

PDT in oncology

26

Proliferation index of keratinocytes in 4-NQO-induced oral dysplasias submitted to 5-aminolevulinic acid-mediated photodynamic therapy

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Introduction: Oral dysplasias (OD) are potential malignant disorders that are currently treated with traditional or laser surgical excision. There is no consensus regarding the efficacy of these procedures mainly in relation to frequency of OD recurrences. Topical 5-aminolevulinic-mediated photodynamic therapy (5-ALA-PDT) has been tested in OD in preliminary clinical studies, demonstrating significant frequency of complete response and low frequency of recurrences. The protocols for these purposes have not been completely established, mainly regarding the frequency of topical 5-ALA-PDT sessions. The aim of this study is to analyze the proliferation index of keratinocytes in 4-NQO-induced OD at times from 6 h to 72 h after 5-ALA-PDT, in order to suggest the intervals for topical PDT sessions based on the cell proliferation recovery in the oral mucosa.

Material and Methods: OD was induced in thirty adult rats (Wistar) using daily topical application of 4-NQO on oral tongue mucosa (3 times per week). The lesions were submitted to a single session of 5-ALA-PDT, which was performed through topical application of 5-ALA 5% ointment on oral tongue for 2 h followed by laser irradiation (diode laser, 660 nm, 40 mW, 90 J/cm², 1.5 min). Six animals were used as control without PDT. After the PDT, the animals were euthanized at 6 h, 24 h, 48 h, and 72 h, and the controls at 72 h. The rat tongues were then submitted to immunohistochemical tests for caspase-3 and proliferating cell nuclear antigen (PCNA). Labeling index (LI) was calculated using manual cell counting method.

Results: None of the animals showed any clinical alterations of the lesions after single PDT session. In PDT group, LI for caspase 3 was significantly higher than in the control group ($p=0.0012$), mainly at 6 h ($LI = 4.5 \pm 0.7$, $p=0.0031$). PDT at 24 h and 48 h showed LI for PCNA significantly lower than the control group (LI at 24 h = 1.7, $p=0.0440$, and LI at 48 h = 1.4 $p=0.0165$), but at 72 h the LI was close to the control group ($LI = 2.5$, $p=0.2312$). LI for caspase 3 was significantly higher than LI for PCNA only at PDT 6 h ($p=0.0223$), indicating that significant apoptosis index was present for the first hours of laser irradiation.

Conclusion: 5-ALA-PDT exerts down-regulation on the cell cycle from 24 h to 48 h after irradiation, with significant apoptosis index at 6 h. Intervals between PDT sessions focused on OD remission must be no longer than 3 days.

27

Lasers and light emitting diodes in diagnostics and treatment of tumors located in ocular adnexa

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Introduction: Laser treatment of tumors, which arise in ocular adnexa, has great potential. It enables to provide a minimally invasive therapy. The aim of this work was to report our experience using various laser and light emitting diode (LED) systems in diagnostic and treatment of different benign and malignant lesions located in ocular adnexa.

Material and Methods: Since 1986 the total of 252 patients with 282 malignant tumors and 1614 patients with more than 3000 benign tumors located in ocular adnexa underwent laser treatment. Surgical laser treatment was applied for the most of patients. 128

patients (153 lesions) underwent photodynamic therapy (PDT). 1/3 PDT was provided for 43 patients with advanced (T₃-T₄) cancer. For 85 patients with T₁-T₂ cancer topical PDT was provided. Fluorescence diagnostics measurements were performed in all 128 patients for whom PDT was applied. This method was applicable for detecting early superficial tumors, margins of tumors and follow-up after therapy. Usually patients underwent PDT 2–5 days after laser surgery. There were only 15 patients treated with PDT alone. 7 different lasers and 2 LED systems were used for diagnostics and treatment of lesions.

Results: The immediate results of the surgical laser treatment revealed a complete disappearance of 98% benign and more than 90% of malignant lesions. Cancer patients treated with laser surgery alone were followed up for the period from 11 to 182 months. The average follow up of these patients was 66 months. During this period the recurrent disease was noticed in 6 patients. As the immediate results of 43 patients with advanced cancer in ocular adnexa for whom PDT was applied – 34 tumors in 34 patients completely disappeared. The patients treated with PDT were followed up for the period from 8 to 184 months. The average follow up of these patients was 68 months. The recurrent disease was noticed in 12 patients. In these cases PDT was repeated. There was progression of the disease in 3 patients and enucleation was provided for them.

Conclusions: Laser surgery is the method of choice for the treatment of ocular adnexa tumors. PDT is the alternative method of treatment for cancer patients with advanced recurrent disease in ocular adnexa. It enables to avoid enucleation. Fluorescence diagnostics has been shown to be highly effective in malignant superficial lesion diagnostics.

28

Photodynamic therapy of dermal metastases of disseminated melanoma

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The aim of research was the development of a method and evaluation of efficiency of photodynamic therapy (PDT) of dermal metastases of disseminated melanoma.

Materials and Methods: We examined 45 patients, 19 men and 26 women at the age of 18 to 76 with cutaneous metastases from disseminated melanoma after surgery, polychemotherapy, immunotherapy, radiation therapy before the PDT. 80 metastatic nodes were treated in total. Ultrasound verification of metastases was done before the treatment. All metastases were divided into intra- and subdermal.

Intradermal metastases 59 focuses were located in epidermal and dermal layers 0.5 centimetre below the skin surface. 21 subdermal metastases, located on the border of reticular layer of the dermal and subcutaneous fat and muscles, below the surface of skin.

Photolon was prepared ex tempore in dose 1.0–1.5 mg/kg and administered intravenously 3 hours before PDT. Laser with wavelength of 662 nm was used for treatment. Parameters of PDT: density of power of laser radiation (Ps) was 0.3–0.5 W/cm², density of energy of laser radiation (E) was 150–300 J/cm². Fluorescent imaging and spectral analysis were used. Fluorescent spot against dark normal tissue was seen before the treatment, it was due to 3–4 times higher accumulation of Photolon in the tumor. Dark spot seen after the treatment was the result of hemorrhagic necrosis. After the treatment the spectral peak had lowered.

The treatment procedure was done step-by-step. At the first stage all metastases of melanoma were treated without the contact with skin. Nodes with diameter 1 cm were irradiated by one field, larger metastases were irradiated by 3–4 overlapped field, which covered about 5 mm of perimeter normal tissue. Polimorphic picture of the treatment, sometimes with unchanged melanoma cells were seen in 3–5 days after the first stage of treatment. More radiation session