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PERIPHERAL BLOOD AND BONE MARROW CHANGES AFTER TREATMENT WITH ATRA AND G-CSF IN AML, APL AND BLAST CRISIS FOLLOWING VAQUEZ'S DISEASE

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ABSTRACT

The aim of the present study was to better understand the possibility of utilizing growth factors of the myelomonocytic line in acute leukemias. The study is an examination of morphological changes and marker behavior in peripheral and bone marrow cells in AML and APL during treatment both with all-transretinoic acid (ATRA) alone and in association with chemotherapy and G-CSF. The same treatment was carried out in a patient who had been diagnosed with Vaquez's disease 15 years earlier and currently presented a bone marrow and peripheral picture of AML (80% myeloblasts) with thrombocytopenia.

We observed that treatment with ATRA, alone or in association with chemotherapy, was followed by a remission of AML and especially of APL, with amelioration of the general condition of the patients. The addition of G-CSF to ATRA at the end of chemotherapy, during consequent pancytopenia, produced a rapid increase in mature peripheral granulocytes and an apparent medullary complete remission, which was more prolonged in APL than in AML; there was no increase in peripheral blasts. Discontinuation of G-CSF was followed by a relapse in the patient with AML. A patient with Vaquez's disease, in remission for 15 years and presenting a progressive increase in bone marrow and peripheral myeloblasts, did not have a positive response to the administration of ATRA; however, the association of G-CSF to ATRA was followed by a complete remission. The morphological changes observed in bone marrow and peripheral granulocytes (with changes in the main cellular markers: CD11b, CD13, CD14, CD15, CD34) seemed to express progressive modification of the single elements towards differentiation, with progressive bone marrow reduction and peripheral disappearance of blasts. The data agree with the changes observed in *in vitro* blasts cultured in the presence of ATRA and G-CSF.

Key words: Vaquez's disease, acute myeloid leukemia, acute promyelocytic leukemia, ATRA, G-CSF

A great amount of recent research concerns the influence that retinoic acid (*cis* and *trans*) and its derivates exert on cell differentiation in normal and pathological conditions (cancers and, above all, acute leukemias). The most significant observation is the ability of RAs to stimulate cellular differentiation in APL. This ability is particularly evident in the presence of a t(15;17) chromosomal alteration, with fusion of the genes encoding PML on chromosome 15 and nuclear retinoic acid receptor-α (RAR-α) on chromosome 17.[1-5]

Frequently positive clinical results have stimulated abundant studies on ATRA (all-transretinoic acid) and its utilization in the treatment of acute leukemias; this is the case not only in APL, but also for other proliferative pathological conditions, such as other acute myeloid leukemias, chronic myeloid leukemias and cancer.[2,5-9] The interesting results obtained in APL have only been partially duplicated in other acute myeloid leukemias, and the positive effects obtained have always been transitory, although evident stimulation of differentiation was docu-

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mented in almost all cases at the beginning of treatment, and also *in vitro*.[10,11] It is always difficult to understand the mechanisms of the modifications obtained, which undoubtedly represent the result of an attempt to normalize behavior in the pathological elements rather than to destroy them.

Our purpose was to better understand the modality of action of ATRA in acute leukemias and to identify the best way of utilizing G-CSF in association with ATRA in these diseases, since even recently some researchers have noticed the appearance of terminal differentiation in chronic myeloid leukemia treated with a combination of ATRA and G-CSF.[11]

Patients and Methods

The study was carried out on peripheral leukocyte CFU from 3 patients with APL and from 5 with AML (3 M1 and 2 M2); age varied from 42 to 82 years. The study also involved 1 patient in blast crisis (BC) that appeared 15 years after the first manifestation of Vaquez's disease.

During first remission one of the M1 patients (see below) was operated for a renal cancer, without complications. Overall survival varied from 3 months to 2 years or more.

Morphological and marker changes were monitored from the beginning of observation and on several occasions during treatment. Specific diagnoses were formulated on the basis of cytochemistry and cellular markers.

