Haematology and Blood Transfusion Vol. 31
Modern Trends in Human Leukemia VII
Edited by Neth, Gallo, Greaves, and Kabisch

Sensitivity of Stromal Elements from Human Bone Marrow Cells to Cytosine Arabinoside In Vitro

E. Elstner, H. Goldschmidt, M. Wächter, and R. Ihle

A. Introduction

Cytosine arabinoside (Ara-C) is one of the most effective agents in the treatment of acute myelogenous leukemia (AML). Recently, low-dose Ara-C therapy has been the subject of topical interest in view of its differentiation-inducing effect on leukemic blast cells. Many patients, however, develop severe aplasia with low-dose Ara-C therapy [1, 6, 10, 11]. The aim of our study was to obtain some information about causes of this aplasia, i.e., to determine whether the lesion is primarily in the hemopoietic stem cells or whether there is any additional damage done to stromal elements.

Bone marrow cells (BMC) were exposed to concentrations of Ara-C in cultures, reflecting the plasma Ara-C concentrations in low-dose-treated patients (10^{-6}–10^{-8} *M*) [9].

University Hospital (Charité), Department of Internal Medicine, Division of Hematology, Berlin, GDR

B. Material and Methods

Human BMC were obtained from normal volunteers. Iliac bone marrow aspirates were used, diluted with the same volume of McCoy's 5A medium containing preservative-free heparin. Cultures of granulocyte-macrophage colony-forming cells (GM-CFC) were performed with mononuclear BMC from eight normal volunteers in double-layer agar cultures [7]. To study hemopoietic stroma (stroma cell layer), we used the Dexter culture [2] modified for human BMC [3, 4]. Briefly, after spontaneous sedimentation of erythrocytes without washing, the diluted BMC were added to the culture medium (McCoy's 5A supplemented with 10% fetal calf serum SIFIN, GDR, and 10% horse serum, Flow) in order to obtain a cell concentration of 5×10^5 cells per milliliter suspension. The cells were cultivated in Petri dishes at 37 °C in 7.5% CO_2 for 14 days without feeding.

All dilutions of Ara-C were freshly made from a stock solution of Alexan (Mack) and were added to the agar or Dexter cultures on

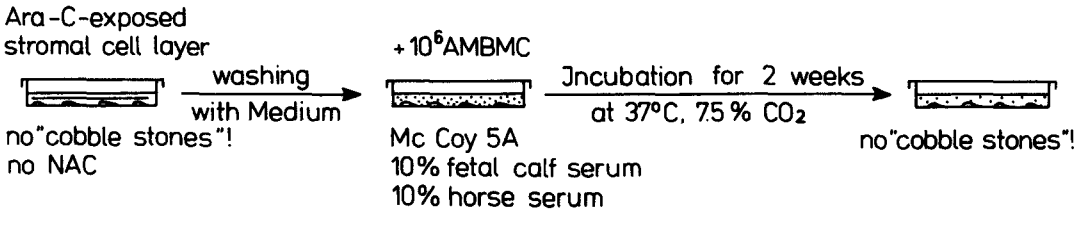

Fig. 1. The influence of Ara-C exposure on the ability of stromal elements to induce active hemopoiesis. Schematic presentation of a cultivation from autologous mononuclear bone marrow cells (AMBMC) over a marrow stromal layer formed during exposure to Ara-C at concentrations of 10^{-6} *M*, 10^{-5} *M*

day 0. The influence of Ara-C on the ability of stromal elements to induce active hemopoiesis was studied in suspension cultures. Fresh autologous mononuclear BMC from five normal volunteers were given over a stromal layer formed during exposure to Ara-C at concentrations of 10^{-6}–10^{-5} *M* (Fig. 1).

C. Results

Figure 2 shows that GM-CFC were damaged at a concentration of 10^{-8} *M* Ara-C and completely disappeared at a concentration of 10^{-5} *M* Ara-C. In the modified 2-week-old Dexter culture, we found a decreased ability of Ara-C-exposed BMC to

