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The Relevance of Cell Kinetics for Optimal Scheduling of 1-β-D-Arabinofuranosyl Cytosine and Methotrexate in a Slow Growing Acute Myeloid Leukemia (BNML)

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Summary. *1-β-D-Arabinosyl cytosine and methotrexate were studied for their antitumor activity in acute myeloid leukemia of the BN rat (BNML), which is characterized by a slow growth rate due to the presence of a high proportion of nonproliferating cells.*

It was found that the two drugs showed the maximal cytotoxic action when given separately. The effect was highly dependent on the interval between the administration of each drug.

The variation of the cell kinetic parameters produced by the recruitment into cycle of the resting population, as determined by labeling indices, correlates well with the antileukemic action of the drug combination.

Introduction

Most antileukemic agents show a strong schedule dependence activity (Venditti, 1971). Several concepts have been developed to explain this schedule dependence (Skipper, 1974). In particular, cell kinetics have been considered of major importance, because most antileukemic drugs are believed to act only against cycling cells (Bruce et al., 1966; Hill and Baserga, 1975; Perry, 1976). Therefore, the antileukemic activity is presumably related to the proliferative status of the neoplastic population.

One of the major features of the growth pattern of human acute leukemias is the presence of a high proportion of nonproliferating cells, which can eventually reenter into cycle to perpetuate the disease (Gavosto and Pileri, 1971; Killman, 1972). When these resting cells are recruited into the cycle, they should theoretically enter a phase sensitive to the specific action of one drug and it should be possible, on this basis, to reduce the tumor load more effectively. However, because of the lack of adequate controls, this hypothesis is difficult to prove in the human situation, although some clinical attempts based on this concept have produced encouraging results (Vogler et al., 1976; Lampkin et al., 1976). Recently Burke et al. (1977) demonstrated pronounced antitumor effects from timed sequential chemotherapy in patients with acute myelocytic leukemia. This therapy resulted in long durations of complete remission without maintenance. However, published data on a possible relation between initial kinetics and the success of a rational leukemic cell-reducing treatment schedule are conflicting (Hart et al., 1977; Vogler, 1975). The relation between labeling index and survival is especially controversial, although most workers agree about the prognostic relevance of a high LI for the period in which complete remission can be achieved.

Adequate experiments can be performed in animal models, provided that their growth characteristics are similar to those of the human. Most experimental leukemias such as the L1210, although useful for studying the basic action of drugs, exhibit a growth pattern which is very different from that seen in the human situation (Skipper and Perry, 1970; Gavosto and Pagliardi, 1975). The L1210 leukemia is in fact characterized by an exponential growth pattern with a doubling time that approximates the cell cycle time; this means that nearly all of the leukemic cells are in cycle. Therefore, the model is not suitable for studies designed to determine whether recruitment of a nonproliferating population can play an important role.

The acute myeloid (promyelocytic) leukemia of the BN rat (BNML) has been proposed as a more reasonable model for comparative evaluation because of the close similarity with human AML (van Bekkum and Hagenbeek, 1977; van Bekkum et al., 1976; Hagenbeek, 1977). The BNML is characterized by:

Abbreviations used: Ara-C = 1-β-D-arabinosyl cytosine; MTX = methotrexate; ^{3}H-TDR = tritiated thymidine; LI = labeling index

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Early suppression of normal hemopoiesis with migration of normal hemopoietic stem cells to extramedullary sites in the second half of the disease. The number of CFU-s in peripheral blood increases 100fold from day 8 to day 18; a slow net growth rate with a growth fraction (GF) of 50% which can be calculated from: $Ts/Ns = Tc/Nc$ where *Ts* is the duration of the S phase (10 h), *Ns* is the number of cells in S, *Tc* is the cell cycle time (14 h) and *Nc* is the total number of cells in cycle (= GF) (Steel, 1968); increase in spleen weight to eight times the normal weight, and in liver weight by a factor of 2 at death.

