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Biliary excretion and pharmacokinetics of 4'epidoxorubicin (epirubicin) in advanced cancer patients*

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Summary. Plasma pharmacokinetics and biliary and urinary excretion of the new doxorubicin analogue, epirubicin, have been studied in three patients with extrahepatic obstruction and percutaneous biliary drainage.

At variance with the reported observations concerning doxorubicin metabolism, conjugation of epirubicin and 13-dihydroepirubicin with glucuronic acid takes place, and corresponding amounts of 4'-*o*-*β*-*D*-glucuronyl-4'-epidoxorubicin and 4'-*o*-*β*-*D*-glucuronyl-13-dihydro-4'-epidoxorubicin can be found in the bile and urine.

The total amount of unaltered drug and metabolites excreted in the bile in the first 4 days after treatment accounts for the 37%, 27%, and 40% of the administered dose; urinary excretion accounts for 19%, 16%, and 26%. Biliary clearance of epiDX (32.5, 8.1 and 21.6 l/h) is higher than renal clearance (15.2, 3.3 and 9.4 l/h).

The relevance of the biliary disposition of epirubicin suggest prudent dose reduction in patients with impaired biliary drainage.

Introduction

Epirubicin (4'-epidoxorubicin, epiDX) is a new anticancer agent, which is closely related to doxorubicin (DX) and is characterized by an improved spectrum of activity and a better therapeutic index [1]. In randomized clinical studies, epirubicin proved to induce less acute toxicities and to be less cardiotoxic than DX when used in equimolar doses [8]. Pharmacokinetic studies following i. v. administration have revealed for this new drug a triphasic decay pattern with a long terminal half-life. This behavior is qualitatively similar to that observed for DX, but crossover experiments demonstrate a more efficient plasma clearance and a shorter mean residence time for epiDX and metabolites [5].

Liver functionality seems to have noticeable relevance in determining epiDX disposition. Patients with liver metastases show a statistically significant reduction in plasma clearance, not generally accompanied by a longer terminal half-life [5].

We report here the results of a pharmacokinetic study conducted in three patients with advanced solid tumors, extrahepatic obstruction and percutaneous biliary drainage.

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Materials and methods

Patients

The subjects in this study were three hospital inpatients suffering from advanced cancer, with extrahepatic obstruction and percutaneous biliary drainage. Relevant clinical data are reported in Table 1.

Two patients had received prior chemotherapy. (Pt 1, 6 courses of CIVV, i. e., cyclophosphamide, 12 mg i. v. on days 1 and 2; hydroxyurea, 25 mg/kg on days 2, 3, 4 and 5; vinblastine, 0.1 mg/kg on day 9; vincristine 0.01 mg/kg on day 9: Pt 2, 2 courses of MF, i. e., methotrexate, 50 mg i. v.; 5-fluorouracil, 1000 mg IV). Chemotherapy was discontinued in patient 1 15 days before and in patient 2, 5 months before the start of this study. Patient 3 had not received previous chemotherapy.

The patients had normal serum creatinine and BUN values, but abnormalities were seen in serum liver function tests (Table 1).

Drug administration

4'-Epidoxorubicin (epiDX) was administered as a single i. v., bolus at a dose of 50 mg/m^2 (Pt 1, 77.5 mg; Pt 2, 85 mg; pt 3, 75 mg total dose). After administration the vein was flushed with 150 ml saline solution.

Sample collection

Blood. Immediately before and at various times after the treatment (15 and 30 min, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, and 144 h) venous blood samples (4–6 ml) were drawn from the contralateral arm with disposable syringes and immediately centrifuged at 1000 rpm for 10 min. Plasma fractions were collected in disposable tubes, carefully protected from light, and kept frozen at −20 °C until analysis, which was performed not later than 24 h after collection.

Bile

Bile was continuously collected by biliary drainage, into 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 discharged.