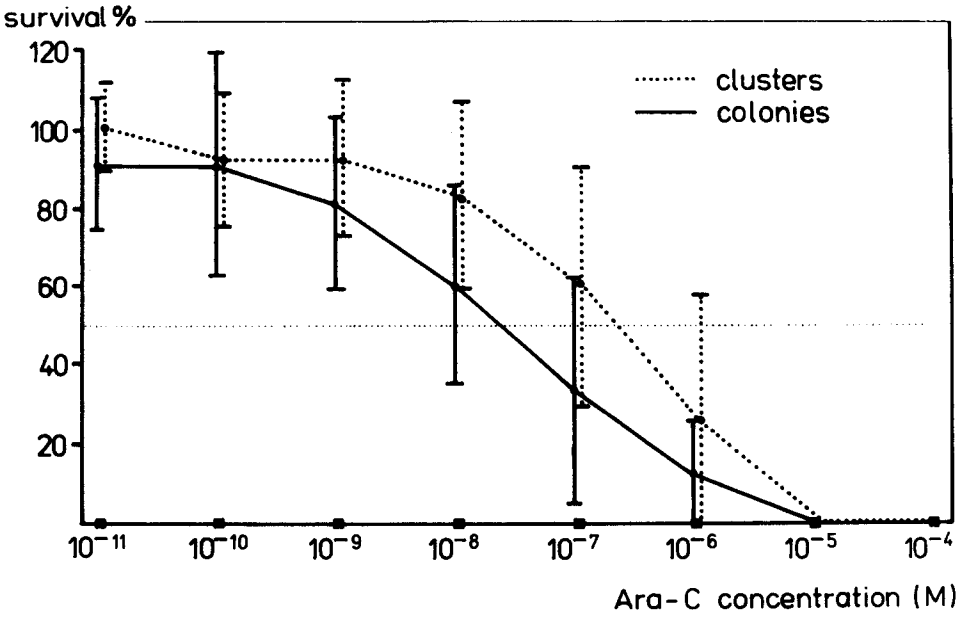

Fig. 2. Sensitivity of GM-CFC from human bone marrow to long-term exposure (7 days) at different concentrations of Ara-C

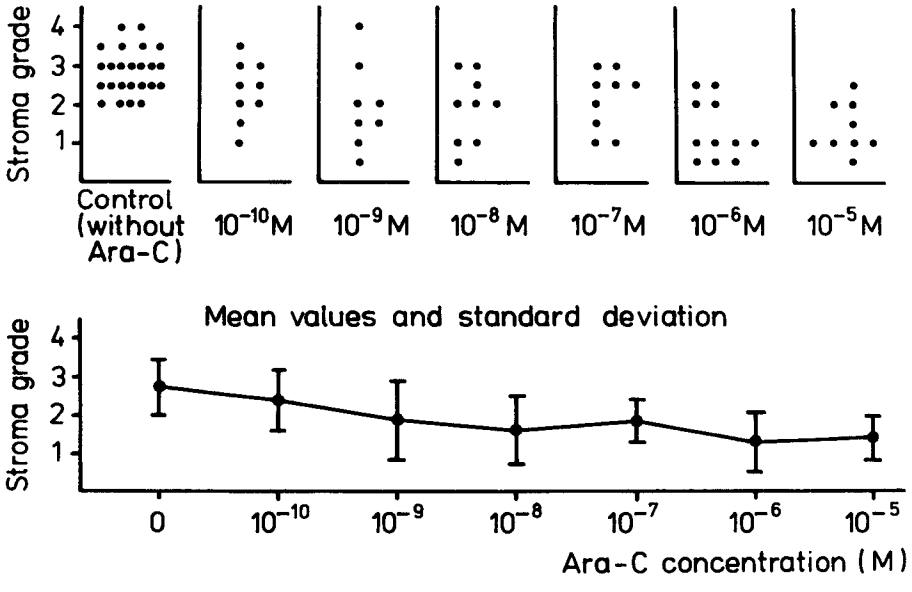

Fig. 3. Sensitivity of stromal layer (Stroma grade) from human bone marrow cells to long-term exposure (14 days) at different concentrations of Ara-C. SG: stromal grade. Each Petri dish was assigned a score from 1 to 4, corresponding to a stromal layer covering from 25% to 100% of the area of the culture dish

Table 1. Effects of different Ara-C concentrations on growth characteristics of bone marrow cells in the modified 2-week-old Dexter culture

Ara-C concentration (*M*)	NAC	F	CS
0	24/24[a]	24/24	24/24
10^{-10}	8/9	9/9	9/9
10^{-9}	4/8	8/8	6/8
10^{-8}	3/9	9/9	2/9
10^{-6}	1/11	11/11	0/11
10^{-5}	0/9	9/9	0/9

NAC, nonadherent cells (trypan-blue – negative); F, fibroblasts; CS, cobblestones.
[a] Number of growth characteristics present/number of investigations.

establish an adherent stromal layer (Fig. 3). The formation of active hemopoietic areas ("cobblestones") was rapidly affected at a concentration of 10^{-8} *M*, and no cobblestones were detectable at concentrations from 10^{-6} *M* Ara-C and upward. Fibroblasts, however, could still be observed at a concentration of 10^{-5} *M* Ara-C (Table 1). After 2 weeks of 10^{-5}–10^{-6} *M* Ara-C treatment, adherent stromal cells were unable to support cobblestone formation from fresh autologous mononuclear BMC added after removal of the drug (Fig. 1).

