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Cytotoxic effects of parthenolide on lymphoid malignancies' cell lines

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Aim: The aim of this study was to evaluate the therapeutic potential of parthenolide (PRT), an NF- κ B inhibitor, on acute lymphoblastic leukemia (ALL) and Burkitt Lymphoma (BL) cell lines and characterize the type of cell death induced and its molecular mechanisms.

Introduction: Playing an important role in the regulation of diverse biological processes such as cell proliferation and survival, nuclear factor kappa B (NF- κ B) is closely associated with various human malignancies. Deregulated NF- κ B signaling has been appointed as one important player in all stages of tumorigenesis. PRT has a dual anti-tumor effect – NF- κ B pathway inhibition and oxidative stress induction – on a wide range of malignancies and could be a valid option for hematological cancer.

Methods: We used one BL (RAJI) and five ALL (697, CEM, JURKAT, MOLT-4 and KOPN8) cell lines. Cells were incubated in absence or presence of different concentrations of PRT in single dose and daily administration. Metabolic activity was assessed by Resazurin Assay. Cell death was analyzed by Optical Microscopy and Flow Cytometry (FC) using Annexin V/7-AAD double staining and JC-1 probe. Apoptotic proteins levels (FAS, FAS-L, BCL-2, BAX and activated caspase 3), cell cycle and oxidative stress parameters (superoxide anion, peroxides and reduced glutathione through the DHE, DCFH2DA and mercury orange probes, respectively) will be evaluated by FC.

Results: Preliminary results showed that PRT reduces the metabolic activity in time, dose and cell line dependent manner, being the KOPN8 and RAJI cells the most sensitive and JURKAT cells the lowest. In fact, the half maximal inhibitory concentration (IC₅₀) at 72 h was 2 μ M for KOPN8 and RAJI, 3 μ M for CEM, 4 μ M for 697, 6 μ M for MOLT-4 and 12 μ M for JURKAT. These results may be related with the cell type and genetic background. Cell death analysis suggested that PRT induced apoptosis in these cell lines. Studies on the cell cycle and oxidative stress are still underway.

Conclusion: Our results suggest that PRT is a potential new targeted therapy in lymphoid malignancies, mainly ALL and BL.

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WNT/ β -catenin and Hedgehog signaling pathways as therapeutic targets in B-cell neoplasms

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Aim: The goal of this study was to evaluate the therapeutic potential of WNT/ β -catenin and Hedgehog inhibitors, IWR-1 and GDC-0449 respectively, alone and in combination, in B-cell neoplasms.

Introduction: B-cell neoplasms include, among others, the B-cell lymphomas and plasma cell disorders, such as multiple myeloma (MM), a malignant neoplasm originated by proliferation of monoclonal plasma cells; and diffuse large B-cell lymphoma (DLBCL), the most common form of non-Hodgkin lymphoma. Inappropriate activation of conserved embryonic signaling pathways, such as WNT/ β -catenin and Hedgehog has been implicated in B-cell neoplasms. Hence, these pathways may constitute new potential candidate targets for MM and DLBCL therapy.

Methods: For this propose, H929 (MM) and FARAGE (DLBCL) cell lines, were cultured in absence and presence of different concentrations of IWR and GDC. Metabolic activity was evaluated using resazurin assay and cell death by optical microscopy (May-Grunwald staining) and flow cytometry (FC) (Annexin V/7-AAD staining). Cell cycle analysis was evaluated by FC, using a PI/RNase solution. Proteins related to apoptosis and some molecules related to WNT and HH signaling pathways were tested by FC. The expression levels of AXIN and SMO genes were analyzed by RT-PCR.

Results: Preliminary results showed that IWR-1 and GDC-0449 reduced metabolic activity in a time-, dose- and cell line dependent manner, when administrated alone or in combination. The IC₅₀ of IWR-1 and GDC-0449 in H929 cells was 40 μ M and 70 μ M, respectively, and 75 μ M and 57 μ M for FARAGE cell line, after 24 h of treatment. These compounds induce cell death mainly by apoptosis and showed an arrest in cell cycle at G₀/G₁. Complementary studies are still ongoing.

Conclusion: In conclusion, results suggest that IWR-1 and GDC-0449 are potential new targeted therapies that could be efficient in MM and DLBCL treatment.

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