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Epirubicin and doxorubicin comparative metabolism and pharmacokinetics

A cross-over study

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Summary. The pharmacokinetics and metabolism of doxorubicin (DX) and epirubicin (epiDX) were investigated in eight cancer patients who received 60 mg/m^2 of both drugs independently by intravenous (i.v.) bolus at 3-week intervals according to a balanced cross-over design. Unchanged DX and epiDX plasma levels followed a triexponential decay. Half-lives (t/2) of the three decay phases were longer for DX (t/2α: 4.8 vs. 3 min; t/2β 2.57 h vs. 1.09 h; t/2γ 48.4 vs. 31.2 h). According to a model-independent analysis, the different plasma disposition kinetics of the two compounds appears to be related to a higher plasma clearance (PlCl) and to a lower mean residence time (MRT) of epiDX (PlCl: 75.0 l/h, range: 35.6–133.4 l/h; MRT: 31.6 h, range: 7.0–41.5 h;) compared to DX (PlCl: 56.8 l/h, range: 24.4–119.5; MRT: 45.6 h, range: 26.0–83.1 h). No statistically significant differences could be detected for the volume of distribution at steady state (Vss) (epiDX, 31.8 l/kg; DX, 33.3 l/kg). Metabolites common to both compounds were detected in plasma: the 13-dihydro derivatives doxorubicinol (DXol) and epirubicinol (epiDXol), together with minor amounts of four aglycones (7-deoxy adriamycinone, adriamycinone, 7-deoxy 13-dihydro adriamycinone, and 13-dihydro adriamycinone). Following epiDX administration, two additional major metabolites were detected: the glucuronic acid conjugates of epiDX (4'-*O*-β-D-glucuronyl-4'-epiDX) and epiDXol (4'-*O*-β-D-glucuronyl 13-dihydro-4'-epiDX). This additional detoxication route appears to account for the more efficient and faster elimination of epiDX than of DX. In the urine collected in the 6 days after treatment, 12.2% of the DX and 11.9% of the epiDX dose was excreted as unchanged drug and fluorescent metabolites. A comparable renal clearance was calculated for DX (4.7 l/h, range 1.4–7.0 l/h) and epiDX (4.4 l/h, range 1.7–7.0 l/h). One patient with hepatic metastases and abnormal bilirubin serum level had percutaneous biliary drainage because of extrahepatic obstruction. The elimination of both drugs was significantly impaired in this patient; nevertheless, elimination of epiDX was still more efficient and faster than that of DX (PlCl: 35.6 vs. 24.4 l/h; MRT: 39.0 vs. 83.1 h; t/2γ: 47 vs. 74 h). This patient's biliary excretion accounted for 35.4% of the epiDX dose and 18.2% of the DX dose.

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Introduction

The new anthracycline antibiotic, epirubicin (epiDX), differs from doxorubicin (DX) in the epimerization of the OH group in position 4' of the aminosugar moiety [1].

Preclinical studies in animal models and extensive clinical trials have proven that this compound is less toxic than DX at similar equipotent therapeutic doses [10]. It is of particular interest that epiDX cardiotoxicity is about 0.7 times the toxicity of DX in mice and rats, 0.8 times in rabbits, and 0.4–0.5 times in humans [16]. It has been suggested [5, 7, 15] that the lower toxicity observed in man might depend on a different metabolic pathway available for this new drug.

Two comparative pharmacokinetic studies are already available in the literature. The crossover study carried out by Martini et al. [13], albeit well designed, does not report a complete analysis of DX and epiDX metabolites; the work by Robert et al. [14] is not a crossover study. In addition, in both studies drug decay was not followed for a sufficient span of time, probably because of a limited sensitivity of the analytical method. It is also interesting that after years of clinical use there is still no general agreement on some basic DX pharmacokinetic parameters such as the terminal half-life or the amount of circulating aglycones [3].

Here we report results concerning the comparative pharmacokinetics and metabolism of DX and epiDX in a group of eight cancer patients who received 60 mg/m^2 of both drugs independently in line with a balanced cross-over design. A specific, sensitive HPLC assay with fluorimetric detection was used for the quantitative analysis of unchanged drugs and their metabolites in biological fluids. Its detection limit (about 0.2 ng/ml) allowed plasma concentrations to be followed for up to 7 days after drug administration for an accurate estimation of the slow decay phase typical of anthracycline antibiotics.

