Short Communication

SENSITIVITY OF NORMAL AND ACUTE MYELOGENOUS LEUKAEMIA MARROW CELLS TO INHIBITION BY CYTOSINE ARABINOSIDE

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CYTOSINE ARABINOSIDE (Ara-C) is a pyrimidine analogue of deoxycytidine (CdR) that produces a marked inhibition of DNA synthesis in replicating cells. Ara-C is converted intracellularly to the triphosphate (Ara-CTP) which is a DNA-polymerase inhibitor that competes with the normal precursor deoxycytidine triphosphate (dCTP).

Ara-C is one of the most effective drugs for the chemotherapy of acute myelogenous leukaemia (AML) but response to treatment varies. The patient's clinical condition and haematological status, together with the [³H]-TdR labelling index (LI), may account for some of this variation (Freireich *et al.*, 1975). Individual differences of some prognostic value are found in the pharmacokinetics of Ara-C (Ho & Frei, 1971) in the rate of its phosphorylation to Ara-CTP (Kessel *et al.*, 1969) and in the rates of deamination of Ara-C and its monophosphate (Ara-CMP) (Steuart & Burke, 1971; Tattersall *et al.*, 1974).

Although intracellular concentrations of Ara-CTP might be predicted from a complete knowledge of the details of Ara-C metabolism, the extent of inhibition of DNA synthesis at any particular Ara-CTP level may also vary between different types of cell (Chou *et al.*, 1977). We have therefore compared the uptake of [³H]-

TdR *in vitro*, as a measure of DNA synthesis, by normal and AML cells in order to detect any differences in their sensitivity to Ara-C.

Patients with AML were studied at diagnosis (new AML, 14) in remission (5) or in relapse (5) and had received no chemotherapy for at least 3 weeks. Marrow aspirates were passed through 26-gauge needles into tubes containing 3 ml medium (Eagle's MEM with Hanks' salts; Flow, U.K.), 1 ml dextran 110 (6% in 0·9% saline) and 200 u preservative-free heparin, and were allowed to settle at room temperature. After 15–30 min the supernatant was centrifuged at 500 revs/min for 5 min; the cells were resuspended in fresh medium and passed through a stainless-steel mesh (36μm apertures). Normal marrow was obtained from ribs taken from patients with non-invasive carcinoma of the bronchus or with cardiac conditions.

Between 2×10^5 and 1×10^6 cells were used in duplicate or triplicate 1·1ml incubations at 37°C containing 5μCi methyl-[³H]-TdR (sp.act. 50–55 Ci/mmol; Radiochemical Centre, Amersham, U.K.) and differing concentrations of Ara-C (Upjohn, Kalamazoo, Michigan). [³H]-TdR incorporation was stopped after 30 min by adding 0·1 ml of 0·15M TdR. After one wash in medium, the cells were lysed with

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deionized water and precipitated by 5%
trichloracetic acid (TCA) at 4°C. The pre-
cipitate was collected on Millipore filters
(0·45 µm), washed with 5% TCA followed
by propan-2-ol. Filters were dissolved in
0·3 ml acetone and the released pre-
cipitate was suspended in 11·5 ml Aquasol
(New England Nuclear, Dreiechenhain,
W. Germany) and gelled with 3·5 ml
water. Samples were counted for 100 min
or to 40,000 counts. Channel-ratio quench
correction was used, and efficiencies
ranged from 14 to 22%.

Cells from parallel incubations without
Ara-C were washed and fixed with 3 parts
methanol to 1 part glacial acetic acid.
Slides were made by an air-drying tech-
nique and stained with aceto-orcein.
Autoradiographs were prepared using
AR10 stripping film (Kodak) and exposed
at 4°C for 5 days. After processing, the LI
was determined as the percentage of cells
labelled (> 5 grains) from samples of 1000
cells.