The therapeutic protocol underwent some changes during the follow-up according to the behavior of the disease. In the initial stage all the patients were treated with transretinoic acid (100 mg/m^2/day); subsequently, due to unsatisfactory response, all but one of the leukemia patients were submitted to a cycle of daunomycin (D) and cytosine arabinoside (A) (D=60 mg/m^2 and A=150 mg/m^2). At the end of chemotherapy, during the period of maximal leukopenia and disappearance of blast cells, they all received 100 mg/m^2 of ATRA/day and 200 µg/m^2 of G-CSF/day. The patient in blast crisis subsequent to Vaquez's disease was only treated with ATRA and G-CSF because the good results obtained made chemotherapy unnecessary. At the beginning of observation and throughout treatment, all subjects were regularly monitored for changes in peripheral leukocyte morphology and for the behavior of the main cellular markers (CD11b, CD13, CD14, CD15, CD34) by means of cytofluorimetry.

Results

A complete remission following ATRA treatment alone was observed in 1 patient with APL. A remission was not obtained in the subject with BC after treatment with ATRA alone, but only after adding G-CSF to ATRA in order to correct concurrent bone marrow hypoplasia. In all the other patients remission was only partial and treatment with ATRA was followed by chemotherapy, which determined a complete remission of variable duration in every case. Only the patient in BC has still not needed chemotherapy to this day. The mean life span of the other patients was 2 years.

Systematic monitoring of peripheral and bone marrow cells of the granuloblastic line revealed singular behavior subsequent to the administration of ATRA and G-CSF. ATRA was generally responsible for the appearance of signs of cell evolution towards maturity in all subjects; in the 2 patients with APL and in the subject with BC, we observed a slow transformation in the peripheral white cellular population, preceded by a reduction in granuloblastic markers

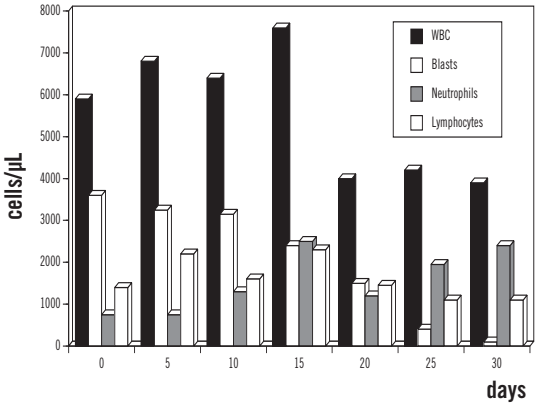

Figure 1. Behavior of the peripheral blood count of the patient in blast crisis during treatment with ATRA and G-CSF.