Patients and methods

Included in this study were eight inpatients with advanced cancer (Division of Oncology, M. Malpighi Hospital, Bologna, Italy). Eligibility criteria were:

A. Progressive neoplastic disease
B. Prognosis better than 2 months
C. Performance status 50% (Karnofsky) or better
D. WBC count $>4000/mm^3$
E. Platelet count $>120{,}000/mm^3$

Table 1.
(a) Patient characteristics

Patient no.	Age	Weight	Body surface	Performance status	Tumor	Metastases
66	52	71.5	1.76	90	Lung	LN(M)
67	58	66.0	1.74	50	Lung	LN(M)
68	68	74.4	1.72	60	RePe	
69	58	50.0	1.50	40	Stomach	Liver LN(IE)
70	72	45.9	1.40	50	H&N	LN
71	42	80.4	1.92	70	OSS	
72	54	90.0	1.90	70	Lung	Lung LN
73	57	75.0	1.80	100	Kidney	

LN, Lymph nodes; LN(M), Mediastinic lymph nodes; LN(IE), Hepatic lymph nodes; OSS, Osteosarcoma; RePe, Retroperitoneum; H&N, Head and neck

(b) Treatment and side effects

Patient no.	Drug	Date of treatment	Dose (mg)	Side effects	Grade (WHO)
66	Doxorubicin	14/11	105	Leukopenia	1
				Na/Vo	1
				Alopecia	4
66	Epirubicin	05/12	105	Alopecia	NV
67	Doxorubicin	18/12	100	Na/Vo	2
				Alopecia	NV
				Anemia	1
67	Epirubicin	26/11	100	Headache	1
				Alopecia	3
				Anemia	1
68	Doxorubicin	20/03	100	Leukopenia	2
				Na/Vo	3
				Alopecia	2
68	Epirubicin	11/04	100	Na/Vo	3
				Alopecia	NV
69	Doxorubicin	15/04	90	Leukopenia	3
				Alopecia	4
				Anemia	1
				Stomatitis	2
69	Epirubicin	07/05	90	Leukopenia	1
				Alopecia	NV
				Anemia	1
70	Doxorubicin	20/06	90	Alopecia	NV
				Anemia	1
70	Epirubicin	30/05	90	Alopecia	4
71	Doxorubicin	01/07	115	Na/Vo	2
				Alopecia	NV
				Stomatitis	2
				Headache	1
71	Epirubicin	05/06	115	Na/Vo	1
				Alopecia	4
72	Doxorubicin	01/08	115	Alopecia	1
72	Epirubicin	12/09	115	Alopecia	1
73	Doxorubicin	28/10	100	Alopecia	NV
				Anemia	2
73	Epirubicin	02/10	100	Alopecia	4
				Anemia	1

F. Bilirubin level <1.5 mg/100 ml; SGOT and SGPT <100 mU/ml
G. Serum creatinine <1.5 mg/100 ml; blood urea nitrogen (BUN) <50 mg/100 ml
H. No other antitumor treatment in the previous 30 days

Patient no. 69, with hepatic metastases, extrahepatic obstruction, and percutaneous biliary drainage, was also included in the study. All the patients were eligible for standard anthracycline therapy and gave informed consent; relevant clinical data can be found in Table 1a.

The study followed a balanced crossover design: on day 1, with DX (or epiDX), 60 mg/m^2, and after 21 days, with the same dose of the other drug. Table 1b reports the date the drugs were given, individual dosages, and toxic side effects. DX or epiDX (Adriblastina or Farmorubicina, lyophilized powder, commercial vials, Farmitalia, Milan) was given as a rapid intravenous (i.v.) infusion over 1–2 min.

Blood samples were drawn before treatment and at 5, 15, 30 and 60 min as well as at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h after drug administration. The urine excreted was collected for 7 days at 24-h intervals. In one patient (no. 69, extrahepatic occlusion and percutaneous biliary drainage), bile was continuously collected in a light-protected plastic bag. At varying intervals, the volume of bile was measured and a 4- to 5-ml sample was collected for analysis, the remainder being discarded.

The plasma was separated after centrifugation and the urine and bile samples were kept frozen at $-20°$ C in light-protected tubes; drug and metabolite analysis was carried out no later than 48 h after sampling.

Materials and methods

Analytical methods. A novel HPLC assay with fluorimetric detection was applied for the quantitative determination of anthracyclines in biological fluids. The method was specific for the simultaneous assay of the unchanged drugs and their known fluorescent metabolites: doxorubicinol (DXol), epirubicinol (epiDXol), 7-deoxy adriamycinone (metabolite C), adriamycinone (metabolite D), 7-deoxy 13-dihydro adriamycinone (metabolite E), 13-dihydro adriamycinone (metabolite F), 4'-*O*-β-D-glucuronyl-4'-epiDX (metabolite G), and 4'-*O*-β-D-glucuronyl 13-dihydro-4'epiDX (metabolite H). A detailed description of the analytical method has been reported by Camaggi et al. [6].

Pharmacokinetic analysis. DX and epiDX decay curves were first computer-fitted with polyexponential equations,

$$C = \Sigma A_i.\exp(-\alpha_i.t),$$

using the PAR program of the BMDP package [9], running on a Digital PDP 11/24 computer system. The nonlinear least-square fit of the experimental data with a triexponential equation was satisfactory for all patients. By using biexponential equations, the long terminal decay phase typical of both drugs cannot be equally well reproduced. It is also of interest that biexponential and triexponential interpolations do not become statistically different if only data relative to the first 24–48 h are used in the interpolation procedure.

The area under the time-concentration curve (AUC),

$$AUC = \int C dt,$$

was then computed for drugs and metabolites with the trapezoidal rule (0–168 h interval) and by analytical integration of the exponential equation (0 to infinity interval) for the unchanged drugs only. The two integration methods gave consistent results.

