Pharmacokinetics and Metabolism of Epidoxorubicin and Doxorubicin in Humans

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Pharmacokinetics of doxorubicin (DOX), epidoxorubicin (EPI), and their metabolites in plasma have been performed in eight patients receiving 40 to 56 mg/m^2 of both anthracyclines as a bolus injection in two sequential cycles. Terminal half-life and volume of distribution appeared to be smaller in case of EPI, whereas plasma clearance and cumulative urinary excretion was larger in comparison to DOX. The major metabolite of DOX was doxorubicinol (Aol) followed by 7-deoxy-doxorubicinol (7d-Aolon). Metabolism to glucuronides was found in case of EPI only. The area under the curves (AUC) of the metabolites of EPI decreased in the order of the glucoronides E-glu > Eol-glu, 7d-Aolon > epirubicinol (Eol). The AUC of Eol was half of the value in its counterpart Aol. In the case of EPI, the AUC of 7d-Aolon was twice the level of that of the corresponding metabolite of DOX. The terminal half-lives of the cytostatic metabolites Aol and Eol were similar, but longer than the corresponding values of their parent drugs. Half-lives of the glucuronides (E-glu, Eol-glu) were similar to the half-life of their parent drug. 7d-Aolon had a somewhat shorter half-life in comparison to both DOX and EPI. Approximately 6.2% of EPI and 5.9% of DOX were excreted by the kidney during the initial 48 hours. Aol was found in the urine of patients treated with DOX, whereas Eol, E-glu, and Eol-glu were detected in urine of patients treated with EPI. The cumulative urinary excretion appeared to be 10.5% for EPI and its metabolites, and 6.9% for DOX and its metabolite. The plasma concentration v time curves of (7d)-aglycones showed a second peak between two and 12 hours after injection, suggesting an enterohepatic circulation for metabolites lacking the daunosamine sugar moiety. The plasma concentrations of the glucuronides were maximal at 1.2 hours for E-glu and 1.9 hours for Eol-glu. All other compounds reached their maximum plasma concentration during the first minutes after the administration of DOX and EPI. Deviating plasma kinetics were observed in one patient, probably due to prior drug administration.

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EPIDOXORUBICIN (EPI; epirubicin Pharmarubicin [Farmitalia Carlo Erba, Milan, Italy]), the 4′-epimer of doxorubicin (DOX; Adriamycin [Farmitalia Carlo Erba]) (Fig 1), is one of the analogs presently being studied in phase II and III trials.[1-3]

From preclinical studies with EPI it was concluded that differences in therapeutic and toxicologic manifestations exist between EPI and DOX, reflecting apparent alterations in pharmacologic properties and possible mode of action.[4] A comprehensive review of clinical activity and adverse effects of EPI was published recently in the *Journal of Clinical Oncology*.[5]

Search for anthracycline analogs is necessary because clinical use of DOX is hampered by unfavorable side effects. Furthermore, human tumors of major importance, such as pancreatic cancer and lung cancer, are not generally responsive to DOX. One of the major drawbacks of DOX treatment is its cardiotoxic effect. The incidence of congestive heart failure (CHF) is 5% at a cumulative dose of 550 mg/m^2 and 800 to 900 mg/m^2 of DOX and EPI, respectively.[6,7]

The differences between DOX and EPI recorded for preclinical and clinical pharmacological behavior are probably attributed to differences in tissue distribution, metabolism, and pharmacokinetics. EPI and DOX do not differ substantially in their affinity to double-stranded DNA, most likely the main biological target of these two anthracyclines.[8]

Human metabolism of DOX involves carbonyl reduction by aldo- keto reductase, the major enzymatic conversion,[9] as well as reductive glyco-

