nature of immune responses induced with PDT and the underlying action mechanisms. Critical for this knowledge is the perception of PDT as an immunogenic cell stress agent.
Methods: PDT-induced oxidative stress is recognized as directly responsible for the responses ranging from regulated immunogenic cell death (ICD) to immunogenic modulation. The PDT-elicited activity in stress signaling networks need to be in the focus of critical experimental approaches.
Results: In ICD, PDT-triggered stress pathways orchestrate release of antigens and danger signals from the dying tumor cells that enable the activation of adaptive antitumor immune response. The provided critical adjuvant signals come in the form of damage-associated molecular patterns (DAMPs) that are responsible for the recruitment and maturation of antigen-presenting cells (APCs). On the other hand, the immunogenic modulation enables enhanced antigen presentation, increased proapoptotic signals, changes in the expression of surface flag molecules by the tumor cells, and other tumor phenotype alterations making tumor more sensitized to the attack of T cell effectors.
Conclusions: PDT can be clinically exploited as immunogenic cell stress agent for treatment of cancerous lesions either using adjuvants that can amplify the elicited immune responses or block negative immunoregulatory responses in tumor microenvironment.

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Comparing two photodynamic therapy protocols for high-grade cervical intraepithelial neoplasia

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Significance: Photodynamic therapy (PDT) is a therapeutical modality which offers a minimally invasive alternative for high-grade cervical intraepithelial neoplasia (CIN) treatment.
Approach: this prospective randomized controlled clinical trial aims to compare two PDT protocols for histopathologically similar high-grade CIN treatment. In protocol 1, only ectocervix is treated; in protocol 2, the patients had both the ectocervix and endocervix illuminated simultaneously with LED plus LASER (a cylindrical diffuser fiber was coupled inside the squamocolumnar junction). In both groups was applied 20% MAL overnight and the irradiance delivered was 120 mW/cm^2, for 21 minutes, in a fluency rate of 150 J/cm^2.
Results: after two years of follow-up, group 1 showed 96% cure and group 2, 87%.
Conclusions: no significant difference was observed between the two protocols. However, the number of patients who completed the two-year follow-up is still low and we expect better conclusions with more patients followed.

Keywords: high-grade CIN treatment, photochemotherapy, HPV, cervical cancer, cervical intraepithelial neoplasia

1. Introduction and Background

Cervical cancer is squamous cell carcinoma often caused by Human papillomavirus (HPV) infection and has been the fourth-most common type of cancer and cause of death in women (1). The high-grade intraepithelial neoplasia (CIN) precedes cervical carcinoma and its major cause has been the persistent infection by HPV, and the vast majority of cervical cancer cases are caused by persistent or chronic HPV infection (2). Aminolevulinc acid (ALA) and methyl aminolevulinate (MAL) mediated photodynamic therapy (PDT) has showed be promising treatments for HPV-related diseases, such as condyloma acuminate and high grade CIN (3) (4) (5).

2. Aims

We conducted a clinical trial designed to evaluate the efficacy and safety of photodynamic therapy (PDT) using a CerCa 150 System® available for the application of intra-canal fiber or no in patients with high grade cervical intraepithelial neoplasia.

3. Methods

3.1 Patient Enrollment

The present study was approved by the Ethics Human Medical Ethics Committee (CEP 827.010, April 2013 CAAE: 90629218.8.0000.5383) and all patients provided informed written consent to participate. Patients with confirmed diagnosis of cervical intraepithelial neoplasia (CIN) grades 2/3 by histopathology were selected and invited to participate in this study. Thirty-seven patients were treated between April 2016 and October 2018 and monitored for two years. The patient inclusion was random and decided by the clinicians involved in the research, depending on the type of the transformation area and the lesion site: PDT LED ectocervix (1 or 2 session); PDT LED + LASER endocervix group (1 or 2 sessions).

3.2 Photodynamic Therapy

When patients were treated two times, the interval between sessions was a week. The methyl aminolaevulinic acid (MAL) 20% cream (PDT-Pharma, Cravinhos, Sao Paulo, Brazil) was administered by the patient in the cervix by 10 hours (overnight). For illumination, the patient in the gynecological position was treated by the equipment CerCa 150 System® (MMOptics, São Carlos, Sao Paulo, Brazil). This device has LEDs (light-emitting diodes) emitting at 630 nm and can be anatomically positioned to illumine the entire ectocervix and also a cylindrical light diffuser (MedLight S.A., model RD30) for homogenous light distribution to the endocervical canal. The illumination was performed by delivering 120 mW/cm^2 for 21 minutes and fluency rate of 150 J/cm^2. Two months after one or two procedures, a conservative Excision of Transformation Zone (ETZ) is performed for histopathological analysis. The viral load test (digene® HC2 HPV DNA, Qiagen) was carried out in two moments: a) before the treatment and b) 60 days after LEEP.