A model-independent pharmacokinetic analysis (statistical moments theory) was used for parent drug levels [11]. The mean residence time was obtained as

$$MRT = \int t.C\, dt / \int C\, dt.$$

The plasma clearance (PlCl) and volume of distribution at steady state (Vss) were calculated from the equations:

$$PlCl = Dose/\int C\, dt$$
$$Vss = (MRT.PlCl)/Body\ weight$$

The data in Table 5 were computed using the $\int$ Cdt and $\int$t.C dt terms obtained by analytical integration of the triexponential equation. Similar results can be obtained using terms computed according to the trapezoidal rule. The renal clearance (RCl) was obtained on the basis of the drug concentration in urine:

$$RCl = [Xu]_{0-168}/AUC_{0-168},$$

where $[Xu]_{0-168}$ is the amount of unmetabolized drug eliminated in the urine during the 0–168 h time interval, and AUC_{0-168} is the area under the plasma time-concentration curve between 0 and 168 h (trapezoidal rule).

Statistical analysis of the results was carried out with the BMDP P3D and P3S [9] programs (matched pairs *t*-test, sign test, and Wilcoxon signed ranks test). Levels of significance reported below were computed according to the Wilcoxon signed ranks test; similar (or better) significance was observed with the other tests.

Results

Mean plasma levels of the unchanged drugs and their known metabolites observed in the eight patients of the present study, following 60 mg/m^2 i.v. administrations of DX or epiDX are shown in Fig. 1. The areas under plasma-level curves (AUC) of all compounds, computed according to the trapezoidal rule in the 0–168 h time interval, are reported in Table 2. For both anthracyclines, the unchanged drug plasma-level profile was characterized by an initial rapid decrease lasting 0.5–1 h. It was followed by an intermediate phase, and finally by a slow terminal elimination phase, involving low drug plasma levels apparently established 12–24 h after dosing.

In addition to the unchanged drugs, several metabolites common to both anthracyclines were detected in the plasma (Scheme 1). The reduction of the keto group in the anthracycline side-chain is a main metabolic pathway for both drugs; the C13-reduced metabolites epiDXol and DXol are formed following epiDX and DX treatment, respectively. Small amounts of aglycones maintaining the anthracycline nucleus but lacking the aminosugar moiety can also be observed (metabolites C, D, E, F). Following epiDX administration, two additional metabolites, the glucuronic acid conjugates of EpiDX (metabolite G) and epiDXol (metabolite H) are observed.

The unchanged drug was the predominant circulating species for both anthracyclines. The lowest plasma levels were observed for the four aglycone derivatives C, D, E, and F; the major metabolites in plasma were, therefore,

