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Pharmacokinetics of 4-demethoxydaunorubicin in cancer patients

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Summary. The clinical pharmacokinetics of 4-demethoxydaunorubicin was investigated in 28 cancer patients who received the drug orally. The majority of the patients were elderly (median age, 72 years). Nine of them also received an i. v. dose, and the bioavailability of the oral dose ranged between 9% and 39%. 4-Demethoxydaunorubicin peak levels were achieved 2–4 h after the oral dose in most patients. The drug was rapidly and extensively metabolized to 4-demethoxy-13-hydroxydaunorubicin, which is probably as active as the parent drug. The metabolite levels were much higher and longer lasting than the parent drug, suggesting that it may play an important role in the drug's pharmacological effects.

Introduction

4-Demethoxydaunorubicin (4-DMDR) is a synthetic analogue of daunorubicin (without the methoxyl group in position 4) [1] that is effective against several tumoral models in vitro and in vivo [4, 6, 7, 17] and is more potent than daunorubicin and doxorubicin [1, 4]. These properties, together with its lower cardiac toxicity [8], suggested a more promising therapeutic profile for 4-DMDR than for the other anthracyclines currently used in clinical practice. Preliminary trials in humans gave good results in acute leukemia [5, 9, 12] and Hodgkin's and non-Hodgkin's lymphomas [3]. In addition, 4-DMDR is the first anthracycline available in the oral form. The interesting preliminary results obtained with this formulation [2, 11, 14] suggested that an investigation of the disposition and pharmacokinetics of 4-DMDR after oral administration would be worth-while. The disposition of 4-DMDR has previously been studied in only small series of cancer patients [10, 13, 16, 18]. As this drug appears promising, further studies in larger series of patients are warranted, particularly in elderly patients, who are the preferential candidates for oral anthracycline therapy. This consideration prompted us to carry out these pharmacokinetic studies in a relatively large group of patients, most of them elderly.

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Patients and methods

Patients. Table 1 reports the main characteristics of the 28 cancer patients studied; only 6 of them were younger than 60 years of age and 16 were older than 70 (median age, 72 years). The Karnofsky performance status (PS) was 40–90. Patients 1, 5, 6, 13, 16, 20 and 25 had undergone chemotherapies including the following drugs: Adriamycin, bleomycin, cytosine arabinoside, cyclophosphamide, cisplatin, methotrexate, nitrogen mustard, prednisone, procarbazine, 6-thioguanine, vincristine and etoposide. Patients 6, 12 and 13 had bilirubin values of 2, 2.2 and 1.5 mg/100 ml (normal value, <1.2 mg/100 ml); patients 6, 12, 13, 16, 20, 26 and 27 had lactic dehydrogenase (LDH) values of 1,110, 761, 5,375, 1,370, 2,430, 570 and 908 IU/l, respectively (normal values, 230–460 IU/l); patients 6, 12, 13, 16, 18, and 20 had γ-glutamyl transferase (γGT) values of 467, 88, 343, 188, 73 and 374 respectively (normal values, 32 and 50 IU/l for females and males, respectively); patients 6, 8, 12, 13, 18, 20 and 26 had alkaline phosphatase values of 473, 557, 365, 2,078, 320, 680 and 323 IU/l, respectively (normal values, 9–279 IU/l).

Drug treatment. 4-DMDR was supplied by Farmitalia Carlo Erba, Milan, Italy. After 12 h fasting, the 28 patients received 4-DMDR p. o., as capsules of 5, 10 and 25 mg at the doses reported in Table 1. For the bioavailability study nine patients received an i. v. push injection. The injectable drug was supplied in 5-mg vials that were diluted with 0.9% NaCl solution just before administration. Patients 1–5 and 9 received an initial oral dose of 4-DMDR and an i. v. dose after an interval of at least 15 days; the sequence was reversed for patients 6, 7 and 8.

Sample collection. Blood samples (5 ml) were collected into heparinized tubes at O (before treatment) and 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96 and 120 h after the oral or i. v. dose. After centrifugation at 2,000 rpm, plasma samples were frozen at $-20°$ C until assayed.

