Investigation of Melanoma Cell Death *In Vitro* with the Combination of Radiotherapy and Photodynamic Therapy

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Melanoma, a skin carcinoma that affects melanin-producing melanocytes, is notorious for its high propensity to metastasize, resulting in low patient survival. Conventional therapies such as surgery, chemotherapy, radiotherapy and immunotherapy face challenges due to tumor resistance. Radiotherapy (RT) and photodynamic therapy (PDT) are medical modalities aimed at eliminating primary tumors and it is known that, associated with immunotherapy, they can enhance the tumor response through mechanisms of immunogenic cell death. The research evaluated the death of B16F10 murine melanoma cells *in vitro* after isolated and/or combined application of RT and PDT. The commercial chlorin Photoditazine® (PDZ) was used as a photosensitizer (PS) irradiated at 660 nm. Fluencies of 2, 6 and 20 J/cm^2 were tested in the standardization phase of the groups subjected to PDT, with PDZ concentrations of 1, 5, 10 and 20 µg/ml with 1 hour of incubation in the dark and evaluated by the MTT after 24 hours. The parameters of the experimental groups for the association of RT and PDT were: RT dose of 4 Gy, PDT with a fluence of 2 J/cm^2 and PDZ at a concentration of 5 µg/ml incubated for 1 hour in the dark. The tested intervals between therapies were 1, 4, 24 and 48 hours for MTT viability analysis and 24 and 48 hours for flow cytometry analyses. Cell viability analysis revealed that the combination of therapies, at longer intervals, favored synergism, with the RT+PDT group with a 48-hour interval between therapies being the most efficient, with a rate of cell death by apoptosis of 37%. The order of application of therapies also proved to be a determining factor, with the RT+PDT sequence demonstrating greater efficacy in inducing cell death compared to the reverse approach (PDT+RT), which presented an apoptotic rate of 11.22%. The present study revealed significant advances in the search for effective therapeutic strategies against melanoma, highlighting the combination of Radiotherapy and Photodynamic Therapy as an especially promising approach for the 48-hour interval. The RT and PDT protocol, 48 hours apart, highlights immunogenic potential by inducing apoptotic death, suggesting stimulation of the immune response. Despite advances, there is a need for additional investigations to understand the underlying mechanisms, especially in the relationship between PDT, cell cycle and immunogenic response. Continuing research aims to contribute to the development of innovative therapeutic strategies against melanoma.