CHANGES IN INTRACELLULAR TAURINE CONTENT OF HUMAN LEUKEMIC CELLS

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ABSTRACT

Intracellular levels of the free amino acid taurine were measured in circulating granulocytes and mononuclear cells from 12 normal subjects and 27 patients with various types of leukemia, and in bone marrow cells from seven acute leukemias in blastic phase and 4 in remission. Leukemic cells have consistently lower taurine levels compared to normal lymphocytes and granulocytes. Although taurine levels in the circulating granulocytes from patients with acute and chronic leukemias were normal, they were significantly lower in the group of patients with atypical AML. The lowest taurine levels were observed in the mononuclear cells from patients with AML and ALL followed by those from patients with CML and CLL, and then those from patients with atypical AML. In AML patients the levels increased to normal values during clinical remission. The bone marrow mononuclear cells of AML in the blastic phase also had lower taurine levels compared to these in remission just same as peripheral blood cells do. These observations on the alteration in taurine content of both mononuclear cells in peripheral blood and bone marrow may be useful as a biochemical marker in diagnosis as well as for prediction of relapse and effectiveness of chemotherapy on patients with various types of leukemia.

Key Words: Taurine, Lymphocytes, Granulocytes, Leukemic cells, Atypical leukemia

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; AMoL, acute monocytic leukemia;

INTRODUCTION

Taurine, a highly acidic sulfur-containing amino acid, was found to have the highest concentration among the free amino acids in normal human leukocytes.[1,2,3,4] This amino acid is the metabolic end product of methionine which is essential in the initiation of polypeptide chains and which is the chief methyl donor for the methylation of DNA and RNA. Leukemic cells from human cell lines in culture were shown to have a greater uptake of ^{35}S L-cystine compared to normal leukocytes.[5,6] The increased requirement by leukemic cells for L-cystine is believed to be due to the tenfold decrease in the activity of the enzyme γ-crystathionase[7] resulting in the depletion of L-cystine and possibly of the end product taurine as well (Fig. 1).

Taurine levels in human leukemic cells were reported to be abnormally low in patients with AML, CML and CLL according to some of the previous studies.[2,3] However, others observed normal taurine content in cells from patients with AML, AMoL, and CML.[8] Non of these reports[2,3,8] showed the differences of the taurine value in lymphocytes and

A Pathway of Taurine Biosynthesis

Methionine

$\downarrow$ $-CH_3$

Homocysteine + Serine

B_6 $\downarrow$ transsulfurase

Cystathionine

B_6 $\downarrow$ cystathionase

Cystine $\rightleftarrows$ Cysteine + Homoserine

$\downarrow$ (O) α-Ketobutyrate

Cysteine sulfinic acid $\xrightarrow{-CO_2}$ Hypotaurine

$\downarrow$ (O) $\downarrow$ (O)

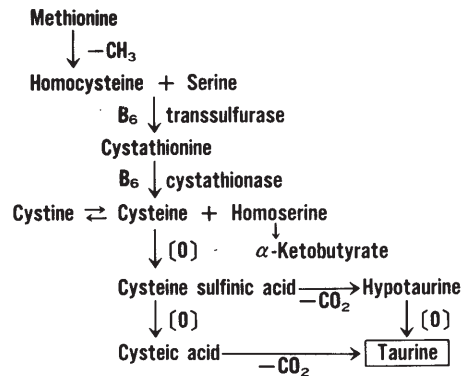Cysteic acid $\xrightarrow{\quad\quad\quad}$ ⌐Taurine⌐
$-CO_2$

Fig. 1

Fig. 1.   Schematic diagram of the taurine
pathway.

granulocytes. Furthermore, they did not show any reason for either the abnormal or the normal value of taurine. Our study was designed to measure taurine levels in leukemic cells in peripheral blood and bone marrow as well as in granulocytes under the disease conditions of various types of leukemia. We used newly developed cell separation method and a much more sensitive and accurate assay method for the amino acid. Our purpose was to explain or speculate about the reason for the abnormal taurine levels in leukemic cells.