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

Table 1. Patient characteristics

	Pt. 1	Pt. 2	Pt. 3
Primary tumor	Kidney	Stomach	Duodenum
Metastatic sites	LFG	LFG	LFG
Age (years)	55	53	67
Performance status (Karnofsky)	50	40	50
Bilirubin (< 1 mg%)[a]	2.4	4.2	1.1
Alkaline phosphatase (< 130 mU/ml)[a]	262	480	203
SGOT (< 41 mU/ml)[a]	24	29	23
SGTP (< 45 mU/ml)[a]	39	37	45

LFG, lymph nodes
[a] Normal values

Urine

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

Analytical methods

Plasma extraction [4, 5]. After addition of 100 µl stock aqueous solution of daunorubicin hydrochloride as internal standard and 1 ml phosphate buffer (pH 8), plasma samples were extracted with 10 ml chloroform:eptanol 9: 1 in a vortex mixer for 5 min, as previously described. The organic layer was collected and extracted with 0.5 ml 0.3 *M* phosphoric acid in a vortex mixer. The acidic phase was carefully removed and washed with 2 ml *n*-exane to remove nonpolar contaminants. Aliquots (10–100 µl) of the acidic phase were analyzed by high-pressure liquid chromatography.

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

Bile and urine

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

Chromatographic peaks attributed to glucuronides were not present in the samples extracted.

For drug concentrations between 100 and 1000 ng/ml, analytical results for epiDX and its C13-reduced metabolite epiDXol were identical following the two procedures. Samples with lower concentrations were determined only after extraction, in order 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 online with a Perkin-Elmer Sigma 10 data system, using internal standard calculations. Chromatographic peaks were identified by comparison with authentic specimens; glucuronides were also identified by deconjugation experiments with glucuronidase [6].

Computational methods

Plasma decay curves were fitted with polyexponential equations:

$$C = \sum_{i=1}^{n} Ai \cdot Exp[-\alpha i \cdot t]$$

using the PAR program of the BMDP biomedical computer programs of the University of California [7]. Areas under the time-concentration curve were computed by analytical integration of the polyexponential equation and by the trapezoidal rule.

Model independent parameters were computed as follows:

Plasma clearance (PlCl) = Dose/$\int$ Cdt;
Mean residence time (MRT) = $\int$ tCdt/$\int$ Cdt;
Apparent volume of distribution (Vss) = PlCl · MRT.
Renal clearance was obtained by determining the concentration of the drug in urinary excretion:

Renal clearance (RCl) = [Xu]/$\int$ Cdt,

where [Xu] is the amount of unmetabolized drug eliminated in the urine during the time interval 0–96 h. Biliary clearance was similarly computed using the amount of unmetabolized drug eliminated in the bile.

Results

Figure 1 reports plasma levels of epiDX and of its metabolite epiDXol measured in the subjects of this study. In patients 1 and 3 the decay followed a triphasic pattern consistent with the well-known pharmacokinetic behavior of this drug. Model-independent parameters determined according to statistical moment theory are reported in Table 2. Plasma clearance and terminal half-life values were in the range previously observed by us for patients with intact liver functions.

In patient 2 the decay curve was less regular, possibly because of the more severely impaired liver functions, and curve fitting with a polyexponential equation was not suc-

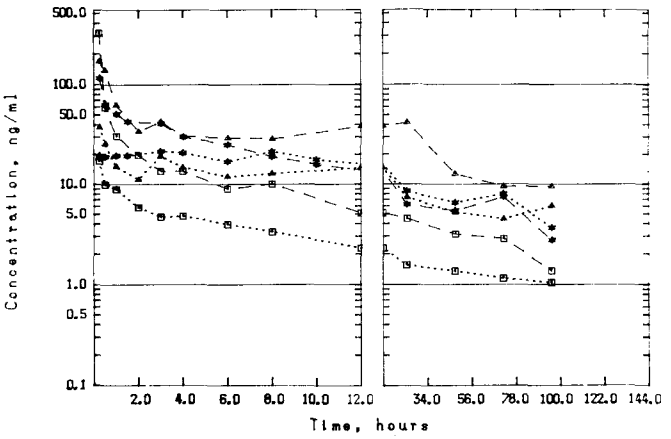

Fig. 1. Plasma levels of epiDX (Pt 1 □-----□; Pt 2 △-------△; Pt 3 ∗-------∗) and epiDXol (Pt 1 □·····□; Pt 2 △·····△; Pt 3 ∗·····∗), observed after a single i. v. bolus of epiDX

cessful. Pharmacokinetic parameters were therefore obtained only by computing the AUC value according to the trapezoidal rule.

