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Short Communication

Plasma levels of 1,4-dihydroxy-5,8-*bis*({2--[(2-hydroxylethyl)amino]ethyl} amino)-9,10-anthracenedione dihydrochloride (DHAD) in humans

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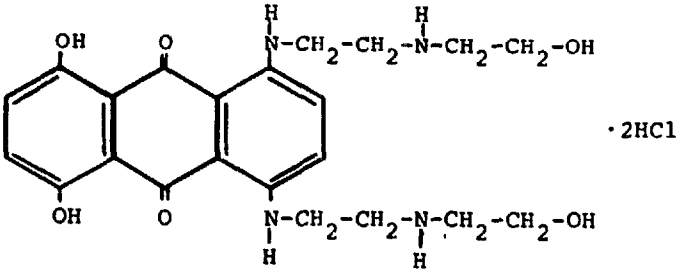
DHAD(I) is a new analog of the anthracycline antibiotic, adriamycin, which has been shown to have significant antineoplastic activity in several animal tumor



systems (Johnson et al., 1979; Wallace et al., 1979; Zee-Cheng et al., 1978). Initial studies have indicated that DHAD is less cardiotoxic than adriamycin (Cheng et al., 1979) and Phase I clinical studies have shown leukopenia and thrombocytopenia to be the dose-limiting toxicities (Von Hoff et al., 1980). Phase II clinical studies are currently in progress, and to date, no reports have appeared in the literature describing the DHAD blood level–time relationship in human subjects. Therefore, the present study was initiated to determine plasma drug levels following i.v. administration of DHAD to human subjects with various malignancies. Plasma levels were determined by HPLC using a recently published method (Reynolds et al.,

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1981) that allows DHAD quantitation in the presence of its chemical degradation products.

Four human subjects received DHAD as treatment for sarcoma, liposarcoma, small cell carcinoma of the lung or renal cell carcinoma. None of the patients had previously received DHAD, but 3 of the 4 had received prior chemotherapy. However, any such treatment had occurred at least 3 weeks prior to DHAD treatment. All patients had normal renal and hepatic functions as determined by levels of serum creatinine (< 2 mg%) and bilirubin (< 2 mg%).

DHAD was supplied by The National Cancer Institute. Each patient was administered DHAD at a dose of 12 mg/m^2 in 100 ml of 5% dextrose in water by i.v. infusion over 30 min. A blood sample was collected prior to infusion, and 10 samples were collected over a period of 150 min starting at the end of infusion (t = 0, 5, 10, 15, 30, 40, 60, 90, 120 and 150 min post-infusion). As the end of this time, drug levels fell below the limits of the assay.

Previous work (Reynolds et al., 1981) has shown that DHAD is unstable in plasma at room temperature ($t_{1/2} \sim 24$ h). In order to minimize DHAD loss during sample work-up (prior to the addition of the stabilizing solution), blood samples (14 ml) were collected in two *pre-cooled* (in ice) 7 ml blood collection tubes containing 0.07 ml of a 15% EDTA solution (Monoject, Sherwood Medical Laboratories, St. Louis, Mo., U.S.A.). The samples were centrifuged at about $3400 \times g$ at 4°C for 4 min in pre-cooled polystyrene centrifuge tubes. Aliquots (5 ml) of the resulting plasma fraction were removed, treated with a solution of ascorbic acid in citrate buffer, and frozen for subsequent analysis as detailed by Reynolds et al. (1981). Under these conditions, no detectable deterioration of the analyte occurred over the storage and work-up periods.

Immediately prior to analysis, each sample was thawed in a 30°C water bath and centrifuged at 2000 rpm for 5 min to precipitate any denatured protein generated by the freeze–thaw cycle. The homogeneous fluid obtained was analyzed for DHAD by the method described by Reynolds et al. (1981). Briefly, the method involves sample clean-up by retention of DHAD on XAD-2 resin, elution with 2-propanol–aqueous phosphate buffer and subsequent analysis by HPLC.

Detection and quantitation was accomplished by UV spectrophotometry at 254 nm. A Spectrum Scientific (Newark, Dela., U.S.A.) model 1021A Filter and Amplifier was used to enhance the signal-to-noise ratio of the detection system and was operated at a gain of unity; 0.01 Hz cut-off frequency was used throughout the study.

The apparent terminal slopes of the plasma concentration–time profiles were calculated both by a least-squares regression analysis of the data using all time points ≥60 min post-infusion, and a non-linear regression analysis of the entire profile using a Simplex algorithm (Nelder et al., 1965).