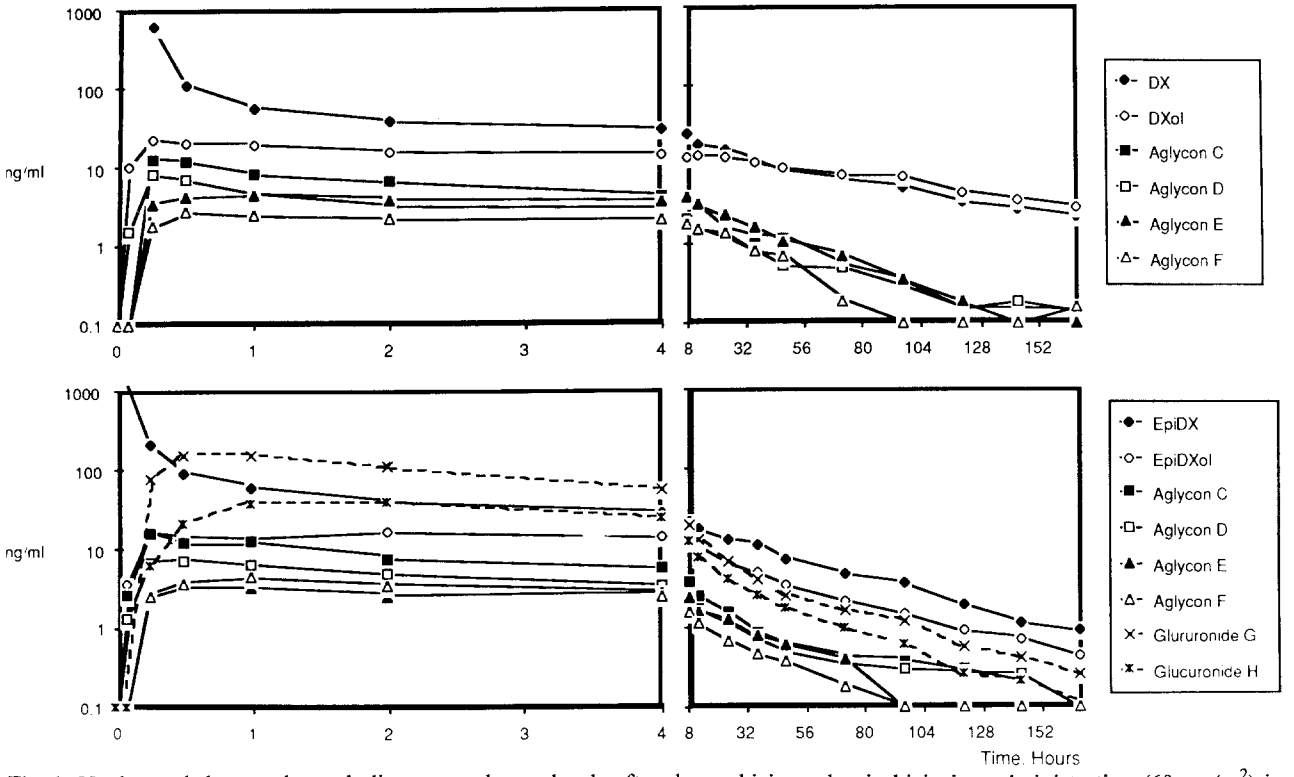


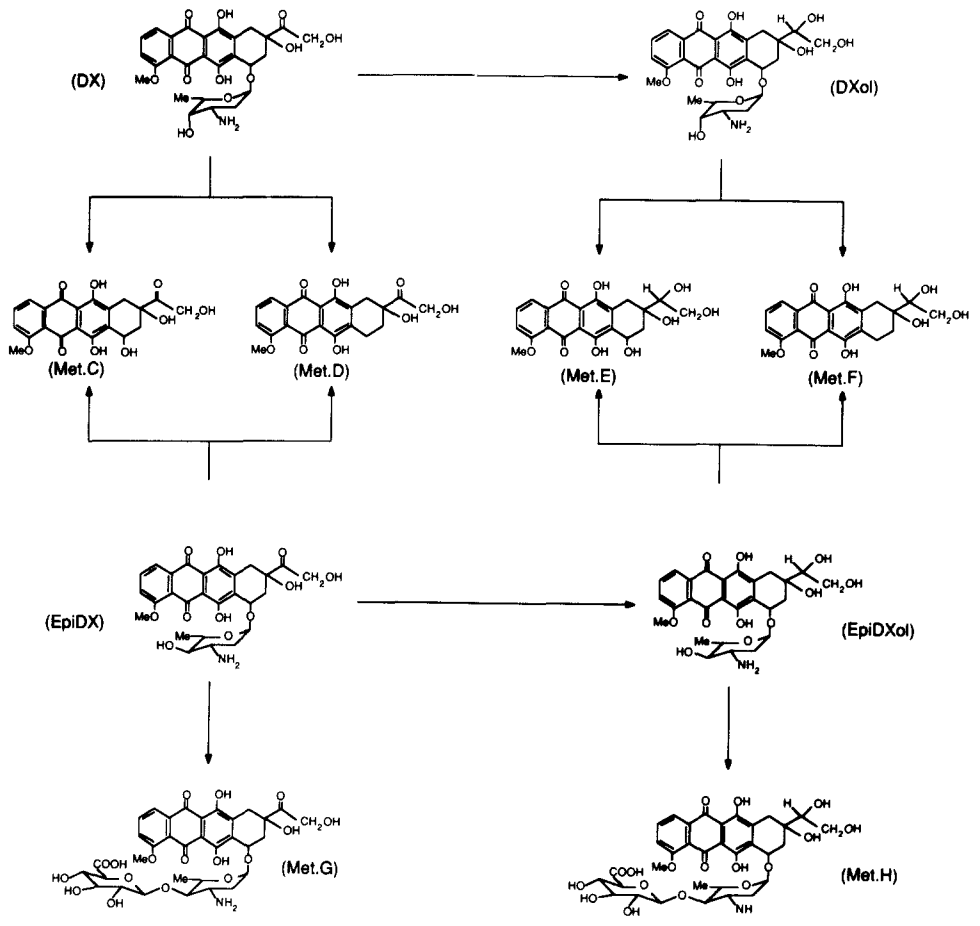
Fig. 1. Unchanged drug and metabolite mean plasma levels after doxorubicin and epirubicin i.v. administration (60 mg/m^2) in eight cancer patients (crossover experiment)

DXol (following DX treatment) and glucuronide G, epiDXol, and glucuronide H (following epiDX administration). As shown in Fig. 1, the apparent elimination rate of all the metabolites during the terminal phase was on the same order or, for glucuronides G and H, faster than that observed for the unchanged drugs in all patients.

The unchanged drug and DXol were the major fluorescent compounds excreted in urine after DX administration (Table 3). Aglycone derivatives were only detected in 3 of 9 patients. On average, the urinary excretion of total fluorescence (0–168 h) accounted for 12.2% of the DX dose, mostly as unchanged drug (9.02%) and DXol (2.49%). A

