Histidine Decarboxylase in Peripheral Lymphocytes of Healthy Individuals and Chronic Lymphoid Leukemia Patients

Possible Involvement of Intracellular Histamine in the Regulation of Lymphocyte Proliferation

Márta BENCSÁTH, Katalin PÁLÓCZI, Csaba SZALAI, Andrea SZENTHE, Júlia SZEBERÉNYI, András FALUS

1Department of Genetics, Cell- and Immunobiology, Semmelweis University of Medicine, Budapest; 2National Institute of Hematology and Immunology, Budapest; 3Pal Heim, Children’s Hospital, Budapest, Hungary

Histidine decarboxylase (HDC), the only enzyme capable of synthesis of histamine, has been found in many proliferating cells and tissues suggesting a role of histamine in cellular proliferation. In this study expression of HDC and the significance of histamine in the proliferation of peripheral lymphocytes of five healthy persons and six patients with chronic lymphoid leukemia (CLL) was examined. Expression of HDC mRNA and HDC protein was proved by reverse transcriptase polymerase chain reaction and by immunoblot, respectively. The role of histamine was studied in proliferation assays in the presence of irreversible inhibitor of the HDC (alpha-fluoromethylhistidine—FMH) and also by competing for the intracellular binding sites of histamine using N,N-diethyl-2,4-phenylmethyl-phenoxy-ethanamine-HCl (DPPE). By inhibiting the HDC enzyme activity by FMH and blocking the intracellular action of histamine by DPPE, a significant decrease in cell proliferation was observed in mitogen stimulated lymphocytes of healthy donors. In CLL patients the proliferation of leukemic lymphocytes was significantly inhibited by blocking the binding of histamine to intracellular binding sites by DPPE, but not by FMH inhibiting only the de novo histamine formation. The observations suggest that HDC has functional relevance in lymphocytes, since mitogen induced lymphocyte proliferation of healthy donors is mainly enhanced by de novo synthesis and subsequent action of intracellular histamine. Alternatively, in constitutively proliferating chronic lymphoid leukemia cells we suggest that the preformed pool but not the de novo synthesized intracellular histamine interferes with cellular proliferation. (Pathology Oncology Research Vol 4, No 2, 121–124, 1998)

Key words: histamine, lymphocyte, cell proliferation, leukemia

Introduction

Many data suggest the expression and activity of histidine decarboxylase (HDC) and the presence of histamine in proliferating tissues both of benign (e.g. embryogenesis, wound healing and hematopoiesis) and malignant (e.g. in colorectal carcinomas, leukemias) tissues. This “intracellular” histamine may be secreted and may bind to histamine receptors in an autocrine way. Alternatively or simultaneously histamine may attach to specific intracelular binding sites dissimilar to plasma H1, H2 and H3 membrane receptors of histamine. The ligands for the intracellular histamine receptor/binding sites appear to be a new class of tumor promoting agents.

In this study we demonstrate evidence of the gene expression and synthesis of HDC in lymphocytes of healthy donors and in lymphocytes of patients suffering from CLL. We present data on the inhibitory effect of the blockade to the binding sites of intracellular histamine by N,N-diethyl-2,4-phenylmethyl-phenoxy-ethanamine-HCl (DPPE) both in lymphocytes of healthy donors and CLL patients. Irreversible inhibition of the enzyme synthesizing intracellular histamine by alpha-fluoromethylhistidine (FMH) was effective on proliferation only during mitogenesis of lymphocytes of healthy donors but not on constitutively proliferating CLL cells.
Materials and Methods

Lymphocytes were separated on a Ficoll density gradient from freshly taken heparinized blood samples of healthy volunteers (n=5) and of chronic lymphoid leukemia (mainly B-CLL, since over 90% of cells were CD19⁺, as proven by cytofluorometry) patients (n=6). After several washes cell viability and count were checked by trypan blue and Turk solutions, respectively. The viability of lymphocytes after Ficoll separation from healthy and CLL blood samples was above 90% during the experimental procedure.

In functional tests, chemicals interfering with intracellular histamine were administered either directly (CLL cells) or after mitogen (40 h to lymphocytes of healthy donors) stimulation by concanavalinA (ConA, Serva) at a concentration of 1 µg/ml, (the optimal concentration was determined in previous experiments).

