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# Pharmacokinetics of 10-ethyl-10-deaza-aminopterin, edatrexate, given weekly for non-small-cell lung cancer

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**Summary.** We studied the pharmacokinetics of 10-ethyl-10-deaza-aminopterin (10-EdAM), edatrexate and its 7-hydroxy metabolite during a phase II trial of treatment in advanced non-small-cell lung cancer. A dose of 80 mg/m$^2$ was given weekly, with dose reduction being undertaken for mucositis or haematological toxicity. A triphasic pattern of plasma elimination was seen, the mean half-lives being $0.10 \pm 0.07$, $0.8 \pm 0.3$ and $7 \pm 7$ h, respectively. The mean plasma clearance was $25 \pm 14$ l/h, with $18\% \pm 11\%$ of the dose appearing unchanged in the urine. The serum concentration at 1 h accurately predicted the area under the curve (AUC) with $r^2 = 0.976$. There was considerable variation of the clearance both within and between patients but there was no evidence of a dependence on time or dose. The 1-h concentration of the drug was shown to be related to the incidence of toxicity requiring dose reduction. The change in WBC due to the initial dose was shown to be related to both the AUC of the drug and that of its 7-OH metabolite.

## Introduction

The antifolate 10-ethyl-10-deaza-aminopterin (10-EdAM) is a 10-ethyl derivative of 10-deaza-aminopterin and is an antifolate that differs from methotrexate in that the pteridine ring is linked to para-amino benzoic acid by a carbon-ethyl rather than a nitrogen-methyl moiety. Studies by Sirotnak and co-workers [10] indicated that the activity of some 10-deaza derivatives against murine tumours was superior to that of methotrexate, the 10-ethyl derivative (10-EdAM) being the most active. As compared with methotrexate, 10-EdAM accumulates to a greater extent in the cells of these tumours [9]. During pre-clinical testing, intestinal toxicity was a major dose-limiting toxicity [4], but in phase I trial in cancer patients, oral mucositis was the major dose-limiting effect [6]. Pharmacokinetic analyses at doses of 5, 30 and 100 mg/m$^2$ showed a triphasic plasma clearance, the elimination half-lives being 12.9 min, 1.5 h and 11.9 h [6]. In a subsequent phase II study, Shum et al. [7] observed 6 major responses in 19 evaluable patients with non-small-cell lung cancer (NSCLC). Mucositis was again the major dose-limiting toxicity.

In the course of a phase II study in 49 patients with NSCLC [11], we conducted serial pharmacokinetics studies. The intention was to establish whether dose-limiting toxicity could be predicted in individual patients by limited sampling and to determine the extent of inter- and intra-patient variability of drug clearance.

## Patients and methods

*Materials.* 10-EdAM (for therapeutic injection) and 7-0H- 10-EdAM were supplied by Ciba-Geigy Pharmaceuticals Ltd. (Basle, Switzerland). The compounds were initially dissolved in 50 mM $KH_2PO_4$ (pH 8.0) and subsequently diluted with 50 mM $KH_2PO_4$ (pH 7.0):acetonitrile (90:10, v/v; buffer C). The acetonitrile was of high-performance liquid chromatography (HPLC) grade (Rathburn Chemicals Ltd., Peebleshire, Scotland). Human serum for internal controls was donated by healthy volunteers.

*Drug assay.* A fluorometric HPLC assay was used as described by Kinahan et al. [5]. As our initial data were obtained using this method, which gave good recoveries and resolution of 10-EdAM and the 7-OH metabolite, we continued to use this procedure when the improved method of Van Tellingen et al. [12] became available. By this method, the drug and metabolites are extracted from serum or urine samples using Sep-Pak $C_{18}$ cartridges (Waters/Millipore). The extract was concentrated into 100 µl of buffer C and 5–20 µl was used for HPLC analysis. The HPLC apparatus (Waters) consisted of two 501 pumps, a 712 WISP autoinjector, a 420-AC fluorescence detector and the Baseline 810 programme on an NEC powermate IV to control pumps and to collect and integrate data.

