

APOPTOSIS INDUCTION WITH PURINE ANALOGS ON FRESHLY ISOLATED CHRONIC MYELOID LEUKEMIA CELLS

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Sir,

chronic myeloid leukemia (CML) is a myeloproliferative disorder with an average duration of 3 years.¹ The fatal course CML patients on conventional therapy (including radiation, splenectomy and chemotherapy) has prompted an investigation of new treatments for the chronic phase of the disease because allogeneic bone marrow transplantation, the only potentially curative treatment modality known, is an option available to only a minority of patients. At present the most promising results have been obtained with α -interferon (α -IFN) as a single agent, which has produced a clinical hematological response and has controlled disease progression in more than two thirds of the patients treated.^{2,3} Furthermore, α -IFN can also induce karyotypic responses in 30-40% of treated CML patients.⁴

Recently, three new nucleoside analogs, fludarabine (FLU), 2-chlorodeoxyadenosine (2-CdA) and pentostatin, have shown marked therapeutic activity against a similar spectrum of indolent lymphoproliferative diseases. In addition, some *in vitro* reports^{5,6} have described the effective role of FLU and 2-CdA on the activation of apoptosis. Here, we report the *in vitro* induction of apoptosis by FLU, 2-CdA and pentostatin alone and in combination with α -IFN on freshly isolated samples obtained from 4 patients with chronic phase CML, in order to evaluate the effectiveness of these nucleoside analogs alone or with α -IFN through the apoptotic pathway.

Four patients with a diagnosis of chronic phase Ph¹⁺ CML who received no therapy dur-

ing the month preceding the trial were selected for this study. Mononuclear cells from bone marrow samples were obtained after centrifugation of the cell suspension over a Ficoll/Hypaque gradient. The following mean percentages were observed in the mononuclear fraction of the samples: lymphocytes 30%, monocytes 20%, myelocytes 20%, metamyelocytes 15%, promyelocytes 10%, and blast cells 5%.

FLU was purchased from Inveresk Clinical Research (Edinburgh, Scotland) and was used at a final concentration of 5 μ g/mL. 2-CdA and pentostatin were a gift from Dr. V.L. Narayanan of the Drug Synthesis and Chemistry Branch of the National Cancer Institute (Bethesda, MD), and were used at a final concentration of 30 μ mol/L and 5 μ M, respectively. α -IFN (provided by Hoffman-La Roche, Basel, Switzerland) was tested at a final concentration of 100 U/mL. The combinations evaluated were the following: FLU (5 μ g/mL) plus 2-CdA (30 μ mol/L), FLU (5 μ g/mL) plus pentostatin (5 μ M), FLU (5 μ g/mL) plus α -IFN (100 U/mL); 2-CdA (30 μ mol/L) plus α -IFN (100 U/mL); pentostatin (5 μ M) plus α -IFN (100 U/mL). The tumor cells were harvested, counted and added at a concentration of 5×10^5 into 25 cm² culture flasks; drugs were added and the cultures were incubated for 3 days at 37°C. The apoptotic assay was performed as previously described.^{7,8}

The results of our assay demonstrated that DNA, isolated from CML cells, presented the characteristic fragmentation pattern of apoptosis shown by electrophoresis in all four samples induced by FLU alone, 2-CdA alone, FLU plus α -IFN, and 2-CdA plus α -IFN. No differences

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in apoptotic response were seen between FLU or 2-CdA alone, or the combination of α -IFN with these deoxyadenosine analogs (Figure 1, for 1 patient). On the contrary, pentostatin alone displayed minimal evidence of inducing programmed cell death in all the samples; in addition, α -IFN alone or in combination with pentostatin also failed to trigger the apoptotic pathway. FLU and 2-CdA together provoked apoptosis, while it appeared that pentostatin in combination with FLU inhibited the apoptotic pathway observed with FLU alone.

The results of several clinical trials with α -IFN in CML patients were encouraging, particularly because of the repeated observation of complete cytogenetic response. In an effort to improve on the incidence of complete and partial cytogenetic responses, a series of trials has recently been undertaken with new drugs: homoharringtonine (HHT) alone,⁹ HHT and cytarabine (data only *in vitro*)¹⁰, cytarabine and α -IFN,¹¹ 2-CdA.¹²

Our observations reveal that FLU and 2-CdA, alone or in combination with α -IFN, directly activate or release an apoptotic program; α -IFN and pentostatin, on the contrary, do not induce

programmed cell death. For this reason, *in vitro* studies and trials of FLU and/or 2-CdA with conventional antineoplastic agents and with α -IFN are needed to establish clearly the place of these promising new agents in CML treatment. In addition, the early evidence of a possible lack of cross-resistance between these different analogs needs to be investigated further in an attempt to improve treatment protocols. Regarding this panel of effective new drugs against CML cells – FLU, 2-CdA, HHT – one of the most important objectives of future pilot studies in CML patients will probably focus on assays that measure programmed cell death.

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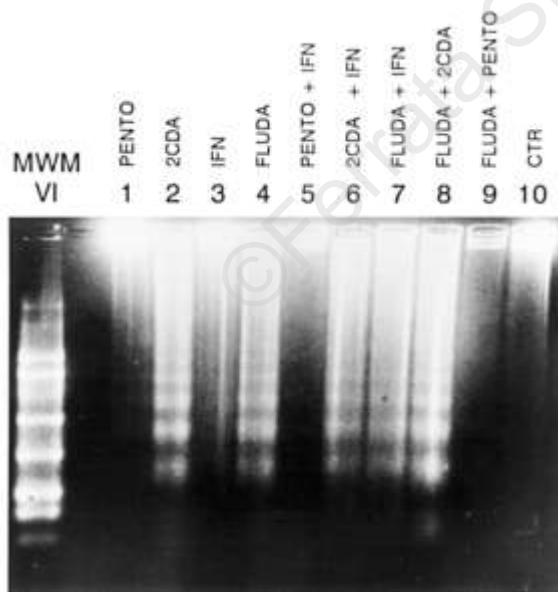


Figure 1. DNA fragmentation of one patient (lanes 1 to 9) and one of normal control cells (lane 10). Cells were incubated with pentostatin (Pento), 2-CdA, α -IFN, FLU, pentostatin + α -IFN, 2-CdA + α -IFN, FLU + α -IFN, FLU + 2-CdA, FLU + pentostatin as described. MWM: DNA molecular-weight marker VI, Boehringer Mannheim, Germany.