ASSESSMENT OF DRUG SENSITIVITY OF HUMAN LEUKAEMIC MYELOBLASTS

II. THE TOXIC EFFECTS OF CYTOSINE ARABINOSIDE ON 125I UdR-LABELLED HUMAN LEUKAEMIC MYELOBLASTS IN MICE

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Summary.—Leukaemia cells from the peripheral blood and bone marrow of patients with acute myeloblastic leukaemia were labelled in vitro with 125I 5-iodo-2′-deoxyuridine (IUdR). The myeloblasts were then injected into groups of mice and the survival of these cells estimated by measuring isotope loss, using whole-body counting. The isotope excretion from mice treated with various doses of cytosine arabinoside (Ara-C) and those not treated with drugs were compared. This comparison showed that the sensitivity of myeloblasts to the drug varies from patient to patient, and in one case was different for myeloblasts from bone marrow and from blood from the same patient. We compare the clinical responses of myeloblasts to Ara-C in 6 patients, who had high peripheral blood myeloblast counts, with the sensitivities of their myeloblasts to Ara-C in mice. This comparison indicates that the assay might be a useful way of predicting the response of leukaemic cells in patients to cytotoxic agents.

Measurement of 125I release from 125I 5-iodo-2′-deoxyuridine (IUdR)-labelled cells has been used to assess cell survival in vivo in a number of animal models (Hughes et al., 1964; Porteous and Munro, 1972). In the previous paper in this series (Falcão et al., 1977) we described a technique for labelling both fresh and cryopreserved human leukaemic myeloblasts with IUdR, and we investigated the fate of the labelled cells after they had been injected into mice. The present study was undertaken to establish, in principle, whether this technique can be used to assess the susceptibility in vivo of human leukaemic myeloblasts to chemotherapy.

Patients

The patients studied had definite acute myeloblastic leukaemia. Brief clinical details are given in the legends to figures. We are extremely grateful to Drs C. Bunch, S. Callender, C. Potter and Professor D. Weatherall for allowing us to study their patients. Five of the patients were treated by low-dose cytosine arabinoside (Ara-C) infusion as part of a pilot study being carried out at Oxford by these workers. The rational basis for this treatment and the reasons for dose selection will be described elsewhere.

Materials and Methods

Myeloblasts.—Myeloblasts were collected from the peripheral blood or bone marrow. Blood was anticoagulated with either acid citrate, dextrose or heparin. Bone-marrow aspirates were flushed into Medium RPMI 1640 containing heat-inactivated 10% foetal bovine serum. Red blood cells and neutrophils were removed from bone-marrow preparations by centrifugation through a Ficoll-Triosil gradient of sp. gr. 1.080. Interface cells were then washed in RPMI before being

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labelled. Myeloblasts from blood were separated from red cells by 1 g sedimentation followed by one wash to remove platelets. Preparations for labelling contained more than 90% myeloblasts by morphological counting of Giemsa-stained films.

Labelling of myeloblasts.—A detailed description of the way suitable conditions for myeloblast labelling was established is given in the preceding paper (Falcão et al., 1977). RPMI 1640 containing 200 u/ml of penicillin, 100 μg/ml of streptomycin, fresh glutamine, 10% pooled human AB serum (heated previously at 56°C for 30 min) and 0.006 μCi/ml of 125IUdR (Amersham, sp. act. 25–35 Ci/mmol) was used for labelling. Myeloblasts were added to flat-bottomed glass culture flasks at 0.7 x 10^5 cells/ml. The culture medium was 1–1.5 cm deep in the flasks during labelling, and the flasks were gassed with 5% CO₂:95% air. Cultures were incubated for 18–20 h at 37°C. The cells were then centrifuged and washed twice in Medium RPMI without serum supplement.

Animals.—CBA/He T6T6 or C3H mice 8–10 weeks old were used in this study. Groups were matched for weight, and animals were of the same strain and sex in each experiment.

Labelling BP8 cells.—BP8 is a C3H-derived fibrosarcoma which is maintained by serial passage as an ascites tumour in C3H mice. BP8 cells were removed from the peritoneal cavity, washed twice in RPMI 1640, counted and suspended at 2 x 10^5 cells/ml in RPMI with 125IUdR at 0.03 μCi/ml. The mixture was incubated for 2 h at 37°C in an atmosphere of 5% CO₂:95% air. The cells were then washed twice to remove free label. The cells were then incubated for a further 30 min at 37°C, rewarshed and suspended at 10^7 cells/ml. Where killed BP8 cells were to be injected, they were freeze–thawed 4 times. Both killed and viable labelled BP8 were injected s.c. at 5 x 10^6/ mouse.

