strong correlation was found between the two variables (r = 0.86; p<0.0001), as suggested by linear regression analysis. The best results were obtained when the number of CD34+ cells in PB was greater than 50 µL, while the worst results were related to a level of less than 10 µL (Figure 1). Only in 5 cases, because of the particular clinical history of the patients, did we perform leukapheresis when circulating CD34+ cells were < 10 µL. None of these reached 0.5×10^6/kg CD34+ cells (mean value collected was 0.23, range 0.05-0.4×10^6/kg). With more than 10/mL CD34+ cells in PB we collected no less than 0.5×10^6/kg, with rare exceptions. When CD34+ cells exceeded 20/mL we usually reached more than 1×10^6/kg. No statistical difference was found in patients with CD34+ cells values ranging from 21 to 50 µL. Finally, in our hands, more than 50 µL circulating CD34+ cells ensured a collection greater than 2×10^6/kg in most patients.

Though the quality of a leukapheresis does not depend only upon the absolute number of CD34+ progenitors present,

our data confirm that daily estimation of the circulating CD34+ cell number by flow cytometry may guide our clinical decisions and may offer a useful tool for predicting the number of procedures to perform.

GIOVANNI D’ARENA
PELLEGRINO MISTO
MARIO CARBONUTTO
Division of Hematology, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy

References


Correspondence: Giovanni D’ARENA, MD, Division of Hematology, IRCCS Casa Sollievo della Sofferenza Hospital, 71013 San Giovanni Rotondo, Italy. Tel. international +39.882.410539. Fax: international +39.882.411705.

All-trans retinoic acid might also induce apoptosis in freshly isolated chronic myeloid leukemia cells

Sir,

we read with interest the recent letters by Martinelli and coworkers’ and Zinzani and coworkers’ on the induction of apoptosis by the nucleoside analogs fludarabine (FAMP), 2-chloro-deoxyadenosine (2-CdA) and 2-deoxycoformycin (DCF), whether used alone or in combination with α-interferon (α-IFN), in freshly isolated leukemic cells from chronic myeloid leukemia (CML). Apoptotic cell death, as demonstrated by electrophoretic gel DNA fragmentation pattern, was induced by both FAMP and 2-CdA, either alone or in combination with α-IFN, whereas DCF, with or without α-IFN, failed to do so. The authors focused on the opportuneness of promoting further in vitro and in vivo studies with these two promising adenosine analogs, possibly employing assays able to measure programmed cell death. We agree with the authors about the timeliness of exploring new effective drugs capable of driving the CML clone into apoptosis, and on this point we would like to provide further evidence in support of their in vitro findings.

We recently tested the in vitro capability of FAMP and all-trans retinoic acid (ATRA) to drive peripheral myeloid cells from untreated Ph+ CML patients in chronic phase into apoptosis. Apoptosis was measured by using simple and reliable flow cytometric methods based on decreased forward light, increased right angle scatter and reduced propidium iodide fluorescence stainability. These methods, as compared to electrophoretic gel DNA fragmentation assays, which allow only bulk apoptosis measurement, are able to detect programmed cell death on a single cell basis. In our model, CML cells cultured alone in standard complete medium (RPMI 1640 plus 10% FCS) showed a low apoptotic cell rate (6.8% at 96 hours of culture) at all the different time points tested (24, 48, 72, 96 hours). By contrast, when cultures were performed in the presence of FAMP (5 µM) apoptosis reached 26.3% and 31.7% at 72 and 96 hours, respectively. Similar results were obtained when FAMP was substituted with ATRA (3 µM). This agent, which was previously shown to induce apoptosis in freshly isolated chronic promyelocytic leukemia and chronic lymphoproliferative disorders, also drove CML cells into apoptotic cell death (28.6% at 72 and 40.5% at 96 hours of culture). Apoptosis occurred mainly via terminal myeloid differentiation of the leukemic clone, as demonstrated by cytospin morphological and cytochemical examinations.

Taken together, these findings further support the importance of focusing on inducers of programmed cell death as promising new agents in the management of chronic phase CML, and provide a rationale for the employment of either purine analogs or ATRA in pilot clinical trials.

References


Correspondence: Fabio Stagno, MD, Istituto di Ematologia, Ospedale Ferrarotto, via S. Citelli 7, 95124 Catania, Italy. Tel. international +39.95.7435968. Fax: international +39.95.365174.

Cryosupernatant in thrombotic thrombocytopenic purpura (TTP): is it really useful?

Sir,

Perroti et al. in their paper entitled Cryoprecipitate-poor plasma fraction (cryosupernatant-CPP) in the treatment of thrombotic thrombocytopenic purpura at onset. A report of four cases’ demonstrated that CPP can induce a more rapid improvement in the clinical manifestations of TTP than fresh frozen plasma, while laboratory parameters show slow normalization. We treated six patients with plasma exchange (PE) and CPP (two relapses at four and six years, respectively; four cases at first diagnosis).

The clinical manifestations of these patients at onset are reported in Table 1.
All patients received the first plasma exchange employing fresh frozen plasma, and CPP was used in the following procedures. PE was performed daily until normalization of the platelet count and serum LDH levels.