Table 2. AUC (ng · h/ml) of unchanged drugs and metabolites

Patient no.-drug	DX or epiDX	DXol or epiDXol	Metabolite C	Metabolite D	Metabolite E	Metabolite F	Metabolite G	Metabolite H
66-DX	2497.7	1032.7	151.8	142.6	166.4	114.2	0.0	0.0
66-epiDX	1343.4	550.4	148.4	118.2	117.9	75.3	1357.6	571.9
67-DX	1771.9	508.7	143.2	54.5	98.3	14.7	0.0	0.0
67-epiDX	1366.6	172.6	127.9	28.6	88.7	14.7	1402.5	401.1
68-DX	1683.1	751.5	158.0	40.6	126.3	48.9	0.0	0.0
68-epiDX	1322.2	277.6	130.3	171.9	84.9	53.2	871.7	293.7
69-DX	3227.0	4022.8	960.3	171.2	953.9	211.2	0.0	0.0
69-epiDX	2315.3	1275.8	961.5	668.8	363.6	154.8	2202.6	929.3
70-DX	2486.2	1018.0	273.6	82.4	225.0	103.1	0.0	0.0
70-epiDX	2146.8	1083.9	138.5	51.0	1.7	65.9	783.1	659.3
71-DX	1815.7	1003.1	181.9	254.9	115.6	105.1	0.0	0.0
71-epiDX	1562.5	661.6	60.9	90.4	1.7	77.6	746.3	308.6
72-DX	949.2	613.2	60.7	36.7	161.8	71.7	0.0	0.0
72-epiDX	890.1	363.0	224.7	131.7	186.2	57.7	730.8	230.6
73-DX	1378.4	1436.9	188.4	144.3	278.6	229.1	0.0	0.0
73-epiDX	1017.0	295.3	212.5	172.8	123.9	85.9	1283.2	682.5
Mean-DX	1973.9	1298.4	264.7	115.9	265.7	112.3	0.0	0.0
Mean-epiDX	1495.5	585.0	250.6	179.2	121.1	73.1	1172.2	509.6



Scheme 1

comparable total fluorescence was recovered after epiDX (11.9%), and the rank order of the major anthracycline species excreted in urine was unchanged drug in free (6.39%) and conjugated (2.11%) form, followed by free (0.93%) and conjugated (0.81%) epiDXol.

The results of the pharmacokinetic analysis on the parent drug's plasma-level data are reported in Tables 4a, 4b, and 5. The half-lives of the three decay phases were higher for DX than for epiDX ($t/2\alpha$: 4.8 vs. 3.0 min, $P = 0.036$; $t/2\beta$: 2.57 vs. 1.094 h, $P = 0.050$; $t/2\gamma$: 48.4 vs. 31.2 h,

Table 3. Cumulative urinary excretion (% of the administered dose) of unchanged drugs and metabolites

Patient no.-drug	DX or epiDX	DXol or epiDXol	Metabolite C	Metabolite D	Metabolite E	Metabolite F	Metabolite G	Metabolite H
66-DX	9.30	3.07	0.00	0.00	0.00	0.00	0.00	0.00
66-epiDX	5.03	1.04	2.06	0.00	0.00	0.00	4.71	1.05
67-DX	12.05	1.33	0.00	0.00	0.00	0.00	0.00	0.00
67-epiDX	9.95	0.38	1.56	0.00	0.00	0.00	5.41	1.07
68-DX	11.14	2.04	0.00	0.20	0.00	0.00	0.00	0.00
68-epiDX	8.58	1.22	0.00	0.00	0.00	0.00	4.87	1.06
69-DX	8.60	4.26	0.65	0.48	0.00	0.42	0.00	0.00
69-epiDX	4.58	0.60	0.10	0.00	0.03	0.00	0.98	0.28
70-DX	13.53	3.51	2.21	0.02	1.26	0.19	0.00	0.00
70-epiDX	9.50	1.41	0.37	0.53	0.00	0.00	3.55	1.42
71-DX	6.75	1.79	0.00	0.00	0.00	0.00	0.00	0.00
71-epiDX	9.35	1.76	0.53	0.00	0.00	0.00	3.52	1.31
72-DX	1.21	0.70	0.00	0.00	0.00	0.00	0.00	0.00
72-epiDX	1.54	0.35	0.15	0.04	0.00	0.02	0.88	0.13
73-DX	9.61	3.28	0.00	0.00	0.00	0.00	0.00	0.00
73-epiDX	2.91	0.62	0.11	0.06	0.00	0.00	1.68	0.43
Mean-DX	9.02	2.50	0.36	0.09	0.16	0.08	0.00	0.00
Mean-epiDX	9.43	0.92	0.61	0.08	0.00	0.00	3.20	0.84