In vivo assay procedure.—Labelled myeloblasts were injected s.c. between the scapulae. Each mouse was given 1–5 x 10^7 labelled myeloblasts, the number of cells depending on the amount of label incorporated by them. Whole-body 125I was estimated each day at about the same time, by counting the mice in a large NaI well counter. Mice received 0.1% KI in their drinking water for 2 days before myeloblast injection, and throughout the duration of each experiment, to block the uptake of released 125I by the thyroid. A suspension of labelled myeloblasts in water was counted at the same time as the mice, to determine isotope decay and compensate for fluctuations in counting efficiency. The activity remaining at any time is expressed as a percent of activity at the time of injection of myeloblasts and is calculated:

\[
\text{percent radioactivity remaining at Day } t = \frac{\text{Counts Day } t \times (\text{Counts standard Day } 0)}{\text{Counts Day 0}} \times 100
\]

All counts had background subtracted.

Results are expressed as the geometric mean ± log₁₀ s.d. of the per cent radioactivity remaining.

![Graph](image.png)

FIG. 1.—The effect of Ara-C on the excretion of 125I from mice injected with IUdR-labelled and killed BP8 fibrosarcoma cells. The cells were injected s.c. and the drug administered i.v. ●, mice injected with Ara-C 24 h before the cells. ▲, mice injected with Ara-C immediately after the injection of cells.

% inhibition of release =

\[
\text{% }^{125}\text{I retained in mouse receiving drug} - \text{% }^{125}\text{I retained in killed cells}
\]

\[
100 \times \frac{\text{% }^{125}\text{I retained in mice receiving live cells}}{\text{drugs}}
\]

Groups of 3 mice were used. The % inhibition of 125I release is calculated from the geometric mean of the % 125I retained by the groups 24 h after the injection of BP8.
RESULTS

The effect of cytosine arabinoside on the clearance of \( ^{125}\text{I} \) by mice injected with killed IUdR-labelled cells

This experiment was carried out using \( ^{125}\text{I} \)UdR-labelled BP8 fibrosarcoma cells which were killed by repeated freezing and thawing before being injected s.c. into mice. The results are shown in Fig. 1. Ara-C was given i.v. at varying doses, either immediately after or 24 h before, the injection of cells.

When Ara-C was given immediately after BP8 cells, there was no slowing in the rate of \( ^{125}\text{I} \) loss from mice. However, 100 or 200 mg Ara-C/m\(^2\), when given 24 h before the cells, caused a slowing in the rate of \( ^{125}\text{I} \) elimination. By 48 h after cell injection, \( ^{125}\text{I} \) retention was not significantly different between treated and untreated mice. Similar results were obtained when this experiment was repeated.

The effect of Ara-C on the excretion of \( ^{125}\text{I} \) by mice injected with IUdR-labelled human leukaemic myeloblasts

The results of these experiments are given in Figs 2, 3 and 4.

In Fig. 2A and B, experiments are shown where there was significant increase in the rate of \( ^{125}\text{I} \) loss from Ara-C-treated mice. The clinical details given in the legends to figures indicate that these patients showed a rapid response to Ara-C infusion. However, the onset of acute marrow necrosis in the patients whose myeloblasts are represented in Fig. 2B complicates evaluation of the drug sensitivity of his myeloblasts.

Fig. 3A and B show myeloblasts which

![Graphs showing excretion of \( ^{125}\text{I} \) after injection of Ara-C in mice](image)

Figs. 2, 3 and 4.—\( ^{125}\text{I} \) excretion from mice injected s.c. with \( ^{125}\text{I} \)UdR-labelled human leukaemic myeloblasts. The effect of an i.v. injection of Ara-C immediately after the injection of cells on \( ^{125}\text{I} \) excretion is assessed. Geometric means and logarithmic s.d. are shown for groups of 3–6 mice.

Fig. 2A.—This 37·5 kg patient’s blood myeloblasts were taken immediately before treatment. At this stage she had \( 56 \times 10^9 \) leukaemic myeloblasts per litre of blood. She was given 20 mg of Ara-C per day by continuous i.v. infusion and by the 12th day the blood myeloblast count had fallen to \( 1 \times 10^9 \)/L. ——, no drug; ———, 200 mg Ara-C/m\(^2\). ——, 400 mg of Ara-C/m\(^2\).