Its slow growth rate, due to the presence of a large proportion of nonproliferating cells, makes the model particularly suitable for studying whether the resting cell population can be recruited into cycle and how kinetic variations can predict the effect of antileukemic drugs.

In the present study, the interaction between Ara-C and MTX, two major antileukemic agents, was investigated. Both of these drugs are believed to exert their cytotoxic action during the S phase, although each achieves its effect through a different mechanism (Hill and Baserga, 1975; Karon and Shirakawa, 1970; Goncharova and Frankfurt, 1976; Hoovis and Chu, 1973). The antileukemic effect of varying the sequential administration of the two drugs was of particular interest.

Materials and Methods

Animals

Male rats of the Brown Norway inbred strain, aged 12–16 weeks (body weight 250–300 g) were used. They were kept four per cage and allowed food and water ad libitum.

Leukemia

Spleen cells of leukemic animals were suspended in Hank's balanced salt solution and 10^7 leukemic cells were transplanted intravenously in a volume of 1 ml. After transplantation, the survival of the animals ranged from 22–28 days.

The experiments started on day 15 after transplantation, when liver and spleen are enlarged, the bone marrow contains more than 90% of leukemic cells, and leukemic cells start to appear in the peripheral blood. The disease is at a stage comparable with that at the time of clinical diagnosis in man.

Labeling Studies

For labeling index studies, ^{3}H-TdR (Radiochemical Centre, Amersham, England), specific activity 24 Ci/mM, was injected i.v. at a dosage of 2 μCi/g. 1 h after injection, the animals were killed by cervical dislocation and smears were prepared from the suspension of bone marrow cells in Hank's solution + calf serum.

Autoradiographs were prepared by the dipping film technique with Ilford K_2 autoradiographic emulsion. After 8 weeks of exposure, the slides were developed and the labeling index was determined by counting at least 1,000 cells per sample. The major characteristics of the growth of the BNML are summarized in Table 1.

Table 1. Kinetics of the BNML at day 15 after transplantation of 10^7 leukemic cells

Labeling index (% ± 2 SD)	Growth fraction (%)	
25.6 ± 3.7	36	Bone marrow
34.6 ± 3.2	48	Spleen
33.6 ± 4.8	46	Liver
21.3 ± 3.4	–	Peripheral blood

Details to be published elsewhere

Tumor Load

Because of their diffuse infiltration by leukemic cells, the spleen, liver, and bone marrow are good indicators of the tumor load. Therefore, the cytotoxic effect of drugs can be measured as a function of the weight of the organs infiltrated by the leukemic process. To test the efficacy of the different schedules, the animals were killed 48 h after the end of the treatment and the spleen and liver were weighed. The femurs were removed and cut in two pieces, and both ends were washed in Hank's balanced salt solution, containing 20% calf serum in order to increase the viscosity and to decrease the cell break. The BM cellularity was estimated by counting the cells in a Bürke hemocytometer.

Drugs

Ara-C was a gift from the Upjohn Company, Ede, The Netherlands, and MTX was purchased from the Lederle Laboratories, Haarlem, The Netherlands. The drugs were dissolved in 0,9% NaCl and injected i.v. in a volume of 0.8–1 ml to animals in a light ether anesthesia. The dosage was 200 mg/kg Ara-Ca and 2 mg/kg MTX. Controls were injected with 0.9% NaCl.

Results and Discussion

The decrease of the liver and spleen weight and of the total cell count of BM cells was used as a function of the efficacy of the treatment with Ara-C and MTX. As shown in Figure 1, the two drugs given simultaneously do not show a higher antileukemic effect in BM than each drug when given alone. The same effect is also observed in liver (Fig. 2) and spleen (Fig. 3). However, since in the BNML the cytotoxic action of MTX is equivalent to the variations in LI when the resting cells are synchronously recruited into cycle after a single injection of Ara-C [as shown by the variation in the labeling index (Fig. 4)], the cytotoxic action of MTX correlates well with the variation of the number of cells in the S phase. Sequential administration results in an en-