[³H]-TdR uptake at each point on the
dose-response curves were compared be-
tween patient groups, using Student's t
test. Further statistical comparisons were
facilitated by fitting cubic curves to each
set of data, using a least-squares method.
Variances were stabilized by conversion
of uptake data to log % of controls, and
goodness of fit was determined by analysis
of variance. Poor fit was generally due to
distortion by terminal values on the
plateau portions of the curve. Removal of
one or two sets of values without encroach-
ing on the sloped portions of the curves
allowed good fit to be obtained. Cubic
curves were considered particularly suit-
able for the present data, as some dose-
response curves showed reversal at the
highest and lowest Ara-C concentrations.

No significant differences were found
between the percentage [³H]-TdR uptake
of normal and remission marrows at each
Ara-C concentration (Fig. 1). A difference
in variance at 1 µM (0·05 > P > 0·02) was
considered acceptable when pooling nor-
mal and remission data.

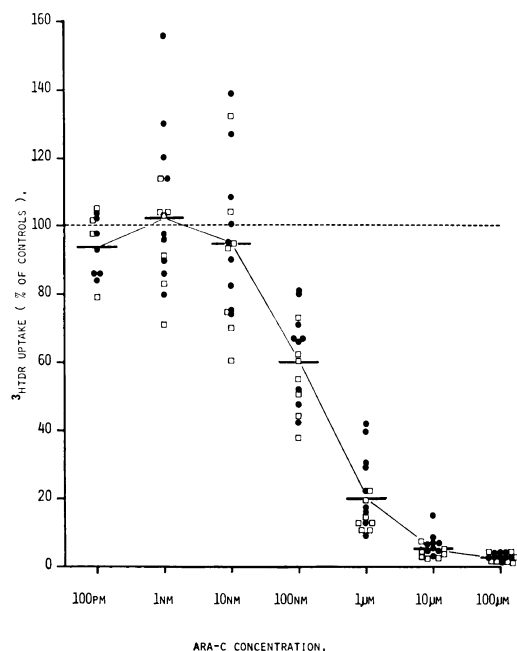Dose–response curves for 14 patients

FIG. 1.—Inhibition by Ara-C of [³H]TdR uptake into marrow cells from normal ribs (●) and patients with AML in remission (□).
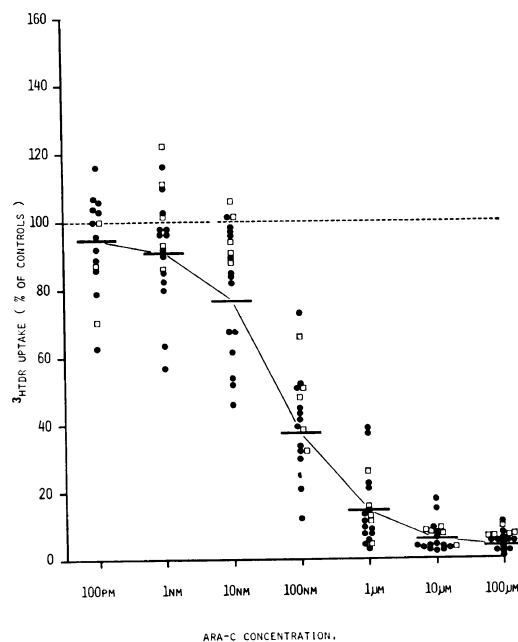

FIG. 2.—Inhibition by Ara-C of [³H]TdR uptake into marrow cells of patients with AML at presentation (●) and at relapse (□).

with AML at presentation (new AML) and 5 in relapse are shown in Fig. 2. [³H]-TdR uptake in relapse marrow was less inhibited than in new AML marrow at 10 nM and 100 μM Ara-C (both $0·05 > P > 0·02$). Relapse marrows were less inhibited than normal or remission marrows by 100 μM Ara-C ($P < 0·001$).

New AML marrows were significantly more inhibited than pooled normal and remission marrows at 10 nM ($0·05 > P > 0·02$) and 100 nM ($P < 0·001$) whilst at 100 μM the variance of new AML was greater ($P < 0·002$). A similar pattern was found when rib and remission data were tested separately. Thus the preliminary analysis shows that there is considerable variation of [³H]-TdR uptake at 100 μM Ara-C, whilst at 10–100 nM the new AMLs are on average more inhibited than normal marrows.

