Laser-induced fluorescence spectroscopy assess the Protoporphyrin IX generated by 5-aminolevulinic acid and its methyl ester in cutaneous malignant lesions

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Photodynamic therapy (PDT) of tumors is a therapeutic modality considered for the treatment of nonmelanoma skin cancers (NMSC) and other cutaneous diseases. PDT is a minimally invasive method, avoiding surgical intervention or the use of ionizing radiation. The cell death induced by PDT results from the local oxidative stress promoted by reactive oxygen species (ROS) induced by a photosensitizing drug activated by light of specific wavelength. The 5-aminolevulinic acid (ALA) and its methylated ester (MAL) as precursors of the endogenous photosensitizer protoporphyrin IX (PpIX) have been widely studied for the topical treatment of NMSC and other cutaneous diseases. However, the detailed role of PpIX generated by ALA/MAL in diseased and normal skin is incomplete. Understanding the PpIX fluorescence in neoplastic skin may be useful for optimization of PDT while minimizing side effects in the surrounding normal tissue. In this way, this study aimed to use laser-induced fluorescence (LIF) spectroscopy to evaluate the generation of PpIX in squamous cell carcinoma using photosensitizer creams based on ALA and MAL. Neoplastic lesions were induced in 20 Swiss mice using a well-established multi-stage chemical-carcinogenesis protocol and the PpIX generated in the mouse skin was monitored non-invasively by the fluorescence signal emitted after application of each photosensitizer. LIF measurements were collected every 30 minutes on the shaved backs of the mice using a bifurcated fiber-optic probe connected to a spectrometer. A diode laser emitting at 405 nm was used as excitation wavelength and fluorescence emission was measured at 635 nm. Measurements were recorded using the SpectraSuite software (Ocean Optics, USA) and the spectra were normalized by the power emitted by the diode laser as well as by the fluorescence intensity at 405nm. A pairwise comparison using T-Student statistical test was performed in each time interval and the data were considered statistically different for p-values < 0.05. Our results showed no statistical significant difference between the groups for 0–90 minutes. On the other hand, the fluorescence intensity for neoplastic skin submitted to ALA was higher than that from tissue submitted to MAL at all times after 90 minutes and showed an increasing behavior with slight fluctuations over time. Considering the lipophilic and charge characteristics from MAL, we concluded that the lowest LIF measurements collected from tissue underwent this prodrug resulted from its higher penetration into the skin, whereas the highest PpIX emission obtained for ALA is an indicative of superficial generation of PpIX and its poor penetration into the tissue.

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