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Clinical pharmacology of oral and intravenous 4-demethoxydaunorubicin*

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Summary. The clinical pharmacology of 4-demethoxydaunorubicin (4-DMDNR) was studied in 28 patients with advanced breast cancer, using a sensitive reverse-phase HPLC technique. All patients had normal renal and hepatic function. The serum levels of 4-DMDNR after a single i.v. bolus injection followed a triple exponential decay curve ($T\frac{1}{2}\alpha = 9.6$ min, $T\frac{1}{2}\beta = 3.2$ h and $T\frac{1}{2}\gamma = 34.7$ h) and conformed to a three-compartment model. Comparison of the area under the curve (AUC) and urinary excretion for the oral and i.v. routes suggests an oral bioavailability of approximately 24%. In patients treated with a schedule of weekly oral administration for periods of up to 12 months there was no significant alteration in either AUC or elimination half-life for the parent drug or its principal metabolite 13-OH4DMDNR. Moreover, there was no evidence of accumulation of the metabolite although measurable amounts were present 7 days after administration of 4-DMDNR.

Introduction

One of the characteristic features of daunorubicin is the presence of a methoxyl group at the C-4 position of the tetracyclic aglycone [16]. This substitution is not, however, present in other anthracyclines, notably carminomycin, which demonstrated considerable antitumour activity in preclinical testing [8]. In order to investigate the properties conferred by the C-4 methoxyl substitution, Arcamone et al. synthesised 4-demethoxydaunorubicin (4-DMDNR) and compared its activity against L1210 and sarcoma 180 with that of daunorubicin [1]. The results showed that 4DMDR was 5–8 times more potent than the parent compound, although their therapeutic ratios were similar. Subsequently it was demonstrated that, in contrast to the parent drug, 4DMDNR was equally effective when administered p.o. to mice bearing Gross leukaemia and sarcoma 180, albeit at doses 3–4 times that given i.v. [7]. In addition, it appears that the major metabolite of 4DMDNR, 13-OH4DMDNR, has a similar antitumour activity to the parent compound in animal models [5] and that this metabolite has a particularly long serum half-life, with significant amounts still present 7 days after drug administration.

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4-Demethoxydaunorubicin has shown activity in advanced breast cancer [3], and we therefore decided to test the oral preparation in a weekly schedule in such patients. The rationale for this regimen was an attempt to simulate a continuous infusion of cytotoxic activity by utilising the long half-life of 13-OH4DMDNR. The present study was conducted in conjunction with the clinical trial to compare the pharmacokinetics of 4-DMDNR given p.o. and i.v. and to determine whether its metabolism alters significantly with prolonged continuous administration.

Methods

All patients entered in the study had advanced breast cancer, a Karnofsky performance status [13] of 60% or more, and no significant hepatic (bilirubin < 25 mmol/l, transaminases < 50% above upper limit of normal) or renal dysfunction (creatinine < 0.15 mmol/l). 4-DMDNR was administered as a single agent at a dose of 15 mg/m^2 weekly. Treatment was given for at least 8 weeks and continued until progression occurred. It was planned to assess serum drug and metabolite profiles at 0, 4, 12, 24 and 52 weeks and to measure the 7-day 13-OH4DMDNR level at each 4-weekly clinic visit. In addition, the pharmacokinetics of oral 4-DMDNR were compared with the same dose given i.v. in five patients.

Blood and urine sampling

4-DMDNR was administered following a light breakfast. For the oral study samples were taken at 0, 0.5, 1.5, 2, 3, 6, 9, 12, 24, 48 and 72 h, and for the i.v. study additional samples were obtained at 2, 5, 10, 20, and 60 min. Blood samples were centrifuged for 10 min at 500 *g*, after which the serum was separated and stored at −20 °C prior to assay.

Urine was collected during time intervals 0–6, 6–12, 12–24, 24–48 and 48–72 h after drug administration. Total volumes were recorded and 5-ml aliquots stored at −20 °C.