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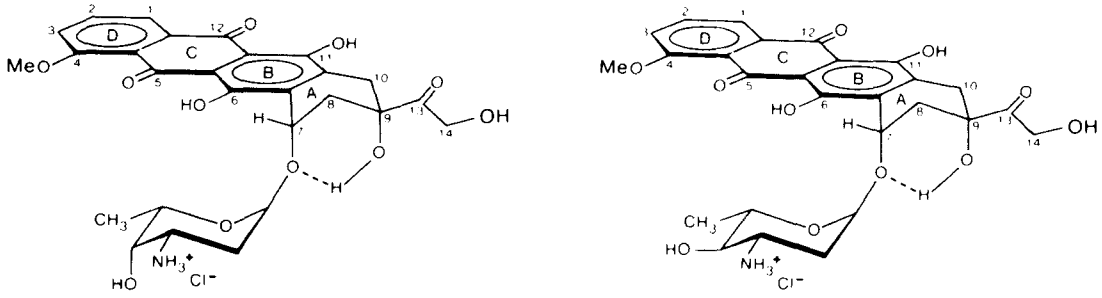

Fig 1. Molecular structures of DOX (left) and EPI (right).

sidic and hydrolytic glycosidic cleavage. Molecular structures of the metabolites are shown in Fig 2. The main metabolite doxorubicinol (Aol), maintains anticancer activity, whereas the aglycones are not active.[10]

The pharmacokinetics of DOX using different schedules is well documented.[11,12] The 4′-glucuronides, only present as metabolites in case of EPI, were first described by Weenen et al.[13] By that time, pharmacokinetic studies of EPI and its metabolites were hampered by low recoveries of the very polar glucuronides due to existing liquid-liquid extraction procedures.[14] This problem was solved by the introduction of a liquid-solid extraction procedure for anthracyclines.[15,16,17] However, description of pharmacokinetic data for EPI has been incomplete with respect to all known metabolites up to now.

A pharmacokinetic study of DOX and EPI in patients with advanced cancer, designed as a crossover study, was implemented to describe the pharmacokinetic behavior of both drugs and their metabolites. The results of this study and their relationship to suggested modes of action are discussed.

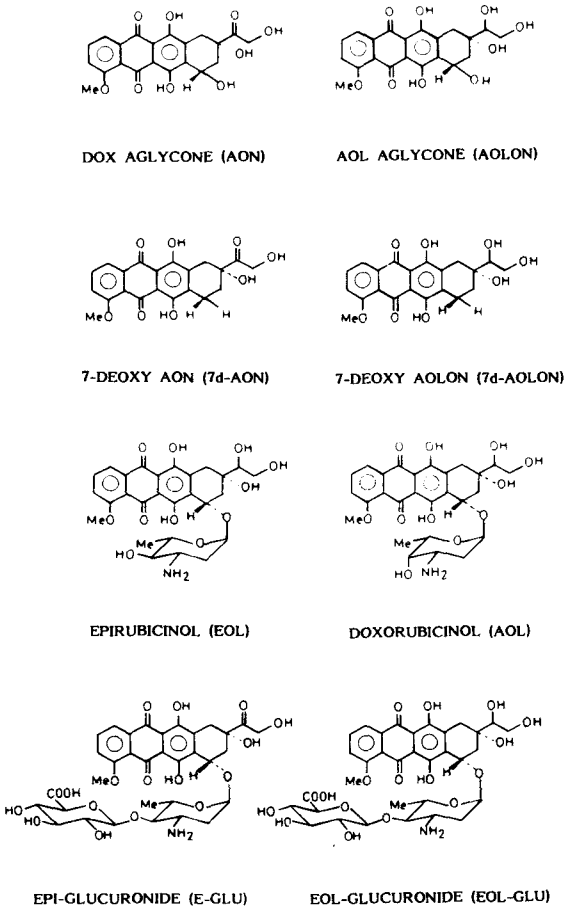

Fig 2. Molecular structure of all measured metabolites derived from DOX and EPI.

PATIENTS, MATERIALS, AND METHODS

Treatment Schedule

EPI and DOX were obtained as a sterile lyophilized powder (50 mg/vial, Farmitalia, Carlo Erba SpA, Italy). The drug was reconstituted with 25 mL sterile water (United States Pharmacopeia). The prescribed dose of DOX or EPI was administered subsequently within one to two minutes through the line of rapid saline infusion. The pharmacokinetic study was performed in patients receiving 40 to 56 mg/m^2 DOX or EPI in a crossover design with an interval of 3 weeks.