Table 4.
(a) Parameters of the triexponential equation**

Patient no.-drug	A	α	B	β	C	γ
66-DX	5411.3	7.377	77.80	0.431	28.68	0.0173
(SD)	(179.9)	(0.272)	(23.38)	(0.187)	(5.22)	(0.0032)
66-epiDX	2618.1	20.061	119.29	0.774	23.93	0.0221
(SD)	(742.9)	(3.582)	(12.11)	(0.109)	(2.09)	(0.0021)
67-DX	4566.9	8.226	27.21	0.188	16.67	0.0143
(SD)	(259.6)	(0.434)	(14.06)	(0.197)	(8.01)	(0.0065)
67-epiDX	6107.8	23.335	101.63	1.222	25.11	0.0220
(SD)	(1335.1)	(2.742)	(12.94)	(0.175)	(1.36)	(0.0014)
68-DX	5070.1	9.178	30.28	0.263	15.22	0.0132
(SD)	(124.9)	(0.200)	(6.01)	(0.106)	(2.66)	(0.0025)
68-epiDX	1276.4	9.396	153.58	1.249	19.00	0.0191
(SD)	(102.6)	(1.151)	(39.67)	(0.281)	(2.24)	(0.0026)
69-DX	1932.1	5.266	113.44	0.239	26.58	0.0093
(SD)	(132.6)	(0.485)	(26.74)	(0.093)	(8.21)	(0.0038)
69-epiDX	10156.6	14.203	115.09	0.305	21.09	0.0147
(SD)	(253.1)	(0.260)	(6.28)	(0.034)	(2.47)	(0.0017)
70-DX	6203.2	8.198	53.07	0.315	23.80	0.0136
(SD)	(118.0)	(0.149)	(8.39)	(0.093)	(2.94)	(0.0018)
70-epiDX	4752.3	13.011	161.69	1.097	43.19	0.0256
(SD)	(162.0)	(0.436)	(18.48)	(0.141)	(2.21)	(0.0015)
71-DX	6352.1	8.121	34.29	0.111	8.99	0.0106
(SD)	(165.3)	(0.183)	(5.94)	(0.043)	(4.19)	(0.0049)
71-epiDX	5310.96	13.438	65.02	0.430	18.78	0.0180
(SD)	(273.4)	(0.540)	(10.29)	(0.123)	(3.13)	(0.0030)
72-DX	2779.1	15.113	21.69	1.076	23.00	0.0303
(SD)	(149.4)	(0.667)	(9.64)	(0.631)	(1.75)	(0.0025)
72-epiDX	2527.65	12.502	89.54	1.239	23.70	0.0404
(SD)	(141.2)	(0.733)	(20.71)	(0.309)	(2.57)	(0.0048)
73-DX	4805.6	15.452	98.95	1.065	22.18	0.0209
(SD)	(179.5)	(0.466)	(10.39)	(0.128)	(1.21)	(0.0013)
73-epiDX	5765.7	16.725	42.86	0.436	20.11	0.0315
(SD)	(175.9)	(0.347)	(3.53)	(0.075)	(1.79)	(0.0024)

SD, Standard deviation
** Triexponential equation: $C(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} + C \cdot e^{-\gamma t}$

(b) Doxorubicin and epirubicin plasma pharmacokinetics: half-lives of the three decay phases (h)

Patient no.-drug	t/2α	t/2β	t/2γ
66-DX	0.094	1.61	39.99
66-epiDX	0.035	0.90	31.34
67-DX	0.084	3.68	48.56
67-epiDX	0.030	0.57	31.43
68-DX	0.076	2.63	52.50
68-epiDX	0.074	0.55	36.19
69-DX	0.130	2.90	74.14
69-epiDX	0.049	2.27	47.04
70-DX	0.085	2.20	50.99
70-epiDX	0.053	0.63	27.06
71-DX	0.085	6.23	65.50
71-epiDX	0.052	1.61	37.64
72-DX	0.046	0.64	22.83
72-epiDX	0.055	0.56	17.17
73-DX	0.045	0.65	33.13
73-epiDX	0.041	1.59	22.00

$P = 0.011$). Moreover, there were statistically significant differences between the two compounds for AUC, PlCl, and MRT, whereas no differences could be detected in terms of Vss and RCl. It is evident from these data that epiDX is cleared more rapidly than DX (PlCl: 75.0 vs. 56.8 l/h, $P = 0.012$; MRT 31.6 vs. 45.6 h, $P = 0.012$). The difference is not accounted for by distribution (Vss: 31.8 vs. 33.3 l/kg, $P = 0.73$) or by renal excretion factors (RCl: 4.4 vs. 4.7 l/h, $P = 0.57$). The differing disposition of the two compounds appears to be related to and explained by the additional epiDX conjugation with glucuronic acid.

In patient no. 69 (liver metastases, extrahepatic obstruction, and percutaneous biliary drainage), it was possible to evaluate the biliary excretion of the two drugs and their metabolites, as well as plasma and urine disposition kinetics. This patient had abnormal serum bilirubin levels before and immediately after the first treatment course with DX, but the liver function improved and bilirubin levels were within the normal range during the second treatment course with epiDX. The time course of serum bilirubin levels (mg/dl; normal values: 0.2–1.2) in this patient during the study was: DX treatment before, 2.3; day 2, 4.8; day 5, 4.7; day 9, 2.2; day 11, 1.6; day 19, 1.3; epiDX treatment (day 23), – day 2, 1.0; day 5, 1.0; day 9, 0.7. The elimination of both compounds was significantly impaired, as demonstrated by the substantially reduced PlCl and increased MRT and t/2 compared to those of the other patients. Even in this patient, however, epiDX elimination was faster than DX elimination. The extent of the difference could partly be due to the improved liver function during the second treatment course (epiDX). The conjugation of epiDX and epiDXol was well maintained, as judged by the relatively high plasma levels of the glucuronides G and H. The fractional biliary excretion of unchanged drug and metabolites observed in this patient is

