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Low dose oral administration of 4-demethoxy-daunorubicin (idarubicin) in advanced cancer patients

A pharmacokinetic study

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Summary. Data relating to 4-demethoxydaunorubicin (DMDR) pharmacokinetics after oral administration (10–15 mg/m^2 per day for 3 days) were collected in a total of 12 patients with advanced breast cancer and melanoma.

Drug absorption took place in the first 2–4 h after administration. Plasma levels of the reduced metabolite DMDRol were higher than those of the parent compound: Peak levels were 4–10 ng/ml for DMDR and 15–40 ng/ml for DMDRol. The dose-corrected area under the time-concentration curve (AUC) was consequently higher for DMDRol (12.3–74.7, mean 32.6 vs 2.4–7.4, mean 4.6 ng/ml.mg for DMDR).

Apparent plasma terminal half-lives after the last dose administered were in the range of 13–36 (mean 23.7) h for DMDR and 30–81 (mean 58.9) h for DMDRol.

Drug and the reduced metabolite accumulated in the blood cells; the ratio of AUC (blood) to AUC (plasma) was 1.40–3.75 (mean 2.80) for DMDR and 1.29–3.50 (mean 2.16) for DMDRol.

The biliary excretion of the drug and of the fluorescent metabolites was studied in two additional patients with extrahepatic obstruction and percutaneous biliary drainage. In the first 7 days of therapy, biliary excretion (DMDR + DMDRol) accounted for 3.7%–4% of the administered dose.

In contrast to our observations with doxorubicin and epirubicin, urinary excretion seems very likely to be more important for this drug than biliary excretion. In these patients urinary excretions were 2.2, 2.9 times (for DMDR) and 1.2, 3.4 times (for DMDRol) the biliary excretion.

Introduction

4-Demethoxydaunorubicin (idarubicin, DMDR) is a new daunorubicin analogue endowed with higher biologic activity and lower cardiotoxicity than the parent compound [1]. Antitumor activity was observed in phase I clinical trials following both IV and PO administration [3, 4, 8, 9, 12]; the suggested dosages for phase II trials were 12.5–15 mg/m^2 for IV bolus administration and 40–50 mg/m^2 for a single administration PO.

A phase II study on the oral use of DMDR in advanced breast cancer and disseminated melanoma is currently in progress at our institute [13]. The cumulative dose proposed following phase I trials is to be administered over 3 consecutive days.

Results relating to DMDR pharmacokinetics and disposition during this study are reported in this paper.

Experimental

Patients and drug administration

The subjects of this study were seven patients with advanced breast cancer and five with disseminated melanoma, all of whom were enrolled in a phase II clinical study carried out of our Institute. All were resistant or no longer responsive to a standard treatment.

DMDR, supplied by Farmitalia Carlo Erba (Milan) in 5-mg and 10-mg capsules was administered at a dose of 15 mg/m^2 daily for 3 consecutive days. It was planned that this cycle should be repeated every 21 days until disease progression.

One of these patients was followed during two cycles with different dosages [patient 9 (CGI), 15 mg/m^2 × 3 and 10 mg/m^2 × 3].

In addition, a study of the biliary excretion of DMDR was carried out in two patients [1 (FE) and 2 (PV)] with gastrointestinal cancer, extrahepatic obstruction, and percutaneous biliary drainage. These two patients underwent gastrectomy.

Sample collection

Blood. Immediately before and at various times (1, 2, 4, 8, 24, 28, 32, 48, 49, 50, 52, 56, 60, 72, 80, 96, 120, and 144 h) after the first treatment, venous blood samples (4–6 ml) were drawn with disposable syringes. Part of each sample was immediately centrifuged at 1000 rpm for 10 min. Whole blood and plasma fractions were collected in disposable tubes, carefully protected from light, and kept frozen at −20 °C until analysis, which was performed within 24 h.

