EFFECT OF PROTEIN KINASE C β SELECTIVE INHIBITION ON ACUTE LYMPHOBLASTIC LEUKEMIA CELL LINES.

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Acute lymphoblastic leukemia (ALL) is the most common leukemia in children and accounts for 20% of acute leukemia in adults. The intensive induction–consolidation–maintenance therapeutic regimens used currently have improved the 5-year disease free survival to around 80% in children and to 25%-40% in adults. The poorer response in adults is basically due to the inability to tolerate the intensive chemotherapy, and to the biology of adult disease which is associated with poor-risk prognostic factors. In the present era of target-specific therapy, PKCβ targeting arose as a new, promising, and well tolerated treatment strategy in a variety of neoplasms, especially in B-cell malignancies. It showed encouraging results in preclinical and clinical studies involving chronic lymphocytic leukemia, diffuse large B-cell lymphoma and multiple myeloma. PKCβ plays a major role in B-cell receptor signaling, but studies describing the role of PKCβ in B-cell ALL are lacking. In the present study, we measured the sensitivity of a variety of B-cell ALL cell lines to PKCβ selective inhibition. Five cell lines were studied: RS4;11 and SEM-K2 [both Pro-B ALL with t(4;11)(q21;q23)], HB-1119 [Pre-B ALL with t(11;19)(q23;p13)] TOM-1 [Ph-positive Pro-B ALL with t(9;22)], and Reh [Pre-B ALL with t(12;21)(p13;q22)]. Cells were tested for PKCβ1 and PKCβ2 expression by immunoblot. Cell viability was measured in the presence of PKCβ-selective inhibitor at concentrations of 1, 2.5, 5, 10, 20 and 30 µM for 48 hours with 10% fetal bovine serum (FBS). MTS assay was performed to quantify cell viability, and TUNEL assay with propidium iodide staining was used to detect apoptotic induction and effect on cell cycle. Results showed that all five cell lines express PKCβ1 and PKCβ2. Treatment with PKCβ-selective inhibitor resulted in a dose-dependent inhibition of cell proliferation. RS4;11 and SEM-K2 cell lines showed the greatest sensitivity with statistically significant cell growth inhibition at inhibitor concentrations as low as 1 µM and an IC50 of 5 µM. Other cell lines required relatively higher inhibitor concentrations, with cell growth inhibition starting at 5 µM and an IC50 of 14 µM. The mechanism of PKCβ-selective cell growth inhibition was shown by flow cytometric TUNEL assay to involve apoptotic induction. No effect on cell cycle progression was observed, except in the Reh cell line. Apoptotic induction in RS4;11 and SEM-K2 cell lines was linked to the inhibition of GSK-3β phosphorylation; flow cytometric analysis showed a 25% decrease in phosphorylated GSK-3β after 24 hours incubation with the PKCβ-selective inhibitor. These results indicate that PKCβ plays an important role in the malignant process in B-cell ALL, and suggest that PKCβ targeting should be considered as a potential treatment, whether in combination with the current regimens used or as a single agent monotherapy. Ongoing studies in our lab will detail the mechanism of PKCβ inhibition and uncover possible relationships between PKCβ signaling and t(4;11)(q21;q23).

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