Table 5. Doxorubicin and epirubicin plasma pharmacokinetics: model-independent parameters

Patient no.-drug	$AUC_{0-\infty}$	Pl/Cl	Vss	MRT	RCl
66-DX	2569.2	40.87	21.36	37.38	3.94
66-epiDX	1367.0	76.81	38.63	35.96	3.93
67-DX	1868.0	53.53	35.91	44.28	6.80
67-epiDX	1483.8	67.40	35.60	34.87	7.03
68-DX	1820.3	54.94	35.62	48.25	6.62
68-epiDX	1251.0	79.93	44.58	41.51	6.50
69-DX	3684.8	24.42	40.61	83.13	2.40
69-epiDX	2524.6	35.65	27.81	39.01	1.78
70-DX	2676.8	33.62	35.44	48.38	4.90
70-epiDX	2200.0	40.92	26.76	30.02	3.98
71-DX	1940.4	59.26	31.62	42.89	4.29
71-epiDX	1566.6	73.41	32.52	35.62	6.88
72-DX	962.0	119.54	34.52	25.99	1.42
72-epiDX	861.9	133.43	25.17	16.98	1.99
73-DX	1464.3	68.29	31.59	34.69	6.97
73-epiDX	1081.6	92.50	23.39	19.00	2.86

$AUC_{0-\infty}$ (ng · h/ml), Area under the time-concentration curve (analytical integration); PlCl (l/h), Plasma clearance; Vss (l/kg), Volume of distribution at steady state; MRT (h), Mean residence time; RCl (l/h), Renal clearance

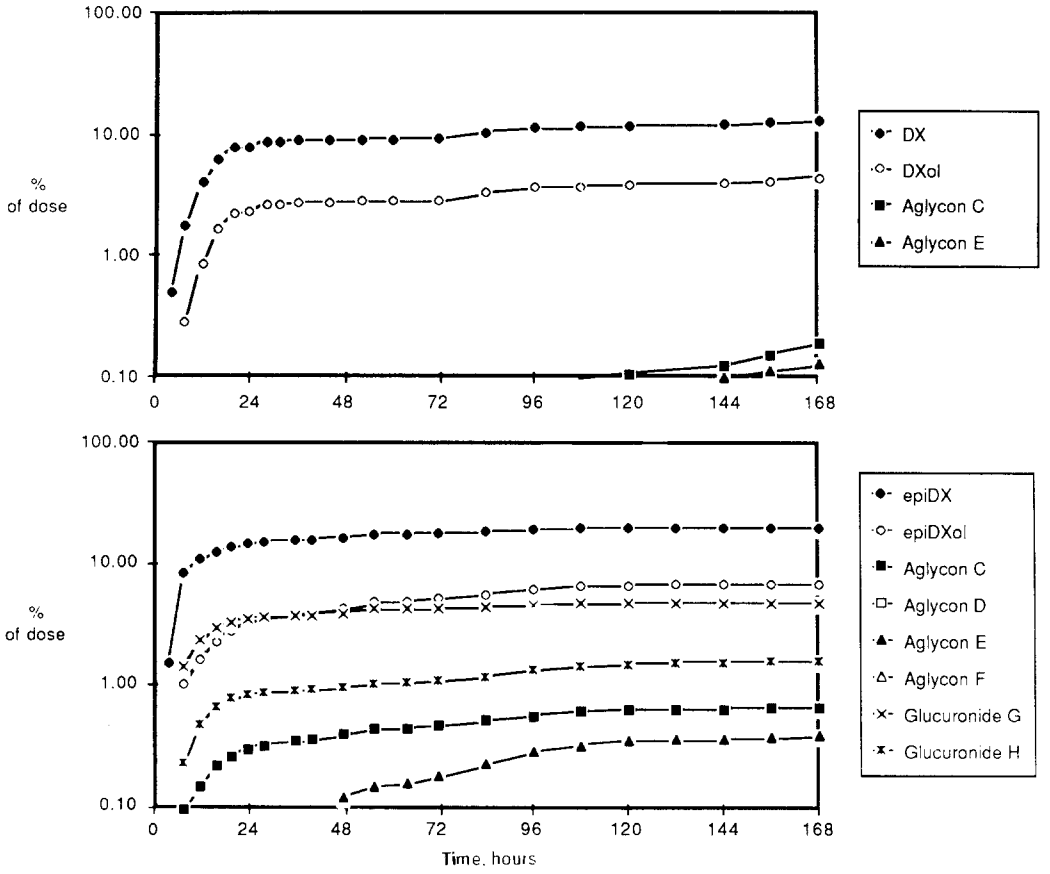


Fig. 2. Biliary excretion of unchanged drug and metabolites after doxorubicin and epirubicin administration in patient no. 69

reported in Fig. 2. The biliary excretion of total fluorescence in 6 days accounted for 18.2% of the DX dose given and for 35.4% of the epiDX dose. After DX administration, unchanged drug and DXol were the major anthracycline species present in the bile, with a negligible contribution of the aglycone derivatives. After epiDX, glucuronic acid conjugates of epiDX and epiDXol were found together with both compounds in free form.

