Effect of hydralazine on CD3-ζ chain expression in Jurkat T cells

Januchowski R, Jagodziński PP*

Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznań, Poland

Abstract

Purpose: Deficient CD3-ζ chain expression in T cells of patients with idiopathic SLE is associated with T cell receptor/ /CD3 complex (TCR/CD3)-mediated signaling defect. Hydralazine (HYD) inhibits expression of DNA methyltransferase 1 (DNMT1) and may cause a lupus-like disease in man.

Material and methods: To explain the HYD effect on intracellular level of CD3-ζ chain in Jurkat T leukemia cells clone E6-1, we employed the flow cytometric analysis.

Results: We observed a dose-dependent increase in cellular content of CD3-ζ chain in Jurkat T cells treated with HYD. Our results suggest that HYD may result in T cells dysfunction different from this observed in idiopathic SLE T cells.

Conclusions: This difference may partially explain distinct disease course in patients with HYD induced and idiopathic SLE.

Key words: hydralazine, T lymphocytes, cell signalling, DNA methylation.

Introduction

Systemic lupus erythomatosus (SLE) is autoimmune disease characterised by abundant production of autoantibodies. Defect in $CD4^+$ T lymphocytes signaling can be responsible for improper immune response development in patients with SLE [1].

* CORRESPONDING AUTHOR:
Department of Biochemistry and Molecular Biology
University of Medical Sciences
ul. Święcickiego 6, 60-781 Poznań, Poland
Fax: +48 61 8659586
e-mail: fagm13@wp.pl (Radosław Januchowski)

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The T cells stimulation is initiated by binding TCR/CD3 with an antigen coupled to the major histocompatibility complex [2]. The ζ chain is component of CD3 complex and plays a major role in intracellular signaling transduction, which activate second messenger and transcription factors [2-4]. T cells stimulation induces cytokines production, increases proliferation and augmentation of effector function of T cells [2].

The defect in T cells signalling can be responsible for improper immune response development in patients with SLE [5].

It has been reported that low methylation of CpG residues in the regulatory sequences of DNA and high level of histone acetylation correlate with transcriptional activity of numerous genes [6-8]. During DNA replication, the CpG pairs of the newly biosynthesised DNA strand are methylated by DNA methyltransferase 1 (DNMT1) [6]. Expression of DNMT1 is partially regulated by extracellular signal regulated kinase pathway (ERK), and activity of this pathway is decreased in T cells from SLE patients [9]. Hydralazine (HYD) is a substance, which is able to induce a lupus-like syndrome in man. HYD inhibits ERK pathway resulting in decrease of DNMT1 expression and DNA hypomethylation [10].

Using the flowcytometric analysis, we evaluated the effect of HYD on CD3-ζ chain content in Jurkat T leukemia cells.

Material and methods

Reagents and Antibodies

HYD and digitonin were obtained from Sigma Chemical Co. (St. Louis, MO). (PE)-conjugated anti-CD3-ζ (6B10.2) mouse monoclonal antibody (MmAb) was purchased from Santa Cruz Biotechnology, Inc. USA.

Cell culture and HYD treatment

Jurkat T leukemia $CD4^+$ cells clone E6-1 were obtained from the American Type Culture Collection (Rockville, MD) and maintained in RPMI1640 (GibcoBRL, Grand Island, NY)

medium containing 10% heat-inactivated foetal bovine serum (FCS), 2 mM glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin. Jurkat T leukemia cells were suspended at a concentration $0.5x10^6$ cells/ml in culture medium and grown for 48 h without, or in the presence of HYD in concentration of 0.5, 5.0 and 50.0 μM.

Flow cytometric analysis

After incubation, the cells were harvested and washed three times in phosphate buffered saline (PBS) supplemented with 2% FCS and 1% sodium azide (PBS/FCS).

The cells were permeabilized with digitonin 10 μg/ml, fixed with 0.25% paraformaldehyde and washed three times with PBS/FCS. The cells were then stained with PE-conjugated anti CD3-ζ MmAb, washed three times with PBS/FCS and immediately analysed on FACSCanto Flow Cytometer (Becton-Dickinson, San Jose, CA). The increase of CD3-ζ chain cellular content were calculated according to (MFx-MFo)/(MFc-MFo) x 100 formula. MF is the mean fluorescence intensity of cells, which were grown in the presence (MFx) or absence (MFc) of HYD and then stained with PE-conjugated anti CD3-ζ MmAb. Control represent fluorescence intensity of cell stained with an appropriate isotope antibody (MFo).