Analytical method

Extraction procedure. Stored serum was thawed and 2 ml removed. To this was added 3 ml methanol and the resulting solution centrifuged at 33000 *g* for 15 min at 4 °C. The supernatant was removed and 4 ml chloroform added. The mixture was then shaken until the chloroform was com-

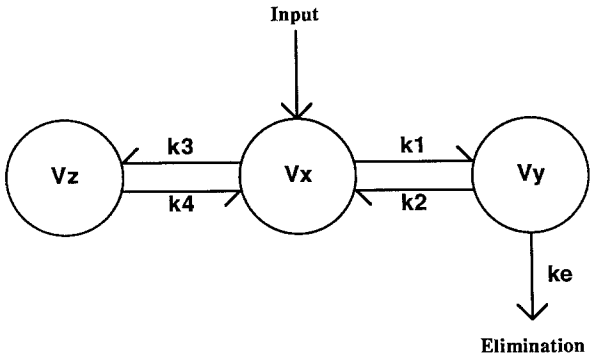

Fig. 1. Three-compartment model for 4-DMDNR pharmacokinetics with input into the central compartment, V_x, and elimination from peripheral compartment, V_y. k_{1-4} represent inter-compartmental rate constants and k_e, the elimination rate constant

pletely dispersed and further centrifuged at 500 *g* for 10 min at 15 °C. The upper layer was then discarded and the chloroform solution passed through a phase separation filter to remove any remaining aqueous phase. The resulting solution was evaporated to dryness and the residue resuspended in 100 µl methanol prior to assay.

Urine was prepared for analysis by the addition of 450 µl mobile phase to 50 µl from each sample. This solution was centrifuged at 11600 *g* for 10 min at 4 °C. Aliquots (50 µl each) of the resulting supernatant were used for analysis.

Standards were prepared by the addition of known amounts of 4-DMDNR and 13-OH4DMDNR to blank serum and urine. The linearity of the standard curves thus obtained was 0.997 ± 0.012, with an interassay variation of 5.6%. The extraction efficiency was 15% for serum and 60% for urine.

HPLC method

Analysis was carried out according to a modification of the method described by Israel et al. for adriamycin [10]. The HPLC assay was performed using a Waters Associates U6K injector and 6000A pump in conjunction with a Technicol ODS Hypersil column (5 µm particle size and 120 Åpore size). The column was isocratically eluted with an acetonitrile/ammonium formate buffer at pH 4 (45/55) at a flow rate of 1.5 ml/min. Fluorescence was monitored with a Schoeffel FS970 detector using an excitation wavelength of 250 nm and a 550-nm emission filter.

Retention times for 13-OH4DMDNR and 4DMDNR were 3 min 40 s and 4 min 30 s, respectively. The limit of sensitivity of the assay was 0.25 ng/ml.

Pharmacokinetic analysis

Data from the i.v. study were analysed using a Nelder and Mead Non-Linear Optomization computer program [4]. The volume of distribution (V_d) was obtained from the equation $V_d = \text{dose}/A + B + C$, where A, B and C represent the intercepts on the *y*-axis calculated for the triple exponential decay curve. For the oral study elimination half-lives were estimated from post-peak concentration data using least-squares regression analysis by the equation $T½ = 0.693/k_{el}$ (k_{el} = elimination rate constant). Total area under the curve to infinity (AUC) was calculated using the trapezoidal rule $+C/k_{el}$, where C represents the concentration at the final data point. Statistical comparison of the mean values for AUC and elimination half-life at 0 and 24 weeks therapy was by the paired *t*-test.

Results

Intravenous study

The plasma concentration of 4-DMDNR following i.v. bolus administration described a triple exponential decay curve with the equation: $Ct = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$.

In addition, the data fitted a three-compartment model with input into the central compartment and excretion from one of the peripheral compartments (Fig. 1). The mean short, intermediate and long half-lives were 9.6 min, 3.24 h and 34.74 h, respectively. The pharmacokinetic parameters following i.v. administration are shown in Table 1 and those following oral administration, in Table 2.

The mean bioavailability for the oral preparation calculated as the ratio of AUC following oral administration to AUC following i.v. administration was 23.7% (range 8.9%–38.9%). The mean ratio of oral 4DMDNR AUC + 13-OH4DMDNR AUC to i.v. 4DMDNR AUC + 13-OH4DMDNR AUC was 40.6% (range 19.1%–58.9%). The elimination half-life and AUC for 13-OH4DMDNR following i.v. administration were 52.1 h and 696 $\mu g \times l.h^{-1}$, respectively. The decay curves for

Table 1. Pharmacokinetic parameters of 4-DMDNR and 13-OH-4-DMDNR following i.v. 4-DMDNR