PATIENTS AND METHODS

Subjects: Peripheral blood of 12 normal subjects and 27 patients with some form of leukemia who were either previously untreated or had not received any form of chemotherapy within two weeks of the taurine assay was studied. The leukemic patients included four with CML, eight with CLL, three with AML in blastic phase and three during remission, two with ALL in blastic phase and three during remission and four patients with atypical AML. Atypical AML was defined as AML which had an earlier dysmyelopoietic phase for more than one year. Bone marrow samples were studied in seven patients with AML in the blastic phase, four of these patients were restudied after they entered a clinical remission phase. The patients' ages ranged from nine to 68 years.

Cell Preparation: Anticoagulated blood by EDTA was diluted by Hanks' balanced salt solution without Ca and Mg (HBSS) to a concentration of $2 - 5 \times 10^6$ cells/ml and was separated into mononuclear cells and granulocytes using Ficoll-Hypaque according to the method described by Boyum.[9] Mononuclear cells (lymphocytes or leukemic blast cells) were separated in the interphase between HBSS and Ficoll-hypaque. The granulocytes were obtained from the bottom of the tube after a removal of RBC by sedimentation using Dextran. These cells were washed twice and resuspended in HBSS. The number of cells was counted using a hemocytometer and smears were prepared from both the mononuclear cell and granulocyte fractions. Separated cells were sonicated for 30 sec. at 250 watts using a

Taurine Levels in Normal and Leukemic Cells
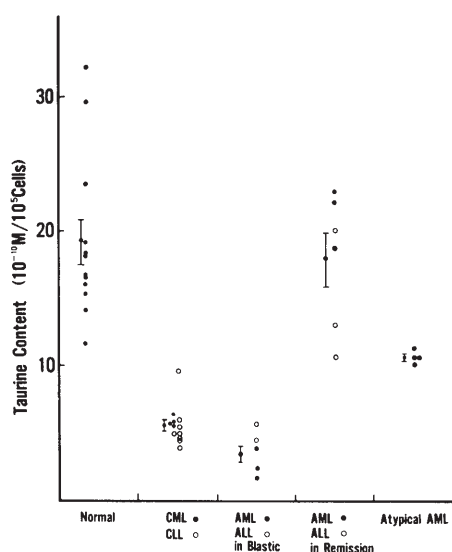**— circulating mononuclear cells —**

Taurine Levels in Normal and Leukemic Cells
— circulating granulocytes —

Fig. 2

Fig. 3

Fig. 2. The mean (±SE) taurine contents of circulating mononuclear cells in each group are: 19.28±1.79 (10^{-10} moles/10^5 cells) in normal lymphocytes, 5.64 ±0.42 in CML & CLL, 3.56±0.68 in AML and ALL in blastic phase, 17.80±2.01 in AML and ALL in remission and 10.70±0.23 in atypical AML.
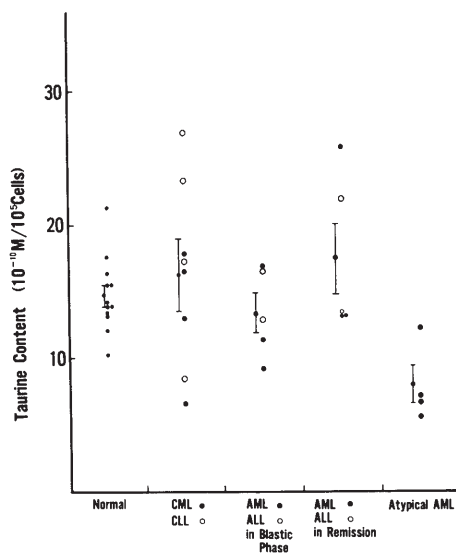
Fig. 3. The mean (±SE) taurine contents of circulating granulocytes are: 14.76 ±0.81 (10^{-10} moles/10^5 cells) in normal granulocytes, 16.24±2.65 in CML and CLL, 13.38±1.48 in AML and ALL in blastic phase, 17.46±2.68 in AML and ALL in remission and 7.9±1.47 in atypical AML.