The separation of 10-EdAM and the 7-hydroxy metabolite was achieved on a Waters $C_{18}$ Nova Pak Radial Pak (4 µm particle size)

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column using 50 mM $KH_2PO_4$ (pH 7.0; buffer A) and 50 mM $KH_2PO_4$ (pH 7.0): acetonitrile (40:60, v/v; buffer B) as the mobile phase at 1.8 ml/min. The initial ratio of A:B was 80:20 (v/v). After 2 min equilibration, a linear gradient from 20% to 40% B was run over 2 min. The mobile phase was then run at this ratio for 4 min, during which time the drug and metabolite were eluted (6.8–7.04 min). Detection was accomplished by fluorescence using an excitation wavelength of 375 nm and an emission wavelength of 460 nm.

Assay standards for 10-EdAM ranged from 50 nM to 10 μM in buffer C. All samples from a single patient were run within a single assay together with control samples of 10-EdAM, which were made up in normal human serum at 64 nM, 320 nM and 5.35 μM. The mean recovery of the controls was 99% ± 6%, 94% ± 4% and 98% ± 5%, respectively; the within-assay coefficient of variation (CV) was 2.4%, 6.3% and 3.3%, respectively; and the between-assay CV was 6.3%, 4.0% and 5.5%, respectively. For a control sample at 2 nM the between-assay CV was 19%, which thus represents the practical approximate limit of detection of the assay.

In the determination of the 7-OH metabolite, assay standards and internal standards were initially unavailable; therefore, a retrospective analysis was used. This was possible because it was found that using controls in the range of 200 nM–10 μM containing equal concentrations of both 10-EdAM and 7-OH metabolite in serum, the areas in their HPLC peaks were in a consistent ratio of 1:1.57 (±0.21 SD). On the assumption of the validity of this ratio for the earlier patient assays it was therefore possible to infer the 7-OH metabolite levels. It is noteworthy that as compared with the parent drug, the 7-OH metabolite had a considerably lower recovery (mean, 35% ± 5%) but had an approximately 2.5 times higher fluorescence.

*Patients.* A total of 49 patients with histologically or cytologically confirmed NSCLC were treated with 10-EdAM. The details are described elsewhere [11]. All patients had normal renal and cardiac function before starting treatment and none had a pleural effusion or ascites. 10-EdAM was given at a dose of 80 mg/m² into a fast-running saline infusion over a mean of 2.3 min. The dose was repeated weekly. Dose reductions and delays were made according to a defined scheme according to haematological toxicity, mucositis and skin rash [11].

Blood samples were collected into plain glass tubes prior to treatment and at 5, 10, 20, 30, 60 and 90 min as well as 2, 3, 6, 12, 18 and 24 h after the end of the infusion. After centrifugation, the serum was collected and stored at –20° C until assay. Patient urine was collected for 24 h following treatment, the volume was measured and an aliquot was frozen and stored at –20° C until assay.

*Pharmacokinetic calculations.* The relationship of the plasma concentration (C) of 10-EdAM against time (t) was fitted to mathematical formulae of the type

$$C = \sum_{i=1}^{n} C_i \exp(-\lambda_i t).$$

This assumes that the dose is given by bolus administration. Fits were tried for bi-exponential ($n = 2$) and tri-exponential functions ($n = 3$) with a weighting proportional to $1/C^2$ using the PCNONLIN nonlinear regression programme (Statistical Consultants, Inc., Edgewood, Ky., USA). The area under the curve (AUC) was calculated as

$$AUC = \sum_{i=1}^{n} \frac{C_i}{\lambda_i}$$

and the clearance (CL) was calculated as CL = D/AUC, where *D* represents the dose.

