Effect of 2-chlorodeoxyadenosine therapy on bone marrow fibrosis in hairy cell leukemia

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The study shows the influence of 2-chlorodeoxyadenosine therapy on bone marrow fibrosis in patients with hairy cell leukemia. Eighteen patients were studied; bone marrow fibrosis was graded 0–4 according to the quantity and pattern of distribution of reticulin. The grade of bone marrow fibrosis was established before the therapy and then in 12- and 24-month intervals. Patients showed complete remission after the therapy and remarkable reduction of bone marrow fibrosis.

Key words: hairy cell leukemia, bone marrow fibrosis, 2-chlorodeoxyadenosine, 2-CdA, complete remission

Hairy cell leukemia (HCL) is less common chronic B-lymphoproliferative disorder, first described as leukemic reticuloendotheliosis in 1958 [2]. The disease was named as histoleukemia, medullosplenic histiolymphocytosis, lymphoid myelofibrosis, and reticular cell leukemia. Leukemic cells have typical "hairy" cytoplasmic projections well seen in electron microscopy and phase contrast microscopy.

2-chlorodeoxyadenosine (2-CdA) is a simple purine nucleoside that has previously been shown to be effective in the treatment of low-grade malignant disorders of lymphoid tissue, including chronic lymphocytic leukemia and non-Hodgkin's lymphoma. 2-CdA is administrated in the dose 0,1 mg/kg/day for seven days in continuous infusion. The administration of 2-CdA results in a higher rate of complete remission than is observed with interferon alfa. Its toxicity is lower than that of 2-deoxycoformycin (2-CDF) [5].

We decided to study bone marrow fibrosis in patients with HCL at the time of diagnosis and in 12-month intervals in patients in complete remission (CR); minimal time interval after the therapy with 2-CdA was 6 months.

Patients and methods

Study group. Eighteen patients with hairy cell leukemia were included in this analysis. Diagnosis of HCL was based on history, physical examination, trephine core bone marrow biopsy, peripheral blood smear, positive TRAP reaction on leukemic cells, and sIL-2R level. Patient selection was based upon the availability of 12-month intervals between consecutive trephine core bone marrow biopsies. Complete remission was defined by the complete absence of circulating leukemic cells, hemoglobin concentration >120 g/l, white cell count >$3.0x10^9$/l, absolute neutrophil count >$1.5x10^9$/l, platelet count >$100x10^9$/l, and the absence of hepatosplenomegaly and lymphadenopathy.

Bone marrow biopsies. A total of 54 trephine core bone marrow biopsies were obtained from the 18 patients at time intervals before 2-CdA therapy, at 12 months, and 24 months. All specimens were fixed in Lowy's fixation-decalcification solution [4], embedded in paraffin, cut into 3 μm thick sections and stained with Giemsa and with Gomori reticulin stain. Grading of reticulin fibrosis (scale of 0 to 4) was made according to the quantity and pattern of distribution of reticulin as described [1]: grade 0 – reticulin fibers absent, grade 1 – scattered reticulin fibers or foci of fine reticular network, grade 2 – diffuse fine reticular network, grade 3 – diffuse fine reticular network, scattered thick fibers, grade 4 – diffuse thick reticular fibers, the presence of collagen fibers.

Sections were obtained from bone marrow core biopsy samples 6–20 mm in length. At least 10 high power fields per

case at magnification of x 400 were examined at equidistant representative areas of the whole specimen.

Results

The characteristics of the 18 patients under study are as follows: Median age was 60.5 years (range, 45–82 years), males represented 78%, 22% were females.

Table 1 summarizes bone marrow study by grade of fibrosis before and after 2-CdA therapy.

Before the start of 2-CdA therapy, reticulin fibrosis was grade 4 in 0 patients, grade 3 in 11 patients (61%), grade 2 in 4 patients (22%), grade 1 in 3 patients (17%), and grade 0 in 0 patients.

Reticulin fibrosis after 2-CdA therapy (12 months after initial bone marrow biopsy, minimum 6 months after the therapy) was grade 4 in 0 patients, grade 3 in 4 patients (22%), grade 2 in 5 patients (28%), grade 1 in 8 patients (44%), and grade 0 in 1 patient (6%).

Reticulin fibrosis 24 months after initial biopsy was grade 4 in 0 patients, grade 3 in 2 patients (11%), grade 2 in 4 patients (22%), grade 1 in 10 patients (56%), and grade 0 in 2 patients (11%).

Following 2-CdA therapy, bone marrow fibrosis increased in 12-month interval by two grades in 1 patient (6%), by one grade in 0 patient, remained unchanged in 8 patients (44%), decreased by one grade in 2 patients (12%), and decreased by two grades in 7 patients (39%).

In 24-month interval, bone marrow fibrosis increased by two grades in 0 patient, by one grade in 3 patients (17%), remained unchanged in 3 patients (17%), decreased by one grade in 3 patients (17%), decreased by two grades in 7 patients (39%), and decreased by three grades in 2 patients (12%).