Branson Cell Sonifier Model 350A (Shelton, CT). Aliquots of 100 μl were separated into microcentrifuge tubes and deproteinized using 400 μl of 100% ethanol followed by 100 μl of 20% ethanol and then with 100 μl of deionized water. The combined extracts were centrifuged for 15 min at 31,000X g. The supernatant obtained was stored in a freezer (−20°C) for further studies.

Taurine Assay: High pressure liquid chromatography was performed on 7 μl aliquots of extracts from 2 − 10 × 10^3 cells using the following: a) Model UK6 injector, b) Model 6000A solvent delivery system, c) Fatty acid analysis column, d) Model 420-C and 420-E fluorometric detector (excitation at 325 nm, emission at 410 nm) from Waters' Associates (Milford, Massachusetts). The sample was dissolved in 0.5% (V/V) acetic acid and 0.05% (W/V) sodium lauryl sulfate in distilled deionized water and passed through the column at a constant flow rate of 0.5 ml/min. The sample was reacted with the derivatizing agent containing 0-phthaldialdehyde (Sigma Chem. Co., St. Louis, MO) with 2-mercaptoethanol in borate buffer of pH 10.4. The analysis time for one sample was 15 min. Standard curves of taurine were made for each experimental day and each value obtained was confirmed in duplicate.

Taurine Levels in AML Cells Comparing
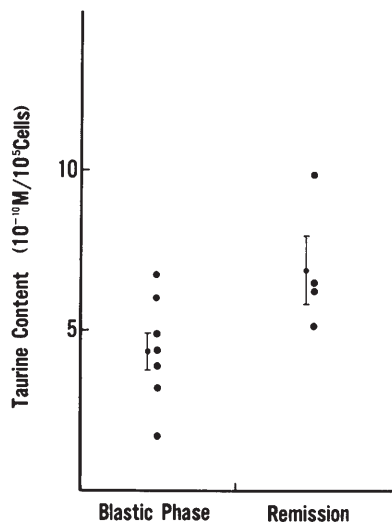### Blastic Phase and Remission
—— bone marrow mononuclear cells ——

Fig. 4

Fig. 4. The mean (±SE) taurine contents of
bone marrow mononuclear cells are
4.35±0.62 (10^{-10} moles/10^5 cells) in
AML in blastic phase and 6.98±1.02 in
AML in remission.

RESULTS

Cell Types Analyzed for Taurine in Normal Subjects and Leukemic Patients

The circulating granulocyte fraction separated by the above methods consisted of
segmented granulocytes in all the samples obtained. The mononuclear fraction yielded cells
into the following groups. The peripheral blood samples from normal subjects or patients
with AML or ALL in remission had 80 to 95% lymphocytes and the remaining cells were
monocytes. The peripheral blood from CLL, AML and ALL patients and bone marrow
samples from patients with AML in the blastic phase were 90 ~ 95% leukemic or CLL cells.
The peripheral blood from patients with CML and bone marrow samples from patients with
AML in remission yielded 50 ~ 70% leukemic or normal immature granulocytes and the
remaining were a mixture of lymphocytes, monocytes and erythroblasts. The peripheral
blood from patients with atypical AML yeilded a wide range (30 ~ 80%) of leukemic cells with
the rest being normal lymphocytes.

Taurine Levels in Circulating Mononuclear Cells of Normal Subjects and Patients with
Leukmia

The mononuclear cells from normal subjects had $19.3 \pm 1.8 \times 10^{-10}$ mol/10^5 cells of taurine
intracellularly. Figure 2 demonstrates a markedly low taurine level ($3.5 \pm 0.7 \times 10^{-10}$ mol/10^5