Drug assay. Plasma levels of unchanged 4-DMDR and its metabolite, 4-demethoxy-13-hydroxydaunorubicin (4-DMDRol), were assayed by an HPLC method using a fluorimetric detector [15]. The method can be described as follows: 2 ml 0.25 *M* borate buffer (pH 8.4) in 1 *M* NaCl and 40 ng doxorubicin (as the internal standard) were

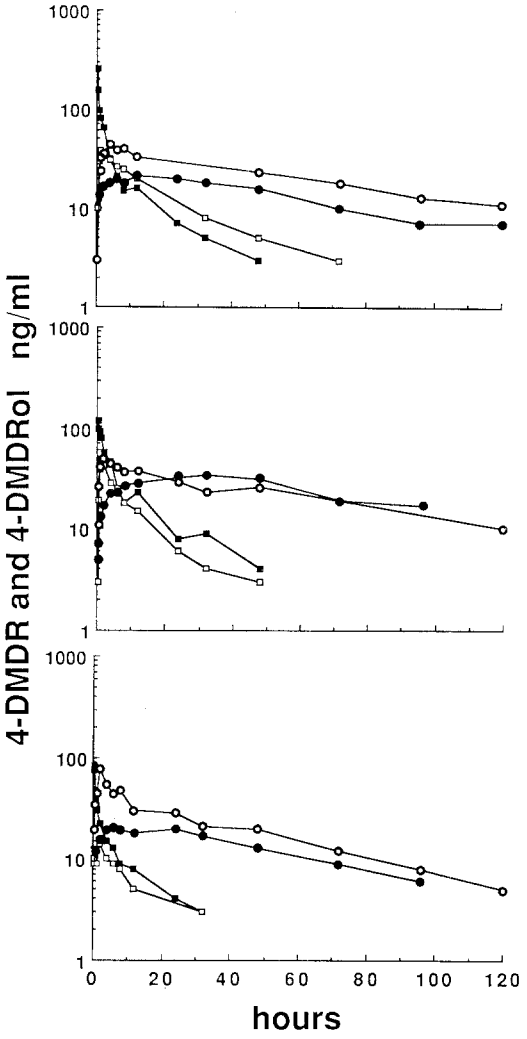

Fig. 1. 4-DMDR and 4-DMDRol plasma decay curves in cancer patients 3 (*top*), 6 (*middle*) and 9 (button), who received 4-DMDR both orally and intravenously. (■), 4-DMDR and (●), 4-DMDRol, after i.v. administration; (□), 4-DMDR and (○), 4-DMDRol, after p.o. administration

added to 1–2 ml plasma. The sample was extracted with 10 ml solvent mixture of chloroform and-1-heptanol (9:1) under 40-min shaking. After centrifugation, the organic phase containing the compounds was washed with 2 ml water and reextracted with 0.3 ml 0.3 *M* phosphoric acid for 10 min. A sample of the aqueous phase was injected into a Perkin-Elmer HPLC equipped with a fluorimetric detector (excitation wavelength, 475 nm; emission wavelength, 580 nm). Separation was achieved with an isocratic solvent system of CH_3CN: KH_2PO_4 0.05 *M* (35:65) at a flow rate of 1 ml/min with a 25-cm-long Waters μBondapak phenyl column.

Pharmacokinetic analysis. The half-lives of 4-DMDR and 4-DMDRol were determined by regression analysis of the log concentration vs time data in the elimination phase of the drugs. The AUC values were computed by the trapezoidal rule from time O up to the last experimental point plus the extrapolated portion of the curve to infinity ($C_{t/\beta}$). The bioavailability, F, was calculated using the following formula:

$$F = (AUC_{p.o.}/AUC_{i.v.})\,(Dose_{i.v.}/Dose_{p.o.})$$

Results

Figure 1 shows the 4-DMDR and 4-DMDRol plasma decay curves in three cancer patients after intravenous and oral doses of 4-DMDR. In all patients and by both administration routes, 4-DMDR rapidly disappeared in the first 24 h, with 4-DMDRol plasma levels persisting longer than those of the parent drug, still being detectable at 120 h. No other metabolites were found in the plasma samples under our experimental conditions.

Table 2 reports the peak plasma levels, $t_{1/2}$ and AUC values for 4-DMDR and 4-DMDRol in 28 patients. In most cases after oral administration, peak 4-DMDR levels were achieved at 2 or 4 h. The $t_{1/2}$ and AUC values varied widely in the subjects studied. Peaks, $t_{1/2}$ and AUC values for 4-DMDRol also varied, but less than those obtained for the parent compound.