Bile. Bile was continuously collected from the biliary drainage in a plastic bag protected from light. At various intervals the total amount of excreted bile was recorded and a sample of 4–5 ml was collected for analysis; the remainder was disposed of.

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Samples were kept frozen at −20 °C in light-protected tubes until analysis.

Urine. Urine was continuously collected and the total urine output was recorded at regular intervals; samples were kept frozen at −20 °C in light-protected tubes until analysis.

Analytical methods

Plasma extraction. Stock aqueous solution of daunorubicin hydrochloride 100 µl as internal standard and phosphate buffer 1 ml (pH 8) were added to the plasma samples in silanized centrifuge glass tubes with PTFE-lined screw caps. The mixture was extracted with 10 ml chloroform : eptanol 9:1 in a vortex mixer for 10 min, then centrifuged at 4000 rpm for 10 min. The organic layer was collected and extracted with 0.3 ml 0.3 *M* phosphoric acid in a vortex mixer. The acidic phase was carefully removed and washed with 2 ml *n*-hexane to remove nonpolar contaminants.

Aliquots (10–100 µl) of the acidic phase were analyzed by high-pressure liquid chromatography.

Following the same procedure, blood bank plasma samples spiked with known amounts of DMDR and metabolites were extracted for calibration of the analytical method.

Blood. Stock solution of internal standard 100 µl, phosphate buffer 1 ml (pH 8), and methanol 1 ml were added to blood samples (1 ml); the mixture was sonicated for 10 min, then centrifuged. The surnatant was then processed as described for plasma samples.

Bile and urine. Bile and urine samples (1 ml) were both extracted following the procedure described above for plasma samples or injected directly in to the chromatographic system after addition of the internal standard and dilution with 1 ml distilled water and 1 ml 0.3 *M* phosphoric acid.

For drug concentration between 100 and 1000 ng/ml, analytical results for DMDR and its oxydrilated metabolite were identical following the two procedures. Samples with lower concentrations were determined only after extraction, to optimize the sensitivity; conversely, drug levels above 1000 ng/ml were only determined after dilution and direct injection.

Chromatographic analysis. Chromatographic analysis was performed on a VARIAN model 5000 liquid chromatograph equipped with a Perkin-Elmer 650/10 LC fluorescence detector (excitation wavelength: 470 nm, slit width 10 nm; emission wavelength: 580 nm, slit width 20 nm). A Waters Bondapak CN reverse-phase column (3.9 × 30 cm; 10 µm) was used; the mobile phase was 20% acetonitrile + 0.03 *M* phosphoric acid, 80% KH_2PO_4 10 m*M*. The flow rate was 1 ml/min. Quantitation was performed on line with a Perkin Elmer SIGMA 10 data system, using Internal Standard calculations. Chromatographic peaks were identified by comparison with authentic specimens. The minimum detectable concentration was 0.1 ng/ml for both DMDR and DMDRol.

Results

The mean plasma and blood levels of DMDR and DMDRol measured in our patient sample are reported in Figs. 1 and 2. Blood levels were determined in only four patients for the dose of 15 mg/m² per day and four patients for 10 mg/m² per day. After oral administration DMDR is readily absorbed and a concentration peak is reached in 2–4 h. No cross-over experiments were carried out with the drug administered IV; no data are therefore available in this study on the absolute amount fo drug absorbed after oral administration.