Figs 1 and 2 show that in some marrows, especially in the normal and relapse groups, [³H]-TdR uptake exceeds control levels at low Ara-C concentrations. This might be expected in some samples, since the final percentage is affected by variation in both experimental and control levels of [³H]-TdR uptake. However, the number of measurements at 100 pM or 1 nM that were significantly different from control levels were no more than would be expected from the number of t tests. To minimize this variability, cubic curves were fitted, the maxima were calculated and the data normalized to these values. Data were omitted from one new AML patients in whom [³H]-TdR uptake was not measured below 10 nM or at 100 μM. The best fit to one set of rib data had significant departure from regression ($0·05 > P > 0·02$) but was not excluded since one such departure could be expected in the fitting of 36 curves.

Calculated inhibition values for Ara-C concentrations between 10 nM and 100 μM were compared between patient groups. Rib and remission data were now indistinguishable, as were new AML and relapse marrow data. Comparison of the 16 pooled normal and pooled AML groups showed a greater inhibition of AML at 10 nM and 100 nM (both $0·01 > P > 0·005$) whilst at 100 μM normal marrow was more inhibited ($0·05 > P > 0·02$). Variances at 10 nM and 100 μM were significantly greater in AML than in normal marrow data ($0·02 > P > 0·002$).

Normal and remission marrows had a similar LI (pooled mean 11·2%, s.d. 2·9%). The LI of pooled AML marrow (mean 8·2%) was not significantly different from normal marrow, although the variation was greater (s.d. 7·2%, range 1·0–32·6%). The LI in each group of marrows was not significantly correlated with the inhibition of [³H]-TdR uptake at any part of the Ara-C dose–response curves.

Inhibition of [³H]-TdR (and ³H-deoxy-uridine) uptake by normal and leukaemic cells incubated for 4 h with Ara-C has been previously described (Wilmanns, 1971) but full dose–response curves were not obtained in the few patients studied. In another study leukaemic cells were pre-incubated with 4 μM Ara-C before addition of [¹⁴C]-TdR or ³H-uridine, and inhibition was positively correlated with prognosis for remission (Zittoun et al., 1975).

In the present study, AML cells were on average more sensitive to Ara-C at low concentrations and exhibited greater variation than normal marrow, although there was some overlap in sensitivity. The rates of cellular deamination and phosphorylation of Ara-C and its derivatives differ between patients with AML (Chabner et al., 1974; Coleman et al., 1975). They also vary with the stage of treatment (Chou et al., 1977) and have some correlation with subsequent attainment of remission (Steuart & Burke, 1971; Tattersall et al., 1974). Variation of Ara-CTP levels in AML cells might account for the differences we have observed in inhibition of [³H]-TdR uptake.

AML cells were less sensitive than normal marrow to 100 μM Ara-C, in contrast to the previously reported studies, but this difference is unlikely to have clinical value as the levels of inhibition are similar (95·48% in relapses vs 96·15% in

new AML and 96·72% in pooled controls). Furthermore, 100 μM Ara-C is seldom reached in the plasma of patients given a standard 2mg/kg i.v. bolus dose, even at peak values (Ho & Frei, 1971).

AML cells differ widely in their cell-kinetic parameters (Skipper & Perry, 1970), and this might be related to the sensitivity to Ara-C. However, there was no correlation between the LI or the absolute uptake of [^{3}H]-TdR in controls and the sensitivity of any group to Ara-C.

Our experiments were performed with cells incubated for 30 min with fixed concentrations of Ara-C, which is analogous to the administration of short infusions *in vivo*. The patients were treated with intermittent boluses of Ara-C together with daunorubicin, so it is not surprising that cell sensitivity to Ara-C was not correlated with subsequent remission. It is possible that the observed *in vitro* differential effect of 10–100 nM Ara-C might be evident in patients given Ara-C in the form of continuous low-dose infusions. Our preliminary experience with such infusions, at doses based on the individual's clearance of Ara-C and the *in vitro* sensitivity of their cells, has been encouraging and clinical studies are in progress.

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