The drug bioavailability was low and variable, ranging between 9% and 39% (Table 3), and the ratios of AUC values after i.v. and oral 4-DMDR were variable as well. Since 4-DMDRol is reported to be as cytotoxic as 4-

Table 1. Patient's main characteristics

Patient Number	Dose p.o. (mg/m^2)	Sex	Age (years)	Performance status	Tumor[a]	Renal function, creatinine (mg/100 ml)
1	30.3	M	66	70	ren.ca.	2.0
2	31.3	M	76	80	MM	1.0
3	34.0	M	76	80	NHL	1.2
4	31.2	M	70	80	NHL	0.7
5	38.1	M	45	70	MM	1.6
6	25.0	F	47	60	AML	0.9
7	38.7	M	67	60	MM	1.4
8	39.3	M	77	60	CLC	0.6
9	35.7	F	73	80	MM	1.3
10	31.3	F	84	60	NHL	1.2
11	29.2	F	71	50	MM	0.6
12	34.4	M	82	50	NHL	2.0
13	28.8	M	55	70	col.ca	0.8
14	30.6	F	70	80	NHL	0.8
15	25.3	M	70	50	SCLC	0.9
16	45.0	M	41	90	AML	1.4
17	33.1	M	80	60	MM	1.0
18	34.9	M	73	90	NHL	1.1
19	35.0	F	80	60	NHL	1.0
20	44.3	M	42	60	NHL	0.8
21	36.0	M	72	90	MM	2.4
22	35.2	F	75	40	MM	1.1
23	34.6	F	76	90	MM	1.2
24	33.3	F	78	50	MM	1.2
25	46.2	F	32	50	NHL	0.8
26	44.3	M	63	80	NHL	0.9
27	38.4	M	61	50	NHL	1.1
28	30.7	M	79	60	NHL	1.4

[a] ren.ca., renal cancer; MM, multiple myeloma; NHL, non-Hodgkins lymphoma; AML, acute myeloid leukemia; CLC, chronic lymphatic leukemia; col.ca., colon cancer; SCLC, small-cell lung cancer

Table 2. Pharmacokinetic parameters of 4DMDR and its metabolite 4-DMDRol in cancer patients after oral and/or i.v. 4-DMDR

Patient number		4-DMDR				4-DMDRol			
		Peak (ng/ml)	Time (h)	$t_{1/2}$ (h)	AUC (ng/ml·h)	peak ng/ml	Time (h)	$t_{1/2}$ (h)	AUC (ng/ml·h)
1	p.o.	7.1	4.00	7.50	131	23.5	2.0	183.0	3,702
	i.v.	106.0	0.25	14.86	350	20.3	12.0	136.7	1,890
2	p.o.	5.3	4.00	7.50	64	11.9	4.0	53.0	828
	i.v.	118.0	0.25	20.65	350	22.4	8.0	62.0	2,526
3	p.o.	38.1	1.00	23.30	905	44.3	4.0	62.0	3,552
	i.v.	256,0	0.25	18.00	762	21.2	12.0	58.7	2,172
4	p.o.	26.3	4.00	16.80	414	22.6	4.0	48.3	1,080
	i.v.	224.0	0.25	14.10	612	23.3	4.0	33.1	1,212
5	p.o.	25.0	2.00	7.60	240	56.0	2.0	44.0	2,076
	i.v.	64.8	0.25	9.01	281	12.5	8.0	39.2	738
6	p.o.	48.8	1.00	10.60	533	5.1	2.0	56.0	894
	i.v.	120.0	0.25	16.00	888	33.7	32.0	68.2	4,122
7	p.o.	8.9	4.00	9.60	136	23.0	6.0	31.8	948
	i.v.	50.2	0.25	7.1	168	10.8	32.0	57.9	1,146
8	p.o.	10.3	2.00	6.3	102	90.5	2.0	68.4	4,332
	i.v.	45.3	0.25	3.4	114	8.2	4.0	144.0	1,074
9	p.o.	14.1	2.00	12.7	241	77.3	2.0	44.2	2,538
	i.v.	83.3	0.25	10.2	351	20.3	6.0	44.2	1,686
10	p.o.	39.5	4.00	18.3	612	47.0	6.0	53.9	2,652
11	p.o.	56.6	4.00	11.1	255	17.5	4.0	46.0	2,178
12	p.o.	10.7	2.00	NE	313[a]	71.4	8.0	45.3	5,142
13	p.o.	57.5	1.00	14.0	451	43.0	2.0	51.7	2,064
14	p.o.	12.0	2.00	8.2	203	29.1	6.0	48.6	1,218
15	p.o.	70.0	1.00	6.7	407	129.0	1.0	47.0	3,120
16	p.o.	32.3	2.00	14.02	585	58.9	4.0	50.7	4,272
17	p.o.	12.6	2.00	5.2	110	20.1	4.0	50.8	774
18	p.o.	32.5	2.00	15.5	454	54.4	2.0	56.3	2,556
19	p.o.	18.1	4.00	6.1	173	38.4	4.0	51.4	1,662
20	p.o.	22.8	4.00	3.3	152	125.6	4.0	31.8	3,702
21	p.o.	5.2	6.50	7.7	74	38.3	6.0	86.6	2,160
22	p.o.	29.7	1.00	18.1	282	128.9	4.0	33.6	4,920
23	p.o.	5.4	2.00	9.4	78	61.9	4.0	55.8	4,122
24	p.o.	5.3	2.00	4.7	43	34.5	2.0	51.6	1,332
25	p.o.	26.9	4.00	8.3	313	93.9	4.0	43.0	3,612
26	p.o.	9.3	2.00	10.1	232	35.8	6.0	29.0	1,392
27	p.o.	15.9	0.75	8.9	199	85.5	2.0	57.0	3,336
28	p.o.	16.3	2.00	NE	90	16.5	4.0	137.0	1,650

