MB02.pdf

Evaluation of the necrosis profile induced by ALA-PDT with the association of tissue micromachining using femtosecond laser ablation

Gustavo Nicolodelli[1], José Dirceu Vollet-Filho[1], Cristina Kurachi[1], Ricardo Elgul Samad[2], Anderson Zanardi de Freitas[2], Nilson Dias Vieira Junior[2] and Vanderlei Salvador Bagnato[1].

[1] *Instituto de Fisica de Sao Carlos, University of Sao Paulo – Av. Trabalhador Saocarlense, 400, São Carlos,SP 13566-590, Brazil*
[2] *Instituto de Pesquisas Energéticas – IPEN/USP*- 05508-000, São Paulo – SP, Brazil

author's e-mail address: nicolodelli@ursa.ifsc.usp.br

Abstract: The aim of this study was the investigation of the 5-aminolevulinic acid (a tissue precursor for protoporphyrin-IX) penetration in rats' liver after tissue micromachining obtained by femtosecond laser ablation.

1. Introduction

Laser ablation of biological tissues, in ultra-short pulses, is a technique that results in a precise ablation without thermal damage due to its characteristic light-tissue interaction [1-3]. Photodynamic therapy (PDT) is a therapeutic technique to treat malignant and pre-malignant lesions, mainly neoplastic ones, which promotes cell death by combining the effect of a photosensitizer irradiated by a proper wavelength in the presence of molecular oxygen [4,5]. A limiting factor of topical ALA-PDT is the drug penetration depth. Therefore, the aim of this study was to investigate if the penetration of 5-aminolevulinic acid (ALA), a precursor of an important photosensitizer used in PDT treatments (protoporphyrin-IX, PpIX), could be improved by the combination of tissue machining of microchannels using ultrashort pulses. The microchannels were obtained at the rat liver surface by laser ablation at the femtosecond regime, before the application of the ALA cream.

2. Materials and Methods

A CPA Ti-Sapphire system composed for Rainbow main oscillator and the Femtopower Compact Pro CE-Phase HP/HR amplifier at 800 nm, emitting pulses of approximately 35 fs, operating at a maximum repetition rate of 1 KHz was used. The beam was steered with highly reflective coated dielectric mirrors and passed through a 20 cm focal lens before reaching the target sample. The Wistar rats were positioned at a Newport-Universal Motion Controller/Driver (Model ESP300) with the liver exposed for irradiation. The microchannels were made at three different areas of the liver, of approximately 0.35 cm x 0.254 cm, spaced of 0.05 cm.

Topical cream of 20% ALA was placed at the liver surface of Wistar rats for 2 hours. A 630 nm diode laser (Ceralas®, Ceramoptec, Germany) was used as a PDT light source. An optical fiber with 300 μm of diameter with coupled microlens was used to obtain a uniform profile of irradiation. Square window (0.5 cm x 0.5 cm diameter) was used for irradiation. Fluence 100 J/cm² were used for a fluence rate (250 mW/cm²).

Normal livers were used to evaluate depth of necrosis variation as a function of the micromachining, obtained by laser ablation. A control region of liver was kept free of ablation for comparison of tissue effects. Animals were anesthetized and photosensitizer was administered at concentration of 1.5 mg/kg of body weight through inferior vena cava reached by surgery. After 2h45min, liver was irradiated. Animals were kept alive for 27 hour to allow necrosis establishment, after which they were killed to remove necrosis tissue for histology investigation.

Necrosis tissue was macroscopically evaluated, and samples were prepared for histological analysis (H-E staining method). Necrotic tissue aspect and depth of necrosis (the measure of necrosis length from the irradiated surface to the borders between necrotic and healthy tissue) were the main analyzed characteristics, which were evaluated using optical microscopy coupled to a micrometer-controlled (at x and y axes) chariot.

3. Results and Prospect

This study is still in progress. At the present moment, liver tissue slides were investigated for depth of necrosis measurements. For each treated region, whether it was micromachined or not, four to six slides of the necrotic region were obtained. For each slide, four depth of necrosis measurements were performed.

Figure 1 shows a micrographic picture of necrotic tissue after PDT (femtosecond laser irradiation parameters: repetition rate of 100 Hz, average power of 6 mW, during 10 s of irradiation time).

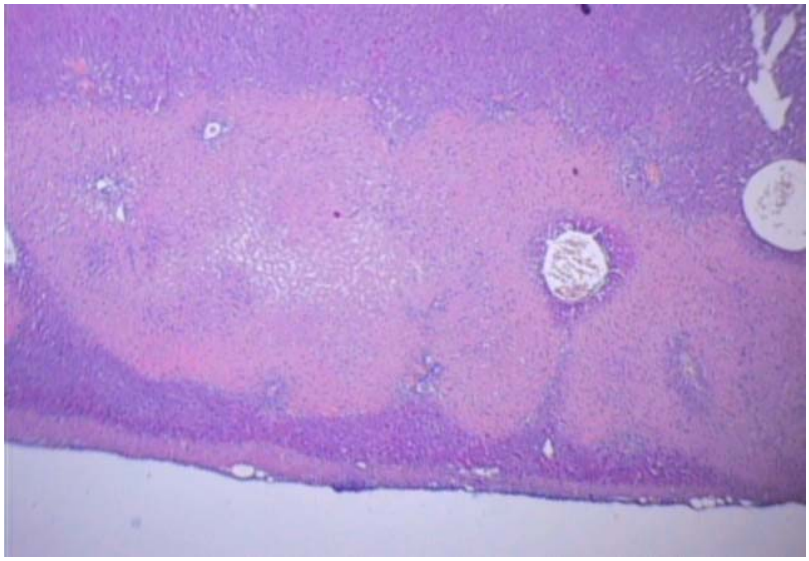

Figure 1 – Histological picture of necrotic liver tissue after PDT, applied in a femtosecond laser-micromachined tissue.

Preliminary results show a healthy tissue region between the irradiated surface and the established necrosis. This result may be related to the femtosecond laser previously applied to obtain tissue micromaching. A variation in depth of necrosis for the micromachined regions of tissue was also observed, which might indicate that laser ablation have influenced ALA penetration. However, since these results are preliminary, more studies that vary conditions of PDT application and femtosecond laser tissue ablation are required to validate them.

4. References

[1] Christopher V. Gabel. “Femtosecond lasers in biology: nanoscale surgery with ultrafast optics.” Contemporary Physics Vol**. 49**, No. 6, November–December 2008, 391–411

[2] J. Neev, L.B. Silva, M.D. Feit, M.D. Perry, A.M. Rubenchik, and B.C. Stuart, “Ultra-short pulse lasers for hard tissue ablation,” *IEEE J. Sel. Top. Quantum. Electron.* **2**(4), 790-800 (1996).

[3] Y. Liu, and M. Niemz, “Ablation of femural bone with femtosecond laser pulses—a feasibility study,” *Lasers Med. Sci*. **22**, 171-174 (2007).

[4] Dougherty, T.J., “An update on photodynamic therapy applications”. Journal of Clinical Laser Medicine & Surgery, 2002. **20**(1): p. 3-7.

[5] Peng, Q., et al., “5-aminolevulinic acid-based photodynamic therapy - Clinical research and future challenges”. Cancer, 1997. **79**(12): p. 2282-2308.