The time profiles of the concentration of the 7-hydroxy metabolite were too irregular to fit any mathematical model. The calculation of the AUC would require a model to predict the portion of the curve from 24 h to infinity. Because this was unavailable, only the AUC (0–24 h) could be calculated and this was done using the linear trapezoidal method. It is interesting to note that the concentration at 24 h, expressed as a ratio of the peak concentration, had a mean of 0.10 (±0.15), so it seems unlikely that the AUC would differ greatly from the AUC (0–24 h).

## Results

Full concentration/time profiles were obtained in 15 patients receiving a dose of 80 mg/m², mainly during their first treatment cycle. It proved possible to fit bi-exponential decay functions to all 15 patients profiles, and tri-exponential functions could be fitted to 14 of these. In these 14 profiles the bi-exponential function generally gave a much poorer fit than did the tri-exponential function. This was evidenced by the considerably higher values of the weighted residual variance for the bi-exponential as compared with the tri-exponential case. The mean ratio of these quantities was 17.6 ± 39 (range, 2.1–157). Table 1 shows the 10-EdAM AUC and clearance values, the percentage of

**Table 1.** Pharmacokinetic values for the 15 patients in whom full concentration/time profiles were obtained

| Patient number | Cycle number | 1-h level (μM) | $t_{1/2}$ (h) | $t_{1/2}$ (h) | $t_{1/2}$ (h) | 10-EdAM AUC (μM h) | 10-EdAM Cl (l/h) | 10-EdAM % in urine (24 h) | 7-OH metabolite AUC (0–24 h) | AUC ratio | Creatine clearance (ml/min) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 11.1 | 0.117 | 1.318 | 29.8 | 43.4 | 7.0 | – | 5.8 | 7.5 | 67 |
| 2 | 1 | 2.88 | 0.029 | 0.425 | 4.21 | 11.9 | 27.0 | 10.4 | 3.6 | 3.3 | 81 |
| 3 | 1 | 1.21 | 0.064 | 0.718 | 5.59 | 5.3 | 57.5 | 25.8 | 0.95 | 5.6 | 100 |
| 4 | 1 | 1.36 | 0.067 | 0.454 | 6.58 | 7.2 | 35.8 | 26.4 | 1.9 | 3.7 | 68 |
| 5 | 1 | 2.27 | 0.071 | 0.679 | 3.62 | 10.7 | 27.0 | – | 2.9 | 3.6 | 65 |
| 6 | 1 | 1.89 | 0.215 | 0.641 | 3.08 | 7.5 | 41.3 | – | 6.1 | 1.2 | 75 |
| 7 | 1 | 5.97 | 0.066 | 1.062 | 7.66 | 24.8 | 13.2 | 27.8 | 15.8 | 1.5 | 47 |
| 8 | 2 | 3.26 | 0.044 | 0.54 | 13.4 | 15.5 | 22.4 | – | 4.1 | 3.8 | 111 |
| 9 | 1 | 7.26 | 0.055 | 1.30 | 6.04 | 28.9 | 9.6 | 27.8 | 12.1 | 2.4 | 45 |
| 10 | 1 | 8.22 | 0.065 | 0.85 | 3.16 | 34.8 | 9.6 | 7.4 | 4.6 | 7.5 | 72 |
| 11 | 3 | 2.41 | 0.179 | 0.737 | 8.25 | 11.3 | 25.8 | – | – | – | 144 |
| 12 | 4 | 1.80 | 0.047 | 0.422 | 2.57 | 10.5 | 26.3 | – | 2.2 | 4.8 | 78 |
| 13 | 1 | 2.24 | 0.103 | 0.784 | 4.52 | 8.2 | 45.4 | – | 0.65 | 12.5 | 111 |
| 14 | 3 | 1.71 | 0.070 | 0.318 | 2.59 | 12.8 | 20.0 | 20.8 | 2.5 | 5.2 | 63 |
| 15 | 1 | 5.13 | 0.270 | 1.230 | – | 24.4 | 10.5 | – | 5.5 | 4.5 | 74 |
| Mean | | 3.91 | 0.096 | 0.76 | 7.2 | 17.2 | 25.2 | 18.3 | 4.90 | 4.81 | 80.0 |
| SD | | 3.0 | 0.069 | 0.33 | 7.1 | 11.0 | 14.7 | 11 | 4.2 | 2.9 | 26 |