Plasma bioavailability of the reduced epiDX metabolite epirubicinol – as expressed by the area under the time-concentration curves – was generally lower than that of the parent compound [4, 5]. In patient 3, after an initial induction period epiDXol plasma levels nonetheless became higher than epiDX levels. In this case, bioavailabilities of parent drug and reduced metabolite were similar.

Biliary and urinary excretion data are reported in Tables 3 and 4. The total amount of unaltered drug and metabolites excreted in the bile in the first 4 days after treatment accounted for 37%, 27%, and 40% of the administered dose; urinary excretion accounts for 19%, 16%, and 26%. Glucuronated metabolites MI (4'-*o*-*β*-*D*-glucuronyl-4'-epiDX) and M2 (4'-*o*-*β*-*D*-glucuronyl-13-dihydro-4'-epiDX) were present in appreciable amounts. These compounds were also observed by Weenen et al. [12] in the plasma of patients receiving epiDX therapy.

Discussion

The use of T-tube drainage in patients interrupts the enterohepatic circulation of biliary constituents; the pharmacokinetic behavior and the elimination of foreign compounds is therefore affected [2]. A decrease in bile flow is to be expected, and the extent of biliary elimination of parent drug and metabolites reported here is likely to be an understimate.

The amounts of epiDX and epiDXol found in the bile in the first 2 days after the i. v. treatment were remarkably similar in the three patients (Table 3). Significant differ-

Table 2. Epirubicin plasma pharmacokinetics

Pharmacokinetic parameter		Pt. 1	Pt. 2	Pt. 3
Dose	(mg)	77.5	85.0	75.0
Dose	(mg/m^2)	50.0	50.0	50.0
AUC, epiDX	(ng h^{-1} ml^{-1})	796.3	2500.0	1065.0
AUC, epiDXol	(ng h^{-1} ml^{-1})	157.3	671.9	1073.0
Plasma clearance	(l h^{-1})	97.3	34.0	70.4
Mean residence time	(h)	31.7		67.8
Volume of distribution	(l kg^{-1})	49.3		91.8
Terminal half-life	(h)	41.1		63.8
Renal clearance	(l h^{-1})	15.2	3.3	9.4
Biliary clearance	(l h^{-1})	32.5	8.1	21.6

AUC, area under the concentration: time curve

Table 3. Biliary excretion of epirubicin (epiDX) and main fluorescent metabolites in three patients with extrahepatic obstruction and percutaneous biliary drainage

Time (h)		0–12	12–24	24–48	48–96	0–96	Percentage of dose	96–144	144–192
epiDX	Pt. 1	13.20	1.82	1.01	0.86	16.89	21.8	0.10	0.00
	Pt. 2	10.39	2.05	2.11	1.77	16.32	19.2	0.61	0.54
	Pt. 3	10.61	2.28	2.90	2.66	18.45	24.6	1.47	0.36
epiDXol	Pt. 1	2.53	0.99	0.83	0.60	4.95	6.4	0.00	0.00
	Pt. 2	1.81	0.70	1.22	1.11	4.84	5.7	0.41	0.37
	Pt. 3	1.37	1.17	1.61	1.39	5.54	7.4	0.94	0.24
M1	Pt. 1	4.60	0.29	0.11		5.00	6.5		
	Pt. 2	0.78	0.15	0.30	0.23	1.46	1.7	0.13	0.08
	Pt. 3	1.43	1.15	1.02	0.62	4.22	5.6	0.33	0.09
M2	Pt. 1	0.79	0.29	0.45		1.53	2.0		
	Pt. 2	0.48	0.05	0.04	0.08	0.65	0.8	0.06	0.01
	Pt. 3	0.20	0.65	0.74	0.51	2.10	2.8	0.26	0.07