The expression of the gene of histidine decarboxylase (HDC) was measured by reverse transcriptase polymerase chain reaction (RT-PCR),⁶ from cellular RNA. The primers spanning exon 10 and 12 of the human HDC gene (sense: 5-ATCTTCAAGCAGCATGTC-3, antisense: 5-CTGGATAGTGCGCCGGATG-3) were applied for amplification. RT was carried out using a Perkin Elmer RT-PCR kit on a Pharmacia Ataq Gene Controller. The reaction mixture (20 µl) consisted of MgCl₂ (25 mM) 4 µl, 10x buffer 2 µl, dNTP-s (4x10 mM) 2 µl, RNase inhibitor 1 µl (20 units), oligo-dT primer 1 µl, Mu-LV reverse transcriptase 0.5 µl (25 units), DEPC-distilled water 3.5 µl containing 1 µg of total RNA. RNA samples (1 µg) were reverse transcribed on 42°C for 30 min then on 99°C for 5 min.

For PCR amplification, a Perkin-Elmer PCR kit was used. The reaction mixture (50 µl) contained 10x buffer 5 µl, MgCl₂ (25 mM) 3 µl, dNTP-s (4 x 10 mM) 5 µl, primers (50 pmol/l sense + antisense) 1 µl, Taq polymerase 0.2 µl, distilled water 31 µl, template (cDNA) 5 µl. The PCR reaction was carried out using the following program for amplification of HDC: 94°C for 4 min, 35 times (95°C for 30 sec., 60°C for 1 min., 72°C for 1 min.), 72°C for 1 min. The 208 bp DNA product was run on agarose gelelectrophoresis (Tris-borate-EDTA buffer pH=8.3) and stained by ethidiumbromide for the UV visualization of the bands.

For immunoblots a newly developed polyclonal chicken anti-human HDC generated against peptides 318-325 of HDC has been applied (Haak-Frendscho et al, submitted). Briefly, total IgY (the avian IgG homologue) was purified from the egg yolks of immunized hens by sequential precipitation as per the manufacturer's instruction using the EGGtracSTM IgY Purification System (Promega Corp., Madison, WI). Specificity of the antibody was proven earlier by the blocking of the immunogenic peptide conjugate and also by using HDC-negative controls (not shown).

For western analysis, extracts of lymphocytes were subjected to SDS-PAGE and transferred onto nitrocellulose membranes. Each gel lane was loaded with 10 µg of total extracted protein. The transfers were blocked with TRIS-buffered saline containing 0.05% (v/v) Tween-20 and 0.1% (w/v) BSA, probed with 100 µg/ml HDC, antibody, washed, incubated with 0.5 µg/ml HRP conjugate and developed with ECL reagent (Amersham Life Science, Buckinghamshire, UK). The identity of the band was controlled by molecular weight standards.

For the inhibition of access of intracellular histamine to its target N,N-diethyl-2,4-phenylmethyl-phenoxo-ethana-
mine-HCl (DPPE, synthesized by dr. F Hudec, Eötvös L.
University, Budapest) was applied in a dose of 5 µM (optimal dose has been selected previously, based on proliferation assays). In the lymphocyte assay system the 5, 24, 48 or 72 h treatment was stopped by the application of the dye thiazolyl blue (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) to be converted into insoluble formazan by mitochondrial dehydrogenase for the assessment of change in cell proliferation.⁶ The changes in MTT extinction parallel the H3-thymidine incorporation, as indicated by previous experiments.

For the irreversible inhibition of HDC activity the lymphocytes were incubated in the presence of alpha-fluoro-
romethyl-histidine⁵ (αFMH, generous gift of dr. Kollo-
nitsch J, Westfield, NJ) in a dose of 100 µM (optimal dose has been selected earlier by proliferation assays). By 5, 24, 48 and 72 h intervals the MTT dye was added to follow changes in cell proliferation (see above).

For statistical analysis two tailed Student „t” test was used.

Results

Figure 1 demonstrates the band of the 208 bp HDC-DNA (A) as obtained by RT-PCR suggesting the presence of HDC-mRNA in lymphocytes of healthy subjects and CLL patients. The identity of the band has been further confirmed by direct sequencing of the PCR product (results not shown). The translation of mRNA to HDC protein is documented by a weak 55 kDa band on western immunoblots (B) in lysates both of lymphocytes from healthy donors and CLL patients. Both in RT-PCR or in western immunoblot system, the specificity of the assays was proved by using HDC-negative cells (not shown). These data suggest no substantial difference between the amount of HDC mRNA and protein between lymphocytes isolated from healthy and CLL patients.