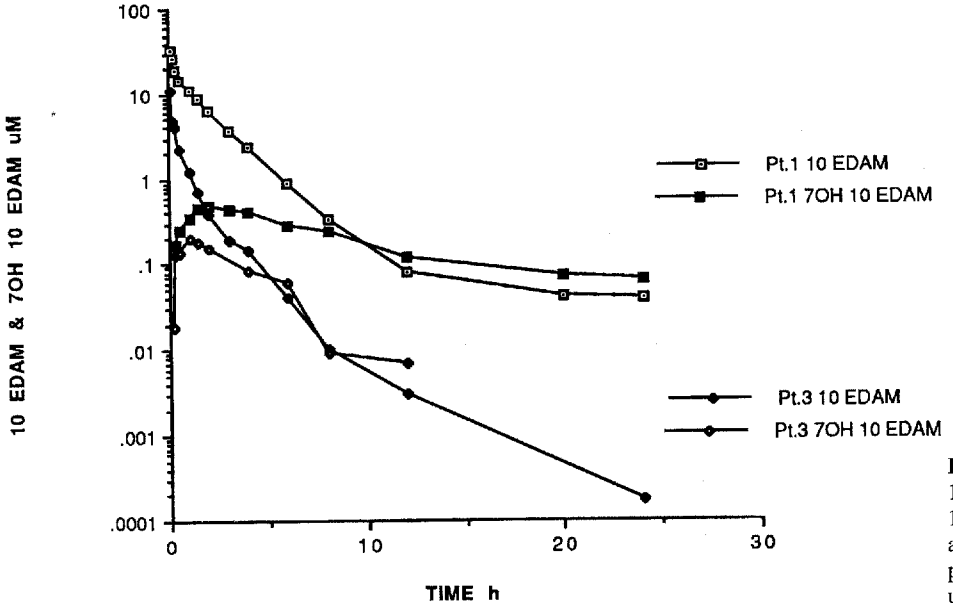


**Fig. 1.** Concentration/time profiles for 10-EdAM and its metabolite (7-OH 10-EdAM) in two patients (*Pt.*) following a dose of 80 mg/m$^2$. The profiles (for patients 1 and 3 in Table 1) represent the upper and lower extremes of those studied

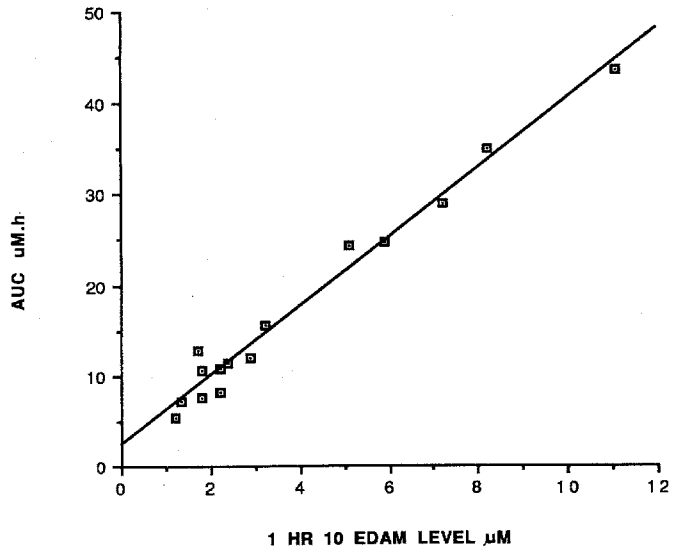


**Fig. 2.** AUC of 10-EdAM plotted against the 1-h concentration. Also shown is the regression line of AUC on the 1-h concentration