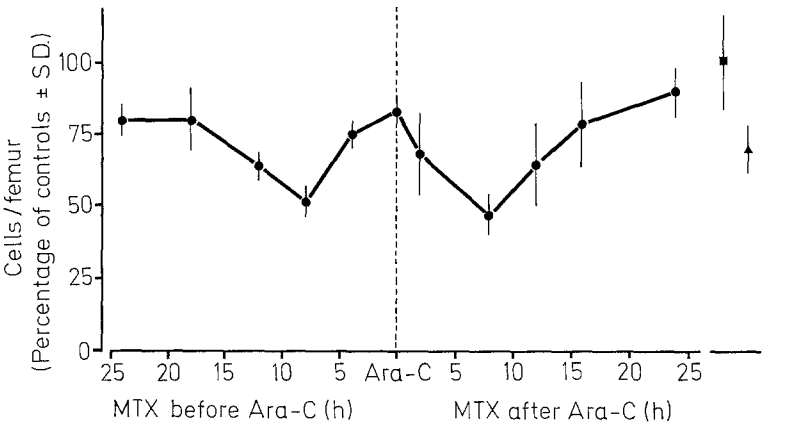

Fig. 1. Variations of bone marrow cellularity following administration of Ara-C and MTX at different intervals. ■: MTX 2.0 mg/kg; ▲: Ara-C 200 mg/kg

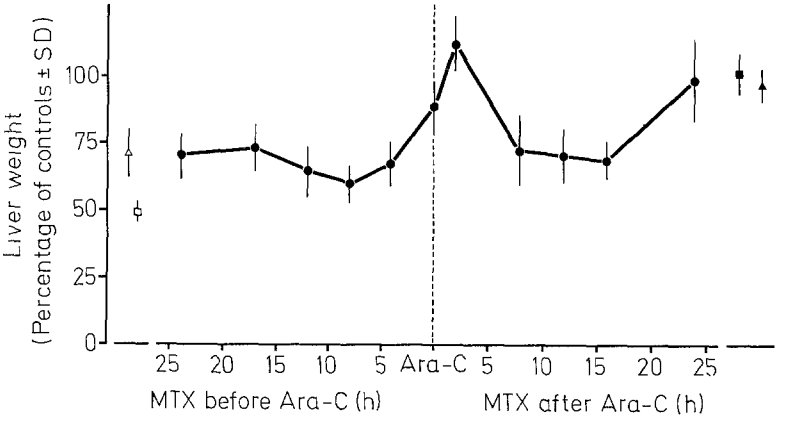

Fig. 2. Variation of liver weight following administration of Ara-C and MTX at different intervals. Extent of leukemic cell infiltration can be derived by comparing liver weight of leukemic untreated animals (100%) with that of normal animals or of leukemic animals after complete remissions (obtained with Ara-C 200 mg/kg × 6; $q = 12$ h). △: liver weight of nonleukemic animals; □ liver weight after complete remission; ■ MTX 2.0 mg/kg; Ara-C 200 mg/kg

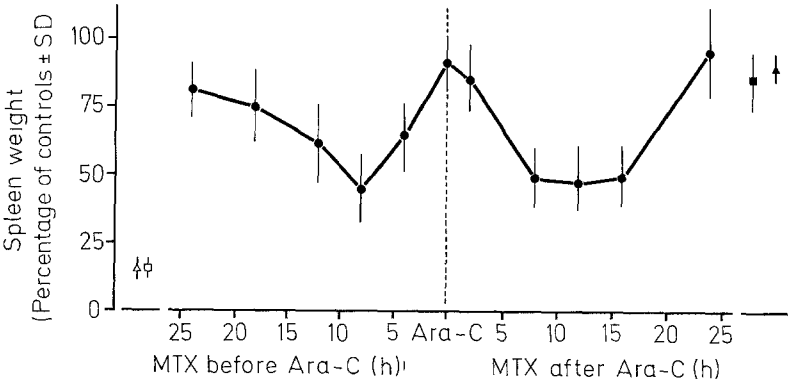

Fig. 3. Variation of spleen weight following administration of Ara-C and MTX at different intervals. Extent of leukemic cell infiltration can be derived by comparing spleen weight of leukemic untreated animals (100%) with that of normal animals or of leukemic animals after complete remission (obtained with Ara-C 200 mg/kg × 6; $q = 12$ h). △: spleen weight of nonleukemic animals; □: spleen weight after complete remission; ■: MTX 2.0 mg/kg; ▲: Ara-C 200 mg/kg