cells) in the blastic phase of the acute leukemias, followed by the chronic leukemias ($5.6 \pm 0.4 \times 10^{-10}$ mol/ 10^5 cells) and then by the dysmyelopoietic syndromes ($10.7 \pm 0.2 \times 10^{-10}$ mol/ 10^5 cells). These differences were significant in statistical sanalysis by t-test ($p < 0.001 \sim 0.025$). The taurine levels did not seem to distinguish the leukemic cell line in our experiment, but probably reflected either the rate of proliferation or the degree of differentiation of cells. The mononuclear cells in atypical AML had lower taurine levels than normal ($p < 0.025$) but were higher compared to cells from patients with chronic leukemias ($p < 0.001$) despite the higher percentage of circulating blast cells. The taurine level did not correlate to the percentage of leukemic blast cells which ranged from $5 \sim 65\%$ in peripheral blood differentials and $30 \sim 80\%$ of the cells in the mononuclear cell fraction in atypical AML patients. Although one patient with atypical AML who had 65% blast cells in the peripheral blood showed morphologically and quantitatively the same blast cells as typical AML, the taurine level of their leukemic cells was different. This patient had an earlier pancytopenic period for one year and also had many erythroblasts in the peripheral blood at the time of transformation. This patient responded poorly to chemotherapy unlike the patients with acute leukemias with cells containing lower taurine levels.

Taurine Levels in Circulating Granulocytes

The taurine levels of normal granulocytes were $14.8 \pm 0.8 \times 10^{-10}$ mol/ 10^5 cells, slightly lower than those of the normal mononuclear cells ($p < 0.05$). The circulating granulocytes in leukemia patients had taurine levels which were not different from normal subjects (Fig. 3) except for the cells from atypical AML patients at $7.9 \pm 1.5 \times 10^{-10}$ mol/ 10^5 cells which were lower than normal ($p < 0.001$). Although the granulocytes in ph^1-positive CML had normal taurine levels, the granulocytes in atypical AML had lower taurine levels compared to normal granulocytes.

Taurine Levels in Bone Marrow of Patients with AML in Remission and in Blastic Phase

Figure 4 shows the taurine levels of the bone marrow cells of acute leukemic patients during remission ($7.0 \pm 1.0 \times 10^{-10}$ mol/ 10^5 cells ($p < 0.05$). Taurine levels in bone marrow mononuclear cells were much lower than those in circulating cells during the same time of the remission phase of acute leukemia in these patients.

DISCUSSION

Our study demonstrated abnormally low taurine levels in cells obtained from patients with leukemia. Taurine is the free amino acid with the greatest concentration in normal human leukocytes and it was reported at levels of 20 to 35 mmol/kg leukocyte water.[1,2,3,4] The water content of leukocytes is presumed to be 75% of the cell mass, and correction of these values approximates to 18.7×10^{-10} mol/ 10^5 cells, assuming a leukocyte count of 8×10^3/mm^3 and leukocyte volume of 1% in the total blood volume of six liters. These levels of taurine in the leukocytes are similar to our data for normal taurine levels. However, in our study, we further separated the leukocytes into mononuclear cells (lymphocytes and monocytes) and granulocytes. By this method,[9] it was impossible to avoid some contamination of RBC in part of the granulocytes and platelets in the mononuclear cell layer. But RBC contamination was lower than half of the granulocytes and the extremely low taurine levels in RBC are already known.[3,4] The taurine content of RBC was determined to be as low as 1×10^{-12}/ 10^5 cells in our experiment. Although another author reported a taurine level for platelets as high as 21 mmol/kg of the wet weight,[4] platelet contamination in the mononuclear cell layer was not a problem in our study. The number of platelets was not more than that of mononuclear cells and the content of taurine in platelets was determined to be only 1/100 that of leukocytes,

if normalized by cell count, in our study. Although one author demonstrated a normal level of taurine in leukemic cells,[8] this discrepancy compared to our result may be due to a difference in the assay method. We previously observed that deproteinization using picric, perchloric, or trichloracetic acid increased the conversion of hypotaurine to taurine resulting in falsely high taurine levels during the assay; therefore we have changed methods by using ethanol to avoid this reaction.[10] The use of high pressure liquid chromatography employing the fluorometric method enabled us to separate individual chromatographic peaks of hypotaurine and phosphoethanolamine from taurine. The assay is sensitive to measures of 5 picomoles of taurine. This was not possible using paper and thin layer techniques or auto amino acid analyzer. these important differences in the assay and the differences in the cell purification method may explain the discrepancies between the findings of the previous report[8] and those of our study.