Eight female patients, not previously treated with anthracyclines, were included in the study after informed consent was obtained. All patients had advanced disease requiring treatment with anthracyclines either as single agent, in combination with fluorouracil and cyclophosphamide for breast cancer, or in combination with mitomycin for adenocarcinoma of unknown primary origin. During the 48-hour sampling period no other cytostatic agents were administered. Thereafter, anticancer treatment was continued according to the treatment protocol.

Patient characteristics and baseline laboratory values are summarized in Table 1. All patients had total bilirubin levels < 15 μmol/L and a normal serum creatinine. Three patients had metastatic lesions in the liver. Only one patient (J.O.) had been

Table 1. Patient Characteristics

Patient	Age (yr)	Primary Tumor	Metastatic Lesion	Creatinine (μmol/L)	Total Bilirubin (μmol/L)	Alkaline Phosphatase (U/L)	γ-GT (U/L)	SGOT (U/L)	SGPT (U/L)	LDH (U/L)	Total Protein (g/L)	Albumin (g/L)
J.O.	62	Breast	Lungs, bones, pleura	63	7	154	17	9	6	315	64	31
K.U.	50	Breast	Liver, bones, pleura	69	8	77	87	55	54	693	63	33
R.O.	50	Breast	Liver	112	10	330	203	36	46	—	72	39
S.T.	41	Breast	Bones	71	6	140	9	7	7	211	68	35
L.A.	53	Unknown	Lungs, bones	65	4	94	7	11	8	201	71	34
B.O.	52	Breast	Lungs, pleura	91	6	43	13	9	8	182	76	33
B.A.	54	Unknown	Lungs, pleura	61	9	111	46	5	10	206	59	33
V.R.	51	Breast	Lungs, liver	86	6	197	93	63	122	399	72	35
Reference values				70-120	9	100	34	5-15	5-15	175	60-80	38-50

Abbreviations: γ-GT, γ-glutamyl transpeptidase. LDH, lactic dehydrogenase.

treated with chemotherapy (cyclophosphamide, methotrexate, fluorouracil) and hormonal therapy (aminoglutethimide, hydrocortisone) before.

Blood and Urine Samples

Blood samples of 10 mL were obtained at −5, 0, 5, 10, 15, 30, 60 minutes, and 2, 4, 6, 9, 12, 24, 36, and 48 hours after bolus injection. Blood was collected in heparinized (150 IU Li-heparine) glass tubes (Terumo, Leuven, Belgium) and immediately centrifuged at 4°C, 4,000 g for 15 minutes. The plasma was transferred to polystyrene tubes. Urine samples were obtained in portions of 6 hours over 48 hours. Plasma and urine samples were maintained at −20°C until analysis. After thawing, all samples were sonicated and centrifuged at 4°C for five minutes with 4,000 g to remove clotted material.

High-Performance Liquid Chromatography Analysis

The high-performance liquid chromatography (HPLC) assay used for detection and quantification of the two parent drugs and their metabolites was recently developed by Maessen et al.[17] Briefly, the chromatographic system consisted of a WISP 710B injection system, a model 6000A solvent delivery system, and a data module with system controller 720 (Waters, Etten-Leur, The Netherlands). This system was provided with a stainless steel HPLC column (4.6 × 100 mm, 3 μm CP MicroSpher, Chrompack, Middelburg, The Netherlands) including a guard column (4 × 4 mm, 5 μm LiChrosorb RP-18, Merck, Amsterdam) and a F-1000 fluorescence detector (excitation wave length, 480 nm; emission wavelength, 580 nm) from Merck-Hitachi, Amsterdam. An isocratic eluent was used consisting of 0.02 mol/L NaH_2PO_4 pH 4/acetonitrile (2.5/1 v/v) at a flow rate of 1 mL/min. DOX, EPI, and their metabolites were extracted from human plasma using C-18 Sep-Pak cartridges (Waters), pretreated with 5 mL methanol, 5 mL H_2O, and 5 mL buffer (0.02 mol/L NaH_2PO_4 pH 4/acetonitrile 9/1, v/v). One milliliter plasma was injected onto the cartridge, subsequently purged with 2 mL buffer, dried with a flow of air, and eluted with methanol/chloroform (3/1 v/v). The eluate was evaporated to dryness at 50° C under a stream of air. The residue was redissolved in 50 μL buffer, of which 30 μL was injected onto the analytical column. EPI was added as internal standard (2.5×10^{-8} mol/L) to all plasma samples containing DOX and its metabolites, whereas DOX was added (2.5×10^{-8} mol/L) to all samples containing EPI and metabolites. Samples were prepared in duplicate. Six spiked plasma samples containing the two drugs and their metabolites (see Fig 2) (5×10^{-10} to 5×10^{-8} mol/L) were included in each series to construct a within-run standard line. A typical chromatogram of a spiked blanc plasma sample is shown in Fig 3. The detection limit of the assay ranged from 1×10^{-10} mol/L for Aolon to 4×10^{-10} mol/L for 7d-Aon and DOX. The other compounds could be detected within this range. Plasma samples were diluted with blank heparinized plasma from a blood donor. For the first two samples

