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Note

Determination of cytosine arabinoside in human plasma by high-pressure liquid chromatography

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Cytosine arabinoside (1-β-D-arabinofuranosyl-cytosine; ara-C) is a pyrimidine analogue with significant anti-tumour activity and has been reported to be therapeutically effective in the treatment of acute myeloblastic leukaemia and other haematological malignancies [1—4].

The anti-metabolite properties of the drug apparently result from selective inhibition of DNA polymerase and incorporation into DNA (or RNA) following the formation of arabinosyl-CTP [5]. However, ara-C is not effective in all treated patients and Baguley and Falkenhaug [6] found that there was a significant correlation between failure of treatment and rapid clearance of the drug from the plasma. If an effective blood concentration is the sole determinant of efficacy, kinetic data could be used to establish the most appropriate dosage schedules. Nevertheless, the availability of active arabinosyl-CTP at tumour sites in appropriate concentration will ultimately determine efficacy and the relationship of this to ara-C blood concentrations needs to be explored.

Previous analytical methods for ara-C in blood have involved radioactive drug administration and subsequent measurement of radioactivity [6, 7], biological assay involving tissue culture [6, 8] or paper chromatography [7, 9]. The methods are complex and most are relatively insensitive. Wan et al. [7] reported that high-pressure liquid chromatography (HPLC) may prove useful in measurement of ara-C but provided little detail of methodology and other characteristics of their assay. The use of a rapid, simple and sensitive HPLC procedure for ara-C is now described in which plasma protein is removed by precipitation and a buffered aliquot of supernatant is injected directly into the chromatographic system.

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METHODS AND MATERIALS

Blood specimens (10 ml) were collected in heparinized tubes containing 100 μg tetrahydrouridine (THU), which prevents deamination of ara-C [5]. These were immediately stored at 4°. Plasma samples (2 ml) were pipetted into tubes containing 100 μl 4 *M* trichloroacetic acid. After mixing for 20 sec on a vortex mixer, these were centrifuged for 20 min at 0° to separate the precipitated protein. A 1-ml volume of supernatant was immediately transferred to another tube and 20 μl 5 *M* ammonium formate buffer (pH 7.0) was added to achieve a final pH of approx. 3.5. Aliquots of 100 μl were then injected into the chromatograph.

Analyses were performed on a cation-exchange column (Whatman Partisil PXS 10/25 SCX; 250 × 4.6 mm) using 0.01 *M* ammonium formate (pH 4.8) at a constant flow-rate of 1.0 ml/min as the mobile phase. A single-piston high-pressure pump (Altex model 110), a sample injection valve containing a 100-μl loop (Chromatronix) and a fixed-wavelength ultraviolet detector with a 20-μl flow cell (Altex model 150) formed the basis of the chromatograph.

Absorbance of the effluent from the column at 254 nm was monitored at a sensitivity of 0.005 a.u.f.s. Peak heights were used for quantitation of the assay.

To prevent contamination of the ion-exchange column by plasma constituents remaining after the protein removal step, a pre-column (50 × 4.6 mm) containing reversed-phase packing (Partisil ODS, 10 μm; Reeve Angel) was incorporated into the system. Ara-C was not appreciably retained by the pre-column. Significant increases in perfusion pressure necessitating a change of pre-column (at 3000 p.s.i.) did not occur until about 100 samples had been passed through the system.

All chemicals used were analytical grade and water was doubly distilled. Ara-C and THU were kindly donated by Upjohn (Kalamazoo, Mich., U.S.A.).

Calibration curves were derived from pooled blood bank plasma.

RESULTS AND DISCUSSION

Typical chromatograms of plasma samples (Fig. 1) show that control samples are free from contaminating peaks. Ara-C was eluted in 11 min. The major metabolite of ara-C, 1-β-D-arabinofuranosyl uracil (ara-U) was eluted with the large initial mass of endogenous material. All calibration curves for ara-C passed through the origin and were linear from the detection limit of 20 ng/ml plasma to the maximum concentration used (1 μg/ml). Concentration of ara-C in the plasma as a result of the precipitation of plasma protein led to a high recovery of the drug throughout the concentration range listed above (mean recovery 109.5%; S.E.M. 0.7%; n=10) in comparison to standard aqueous solutions of ara-C. The coefficient of variation for the analysis was determined to be 1.7% (n=10) at a concentration of 200 ng/ml.

Blood samples from six hospitalised leukaemic patients receiving constant intravenous infusions of ara-C over 7 days were taken twice between days 2 and 7 to determine the plasma levels likely to be obtained in a steady-state situation (Table I). There was no apparent correlation between the dose of ara-C admin-

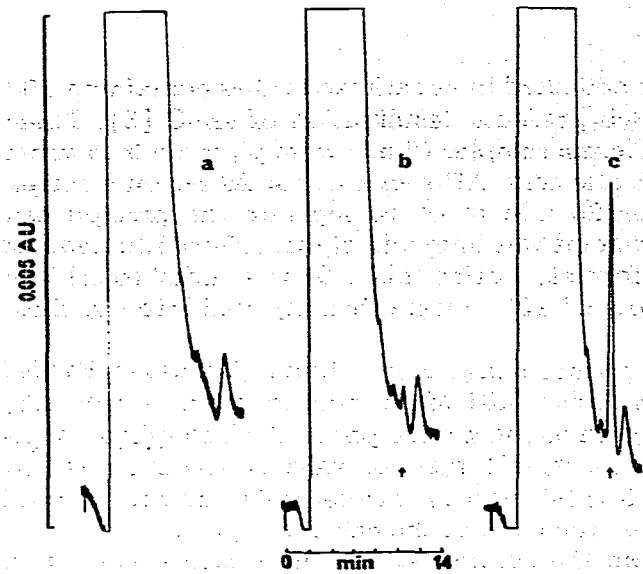

Fig. 1. Typical chromatograms for ara-C in deproteinized plasma. The ordinate represents absorbance units (AU). The arrows indicate the peaks eluting as ara-C. a, blank; b, 50 ng/ml ara-C; c, 500 ng/ml ara-C.

TABLE I

STEADY-STATE PLASMA CONCENTRATIONS OF ARA-C IN SIX PATIENTS WITH ACUTE MYELOID LEUKEMIA

Patients received constant intravenous infusion of ara-C for 7 days. Blood samples from each patient were taken on separate days. Each level represents the mean of duplicate estimations.

Patient	Surface area (m^2)	Dose (mg/m^2/day)	Ara-C plasma concentrations in steady state (ng/ml)
E.C.	1.9	70	241, 223
J.T.	1.6	70	168, 170
R.B.	1.6	70	88, 79
L.P.	1.4	70	76, 80
R.T.	1.6	130	105, 114
B.J.	1.9	150	153, 135

istered daily and the plasma concentration attained in these patients. This is probably a reflection of pharmacokinetic variability and emphasizes the difficulties in establishing appropriate dosage schedules. Routine measurement of ara-C in plasma may prove useful in this regard.

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