

PROTOCOLOS DE INVESTIGACIÓN - BIOMEDICINA

EVALUATION OF THE INFLAMMATORY INFILTRATE IN ORAL SQUAMOUS CELL CARCINOMA AFTER 5-ALA MEDIATED PHOTODYNAMIC THERAPY: AN IN VIVO STUDY

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Photodynamic therapy (PDT) uses light and a photosensitizer (PS) to generate reactive oxygen species (ROS) and eliminate tumor cells, such as those of oral squamous cell carcinoma (OSCC), an aggressive cancer type, frequently associated with resistance to conventional therapies and poor. However, the in vivo effects of PDT in OSCC remains unclear. Thus, this study aims to investigate the effects of PDT in an in vivo experimental model of OSCC. Forty male C57BL/6 mice will be randomly distributed into four groups: control (n=10), 5-ALA (n=10), laser (n=10), and PDT (n=10). A total of 1×10^5 MOC2 cells will be subcutaneously inoculated into the right dorsal area of the animals. Tumor growth will be assessed three times a week using a digital caliper. Once tumors

reach 100 mm³, animals will be treated according to their group. The 5-ALA and PDT groups will receive 100 mg/kg of 5-ALA (Sigma-Aldrich) diluted in water, administered intraperitoneally, and kept in the dark for 4 hours. The laser and PDT groups will undergo a single irradiation session using the Photon Laser device (DMC, Brazil) with the following parameters: 660±20 nm, 100 mW, 18 J, 90 J/cm², 180 s. Euthanasia will be performed at 7 and 15 days post-treatment. Tumors will be removed, fixed in 10% formalin, and processed for immunohistochemistry to evaluate the expression of CD8, CD4, CD163, CD56, and elastase. Sections of 3 µm will be deparaffinized, rehydrated, and subjected to antigen. After blocking endogenous peroxidase, samples will be incubated for 18 hours at 4 °C with primary antibodies. The EnVision®+ Dual Link System kit will be applied followed by DAB development and counterstaining with Mayer's hematoxylin. Five fields (200x) will be photographed and analyzed using ImageJ software and the data will be submitted to statistical analysis.

Palavras-chave: oral squamous cell carcinoma; photodynamic therapy; inflammatory infiltrate.