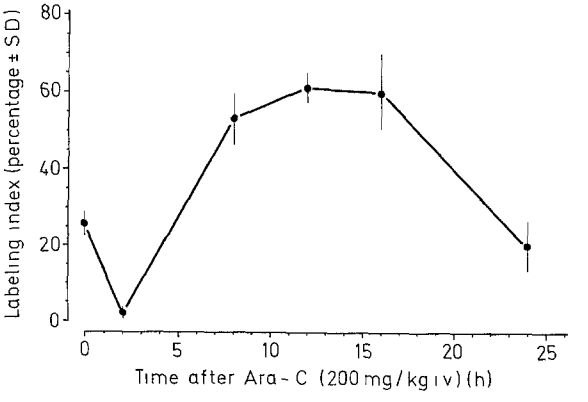

Fig. 4. Variations in LI of leukemic cells after single injection of Ara-C

hanced cytotoxicity in BM, liver, and spleen. Data on the variations in the proliferative status of the leukemic cells after MTX are not available in this model. Here, no significant influence of the sequence can be demonstrated. In both cases, (Ara-C given before or after MTX), the effectiveness is dependent on the interval: the maximal cytotoxic activity is produced by a sequential administration with an interval of 8–12 h. With a longer interval the synergistic effect decreases and virtually disappears within 24 h. The antileukemic effect of the second drug is evident when the cells recruited by the first drug are in the first part of the S phase. This correlates well with the S phase-specific antitumor action of both Ara-C and MTX. However, when cells have already passed the early S phase, the antileukemic action of the second drug decreases. Although no survival studies were performed, it appears from other studies concerning recruitment and synchronization with high doses of Ara-C (which will be published elsewhere) that the toxicity for the normal hemopoietic system is not significantly increased by rational scheduling.

These data are in contrast with those of Edelstein et al. (1975), who found the maximum activity against L1210 LCFU-s when the two drugs were given simultaneously; sequential administration resulted in a decrease in activity, which was proportional to the interval of time between administration. Their results are in contrast with those expected for two agents believed to kill cells in the same phase of the cell cycle. The authors suggested that biochemical interactions resulting in variation in the nucleoside pool after Ara-C and of the nucleotide pool (mainly CTP) after MTX are more important than cell kinetic interactions. They concluded that, if synergistic effects depend on a biochemical basis more than on a cell kinetic basis, the translation from experimental models to the human situation is easier to perform.

When MTX was given together with or before Ara-C, an increased cytotoxic effect, probably due to biochemical interaction, was observed by Hoovis and Chu (1973) on the exponentially growing L5178Y leukemic cells in vitro. However, the synergism was evident for only 60 min after the cells were put into MTX-free medium.

Therefore, biochemical interactions seem to play an important role when tumor cells are growing exponentially as in the L1210 in vivo or in the L5178Y in vitro.

However, as discussed in the introduction, the proliferation pattern of human acute leukemias is quite different from that in these models, because of the high proportion of noncycling cells. Therefore, as in the human situation, one has to investigate a model in which the proliferative status of the leukemic population is modified by the recruitment of resting cells after the start of the treatment.

Because of the similarities in phase-specific activity between Ara-C and MTX, a pattern similar to that observed after Ara-C also seems likely for MTX and a similar kinetic explanation can be used to interpret the higher antileukemic effect of sequential combination of MTX and Ara-C as compared to simultaneous combination.

In the BNML it seems likely that, when a large number of nonproliferating cells is present, the antileukemic action of the combination of Ara-C and MTX is more dependent on cell kinetics than on biochemical variations.

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