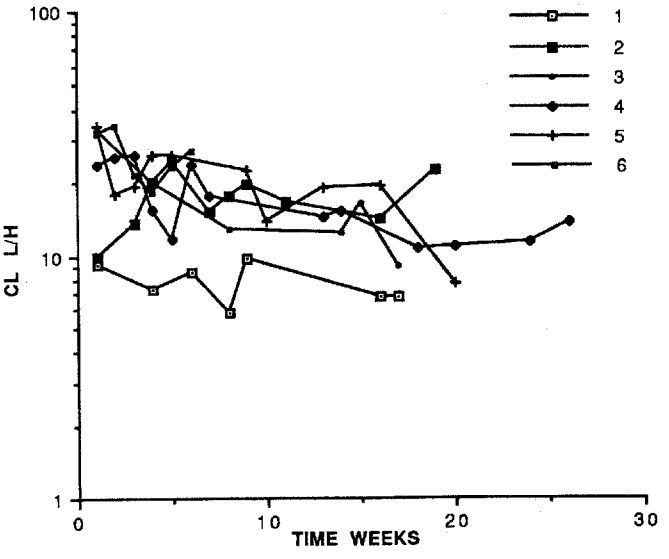


**Fig. 3.** Plasma clearance of 10-EdAM plotted against time in 6 patients

the dose recovered in the urine in 24 h and the $t_{1/2}$ values for the three phases. The mean $t_{1/2}$ values were $0.1 \pm 0.07$, $0.8 \pm 0.3$ and $7 \pm 7$ h, with $18\% \pm 11\%$ of the dose appearing in the urine as unchanged compound during the first 24 h. The AUC (0–24 h) of the 7-OH metabolite for the first 24 h was smaller by a mean factor of $5 \pm 3$ than the AUC of the parent drug. During the first 24 h, $1.1\% \pm 1.2\%$ of the dose appeared in the urine as the 7-OH metabolite. Examples of the concentration/time curves showing the fastest and slowest clearance are shown in Fig. 1.

Creatinine clearance was estimated from the pre-dose level of serum creatinine by the method of Siersbaek-Nielsen et al. [8] and is included in Table 1. No correlation could be demonstrated between 10-EdAM clearance and creatinine clearance or serum albumin levels.

For 10-EdAM the AUC was closely correlated with the 1-h concentration (Fig. 2; $r^2 = 0.976$, $n = 15$, $P < 0.001$), presumably due to the occurrence of approximately half of the AUC on either side of the 1-h time point. The regression line of AUC on the 1-hour concentration was AUC = 2.28+3.80 C. If this equation were used to predict the AUC for a new patient from the same population, the estimated standard deviation of the AUC would be 1.93 µM h at 17.2 µM h (i.e. representing 11% of the mean AUC).

Serial 1-h concentrations were measured in seven patients. From these measurements the clearance of 10-EdAM was calculated as dose/AUC, the AUC having been estimated from the regression equation shown above. The resulting clearances are plotted against time in Fig. 3. As required by the protocol, these patients received varying