The correlation between initial and later fibrosis grade on 2-CdA therapy is shown in Table 2a and 2b.

Table 1. Study group of patients and degree of fibrosis

Patient (initials)	Gender	Age	Fibrosis at the time of diagnosis (0–4)	Fibrosis after 12 months (0–4)	Fibrosis after 24 months (0–4)
1. Z.L.	M	52	3	3	1
2. S.D.	M	79	2	2	2
3. J.Z.	M	63	3	1	1
4. S.Z.	M	58	3	1	1
5. J.V.	M	70	3	3	3
6. J.F.	M	82	2	2	1
7. V.S.	M	71	1	1	1
8. J.C.	F	56	1	1	2
9. O.N.	M	77	3	1	2
10. B.L.	F	65	3	2	0
11. K.G.	M	56	3	1	1
12. A.C.	M	51	2	2	3
13. J.Je.	M	45	2	0	1
14. B.N.	M	72	3	1	1
15. J.Jo.	M	73	1	3	2
16. Z.S.	M	47	3	2	1
17. B.B.	F	52	3	3	0
18. V.S.	F	53	3	1	1

M – male, F – female.

Table 2a. Bone marrow fibrosis study results – change in fibrosis grade on 2-CdA therapy between biopsies (12-month interval)

Change in fibrosis grade	No.	(%)
+2 or worse	1	6
+1	0	0
no change	8	44
–1	2	12
–2	7	39

Table 2b. Bone marrow fibrosis study results – change in fibrosis grade on 2-CdA therapy between biopsies (24-month interval)

Change in fibrosis grade	No.	(%)
+2 or worse	0	0
+1	3	17
no change	3	17
–1	3	17
–2	7	39
–3	2	12

Discussion

Hairy-cell leukemia is a proliferation of clonal B-lymphocytes with features of activation. The disease has a number of distinctive characteristics, prominent among which is the fine reticulin fibrosis invariably present in the bone marrow. However, fibroblast infiltration has never been noted in the marrow and the origin of the fibrosis has not been established. The present studies show that the hairy cells (HCs) of HCL produce an insoluble matrix of fibronectin (FN) *in vitro*. FN synthesis was shown by the appearance of cellular FN on the surface of cells cultured in serum-free medium and by immunoprecipitation of the metabolically labeled protein from HC aggregates. Moreover, the HCs were shown to assemble FN into disulphide-bonded multimers. This assembly was blocked by a 70-kD amino-terminal fragment of the molecule that blocks FN multimer formation by fibroblasts. HCs expressed abundant VLA-5, an FN receptor not present on normal circulating B lymphocytes, but important in matrix formation. Furthermore, HCs were shown to adhere to an FN fragment containing the VLA-5 binding site. It is therefore suggested that the VLA-5 of HCs

is implicated in their assembly of FN matrix. The *in vivo* relevance of the findings was established by the demonstration of FN in association with infiltrating HCs in bone marrow sections from patients with HCL. It is concluded that the HCs synthesize and assemble an FN matrix and that this is, at least, partly responsible for the bone marrow fibrosis so characteristic of the disease [3].

2-CdA is a nucleoside analog with substituted halogen atom at position 2 in its purine ring that makes it resistant to deamination by adenosine deaminase. 2-CdA is the drug of choice in the treatment of hairy cell leukemia, but it is also highly active in other low grade lymphoid malignancies including chronic lymphocytic leukemia (CLL). The results of the studies presented so far have shown that 2-CdA gives similar complete response rate and overall response rate to fludarabine but the influence of both agents on survival times of the patients with CLL is still uncertain. CR rate induced with 2-CdA is significantly higher than in the patients treated with conventional chemotherapy. In refractory or relapsed patients 2-CdA induces 31 to 68% of overall responses including CR in 4 to 31%. In previously untreated patients overall remission rates of about 56–82% have been achieved with 2-CdA alone. When 2-CdA was used as primary therapy the CR rate was also significantly higher and ranged from 10% to 47%. Patients who received 2-CdA as their initial therapy and experienced a response lasting at least a year may be successfully treated subsequently with the same agent. Bone marrow suppression with anemia, neutropenia, and thrombocytopenia are the dose limiting factors for 2-CdA use [6].

The influence of 2-CdA therapy on bone marrow fibrosis has not been well investigated. 2-CdA has a potent myelosuppressive effect, and it has been shown to be toxic to malignant myeloid cells both *in vitro* and *in vivo* [7].

In our experience, bone marrow fibrosis was remarkably reduced in patients treated with 2-CdA; this feature is probably due to reduced number of HCs in bone marrow and thus lower FN synthesis.

In conclusion, 2-CdA therapy demonstrated reduction of bone marrow fibrosis in patients with significant fibrosis before the start of therapy.

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