Figure 2 shows decrease of proliferation of peripheral lymphocytes after 48 hours from healthy cells treated with ConA in the presence of αFMH (a) p<0.01 at 48 h and p<0.05 after 72 h. Similarly, after DPPE (b) treatment significant (p<0.01) decrease of proliferation was found after 48 and 72 h.

PATHOLOGY ONCOLOGY RESEARCH
Figure 3 demonstrates, that the proliferation of CLL lymphocytes is not affected by αFMH (a), while significant inhibition in cell proliferation upon the action of DPPE (b) was detected at five (p<0.01), 24 (p<0.01), 48 (p<0.05) and 72 (p<0.01) hours.

Discussion

The main purpose of our study was to obtain further data on the functional significance of histamine generated and acting within lymphocytes of healthy subjects and CLL patients. Our results provide evidence on the presence of HDC mRNA and weak but definite immunoreactive HDC in lymphocytes of both healthy donors and CLL patients.

Our present data with the inhibitory potential of specific HDC inhibitor αFMH suggest, that in the ConA stimulated lymphocytes of healthy subjects the de novo production of histamine has certain role in cell proliferation. In contrast, constitutive proliferation of malignant CLL cells was not affected by αFMH, acting only on de novo histamine production. However the role of intracellular histamine in the proliferation of both healthy and CLL lymphocytes is strongly suggested by the similar inhibitory potential of DPPE, blocking the intracellular targets of histamine. While there are several explanations of these data, the most probable one is the de novo activation of HDC upon mitogen stimuli in lymphocytes of healthy persons and a presence of preformed histamine pool in CLL cells. We have preliminary HPLC data (unpublished) on the presence of a small, but permanent quantity of histamine in CLL cells, but not in lymphocytes from healthy donors. However, due to the presence of relatively high histamine catabolising activity of diaminoxidase (DAO) in various malignant cell lines and tissues (Darvas et al, unpublished), quantitative histamine measurements should be cautiously evaluated. Moreover, our mRNA and western immunoblot data (Figure 1a and 1b) show slight if any differences in the level of HDC gene expression and in amount of HDC protein. The other, less probable explanation is active uptake of histamine by CLL cells from the plasma.

DPPE is considered to occupy specifically the intracellular histamine binding sites (dissimilar to H1 or H2 receptors on the cellular plasma membrane) located close to the microsomal portion containing the antiestrogen binding sites in the cell.3,10,11 We suggest, that due to the competitive DPPE action for the intracellular histamine binding sites in CLL cells the effect of locally available histamine is inhibited. Otherwise, through acting as a growth inhibitor in vitro, DPPE has tumor promoting effects in vivo.12,13 However, DPPE also selectively sensitizes human breast cancer or

![Figure 1](image1.png)

**Figure 1.** (a) RT-PCR amplification of HDC mRNA in lymphocytes of healthy persons (lanes 1-4) and those of CLL patients (lanes 5-7). Lane 8: HDC cDNA. The arrow indicates the position of cDNA corresponding to reverse transcribed HDC mRNA. (b) Immunoblot for HDC in lysates of lymphocytes of healthy persons (lanes 1-3) and those of CLL patients (lanes 4-6).

![Figure 2](image2.png)

**Figure 2.** Proliferation of ConA (1 μg/ml) stimulated lymphocytes (40 hours) of healthy persons treated with 100 μM of FMH (a) and 5 μM DPPE (b) for the indicated time periods. 100 corresponds to ConA stimulation, alone. Mean (n=5) ± s.e.m values are indicated. * p<0.05; ** p<0.01

Vol 4, No 2, 1998
prostate cancer cells for chemotherapy. The action of DPPE while replacing intracellular histamine at its cellular binding sites does not interfere with the signalling route of protein kinase C, but is parallel in effect with cyclooxygenase inhibitors at least in platelet aggregation studies.

We do not present in this paper our direct HPLC data which already showed the higher amount of of histamine in CLL cells compared to lymphocytes from healthy persons, since due to the relatively high activity of diamineoxodase (DAO) catabolizing histamine only the actual balance of histamine can be given. Further studies are in progress to measure simultaneously HDC, DAO and histamine in lymphocytes from leukemic patients of various origin and stages.

The intracellular target of histamine has not been molecularly characterized yet. Experiments are in progress in our laboratory on cytochrome P molecules and nuclear components as possible candidates as intracellular targets of histamine.

Acknowledgement

The authors thank the technical help of Kristina Nagy. The work has been supported by grants OTKA T21175 of Hungarian Academy of Sciences and MKM No. 90 of Ministry of Health and Welfare.

References