Fig. 2B.—This 72·5 kg patient presented with a blood myeloblast count of \( 63 \times 10^9 \)/L. Bone marrow aspiration at that time only yielded dark brown necrotic tissue at three sites. He was treated with continuous i.v. infusion of Ara-C 20 mg/day for 2 days and 80 mg/day for 5 days, before his blood myeloblast count fell to \( 1 \times 10^9 \)/L. Blood myeloblasts were studied immediately before treatment. ——— no drug. ——— — 50 mg/m\(^2\) Ara-C. ——— — 100 mg/m\(^2\) Ara-C.
have a high spontaneous rate of IUdR release. In each case, about 90% of injected radioactivity is lost within 48 h. This high rate of isotope release is probably due to rapid death of the labelled cells. It is gratifying to see that despite this, a significant cytotoxic effect of Ara-C is demonstrable in mice, against the myeloblasts of the patient depicted in Fig. 3A. Fig. 3B and C indicate that different characteristics may be seen in myeloblasts from a single individual taken from different sites. The marrow myeloblasts from this patient represented in Fig. 3C have a low rate of $^{125}$I loss, and these cells appear sensitive to damage by Ara-C. On the other hand the same patient’s peripheral blood myeloblasts (Fig. 3B) show more rapid spontaneous release of isotope, and this is not increased further by the administration of Ara-C. We have compared marrow and blood myeloblasts in 2 other patients. In these patients the drug sensitivity and spontaneous rate of isotope release did not differ between cells from the two sites. In all 3 patients the number of counts taken up by marrow cells was greater than that by blood cells.

Fig. 4 shows the rate of excretion of $^{125}$I from mice injected with IUdR-labelled myeloblasts from 2 further patients. The spontaneous rate of isotope release is not particularly high from the myeloblasts from either patient, but in neither case did Ara-C increase isotope release. The clinical history of the response to Ara-C in these 2 patients, which is given in the legend to Fig. 3, shows that neither patient was markedly sensitive to this drug.

**DISCUSSION**

Our results, although obtained in a small group of patients, indicate that it is feasible to measure the susceptibility of leukaemic myeloblasts to lysis by chemotherapeutic agents, using an assay in which...
Fig. 4A.—Blood myeloblasts from this 70-kg patient were taken after the patient had received 7 5-day courses of treatment in 13 weeks. Each course consisted of 95 mg of rubidomycin i.v. on Day 1 and 120 mg of Ara-C i.v. on Days 1–5. The blasts were taken 3 days after the last course of treatment, when the myeloblast count was rising. After an initial response to therapy this patient showed progressive increase in resistance to these courses of treatment. The separate lines represent geometric means of mice treated with no drug (---), 50 (– - - - ), 100 ( . . . . ) and 200 ( - - - ) mg/m² Ara-C.

Fig. 4B.—Results with labelled blood myeloblasts taken from a 42-kg patient before treatment by continuous Ara-C infusion. These myeloblasts were cryopreserved over liquid N₂ before being rapidly thawed for labelling. Conditions of cryopreservation as in Falcão et al. (1977). The patient was treated with 32 × 10⁹ myeloblasts/l of blood. She was treated by i.v. infusion of Ara-C at 10 mg/day for 5 days, 20 mg/day for 3 days, 40 mg/day for 9 days, and finally 80 mg/day for 11 days, before the blood myeloblast count fell below 1 × 10⁹/l on Day 27 of the infusion. No treatment (-----) and 200 mg/m² Ara-C ( - - - - ).

cell death is assessed by ¹²⁵I excretion. Such a result is in agreement with earlier studies in which ¹²⁵I elimination was used to study the response of a murine leukaemia to methotrexate (Hofer et al., 1969; Hofer, 1972). Evaluation of our results was facilitated by the fact that all the patients studied had relatively high peripheral myeloblast counts at presentation, and that 5/6 patients described were treated only with Ara-C.

The use of the thymidine analog, IUdR, to label myeloblasts, means that the label is only incorporated into cells actively synthesizing DNA. The growth fraction of myeloblasts is far from 100% (Crowther et al., 1975) and so in this assay the sensitivity is not being assessed for all leukaemic cells from a patient. However, this theoretical objection is to some extent academic if the assay we describe is shown to correlate consistently with the clinical response to the drugs.

Myeloblasts die, as measured by ¹²⁵I excretion, 2–5 days after injection into mice. This interval is sufficient to measure the effect of drugs on myeloblast viability. The relative contribution of host rejection and spontaneous death of myeloblasts to the rate of ¹²⁵I loss is discussed in some detail in our previous publication in this issue (Falcão et al., 1977).

The assay may be of particular value in three contexts: in testing the susceptibility of neoplastic cells to specific agents where multiple drug therapy is contemplated; in the selection of second-line drugs in cases where primary therapy has failed;
and finally in the preliminary investigation of new cytotoxic agents and schedules for anti-leukaemic effect.

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