	4-DMDNR														13-OH-4-DMDNR	
Pt	V_x lt	V_y lt	V_z lt	k_1	k_2	k_3	k_4	k_e	$T½_\alpha$ (h)	$T½_\beta$ (h)	$T½_\gamma$ (h)	V_d (lt)	AUC µg/l.h^{-1}	Cl l/h	$T½_{el}$ (h)	AUC (µg/l.h^{-1})
1	252.4	1150.3	844.7	16.8	2.6	57.6	106.7	1.93	0.17	3.0	46.2	263.4	337.1	74.1	73.7	814.1
2	652.1	152.3	844.7	25.1	4.1	201.5	22.1	5.69	0.16	1.0	19.3	654.4	190.1	131.4	69.3	596.7
3	214.8	613.7	1074.5	29.2	4.5	65.4	161.1	1.26	0.12	1.6	39.6	215.0	489.5	61.2	10.6	623.3
4	216.8	1930.6	439.5	11.1	2.3	59.7	32.2	5.22	0.17	6.3	30.1	217.0	391.4	76.6	49.5	839.6
5	456.9	2307.0	3640.5	19.6	4.2	87.2	382.3	4.75	0.21	4.3	38.5	458.7	243.9	102.4	57.7	609.4
Mean	358.6	1230.7	1265.6	20.3	3.5	94.2	140.8	3.77	0.16	3.2	34.7	361.7	330.4	89.2	52.1	696.6
SEM	85.9	394.8	608.8	3.1	0.4	27.3	65.4	0.90	0.01	0.9	4.6	85.7	53.0	10.2	11.2	53.4

V_x, volume of central compartment;
V_y, volume of first peripheral compartment;
V_z, volume of second peripheral compartment;
V_d, volume of distribution;
k_{1-4}, inter-compartmental rate constants (Fig. 1);
k_{el}, elimination rate constant;
AUC, area under the curve;
Cl, total-body clearance

Table 2. Pharmacokinetic parameters following oral 4-DMDNR

4-DMDNR				13-OH	
Pt	$T\frac{1}{2}_{el}$ (h)	AUC ($\mu g/l.h^{-1}$)	Bioavailability %	$T\frac{1}{2}_{el}$ (h)	AUC ($\mu g/l.h^{-1}$)
1	21.6	79.3	23.5	63.0	599.4
2	18.3	36.1	18.9	49.5	236.5
3	18.7	190.6	38.9	21.6	259.7
4	16.1	35.2	8.9	33.4	200.2
5	27.7	69.7	28.9	34.6	358.3
Mean	20.4	82.2	23.8	40.4	330.8
SEM	2.0	28.5	5.0	7.1	72.0

4-DMDNR and 13-OH4DMDNR after i.v. bolus are shown in Fig. 2.

Urine recovery of unchanged drug was 0.4%–0.7% after oral and 2.3–3.3% after i.v. administration, results consistent with oral absorption in the region of 25%. The mean ratio of 13-OH4DMDNR to 4-DMDNR detected in the urine was 3.1:1 for the i.v. and 6.0:1 for the oral route.

Study of repeated oral administration

Serum profiles were assayed in ten patients at the start of therapy. Subsequent measurements were obtained in two patients at 4, 12, 24 and 52 weeks, in two at 4, 12 and 24 weeks and in four at 4 weeks only. Figures 3 and 4 show the change. in $T\frac{1}{2}_{el}$ and AUC with time in patients receiving treatment for 6 months or longer. The mean AUC for 4-DMDNR at week 0 was 57 $\mu g \times l.h^{-1}$ and at week 24, 64 $\mu g \times l.h^{-1}$ ($P > 0.1$). The corresponding elimination half-lives were 23.1 h and 22.9 h ($P > 0.5$). The AUCs for 13-OH4DMDNR at 0 and 24 weeks were 366.3 $\mu g \times l.h^{-1}$

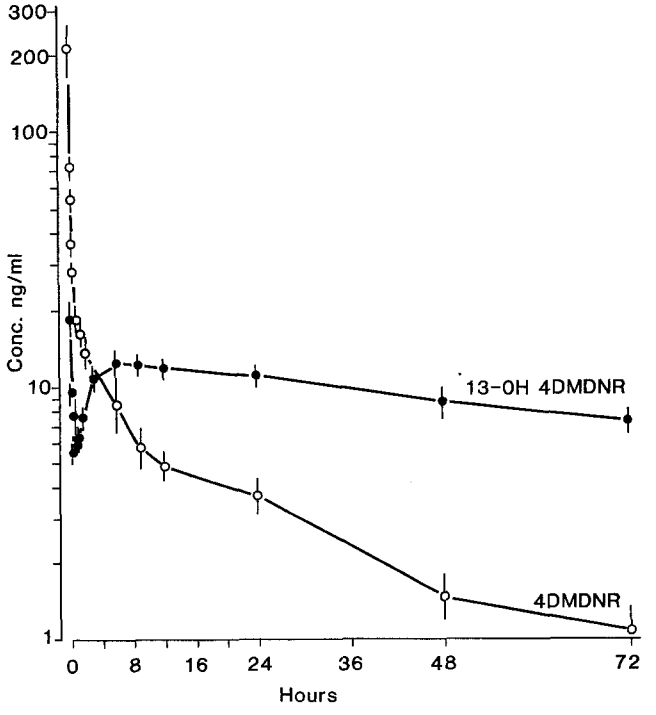

Fig. 2. Plasma decay curves of 4-DMDNR and 13-OH4DMDNR for 5 patients following i.v. administration. Mean ± SEM

