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The expression of caspase 3 in chronic lymphocytic leukemia

KEYWORDS: Leukemia, Lymphocytic, Chronic; Caspases; Apoptosis; Chlorambucil

INTRODUCTION

B - Chronic lymphocytic leukemia (B-CLL) represents the most common type of leukemia of adults in Western countries. It is a neoplastic disease characterized by the accumulation of morphologically mature but immunologically dysfunctional monoclonal CD5⁺ B lymphocytes in the blood, bone marrow and lymphatic organs. B-CLL is an example of human malignancy caused by alternations in the pathways of programmed cell death - apoptosis. Programmed cell death or apoptosis is a special type of cell death essentially different from necrosis in nature and biological significance. Apoptosis is an active process of genetically regulated cell autodestruction and in most cases has a homeostatic function. A central component of the apoptosis machinery is a family of caspase proteases organized in a branched proteolytic cascade. More than 10 caspases have been identified. Some of them (e.g., caspase 8 and 10) are involved in the initiation of apoptosis, others (e.g., caspase 6 and 7) execute the death order by destroying essential proteins in the cell. The caspase 3 is speculated to have a crucial role in apoptosis and is responsible for the cleavage of many critical cellular substrates, leading to characteristic morphological changes in apoptosis such as chromatin condensation, nucleosomal DNA fragmentation and formation of apoptotic bodies (3). In recent years, many experimental studies demonstrated capability of different antineoplastic agents to induce apoptosis (4). Chlorambucil is able to induce typical features of apoptosis in B-CLL cells (5). The aim of our study was to investigate possible Caspase 3 induction potential of chlorambucil as an antineoplastic agent.

MATERIALS AND METHODS

Our study includes 20 patients with B-CLL and 20 healthy persons as control group. The patients with B-CLL were treated by high dose - chlorambucil (HD-CLB). Samples of peripheral blood mononuclear cells from all patients and healthy controls were homogenized in 2 volumes (wt/vol) of 50 mM Tris - HCl (pH 7.0), 150 mM NaCl, 1% sodium dodecyl sulfate, 1% Nonidet P-40,

5mM EDTA and one tablet per 10ml of buffer of protease inhibitor cocktail (Complete Mini; Boehringer Mannheim, Indianapolis, IN, U.S.A.) and processed for immunoblot as described previously (6). The CPP32 polyclonal antibody (1:500 dilution; Pharmingen) and, as an internal control, a monoclonal antibody to β -actin (1:5,000, Sigma) were used. Films were scanned on HP-4C Scanjet, and bands were quantified by using the NIH Image 1.62 software. The data obtained in these experiments were evaluated using the ANOVA test. When ANOVA showed significant differences, pairwise comparisons between means were tested by Student's t-test. In all analyses, the null hypothesis was rejected at the 0.05 levels. All statistical analyses were performed using SigmaStat for Windows (version 2.0, Jandel Corp., San Rafael, CA, U.S.A.).

RESULTS

All patients with B-CLL showed good response to HD-CLB. After 6-8 weeks of treatment, 3 patients were in complete remission while in 17 patients partial remission was archived. In all treated patients elevated levels of Caspase 3 were present. The level of caspase 3 protein was significantly increased ($p = 0.083$) in all analyzed patients compared to controls. There were no statistically significant differences in Caspase 3 protein expression between B-CLL patients in the HD-CHB treated group.

DISCUSSION

Our previous investigation showed that chlorambucil induced apoptosis of B-CLL cells as a positive prognostic parameter in CLL treatment (5, 7). In the same time, numerous cellular mechanisms as well as great number of genes and proteins are involved in the regulation of apoptosis. As a starting molecular sign of execution phase of apoptosis, activation of Caspase 3 was proposed (8). Since most evidences indicates that B-CLL is caused by dysregulation of apoptosis, the expression of Caspase 3 may represent example of aberrant gene expression associated with the pathogenesis of B-CLL (8,9). The results of this study showed increased level of expression of Caspase 3 in peripheral blood of B-CLL patients treated by chlorambucil. These results approved proposed capability of chlorambucil to induced B-CLL cells apoptosis via activation of Caspase 3. The future of cancer therapy will be characterized by personalized treatment and the careful selection of therapeutic targets. According to our investigation we concluded that Caspase 3 may represent a logical molecular target for new approaches to over-coming drug resistance.

REFERENCES

1. Matus E, Polliack A. Morphological and immunophenotypic features of chronic lymphocytic leukemia. *Rev Clin Exp Hematol* 2000; 4(1):22-47.
2. Chiarugi V, Cinelli M, Magnelli L, Dello SP. Apoptosis: molecular regulation of cell death and hematologic malignancies. *Mol Biotechnol* 2002; 20(3):305-14.
3. Kolenko VM, Uzzo RG, Bukowski R, Finke JH. Caspase-dependent and -independent death pathways in cancer therapy. *Apoptosis* 2000; 5(1):17-20.
4. Makin G, Hickman JA. Apoptosis and cancer chemotherapy. *Cell Tissue Res* 2000; 301:143-52.
5. Brajušković G, Marjanović S, Škaro Milić A. In vivo monitoring of therapy induced apoptotic process in patients with chronic lymphocytic leukemia and acute non-lymphoblastic leukemia. *Archive of Oncology* 2002; 10(2):55-60.
6. Ara J, Przedborski S, Naini AB, Jackson-Lewis V, Trifiletti RR, Horwitz J, Ischiropoulos H. Inactivation of tyrosine hydroxylase by nitration following exposure to peroxynitrite and 1 - methyl - 4 - phenyl - 1,2,3,6 - tetrahydropyridine (MPTP). *Proc. Natl. Acad. Sci. USA* 1998; 95: 7659-7663.
7. Brajušković G, Marjanović S, Škaro Milić A. Dejstvo hlorambucila na limfocite B periferne krvi bolesnika sa hroničnom limfocitnom leukemijom - ispitivanje ultrastrukture ćelija. *Vojnosanitetski pregled* 2003; 60(2):175-180.
8. Zimmermann KC, Bonzon C, Green DR. The machinery of programmed cell death. *Pharmacology & Therapeutics* 2001; 92:57-70.
9. Kitada S, Andersen J, Akar S, Zapata JM, Takayama, Krajewski S, Wang HG, Zhang X, Bullrich F, Croce CM, Rai K, Hines J, Reed JC. Expression of Apoptosis - Regulated Proteins in Chronic Lymphocytic Leukemia: Correlations With In Vitro and In Vivo Chemoresponses. *Blood* 1998; 9(1):3379-89.

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