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ORIGINAL ARTICLE

Endoplasmic reticulum stress associated with caspases-4 and -2 mediates korbazol-induced B-chronic lymphocytic leukemia cell apoptosis

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Summary

Purpose: *B-cell chronic lymphocytic leukemia (B-CLL) is an incurable disease that rapidly develops drug resistance. Therefore there is a need for identifying new agents that will improve the therapeutic outcome. Korbazol is a natural product known to exert cytotoxic effect on the in vitro survival of leukemic cells. The aim of this study was to investigate the mechanism of korbazol-induced apoptosis in B-CLL leukemic cells.*

Methods: *Peripheral blood mononuclear cells from 10 B-CLL patients were used for assessing the effect of caspase inhibitors and chelator of intracellular Ca^{2+}.*

Results: *Cell death rate induced by the tested compound was decreased with the caspase-3 inhibitor Ac-DEVD-CHO, and the inhibitors of caspase-2 (Z-VDVAD-FMK) and -4 (Z-YVAD-FMK), but not with the caspase-9 inhibitor z-LEHD-FMK and caspase-8 inhibitor z-IETD-FMK. No significant release of cytochrome C (cyt C) from mitochondria to the cytosol of B-CLL cells treated with korbazol was observed. Moreover, chelating of intracellular Ca^{2+} with BAPTA-AM almost completely abolished the cytotoxic effect of korbazol.*

Conclusion: *Engagement of caspases-2 and -4 and mobilization of intracellular Ca^{2+} indicate involvement of endoplasmic reticulum (ER) stress in apoptosis induced by korbazol.*

Key words: apoptosis, chronic lymphocytic leukemia, endoplasmic reticulum stress, korbazol

Introduction

B-CLL is a hematopoietic disorder that results in the accumulation of clonal CD5-positive B-cells with a low proliferation rate in the blood, bone marrow, lymph nodes and spleen [1]. In most patients, the circulating mature B-lymphocytes are largely quiescent G_0 phase cells, which accumulate due to their longer survival compared with normal B cells rather than to increased proliferation [2]. B-CLL represents an example of a malignancy caused by failure of programmed cell death and the defects in the apoptotic machinery not only increase spontaneous cell survival but also contribute to chemoresistance of the neoplastic clone.

Killing of cancer cells by various cytotoxic approaches such as anticancer drugs is predominantly mediated through induction of apoptosis in target cells [3].

Apoptosis or programmed cell death is a tightly regulated physiological process defined by orderly and characteristic sequence of structural changes resulting in cell detachment and rounding, chromatin aggregation, nuclear and cytoplasmic condensation, and eventual fragmentation of the dying cell into a cluster of membrane-bound segments (apoptotic bodies) that often contain morphologically intact organelles. The execution of apoptosis is mediated through consecutive activation of the members of the caspase family by cleaving specific substrate molecules [4].

The caspases, key components of this self-destruction machinery, are a group of intracellular cysteine proteases that are synthesized in the cells as inactive zymogens (procaspases) containing an N-terminal regulatory prodomain and a large (20 kDa) and a small (10 kDa) enzymatic subunit. Caspase recognition sequences exist between each of these domains, permitting proteolytic activation to occur either by self-processing or through

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