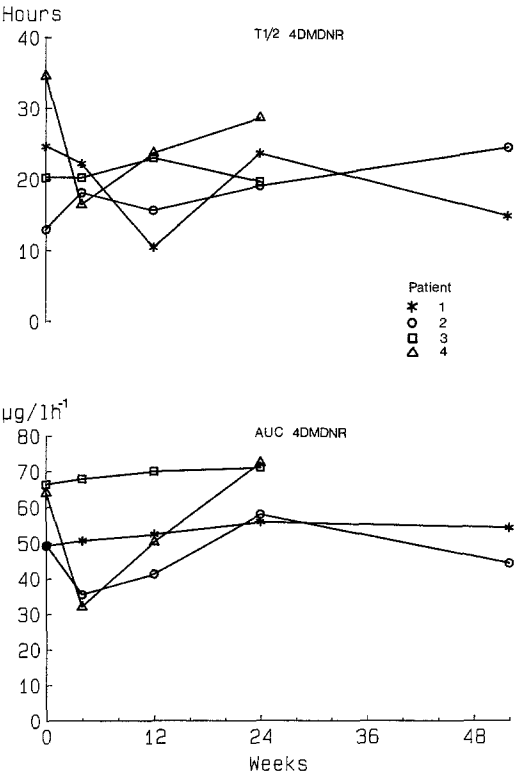

Fig. 3. $T\frac{1}{2}_{el}$ (elimination phase half-life) and AUC (area under the curve) for 4-DMDNR during successive course in four patients receiving at least 6 months' therapy

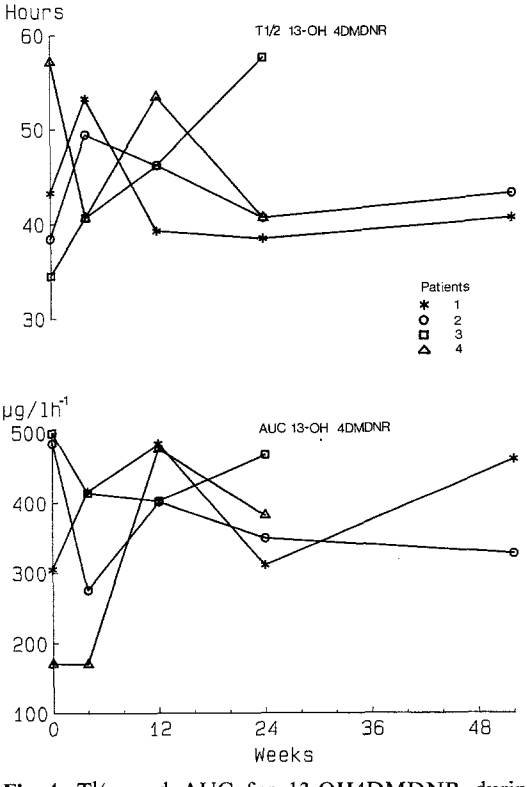

Fig. 4. $T\frac{1}{2}_{el}$ and AUC for 13-OH4DMDNR during successive course in four patients receiving at least 6 months' therapy

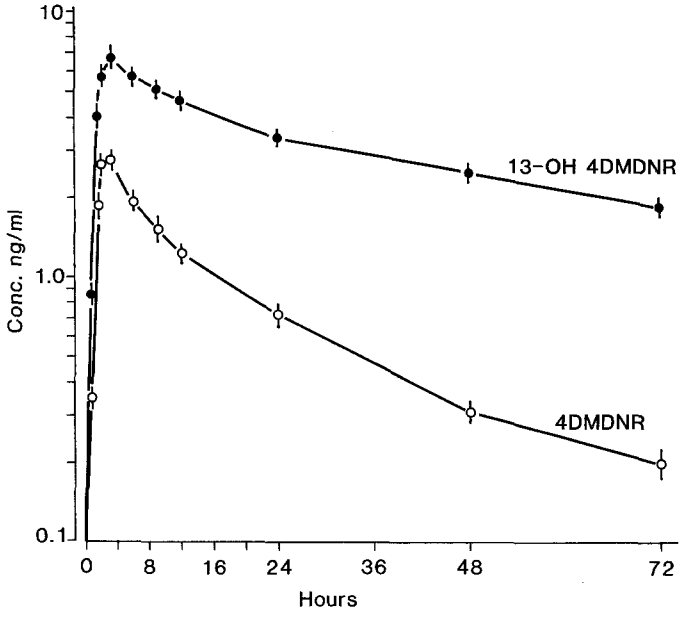

Fig. 5. Plasma decay curves of 4-DMDNR and 13-OH4DMDNR for five patients following oral administration. Mean ± SEM