4. Results

The average age was 38,52±9,79 years to women treated with LED and 33,06±8,84 years to women treated with LED+LASER. LED was applied to 21 women (11 women received one session and 10 women received two sessions) and LED+LASER was applied to 15 women (5 women received one session and 10 women received two sessions).
60 days after treatment, the PDT performed on the ectocervix, LED, showed 52% of positive results in the cytological exams and 19% in the LEEP product. On the other hand, women treated intracanal, LED+LASER, had a 40% positive overall response in cytological exams and 13% in histopathological analysis.
The decrease in viral load, analyzed 60 days after one or two sessions of PDT, showed exactly the same percentage of decrease, comparing the two protocols. No protocol showed complete elimination of the viral load. This result generates an important discussion because this test, despite being recommended and the gold standard, may present flaws that depend on factors such as sample collection and storage.
Considering all lesions with positive responses to PDT with follow-up of two years, there were 96% positive responses (n=20) with PDT in patients treated for LED and 4% recurrences (n=1). The intracanal

LED+LASER treatments showed 86.66% (n=13) of positive response and 13.33% of lesion recurrence (n=2).

5. Conclusion

This retrospective study was postponed due to the coronavirus (Covid-19) pandemic. We concluded that just illuminating the cervix from the outside (ectocervix) with LEDs promoted a higher cure rate than the protocol that associates LED lighting with LASER (ecto + endocervix). We also concluded that the follow-up of this type of treatment should always be long-term (two years at least).

Disclosures if required

The authors declare no conflict of interest.

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The role of pigmentation in tumor treatment with the investigational virus-like drug conjugate belzupacap sarotalocan in an in vitro and in vivo murine model

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Purpose: Tumor pigmentation is known to be a barrier for applying photodynamic therapy in ocular melanoma. We investigated the role of pigmentation in tumor behavior and compared the anti-tumor efficiency of belzupacap sarotalocan (bel-sar, AU-011) treatment using a pigmented (wt) and non-pigmented (TYR knockout, ko) in an in vitro and in vivo murine melanoma model.
Methods: A tyrosinase gene knockout B16F10 cell line was developed using CRISPR/Cas9. Following bel-sar binding and light activation (wavelength 690 nm) in vitro, cellular cytotoxicity and damage-associated molecular patterns (DAMPs) were measured by flow cytometry (FACS). Bel-sar treated tumor cells were co-cultured with antigen-presenting cells (APC) to assess phagocytosis and maturation. The B16F10 wt and ko cell lines were grown in syngeneic mice and treated with bel-sar and light.
Results: TYR knockout in B16F10 led to the loss of pigmentation which had no effect on in vivo tumor growth. Bel-sar treatment induced near complete cell death, accompanied with exposure of DAMPs, resulting in enhanced phagocytosis and maturation of DCs, regardless of pigmentation level. Bel-sar treatment delayed tumor growth in both tumor models. Pigmented tumors had more infiltrating M1 and less M2 macrophages compared with non-pigmented tumors. Bel-sar treatment stimulated more M1 influx in both models. Following treatment, CD8+T cells were upregulated, particularly in the pigmented tumors, and more mature dendritic cells were detected in the tumor draining lymph nodes.
Conclusions: Pigmentation influences the type of infiltrating macrophages in the tumor. Bel-sar treatment induced immunogenic cell death and a positive anti-tumor response in vivo with inhibition of growth in both pigmented and non-pigmented models, and stimulates the influx of M1 stimulating macrophages.

Key word: Pigmentation, melanoma, photodynamic therapy, AU-011, belzupacap sarotalocan, bel-sar

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Photodynamic therapy for nodular basal cell carcinoma up to 5mm located on high-risk area: effectiveness and long-term follow-up results

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Significance: Response rates evaluation of photodynamic therapy (PDT) for nodular basal cell carcinoma (BCC) treatment located on high-risk and low-risk areas of the face.
Approach: Two groups of nodular BCC were selected, debulked, and received 20% methyl aminolevulinate cream. After 3h, the first irradiation was performed (20min, 150J/cm2). Then, the cream was re-applied, and a second irradiation was performed after 1.5h (20min, 150J/cm2). Clearance at 30 days and recurrence-free survival rate were evaluated.
Results: The clearance at 30 days after PDT was 89% for the low-risk area group and 87% for the high-risk group. The recurrence-free survival at 60 months was 82% and 85% for the high-risk and low-risk groups, respectively.
Conclusions: No significant differences were observed between groups, nor for clearance at 30 days, nor recurrence-free follow-up. These results make the PDT an option for nodular BCC less than 5 mm located in high-risk areas.

Keywords: nodular basal cell carcinoma, single visit treatment, photodynamic therapy, methyl aminolevulinate