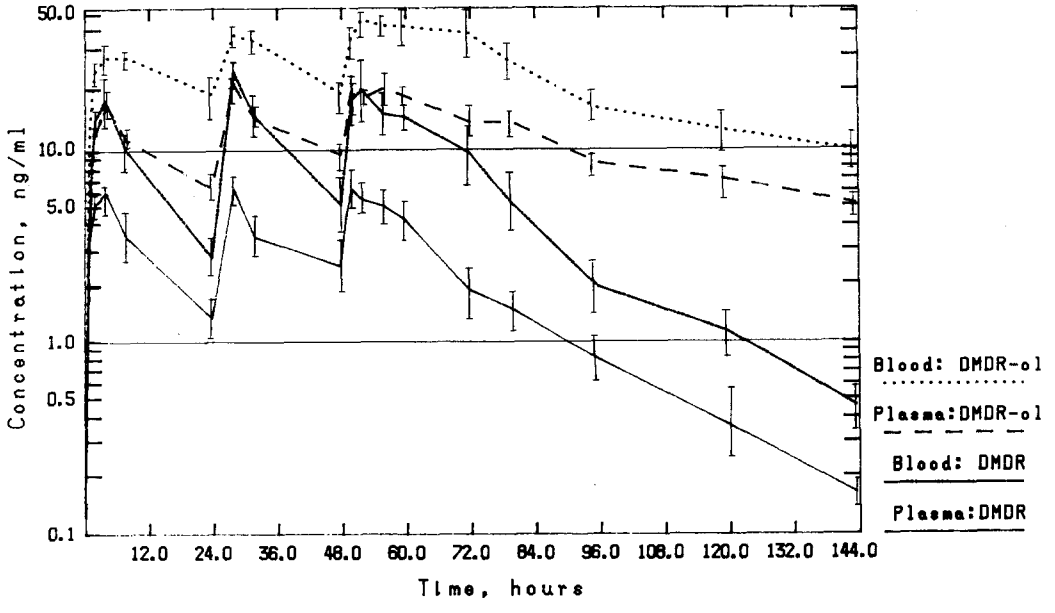

Fig. 1. Mean plasma (9 pts) and blood (4 pts) levels of DMDR and its metabolite DMDRol after three daily doses of 15 mg/m² DMDR PO

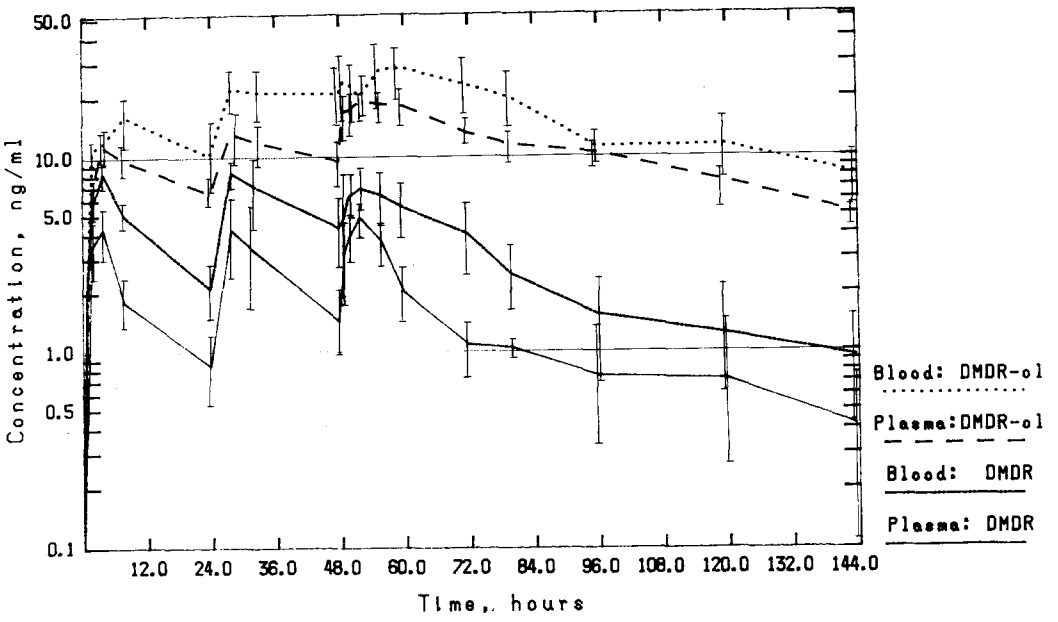

Fig. 2. Mean plasma and blood levels (4 pts) of DMDR and its metabolite DMDRol after three daily doses of 10 mg/m² DMDR PO administration of DMDR