Amounts excreted are expressed in milligrams
M1, epiDX glucuronate; M2, epiDXol glucuronate

Table 4. Urinary excretion of epirubicin (epiDX) and main fluorescent metabolites in three patients with extrahepatic obstruction and percutaneous biliary drainage

Time (h)		0–12		12–24	24–48	48–96	0–96	Percentage of dose	96–144	144–192
epiDX	Pt. 1	4.78		0.88	0.91	1.31	7.88	10.2	0.26	0.13
	Pt. 2	4.07		1.05	0.91	0.66	6.69	7.9	0.16	0.22
	Pt. 3	–	4.41	–	1.76	1.89	8.06	10.7	0.69	0.10
epiDXol	Pt. 1	0.29		0.27	0.40	1.02	1.98	2.6	0.11	0.05
	Pt. 2	1.65		1.00	0.83	0.70	4.18	4.9	0.13	0.09
	Pt. 3	–	1.81	–	0.69	1.09	3.59	4.8	0.78	0.05
M1	Pt. 1	2.02		0.37	0.57	0.54	3.50	4.5		
	Pt. 2	–	0.80	–	0.50	0.35	1.65	1.9		
	Pt. 3	–	1.96	–	1.61	1.90	5.47	7.3	0.76	0.04
M2	Pt. 1	0.34		0.11	0.19	0.30	0.94	1.2		
	Pt. 2	–	0.30	–	0.20	0.15	0.65	0.8		
	Pt. 3	–	0.86	–	0.69	0.82	2.37	3.2	0.38	0.02

Amounts excreted are expressed in milligrams
M1, epiDX glucuronate; M2, epiDXol glucuronate

ences were observed in the excretion of the glucuronated epiDX metabolite, in contrast: high plasma levels of epiDX correspond to low amounts of M1 in the bile or urine.

Patient 2, for instance (elevated serum bilirubin and alkaline phosphatase), is characterized by relatively high epiDX plasma levels and by a consequently low plasma clearance (34 l/h, 0.35 times that determined in Pt 1). The amount of epiDX and epiDXol excreted in the bile of patient 2 are 0.91 and 0.86 times the amounts excreted by patient 1. Patient 3 is in an intermediate position. The observed differences in plasma drug clearance are therefore better explained by the different amounts of M1 excreted.

Conjugation reactions in general lead to the detoxification (and elimination) of xenobiotics. Interspecies variations in drug toxicity owing to defects in conjugation reactions are well documented [3]. Doxorubicin [11] (like idarubicin [10]) is not excreted in the bile or urine in the form of conjugated metabolites. The lesser toxicity of epirubicin at equimolar doses may actually depend on the availability of this detoxification pathway, rather than on a different mechanism of action.

As far as the plasma pharmacokinetic is concerned, delayed recirculation of the drug has been proposed to rationalize the long terminal half-life of drugs subject to enterohepatic reabsorption. The terminal half-life of epiDX in patients with normal enterohepatic circulation is in our experience [4] 39.4 h (14 pts; range 21.1–64.6), and as can be observed in Table 2, it seems not to be significantly affected by the T-tube drainage. Plasma clearance itself is in the range of the values generally recorded for epiDX; enterohepatic reabsorption may be therefore not particularly relevant for determination of the pharmacokinetic behavior of epiDX, and the long terminal half-life of the drug is better explained by a slow release of epiDX from extraplasmatic compartments.

Finally, the significant amounts of parent drug and metabolites excreted in the bile strongly suggest that changes should be made in epiDX dosages when patients with biliary tract obstruction are treated. The therapeutic index of antineoplastic drugs is unfortunately quite low, and even small changes in drug elimination can result in a rise in the plasma drug level to a toxic level.

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