

OXIDATIVE STRESS MARKERS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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MARKERI OKSIDATIVNOG STRESA U HRONIČNOJ LIMFOCITNOJ LEUKEMIJI

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Received/Prilijen: 03.02.2006. Accepted/Prihvaćen: 09.06.2006.

ABSTRACT

Chronic lymphocytic leukemia is characterized by the progressive accumulation of small immature lymphocytes which do not proliferate and that remain predominantly (more than 95%) in the G0 phase of the cell cycle. Expansion of malignant cell clone appears to be due to an underlying defect in its ability to undergo apoptosis. One of the potential mechanisms of defective apoptosis could be irregular oxidative stress. The goal of our investigation was to determine the plasma level of nitric oxide, superoxide anion and malondialdehyde in patients with chronic lymphocytic leukemia as markers of oxidative stress. Thirty patients with untreated chronic lymphocytic leukemia in the A stage of the disease classified by Binet and thirty healthy volunteers were examined. Nitric oxide (its stable metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), superoxide anion and malondialdehyde were measured by spectrophotometry in plasma of both investigated groups which were obtained from heparinized whole blood after centrifugation.

Our results showed that the plasma levels of nitrate/nitrite (32.21 ± 7.25 vs 28.42 ± 7.27 nmol/ml, $p < 0.01$), superoxide anion (10.34 ± 9.40 vs 8.52 ± 6.29 nmol/ml, $p < 0.01$) and malondialdehyde (2.76 ± 1.61 vs 1.37 ± 0.90 nmol/ml, $p < 0.001$) were increased in patients with chronic lymphocytic leukemia than in the group of healthy volunteers. These data suggest that there is more intensive oxidative stress in patients with chronic lymphocytic leukemia. It could be one of the potential mechanisms in the pathogenesis of irregular apoptosis of malignant lymphocytes and it could take part in the genesis of chronic lymphocytic leukemia.

Key words: chronic lymphocytic leukemia, nitric oxide, superoxide anion, malondialdehyde.

Abbreviations: CLL – chronic lymphocytic leukemia, NO – nitric oxide, MDA – malondialdehyde, ROS – reactive oxygen species, IL-4 – interleukin 4, CD – cluster differentiation molecule, SOD – superoxide dismutase

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is predominantly clonal B-cell neoplasm of small, resting, long-lived B-cells. Despite recent advances in understanding of genetics (1), biology (2), clinical behavior (3) and treatment (4), CLL has not been cured yet and its progression and outcome are highly unpredictable. CLL cells co-express low levels of surface membrane molecules CD5, CD23 and weak CD22 and surface membrane immunoglobulin (5). Expansion of these malignant cells leads to the accumulation in the peripheral blood, bone marrow and many tissues. These cells are functionally defective and immunologically distinct from normal B cells (6). The clinical course of B-CLL is highly heterogeneous, ranging from less than two years in symptomatic patients with the advanced disease to more than twenty years for patients with an early stage and non-progressive

SAŽETAK

Hronična limfocitna leukemija je progresivna maligna bolest koja nastaje proliferacijom i akumulacijom klonalnih imunskih nekompetentnih limfocita u kostnoj srži, limfnim žlezdama, slezini i drugim organima. Smatra se da je osnovni mehanizam nastanka bolesti smanjene apoptoze limfocita in vivo. Uzroci ovog poremećaja nisu potpuno istraženi, ali se smatra se da jedan od mogućih mehanizama može biti i oksidativni stres.

Cilj ovog istraživanja bio je određivanje plazmatskih koncentracija azotmonoksida, superoksida anjona i malondialdehida kao markera oksidativnog stresa. Ispitivanu grupu sačinjavalo je 30 obolelih od hronične limfocitne leukemije koji nisu lečeni antineoplastičnom terapijom i koji su bili u A stadijumu bolesti po Binet-u, dok je kontrolnu grupu sačinjavalo 30 zdravih ispitanika slične polne i starosne strukture. Posle centrifugiranja heparinizovane venske krvi svih ispitanika izdvajana je plazma u kojoj su određivane koncentracije markera oksidativnog stresa.

Koncentracije azotmonoksida (32.21 ± 7.25 nmol/ml), superoksid anjona (10.34 ± 9.40 nmol/ml) i malondialdehida (2.76 ± 1.61 nmol/ml) u plazmi bolesnika obolelih od hronične limfocitne leukemije visoko statistički značajno veće od koncentracija istih molekula u plazmi ispitanika kontrolne grupe (NO 28.42 ± 7.27 nmol/ml; superoksid anjon 8.52 ± 6.29 nmol/ml; malondialdehid 1.37 ± 0.90 nmol/ml). Ovi podaci ukazuju da je oksidativni stres bolesnika znatno veći u poređenju sa kontrolnom grupom i da bi smanjenje apoptoze limfocita obolelih od hronične limfocitne leukemije moglo biti povezano sa modifikovanim oksidativnim stresom.

Ključne reči: hronična limfocitna leukemija, azotmonoksid, superoksid anjon, malondialdehid, oksidativni stres.

Skraćenice: CLL – chronic lymphocytic leukemia, NO – nitric oxide, MDA – malondialdehyde, ROS – reactive oxygen species, IL-4 – interleukin 4, CD – cluster differentiation molecule, SOD – superoxide dismutase

disease (7). Although the pathogenesis of B-CLL has not been fully elucidated, the progressive increase of lymphocyte count coupled with the very low proportion of proliferating cells has led to the notion that B-CLL may be determined by defective apoptosis (8). Precise mechanisms underlying apoptosis have still remained unknown. Dysregulation of the p-53, c-myc and bcl-2 oncogenes can be the cause of defective apoptosis in B-CLL and even though the B-CLL cell molecular alterations involving different oncogenes and tumor suppressor genes have been established, the role of oxidative stress in the pathogenesis of this disease is poorly understood and it is a matter of interest (9).

Oxidative stress is a well-known phenomenon in the body which plays an important role in the pathogenesis of various diseases and syndromes (10). Reactive oxygen species (ROS) consist of superoxide anion (O_2^-),