Table 1. 4-Demethoxydaunorubicin (idarubicin, DMDR) pharmacokinetics: Terminal half-lives and dose-corrected areas under the time-concentration curve

Pts		Dose mg/m² × 3	Terminal half life (h)				AUC (ng · h/ml · mg)			
			DMDR		DMDRol		DMDR		DMDRol	
			Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood
1[a]	FE	15	27	–	30	–	2.6	–	9.2	–
2[a]	PV	15	29	–	77	–	2.8	–	10.5	–
3	PE	15	21	–	–	–	5.3	–	10.5	–
4	SA	15	14	–	–	–	3.2	–	20.2	–
5	MV	15	16	–	60	–	–	–	–	–
6	CGU	15	29	–	50	–	6.4	–	27.7	–
7	VN	15	17	–	75	–	3.7	–	13.9	–
8	RB	10	13	24	45	61	6.3	18.3	74.7	170.1
9[a]	CGI	15	35	13	75	72	4.0	14.4	35.7	88.2
9[b]	CGI	10	22	18	71	77	3.9	13.5	63.6	82.1
10	ZA	10	34	15	58	60	3.0	4.2	12.3	43.1
11	FG	10	–	58	43	44	–	23.7	33.7	48.4
12	BL	15	–	31	–	64	–	21.8	–	53.1
13	PR	15	15	–	46	46	7.4	12.8	21.7	41.8
14	FU	15	36	27	55	85	2.4	9.0	22.1	49.0
Mean			23	27	58	64	4.6	14.7	30.6	72.0

[a] Gastrectomized patients; not included in the mean

Plasma and whole blood levels of the C-13-reduced metabolite DMDRol were higher than those of the parent drug in all the samples analyzed.

Apparent terminal half-lives were computed by nonlinear least-square fit (BMDP PAR program [10], biexponential equation) of the experimental data following the third administration and are reported in Table 1.

This determination is biased by the small number of samples in the terminal decay phase and by the extremely low concentrations of both compounds observed after the last day of the treatment. Data in Table 1 must therefore be considered as only indicative; better determination of half-lives could be achieved by analyzing concentration data following IV treatment. In five cases it was not possible to achieve a satisfactory correspondence between the experiment and computed data.

Interpatient spread of drug bioavailability is also documented in Table 1, reporting the dose-corrected areas under the time-concentration curve (t = 0 to infinity) relative to DMDR and DMDRol. These data were computed following the trapezoidal rule in the 0–144 h time interval;

Table 2. Urinary and biliary excretion of 4-demethoxydaunorubicin (idarubicin, DMDR) after three daily oral administrations (15 mg/m² × 3)

Time (h)	Bile excreted (μg)				Urine excreted (μg)					
	Patient 1		Patient 2		Patient 1		Patient 2		Mean, 5 Pts	
	DMDR	DMDRol	DMDR	DMDRol	DMDR	DMDRol	DMDR	DMDRol	DMDR	DMDRol
0– 8	43.9	199.8	35.0	522.5						
8– 24	16.0	224.0	45.0	270.0						
24– 48	66.0	574.0	123.0	770.0						
0– 48	125.9	997.8	203.0	1526.5						
48– 72	83.7	854.9	95.5	425.5	180.0	735.0	238.0	1204.0	450.0	1853.0
72– 96	32.0	592.0	16.0	274.0	42.0	448.0	32.0	664.0	164.6	1097.0
96–120	0.0	186.0	2.5	77.5	28.0	434.0	33.0	680.0	34.7	612.6
120–144	0.0	130.0	0.0	120.0	0.0	525.0	22.0	528.0	21.6	593.1
48–144	115.7	1762.9	114.0	897.0	250.0	2142.0	325.0	3076.0	670.9	4155.7

the 144-h to infinity term was obtained as the ratio C (144)/(slope of the terminal decay phase) [11, 15].