Toxic side effects of the treatment are reported in Table 1b. Epirubicin was better tolerated as both a first- and second-line drug. Patient no. 71 was characterized by a particularly fast elimination of drugs and metabolites, and was remarkably free of unwanted side effects. On the other hand, patient no. 69, who showed impaired elimination of both drugs, was subjected to more severe toxicities.

Discussion

DX and epiDX are significantly metabolized in cancer patients (Scheme 1). Both drugs are reduced to the 13-hydroxy derivatives, DXol and epiDXol, respectively. In each patient, DXol is the main DX metabolite both in plasma and in urine.

The metabolic pathway leading to compounds lacking the aminosugar moiety does not appear to be particularly important in the elimination of these drugs. The concentration of the aglycone derivatives C, D, E, and F was always rather small, at least one-tenth compared to unchanged DX and epiDX. The relative concentration of aglycones was even lower in urine, where they were occasionally detected in only a few patients. Our results are at variance with the literature data, which often report much higher levels of aglycones. In our trials, we have observed a rapid and significant increase of aglycone levels when urine and plasma samples are not rapidly processed and are kept in nondeactivated glassware.

An additional metabolic pathway is available for epiDX. Conjugation with glucuronic acid takes place at the hydroxyl group in the 4' position of the aminosugar, and the glucuronides G and H represent the 15.9%–38.9% (mean, 27.4%) and 8.2%–17.6% (mean, 11.5%), respectively, of the epirubicin-derived circulating species. The glucuronide of epiDX is in fact the major metabolite of the drug in plasma as well as in urine [7, 15]. The main pharmacokinetic consequence of this additional metabolic pathway of epiDX is a more efficient and faster elimination of the unchanged drug compared to DX, as demonstrated by the statistically significant differences between the two compounds in terms of AUC, PlCl, MRT, and t/2 of the slow disposition phase.

Moreover, epiDXol plasma levels were significantly lower than those of the corresponding 13-dihydro derivative of DX (AUC: 585 vs. 1298 ng.h/ml, $P = 0.017$). The difference between reduced metabolite levels was actually higher than what was observed for the unchanged drugs [AUC: 1974 (DX) vs. 1495 ng.h/ml]. This probably reflects the combined effects of the higher clearance of unchanged epiDX and of the additional conjugation of epiDXol.

The triexponential decay of epiDX and DX plasma concentration observed in the present study is consistent with our previous findings [4]. Half-lives of the three DX

decay phases are longer than the corresponding epiDX parameters; this appears to be explained by the more efficient elimination of epiDX rather than by distribution factors. In fact, the apparent volume of distribution (Vss) was similar for the two compounds, whereas in each patient epiDX plasma clearance was higher, and MRT lower, than the corresponding DX parameters.

Both drugs were efficiently cleared from systemic circulation and their plasma clearance was on the same order of magnitude as the expected plasma flow to the liver. The slow elimination documented by the $t/2\gamma$ and MRT values is therefore the outcome of a rapid and extensive tissue distribution (Vss: 31–33 l/kg) and not of a poor elimination efficiency.

It should be pointed out that unless epiDX and DX plasma levels are followed up for a sufficient time, both model-dependent and independent parameters tend to be meaningless. After a short follow-up (24–48 h), bi- or tri-compartment models are not statistically different; the terminal half-life is underestimated (it does not represent the true half-life, but a weighted mean between $t/2\beta$ and $t/2\gamma$); Vss and MRT are severely affected by the error in the projected estimation of the unmeasured terminal decay. All of this can explain the differences between our pharmacokinetic results and some of the data reported in the literature [14]. In addition, a true comparison of epiDX and Dx pharmacokinetics can additionally be obtained only in crossover experiments, where interpatient variability in drug metabolism is taken into account.

A distinct pharmacokinetic behavior was observed in one patient who had liver metastases, extrahepatic obstruction, and percutaneous biliary drainage. The elimination of both drugs and their main metabolites was significantly impaired, particularly following DX treatment, when serum bilirubin levels were abnormally high. Relatively high DXol plasma levels were found in this patient, in agreement with Chan and co-workers [8] observations in cirrhotic patients.

EpiDX and epiDXol disposition were affected to a lesser extent by the hepatic dysfunction. The additional conjugative metabolism leading to glucuronic acid conjugates was well maintained in patient no. 69, although the interpretation of the data observed after epiDX might have been partially influenced by the improved liver function seen in terms of serum bilirubin level. The biliary recovery of unchanged drugs and metabolites in this patient amounted to 18.2% and 35.4% of the DX and epiDX doses, respectively. The incomplete recovery might reflect further biotransformation to currently unknown metabolites with lost or vastly reduced fluorescence.

The data of the present study indicate that epiDX in humans is more rapidly and efficiently cleared and eliminated than DX, because of a significant conjugated with glucuronic acid occurring at the equatorial OH group in the 4' position. The lower toxicity of the new analogue when given at the same dose as DX, well documented in the literature [2, 12, 16] and by the tolerance data in Table 1b, appears to be related to the above-mentioned pharmacokinetic factors rather than to a different mode of action.

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