger dynamic cytotoxic effects is the sensitizing molecule, it becomes very important to verify that the sensitizing molecule to be used for treatment is deposited in sufficient quantity in the target region. These measurements may be feasible non-invasively using imaging when the tumor is non-pigmented. However, when the tumor lesion is pigmented, the PpIX quantification measurements using fluorescence images are not accurate. So, this work aimed to assess the PpIX deposition in melanoma tumors by in vitro fluorescence measurements and chemical extraction method. (1) For this purpose, female BALB/c nude mice were inoculated with 10^6 murine melanoma cells (B16-F10) by intradermal injection on the right flank of the animal. When the tumor volume reaches 100 $\pm$ 10 mm^3, animals were subjected to ALA administration following 5 different protocols: Topical administration using a cream formulation containing 20% Methyl ALA (4 UI, incubation time: 12 h), Intratumoral ALA administration (Dose: 200 mg/Kg b.w., incubation time: 2 h), and intraperitoneal ALA administration (Dose: 200 mg/Kg b.w., incubation time: 3, 4 and 5 h). The PpIX fluorescence spectra, in healthy skin and tumor region, were collected by in situ, ex vivo and in vitro measurements by Steady-state fluorescence spectroscopy. For *in vitro*.measurements, tissue samples (healthy skin and tumor) were excised, homogenized, and put into 1 mL methanol for 24 h at - 20 °C. After this time, samples were thawed and centrifuged, and the supernatants were collected for fluorescence measurements. (2) The amount of PpIX extracted from the melanoma tumor was compared with that extracted from a non-melanoma tumor model (A431) where the PpIX synthesis was induced by a clinical protocol of ALA administration for the application of photodynamic therapy. The *In vitro*.measurements showed that the intraperitoneal (3 h) and intratumoral protocols induced a greater PpIX accumulation (0.34$\pm$0.02 μM) in the tumor region than the other protocols, but it was observed a high sensitization of the healthy skin. By means of the topical protocol, it was possible to avoid the healthy region sensitization, and the PpIX concentration in the tumor (0.11$\pm$0.01 μM) was almost the same amount as that generated by the clinical protocol (0.16$\pm$0.01 μM). Unlike in vitro measurements, ex vivo measurements showed no PpIX fluorescence in the tumor. This discrepancy is due to the light absorption of the pigmented tissues, so, the direct fluorescence readings are misleading. The results suggested that a photosensitive amount of PpIX can be synthesized within the melanoma tumor by the Intratumoral, intraperitoneal (3 h), and topical protocols.

Palavras-chave: Protoporphyrin IX. 5-Aminolevulinic acid. Cutaneous melanoma.

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