Appreciable drug absorption took place even in the two patients subjected to gastrectomy [Fig. 1; Pt 1 (FE) AUC/dose = 2.6 (DMDR) and 9.2 (DMDRol); Pt 2 (PV) 2.8 (DMDR) and 10.5 (DMDRol)]; the means for the remaining patients were 4.56 for DMDR and 32.6 for DMDRol.

Table 2 shows urinary and biliary excretion data for both DMDR and DMDRol. Unfortunately, in the original protocol urine sampling was not programmed to start until 48 h after the first treatment, and a complete determination of urinary excretion was therefore not possible. Direct analysis of both urine and bile samples did not reveal the presence of appreciable amounts of other fluorescent metabolites besides DMDRol.

In the two patients with extrahepatic obstruction and percutaneous biliary drainage, in the time interval observed urinary excretion was more important than biliary excretion.

Discussion

The new anticancer antibiotic 4-demethoxydaunorubicin is readily absorbed after oral administration; appreciable drug absorption took place even after gastrectomy in two patients. The bioavailability of the C-13-reduced metabolite DMDRol is 2–16 times (mean 7.0, 10 pts) the availability of the parent drug, as expressed in terms of areas under the time-concentration curves. This metabolic pathway is quantitatively different in the closely related antibiotics doxorubicin and epirubicin. The plasma concentration of doxorubicinol can actually exceed that of doxorubicin 2–3 days after drug administration, but the AUC ratio remains in the range of 0.57–0.77 (mean 0.69 4 patients) [6]. In our experience, plasma levels of epirubicinol are never higher than those of the unmetabolized drug, and the AUC ratios between reduced metabolite and parent drug are in the range of 0.15–0.87 (mean 0.33, 26 pts). The main metabolic pathway for this drug is the glucuronation of the oxydril in the daunosamine moiety [6, 7].

DMDRol was reported to be active as an anticancer agent [2]. In view of the high levels of this compound observed in cancer patients following DMDR therapy, a better understanding of its pharmacological properties is hoped for.

DMDR and DMDRol blood concentrations are consistently higher than plasma concentrations, indicating appreciable drug complexation to blood cells. Drug and metabolites bound to RBCs are not easily available for hepatic metabolism or antitumor activity.

By dividing the DMDR cumulative dose into three subsequent oral daily treatments, the high initial concentration peak typical of IV bolus administration or – to a lesser extent – of a single oral dose was avoided.

The incidence of cardiac toxicity in anthracycline therapy is generally correlated with cumulative dosages, rather than with drug plasma concentrations. This notwithstanding, it is worth remembering that two generally accepted mechanisms rationalizing cardiac toxicity, i.e., anthracycline-induced perturbation of calcium flux and anthracycline-induced excess of free radicals, have been demonstrated in vitro only in the presence of quite high drug concentrations. These high levels are more similar to those observed in the initial concentration peak of a conventional treatment than to those detected in our study.

Results concerning the clinical efficacy and toxicity of this treatment schedule are to be published elsewhere [14]. At present, among a total of 25 advanced breast cancer patients 7 partial remissions were achieved, with acceptable conventional toxicity. Only in two cases were minor ECG alterations detected.

Finally, we are currently undertaking studies on the influence of renal and liver functions on DMDR pharmacokinetics.

Our data concerning epirubicin pharmacokinetics indicate that hepatic metabolism is more important than renal excretion. The influence of liver impairment was therefore found to be of fundamental importance in determining drug clearance, whereas renal functions were relevant only in the case of severe renal failure [5].

Our findings on DMDR excretion, albeit incomplete, suggest the possibility of a different relevance of renal functions for drug disposition.

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