and 378.1 $\mu g \times l.h^{-1}$ ($P > 0.5$). The elimination half-lives at these times were 43.5 h and 44.4 h ($P > 0.5$). Following oral administration the peak serum 4-DMDNR concentration was 3.1 ± 1.5 ng/ml, occurring 2.7 ± 1.0 h after ingestion. The peak 13-OH4DMDNR concentration was 7.1 ± 3.7 ng/ml, occurring 3.6 ± 1.8 h after ingestion.

The decay urves for 4-DMDNR and its metabolite after oral administration are shown in Fig. 5. The 13-OH4DMDNR 7-day level was measured 4-weekly in 23 patients. The mean value was 0.8 ng/ml (range 0.18–2.48 ng/ml), and there was no evidence of accumulation (Fig. 6).

There was no correlation between peak drug concentrations or 7-day metabolite levels and response to therapy in this group of patients.

Discussion

The limit of sensitivity for the HPLC system used in this study was 0.25 ng/ml. This represents a 10-fold improvement over previous techniques, with which measurement has not been possible below 2 ng/ml [2, 6, 11, 12]. We have therefore been able to show that 4-DMDNR is present in the blood for up to 72 h after both oral and i.v. administration and to define its pharmacokinetics more accurately.

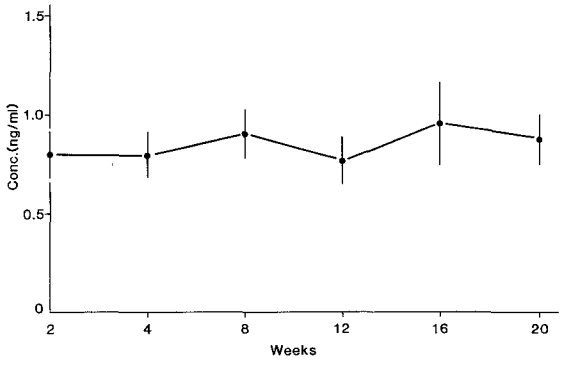

Fig. 6. Levels of 13-OH4DMDNR measured 7 days after the last dose in 23 patients during continuous weekly therapy. Mean ± SEM

Comparison of the AUCs for the i.v. and oral regimens indicates that 4-DMDNR has an oral bioavailability of 25%, a result confirmed by the values obtained for urinary recovery. This figure is slightly lower than previous estimates of approximately 30% [2, 6, 11, 12]. However, our study is likely to be more accurate, since comparisons of oral and i.v. administration were made in the same patients to minimise the known interpatient variability in the handling of anthracyclines. In common with other authors [2, 6, 11], we found that the blood levels of 4-DMDNR followed a triphasic decay pattern ($T^1/_{2\alpha}$, 9.6 min; $T^1/_{2\beta}$, 3.2 h and $T^1/_{2\gamma}$, 34.7 h). The short alpha half-life and high volume of distribution indicate an early egress from the extracellular fluid, although the former is also attributable in part to rapid conversion to 13-OH4DMDNR.

It is interesting to note that the ratio of AUC metabolite to AUC 4-DMDNR after i.v. administration was 2.1:1, as opposed to 4.0:1 after oral administration. Thus, considerably more 13-OH4DMDNR is formed following oral administration, probably as a result of a first-pass effect. This may explain the higher incidence of gastrointestinal side-effects occurring when the drug is taken by mouth [3]. When vomiting does occur this is usually 2–4 h after administration, coinciding with the peak levels of both drug and metabolite.

The results of the oral study show that there was no significant alteration in the metabolism of 4-DMDNR with continuous weekly treatment. In particular, there was no accumulation of 13-OH4DMDNR and no reduction in $T^1/_{2el}$ for the parent drug or metabolite. Although there was some intrapatient variability the absorption of 4-DMDNR, as assessed by the AUC, remained constant in the majority of patients.

Studies of the pharmacokinetics of doxorubicin and 4′epiadriamycin during successive courses of therapy at 21-day intervals have demonstrated a small increase in clearance and a shortening of the alpha half-life but no change in the elimination half-life for these drugs [9, 14, 15]. The present study shows that even with a weekly schedule the pharmacokinetics of 4-DMDNR also change little with time.

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