The granulocytes in acute and chronic leukemias had normal taurine levels even though evidence indicates that CML granulocytes are derived from the abnormal clone. However, granulocytes of patients with atypical leukemia demonstrated low taurine levels indicating that these cells had a defective taurine metabolism not only in blastic cells but also in matured granulocytes. Although this is a significant finding, further accumulation of cases is needed to confirm these results since the number of cases used in our study was too few.

The mononuclear cells demonstrated a range of taurine depletion among the different types of leukemia. The acute leukemic cells regardless of cell origin had the lowest levels of taurine which reverted to normal during clinical remission. Patients who appeared to be in acute transformation with a history of refractory anemia and cytopenia had higher taurine levels in their cells than patients with typical acute leukemias despite the presence of a high percentage of myeloblasts. This finding indicates that the clinical transformation of dysmyelopoietic syndrome caused less alteration in taurine metabolism than the blastic phase of AML. The cells from patients with atypical leukemia contained moderately depleted taurine levels both in the mononuclear (blastic) cells and granulocytes and the cells from patients with typical acute leukemia contained much lower taurine in their blastic cells and higher (normal) taurine levels in their granulocytes. According to these observations, we speculated that an abnormal hemopoietic clone, in which the taurine metabolism has been moderately disturbed, is present in atypical leukemia, while a normal and leukemic hemopoetic clone may co-exist in typical acute leukemia. A similar finding of clones was reported in AML and preleukemic cells in case of CFC (granulocytic colony-forming capacity) and CSA (cloney stimulating activity).[11] These observations may have some clinical relevance, since most acute transformation of dysmyelopoietic syndrome responded poorly to chemotherapy unlike typical acute leukemia.[12] Further studies concerning the role of taurine levels as a leukemogenic factor or a predicting factor for treatment of acute leukemia should be conducted.

The abnormality in taurine levels may be due to the observation of low activity of γ-crystathionase in leukemic cells[7,13] resulting in an abnormal methionine-cysteine pathway (Fig. 1). The activity of γ-cystathionase was decreased to similar levels in leukemic cells from both ALL and AML[14] which could explain the lack of difference in the taurine levels in cells from these patients in our experiments. Unfortunately, the activity of the enzyme has not been measured in dysmyelopoietic syndrome and the chronic leukemias.

The possibility of other taurine pools or metabolic pathways should also be considered since low cystathionase activity in vitamin B_6 deficiency does not always lead to low taurine levels in tissue.[15] Another alternative explanation for the low taurine levels in leukemic cells may be due to a high turnover rate or decreased uptake of this amino aicd. The latter is not likely since most tumor cells studied in vitro were observed to accumulate taurine selectively

to a high degree.[16,17,18] The decreased activity of γ-cystathionase is not unique to leukemic cells and has been observed in other malignant cells[19,20,13] and fetal tissue.[14,21]

These observations including our data suggest the possibility that low taurine levels may be a common characteristic of neoplastic cells and immature growing cells rather than a specific finding in leukemic cells. Depletion of the taurine levels does not always correlate with the degree of maturation of cells as in the patients with atypical AML and CML in transformation and may offer clues to the biochemical changes in leukemogenesis occurring in the hematopoietic cells. The role of taurine in the cell growth and proliferation of leukemic cells has been studied[22] but should be further delineated. In our experiments, the number of cases of each type of leukemia was not enough to give a definitive conclusion. Alteration of taurine levels demonstrated the possibility of a good biochemical marker in the differential diagnosis of acute transformation of the dysmyelopoietic syndrome or that of CML from the acute leukemias and the following response of these patients to therapy. Furthermore, the potential for a possible therapeutic approach based on taurine depletion or of its analogue administration similar to the approach using L-cysteine and L-cystine in leukemia by other investigators[13,23] may improve the current treatment programs.

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