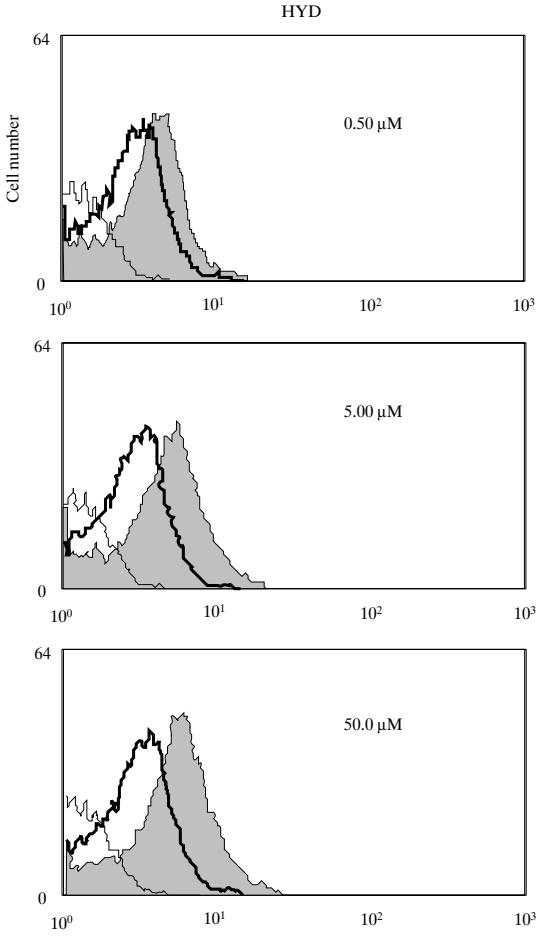
Results and discussion

In order to explain the HYD effect on intracellular level of CD3-ζ chain in Jurkat T leukemia cells, we employed the flow cytometric analysis. We observed that the HYD increased intracellular contents of CD3-ζ chain in Jurkat T cells in dose dependent manner. We have shown that the percentage increase of CD3-ζ chain formation achieves 17±3%, 70±13% and 238±40% in the presence of HYD concentration of 0.5, 5.0 and 50.0 μM, respectively (*Fig. 1*).

HYD is used to reduce the blood pressure but may also cause a lupus-like disease in man [10]. The etiology of idiopathic and HYD induced SLE is still enigmatic. Patients with idiopathic and HYD induced SLE exhibit different disease course. However, the T cells from idiopathic and drug induced SLE patients may display certain common defects at molecular level [11-13]. HYD induces biosynthesis of leukocyte function associated-1 (LFA-1) in T cells [10]. The biosynthesis elevation of LFA-1 is also observed in T cells from patients with idiopathic SLE [7]. T cells from this patients also exhibit TCR/CD3-mediated signalling aberrations, which are associated with cellular decrease of CD3-ζ transcript and protein contents.

The HYD inhibits DNMT1 expression and cause hypomethylation of regulatory DNA sequences that makes DNA template available for transcription [10,14]. The HYD causes hypomethylation of regulatory sequences of LFA-1 gene in T cells, which was also observed in the same region of DNA of SLE T cells [15]. However, we observed that HYD increased CD3-ζ protein but not transcript content (results not shown) in Jurkat leukemia $CD4^+$ T cells (*Fig. 1*). The reason for such an HYD effect on CD3-ζ chain translation is currently not known. We presume that HYD may effect on factors involved in positive control of translation or posttranslational modification of CD3-ζ chain.

Figure 1. **The representative picture of flow cytometric analysis of intracellular contents of CD3-ζ chain in Jurkat T leukemia cells incubated in the presence of HYD.**
Jurkat T leukemia cells clone E6-1 were suspended at a concentration of $0.5x10^6$ cells/ml in culture medium and were grown for 48 h either without or in the presence of HYD (0.5, 5, 50 μM). After incubation the cells were harvested, washed with PBS/FCS, permeabilized with digitonin 10 μg/ml and fixed with 0.25% paraformaldehyde. The cells were then stained with PE-conjugated anti CD3-ζ MmAb and immediately analyzed on FACSCanto Flow Cytometer (Becton-Dickinson, San Jose, CA). (—) and (–)represent expression of CD3-ζ chain in cells incubated without or with HYD. Shadow lines represents the cells stained with an appropriate isotype control antibody



Increase of CD3-ζ chain content in T cells may partially explain different disease course in patients with HYD induced and idiopathic SLE. HYD may also change other elements of TCR signaling pathway resulting in dysfunction of T cells.

The further investigation of HYD effect on expression of other signaling molecules may provide valuable information about etiology of T cells dysfunction in patients with HYD induced SLE.

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