Two patients (B.S. and P.M.L.) died 8 and 5 days, respectively, after beginning plasma exchange, and the addition of high-dose iv immunoglobulins in one case did not produce any effect. Clinical remission was reached in four patients. In one case, neurological disorders at onset such as amnesia and paresis improved after three PE procedures.

Normalization of platelet count was obtained after 7, 11, 13 and 17 plasma exchange procedures, respectively. Our experience confirms the data reported by Perotti et al. regarding hematological recovery, while the neurological disorders that appeared during the treatment in two patients did not seem to be influenced by the addition of CPP.

These observations seem to limit the efficacy of CPP as compared to fresh frozen plasma in the treatment of TTP.

Riccardo Centurioni
Cristina Rife*
Alessandro Cecconelli
Istituto di Clinica Medica Generale, Ematologia ed Immunologia Clinica, Università di Ancona and *Servizio di Immunotematologia e Trasfusionalità, Ancona, Italy

References

Correspondence: Riccardo Centurioni, MD, Istituto di Clinica Medica, Ospedale di Torrette, 60020 Ancona, Italy.

Lymph node myeloid metaplasia associated with chronic neutrophilic leukemia

Sir,

chronic neutrophilic leukemia (CNL) is a rare disorder characterized by neutrophilia due to mature elements and organ infiltration, including both hepatosplenomegaly and lymph node infiltration. Around 78 cases have been reported up to now and no one else has described the presence of myeloid metaplasia in lymph nodes as we have in this study. A 68-year-old man presented weight loss, enlarged lymph nodes, hepatosplenomegaly and ascites. WBC count was 156 x 10^9/L (84% neutrophils, 1% monocytes, 2% basophils, 3% bands, 2% atypical lymphocytes, 5% metamyelocytes, 5% myelocytes). Hemoglobin was 8.7 g/dL, platelets 68 x 10^9/L, uric acid was 15.8 mg/dL, alkaline phosphatase 2457 U/L, LDH 915 U/L, granulocytic alkaline phosphatase 300 U. Renal and liver function was normal. Paracentesis revealed ascitic liquid with 3.6 x 10^9/L cells (85% mature neutrophils). Bone marrow biopsy displayed increased cellularity and granulocytic hyperplasia compatible with CNL. Cytogenetic study was normal (46,XY). Molecular biology did not demonstrate a bcr-abl translocation. A lymph node biopsy showed massive substitution of the normal lymph node architecture by hematopoietic cells, including elements of all three series; within the granulocytic line numerous polynuclear cells were conspicuously intercalated among immature cells. Megakaryocytes were also frequent, and some of them presented phagocytic phenomena. The patient was treated with hydroxyurea, which provided good WBC count control; however, two months later he developed hepatorenal failure and died on day +71 after diagnosis.

Myeloid metaplasia has not been previously described in CNL. Chronic neutrophilic leukemia was recently characterized as a distinct myeloproliferative disease with a specific molecular marker (bcr/abl with C3/A2 junction). Our finding of myeloid metaplasia is consistent with the myeloproliferative nature of CNL.

José A. Pérez-Simón*
Julio M. Hernández-Rivas*
Teresa Flores*

Servicio de Hematología, *Servicio de Anatomía Patológica, *Hospital Universitario de Salamanca, Paseo de San Vicente 58-182, Salamanca, Spain

Prolonged low doses of oral etoposide may be effective in individual patients with advanced lymphoproliferative disorders refractory to aggressive chemotherapy

Sir,
etoposide, a semisynthetic podophyllin derivate, is currently employed in the treatment of several malignancies. The antitumor efficacy of etoposide is highly schedule dependent and it has been demonstrated that five-day administration is superior to single day administration. Oral etoposide has greatly facilitated the use of multiple-day schedules and the drug displays good activity in many extrahematological malignancies as well as in lymphomas. From December 93 to May 94, we treated with prolonged low doses of oral etoposide 24 patients with advanced hematological malignancies not eligible for further intensive approaches due to age >60 years and/or severe previous infective complications. Of these, ten patients had ALL, 8 AML, 4 NHL and 2 had CML.

Etoposide (50 mg/m² per day) was administrated orally for 21 consecutive days; patients who showed a response or stable disease on day 28 received a second and a third cycle of the same treatment. Responder patients received maintenance etoposide at the same dosage 10 days/month until relapse. Three patients (12.5%) died during the first cycle of intracerebral hemorrhage (1 patient) or infective complications (2 patients); no other toxic deaths were observed. All other patients received at least 2 cycles. Two ALL and 1 NHL patient (12.5%) obtained a complete remission of short duration (2, 3, 7 months); 1 ALL and 2 NHL patients (12.5%) achieved a partial response. No patient with myeloproliferative disease attained a response. Data from responder patients are shown in Table 1.

As for toxicity, 15/24 patients (62.5%) suffered febrile episodes during treatment (4 sepsis, 6 bronchopneumonia, 1 fungal sinusitis and 4 fever of unknown origin). One patient developed an intracerebral hemorrhage, while 8/24 patients (33.3%) displayed cutaneous hemorrhagic manifestations. Ten patients (41.6%) had mild nausea (WHO < 2) and vomiting, and 6/24 (25%) mucositis (WHO 2).

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<th>Patient</th>
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