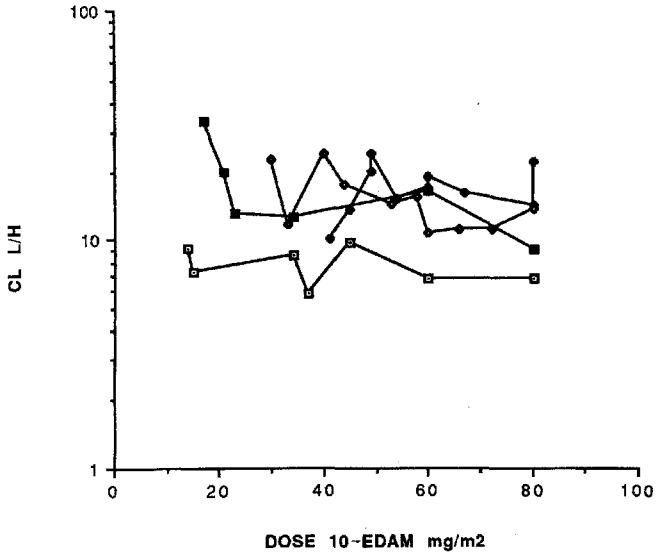


**Fig. 4.** Plasma clearance of 10-EdAM plotted against dose in 4 patients

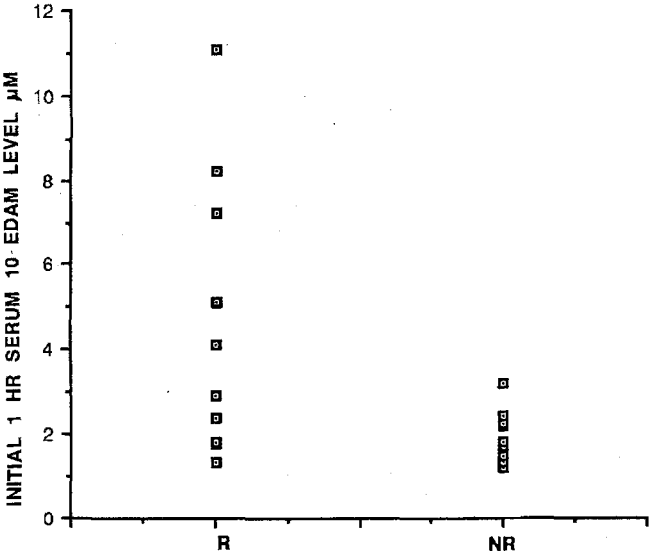


**Fig. 5.** The 1-hour serum concentration of 10-EdAM for a dose of 80 mg/m$^2$ as plotted for patients who required dose reduction (*R*) during the first four cycles of treatment and for those who did not (*NR*)

doses during the period of observation, and the same values of clearance are plotted against dose in Fig. 4. In the relationship of clearance both to time and to dose it is evident that there are considerable variations in clearance both within and between patients, but no discernible trends are apparent.

An attempt was made to correlate the degree of haematological toxicity after the first cycle with the AUC of 10-EdAM and the 7-OH metabolite. The effect on blood haemoglobin and cell counts was examined by first calculating the fractional change, that is, the difference between the pre-dose value and that obtained 1 week later, expressed as a fraction of the mean of the two values. Significant ($P < 0.05$) correlations were found between the AUC of 10-EdAM and the fractional fall in haemoglobin ($r^2 = 0.30$, $P < 0.05$), total WBC ($r^2 = 0.38$, $P < 0.02$) and neutrophil count ($r^2 = 0.36$, $P < 0.02$; $n = 15$ in all cases). The relationship of the reduction in platelet count to the AUC of 10-EdAM was found to fall short of significance ($r^2 = 0.19$, $n = 15$, $0.10 < P < 0.20$). Almost identical values were obtained when the 1-h concentration was substituted for AUC.

Multiple linear regression was used to examine the effect on the change in WBC count of the 10-EdAM AUC and the 7-OH metabolite AUC together. Both of the independent variables explained significant reductions ($P < 0.05$ and $P < 0.01$, respectively) in variance, giving an overall $r^2$ value of 0.76. The resultant equation had the following form:

$$\text{Fractional fall in WBC} = 4.25 \times 10^{-3}\, X_1 + 1.54 \times 10^{-2}\, X_2,$$

where $X_1$ is the AUC for 10-EdAM and $X_2$ is the AUC (0–24 h) for the 7-OH metabolite.

A more general index of toxicity was provided by the weekly assessment of the patients as defined in the protocol for the basis of any subsequent dose reduction. Of 19 patients 10 underwent dose reduction during the first 4 weeks of treatment, and the mean 1-h concentration in these patients was significantly higher than that in the remainder ($t = 2.20$, $P < 0.05$). The 1-h concentrations for the two groups are plotted in Fig. 5. An attempt was made to discover a relationship between the clinical response and the AUC of 10-EdAM (or the 1-h serum concentration), but no evidence was found for this.