D. Discussion

During the last 5 years, low-dose Ara-C therapy of patients with AML and preleukemic syndromes has been assessed optimistically and with increasing interest among clinicians, because Ara-C in low dosage might exert a differentiation-inducing effect on leukemic blast cells, in addition to its cytotoxic action. Many patients, however, develop severe aplasia with this therapy. Although the dose of Ara-C in low-dose therapy is about ten times lower than conventional intravenous dosages, it is, however, administered for a much longer period. Furthermore, the effect of Ara-C on GM-CFC in vitro is more dependent on time than on dosage [8]. We showed that long-term exposure (7 days) to Ara-C concentrations of 10^{-8}–10^{-6} *M* produces toxic effects on GM-CFC. The latter completely disappear at an Ara-C concentration of 10^{-5} *M*.

There is some evidence that normal hemopoiesis is dependent on intact stromal cells, and vice versa. Thus, busulfan-related aplasia is connected with damage to the stroma cells [5]. It has also been demonstrated that the ability of BMC from patients with aplastic anemia to form a stromal layer in Dexter culture is defective [3].

The results presented here indicate that Ara-C concentrations reflecting the plasma Ara-C concentrations in low-dose-treated patients produce toxic effects on the ability of BMC to form a stromal layer. Moreover, the marrow stromal cell layer formed by BMC continuously exposed to Ara-C at concentrations of 10^{-5} or 10^{-6} *M* lost the ability to support normal hemopoiesis. Whether our results can be extrapolated to the in vivo situation has still to be proved, because the stromal progenitors with usually low proliferation in vivo proliferate during Ara-C exposure in our system.

References

1. Degos L, Castaigne S, Tilly H, Sigaux F, Daniel MT (1985) Treatment of leukemia with low-dose Ara-C. A study of 160 cases. Semin Oncol [Suppl 3] 12(2):196–199
2. Dexter TM, Allen TD, Lajtha LG (1977) Conditions controlling the proliferation of haemopoietic stem cells in vitro. J Cell Physiol 91:335–344
3. Elstner E, Schulze E, Ihle R, Stobbe H, Grunze S (1985) Stromal progenitor cells in bone marrow of patients with aplastic anemia. In: Neth R, Gallo R, Greaves MF,

Janka G (eds) Haematology and blood transfusion. Springer, Berlin Heidelberg New York Tokyo, pp 168–171 (Modern trends in human leukemia 6)
4. Gartner S, Kaplan HS (1980) Long-term culture of human bone marrow cells. Proc Natl Acad Sci USA 77:4756–4759
5. Hays E, Hale L, Villarreal B, Fitchen JM (1982) "Stromal" and hemopoietic stem cell abnormalities in long-term cultures of marrow from busulfan-treated mice. Exp Hematol 10:383–392
6. Jehn U, Bock R de, Haanen C (1984) Clinical trial of low-dose Ara-C in the treatment of acute leukemia and myelodysplasia. Blut 48:255–261
7. Pike BL, Robinson WA (1970) Human bone marrow colony growth in agar-gel. J Cell Physiol 76:77–84
8. Raijmakers R, Witte T de, Linssen P, Wessels J, Haanen C (1986) The relation of exposure time and drug concentration in their effect on cloning efficiency after incubation of human bone marrow with cytosine arabinoside. Br J Haematol 62:447–453
9. Spriggs D, Griffin J, Wisch J, Kufe D (1985) Clinical pharmacology of low-dose cytosine arabinoside. Blood 65:1087–1089
10. Tilly H, Castaigne S, Bordessoule D, Sigaux F, Faniel M, Monconduit M, Degos L (1985) Low-dose cytosine arabinoside treatment for acute nonlymphocytic leukemia in elderly patients. Cancer 55:1633–1636
11. Tricot G, Bock R de, Dekker AW, Boogaerts MA, Pettermans M, Punt K, Verwilghen RL (1984) Low-dose cytosine arabinoside (Ara-C) in myelodysplastic syndromes. Br J Haematol 58:231–240