The maximum plasma levels of DHAD in the 4 patients studied following an i.v. dose of 12 mg/m^2 (infused over 30 min) ranged from approximately 400 to 960 ng/ml (mean 630 ± 223 ng/ml). A representative profile for one patient is shown in Fig. 1. This figure shows a very rapid initial decline in DHAD plasma levels, resulting in the loss of approximately 85% of the drug from the plasma within 30

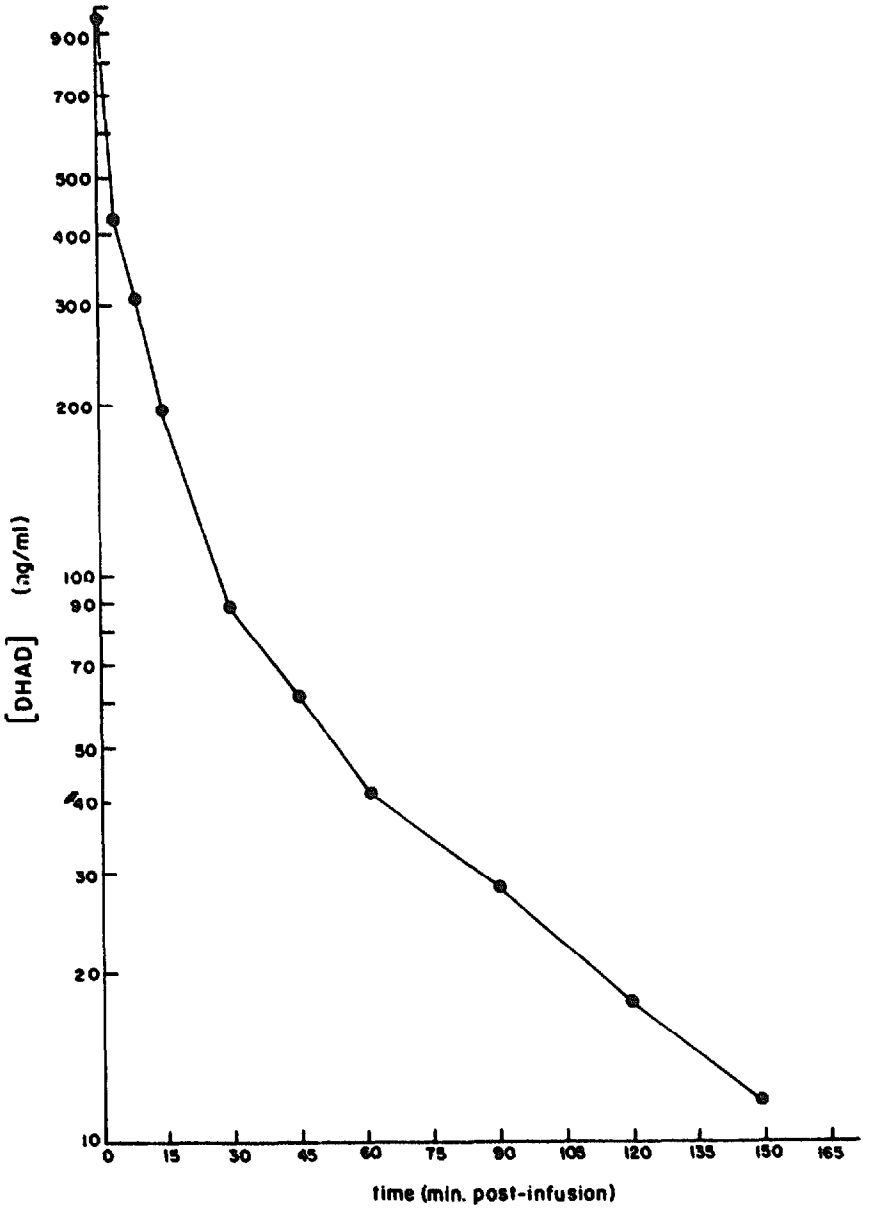

Fig. 1. A plot of plasma concentration of DHAD vs time for a single subject.

min after the end of infusion. With such a rapid initial decline of plasma drug concentration, any delay in sampling will result in a substantial underestimate of the actual plasma drug level at that time. Since it was necessary to collect relatively large blood samples in this study, it is likely that the reported plasma concentrations at early times are probably somewhat low. For this reason, half-life values for the initial phase were not calculated.

Semi-logarithmic plots of plasma concentration–time data show that the profile reached an apparent terminal slope at about 60 min post-infusion. At this point, plasma levels of DHAD were $\leq$10% of the maximum levels achieved. The terminal

TABLE I

THE RATE CONSTANTS FOR THE APPARENT ELIMINATION PHASE OF DHAD IN 4 HUMAN SUBJECTS

Patient	k_{el} (min^{-1}) [a]	r^2
A	0.014	0.999
B	0.013	0.998
C	0.008	0.946
D	0.014	0.858
	mean 0.0125 ± 0.0032	

[a] Calculated by a least-squares regression analysis of all points $\geq$60 min post-infusion.

plasma elimination rate constants for the 4 patients are listed in Table I, along with the mean and associated standard deviation. The mean value corresponds to a half-life of 56 min. These values are in agreement ($\pm$ 10%) with the values obtained by a non-linear regression analysis of the entire profile.

From the data generated in this preliminary study it is clear that DHAD is rapidly lost from plasma. Since urine levels could not be measured (due to analytical limitations), it cannot be deduced whether the loss of DHAD from plasma is due to renal clearance or metabolism. However, in view of the in vitro instability of DHAD in plasma (Reynolds et al., 1981) it is probable that the drug would be subject to extensive non-enzymatic and enzymatic degradation. Further work is needed to elucidate more fully the pharmacokinetic characteristics of DHAD including distribution and metabolism and their clinical significance. In the meantime, the unusually rapid distribution and elimination characteristics of the drug should be taken into consideration in designing future clinical studies.

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