[a] Experimental AUC (not extrapolated to infinity)
NE, not evaluable

DMDR [8], we calculated the sum of the AUC values for 4-DMDR and its metabolite, which is probably better related to the pharmacological effects than is the AUC of the parent drug. The "total bioavailability" (drug plus metabolite) is also shown, ranging between 15% and 142%. No significant difference in any pharmacokinetic parameter of the drug or its metabolite, 4-DMDRol, was found between patients with normal and altered hepatic function.

Discussion

Several reports show that 4-DMDR is an anthracycline that is clinically effective when given orally. This finding stimulated interest in further investigating the pharmacological and therapeutic properties of this drug. Compared with other clinically used anthracyclines, it is unique in at least two features: (1) it is absorbed when given orally; and (2) it is extensively metabolized to 4-DMDRol, which is eliminated very slowly from the body.

Our study essentially confirms and extends the published clinical pharmacokinetics of 4-DMDR. Gillies et al. [10] investigated the bioavailability of 4-DMDR in nine patients and reported a mean bioavailability of 29%, with a range of 4%–77% Smith et al. [16] found a bioavailability of 23.8% in five cases, with a range of 8.9%–38.9% and Lu et al. [13] found that of 38.7% in ten patients (range, 7.2%–97.6%). In the present study involving a population of elderly patients we found the bioavailability of 4-DMDR to be in the same range as previously reported in younger patients [10, 16, 18].

Our study also confirms that the metabolite 4-DMDRol remains in plasma at a relatively high concentration for a long time after 4-DMDR administration. Since the metabolite appears to be as cytotoxic as the

Table 3. Bioavailability of 4-DMDR in cancer patients after oral and i. v. 4-DMDR

Patient number	Dose i. v. (mg)	Dose p. o. (mg)	Bioavailability 4-DMDR (%)	Bioavailability 4-DMDR + 4-DMDRol[a] (%)
1	25	50	19	86
2	25	50	9	15
3	20	60	39	51
4	24	50	32	38
5	18	80	19	51
6	24	39	37	17
7	21	65	27	27
8	21	55	35	142
9	20	60	23	45

[a] Bioavailability (4-DMDR + 4-DMDRol) was calculated by the following formula:

$$\frac{\text{AUC 4-DMDR + AUC 4-DMDRol (after p. o. 4-DMDR dose)}}{\text{AUC 4-DMDR + AUC 4-DMDRol (after i. v. 4-DMDR dose)}} \times \frac{\text{i. v. dose of 4-DMDR}}{\text{p. o. dose of 4-DMDR}}$$

parent drug [7], it probably plays a major role in the antineoplastic activity of 4-DMDR. The mean half-life of 4-DMDRol found in our study (58 ± 31.60 h) was similar to that described in previous studies [10, 16]. However, the elimination rate of this metabolite appeared to vary more widely in our population of patients. In contrast to previous reports, we did not find reproducibly higher levels of the metabolite when the drug was given orally. It may be that the metabolic efficiency at first passage through the liver was not efficient in all cases; again, this might have been influenced by the patients' age, which was higher in our group.

No changes in the kinetics of 4-DMDR or its metabolite were observed in patients with abnormal hepatic tests. Differences may have been hard to detect because of the wide variability. In addition, our patients were mostly suffering from mild liver insufficiency; thus, we cannot exclude that clearance of the drug and its metabolite may be affected in patients with severe hepatic dysfunction. In mild renal insufficiency as well no clearcut changes were found in the kinetics of 4-DMDR and 4DMDRol.

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