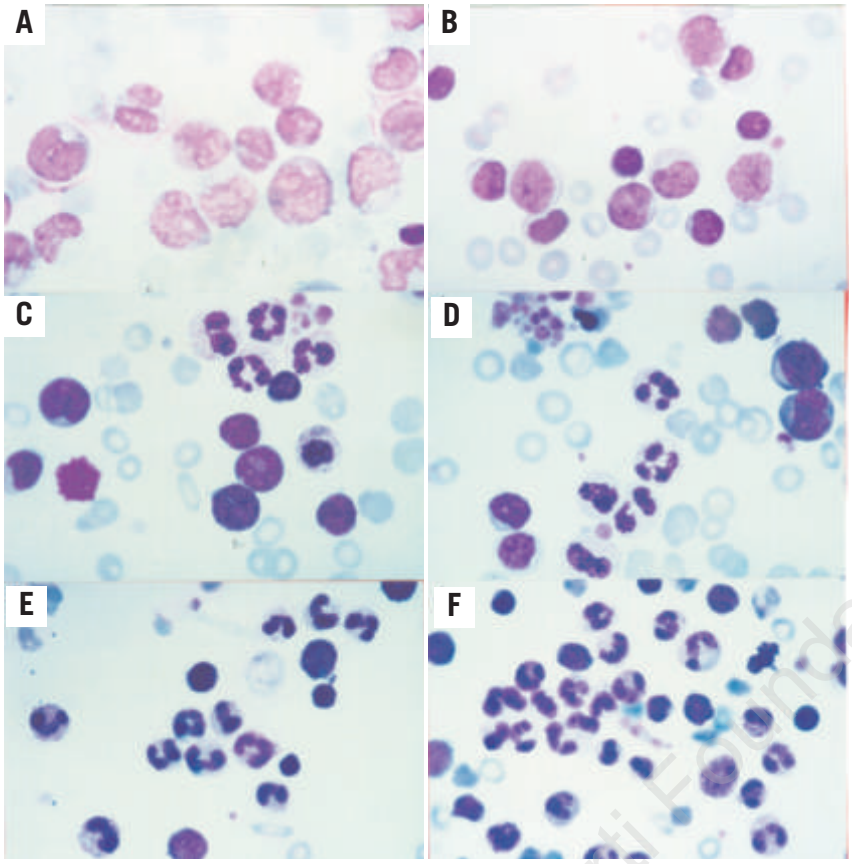

Figure 2. Enrichment of peripheral WBC from a patient in blast crisis at different stages during the treatment with ATRA and G-CSF: a) before treatment with G-CSF; b, c, d, e, f) after 10, 15, 20, 25 and 30 days, respectively. The gradual passage from undifferentiated blast cells to mature granulocytes is evident.

(CD34) and an increase in markers of mature cells. In APL without other treatment, we noted the gradual appearance of myelocytes, metamyelocytes and granulocytes, with progressive disappearance of circulating promyelocytes. Remission in these cases, however, normally lasted for only a few months.

The patient in BC who also presented bone marrow hypoplasia and 75% blasts in circulating leukocytes did not respond to ATRA alone. However, after the addition of G-CSF (300 μg/day) he showed a strong reduction in blasts, with an increase in the more mature cells of the granuloblastic line and, above all, in granulocytes (Figures 1 and 2). We also observed corresponding changes in cellular markers and no increase in the total number of peripheral leukocytes. The G-CSF and ATRA doses were gradually reduced to minimal maintenance levels without compromising the results obtained.

Subsequent to the administration of ATRA alone, the 3 patients with AML (M1 and M2) showed only a slight modification of cellular markers and no changes in the cellular population. The treatment was discontinued and the patients were submitted to a regime of daunomycin (60 mg/m^2 on the 1st and the 8th day) and cytosine arabinoside (150/m^2/day on days 1-5). The same treatment was applied to the 2 APL patients when they were in relapse. At the end of the chemotherapy cycle, during the period of maximum leukopenia, ATRA was restarted in association with G-CSF at the doses reported above. We noted a rapid increase in peripheral granulocytes to normal values, an increase that was preceded by the appearance of intermediate forms of maturation of the granuloblastic line, by the complete disappearance of blast cells and a progressive increase in markers of mature cells. The total number of circulating WBC did not surpass 20×10^9/L. Remission ranged from 5 to 8 months and 1 patient with AML underwent nephrectomy as a result of renal cancer without complications. Subsequent remissions after relapses were always less prolonged, and the treatment proved to be progressively less effective.

Conclusions

The results obtained permit some interesting considerations. First of all, we noted the ability of ATRA to stimulate cell differentiation in acute leukemias of the myelomonocytic line; however, it was only in APL that we were able to achieve a complete remission, which was nonetheless of variable duration. These data were confirmed by monitoring cellular markers. The addition of G-CSF to ATRA in the treatment of the acute leukemias enabled a more rapid recovery following chemotherapy, did not cause any increase in the number of circulating blasts, and was even compatible with good remission in AML. In the BC patient, the addition of G-CSF to ATRA did not modify the total number of peripheral leukocytes but was able to normalize them. This treatment also improved the bone marrow picture, RBC and platelet values. The appearance of intermediate forms of maturation during ATRA+G-CSF treatment seems to be the expression of a tendency towards normalization in pathological cells. This fact seems to be confirmed by the early appearance of normal granules in the cytoplasm of myelocytes and by the progressive increase of normal granulocytes.

Of particular interest is the positive response to G-CSG and ATRA, without other treatment, of the patient in blast crisis. This result could modify the interpretation of the genesis of BC in Vaquez's disease and probably in other myeloproliferative conditions as well.

In short, the association of a differentiating agent (in this study ATRA) makes it possible to utilize G-CSF in acute leukemias of the granuloblastic line to ameliorate the quality of life of the patients and to prolong the remission time of the diseases. Moreover, the association of ATRA and G-CSF may determine a complete remission in the blast crisis of myeloproliferative diseases without the need to use the common cytotoxic drugs.

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