## Discussion

The phase II studies of 10-EdAM in NSCLC reported by Shum et al. [7] and ourselves [11] indicate that major responses occur in approximately 17% of patients treated weekly at doses of 80 mg/m$^2$. A weekly drug regimen is inconvenient, and mucositis and myelosuppression may result from continued treatment. The present study confirms the pharmacokinetic findings of Kris et al. [6], showing 10-EdAM to undergo tri-phasic elimination from plasma. The mean values for the three phases, $0.10 \pm 0.07$, $0.8 \pm 0.3$ and $7 \pm 7$ h, respectively, differ somewhat from the mean values (0.215, 1.5 and 11.9 h) obtained by Kris et al. [6] in nine subjects. As the half-life (mean, 0.1 h) of the initial phase of the tri-exponential function is comparable with the injection times (mean, 2.3 min) and, indeed, with the circulation time, the theoretical assumption of a bolus dose implicit in the model is an over-simplification. In view of the limited number of experimental points in the initial phase, it would have been unrealistic to attempt to fit the data to a more detailed model. The infusion time was not stated in the work of Kris et al. [6], and this may be the source of some of the difference in the half-life values for the fast phase. The half-lives for the slow phase were derived by Kris et al. [6] from observations extending to 120 h and are likely to be more reliable than our values, which are based on sampling to 24 h. The limited data (Table 1) on the 24-h cumulative urinary excretion of 10-

EdAM yields a mean value of 18% ± 11%, which is compatible with the cumulative 8-h and 72-h values reported by Kris et al. [6], thus confirming the difference between the behaviour of 10-EdAM and that of methotrexate, for which the percentage of drug found in the urine usually ranges between 50% and 100% [3]. No relationship between the clearance of 10-EdAM and that of creatinine was apparent.

The present study shows that the 10-EdAM AUC is accurately predicted by the 1-h plasma concentration and that the use of such limited sampling can give good estimates of clearance. Figure 3 shows the considerable variation in clearance observed both within and between patients using this approach. It is reassuring, however, that in spite of such variability, there was no evidence of a systematic change in clearance over many weeks of treatment. Similarly, there was no evidence of a change in clearance with dose (Fig. 4). Therefore, there was no evidence of saturation or induction of the elimination or distribution processes.

Although there do appear to be significant relationships between the 10-EdAM AUC (or the 1-h concentration) and three of the measures of haematological toxicity, they are of limited predictive value. Considering the fractional reduction in the WBC, the correlation coefficients indicate that the between-patient variance in this quantity could be reduced by about 30% and 35% respectively, of the original value by a knowledge of the 10-EdAM AUC and 1-h concentration. This small reduction contrasts with the results reported by Egorin et al. [2] for menogaril, whereby a knowledge of the AUC allowed a reduction in the variance of the fractional reduction in WBC by an amount estimated to represent about 80% of the original value, and for carboplatin [1], whereby such knowledge allowed a similar reduction in the variance of the fractional reduction in platelet count. It is interesting, however, that our data suggest that if predictions of the WBC were based on the AUCs of both 10-EdAM and the 7-OH metabolite, these would have an approximately equal and additive effect and would give a reduction in variance of 76% of the original value. In view of the uncertainties involved in the assay of the 7-OH metabolite, this suggestion must be regarded as tentative.

For the more general measure of toxicity represented by dose reductions during the first 4 weeks of treatment, in a sample of 19 patients we observed a significantly higher 1-h concentration of 10-EdAM in those who underwent dose reduction (Fig. 5). In view of the additive effect of the AUCs of both the parent drug and the metabolite on the WBC, we deemed it interesting to determine whether a comparable effect could be shown on the incidence of dose reduction. However, the small number of patients (13) on whom the relevant data were available was too small to provide firm evidence of such an additive effect.

The present study therefore shows that the 1-h plasma concentration of 10-EdAM gives a good estimate of the AUC and may be helpful in identifying patients at risk of developing toxicity. When the drug is used alone or in combination, its toxicity might be minimised by identifying patients at risk and either undertaking a dose reduction for subsequent cycles or initiating folinic acid rescue.

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