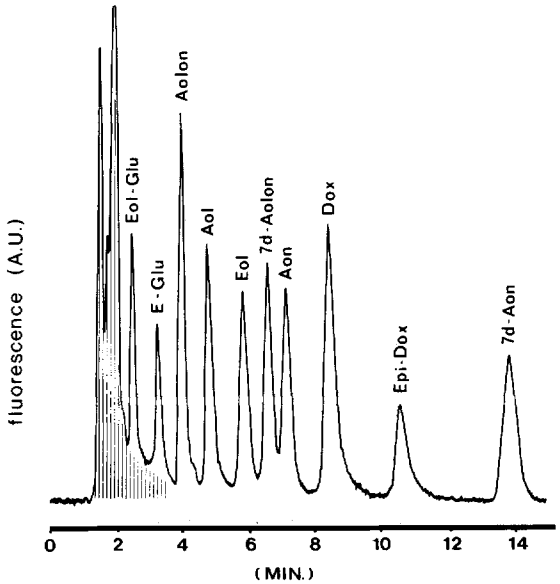

Fig 3. Typical chromatogram after extraction of a plasma sample spiked with DOX and EPI and their metabolites and separation of all compounds. The peaks in front of Eol-glu (hatched area) represent co-extracted plasma material with fluorescent properties.

(t = 0 and 5 minutes) various dilutions were prepared in order to determine all metabolites as accurately as possible because of the large differences in drug and metabolite concentrations. The urine samples were sonicated after pH adjustment to 2.5 and subsequently centrifuged. After addition of an external standard (DOX, respectively, EPI), samples were directly injected onto the column.

Pharmacokinetic Analysis

Each set of concentration time [c(t)] values of DOX and EPI in plasma was fitted to the appropriate polyexponential equation using the program, NONLIN.[18] The final decision to describe the results according to a three compartment model was performed by AUTO AN.[19] The r^2 of NONLIN least-squares fit was always better than .999, with one exception (Patient J.O., r^2 = .97). All fittings were performed to the three exponential equation:

$$(1) \qquad c_p = A \times e^{-\alpha t} + B \times e^{-\beta t} + C \times e^{-\gamma t}.$$

The pharmacokinetic parameters for the parent drugs were calculated using the following equations:

$$(2) \quad AUC = A/_{\alpha} + B/_{\beta} + C/_{\gamma} \text{ (nmol} \times L^{-1} \times \text{min)};$$

$$(3) \quad Cl_p = D/AUC \text{ (L} \times \text{min}^{-1}\text{) (normalized to 1.74 m}^2\text{)};$$

$$(4) \quad V_d = D \times \frac{\frac{A}{\alpha^2} + \frac{B}{\beta^2} + \frac{C}{\gamma^2}}{AUC^2} \text{ (L) (normalized to 1 kg)};$$

$$(5) \qquad t_{1/2} = 0.693/k \times 60 \ (k = \alpha, \beta, \gamma).$$

The abbreviations used are AUC, area under the curve; Cl_p, plasma clearance; V_d, volume of distribution; D, dose; $t_{1/2}$, half-life; and k, rate constant. The terminal half-lives of DOX and EPI were also analyzed by the curve-stripping method using all time points from four hours onward. Additionally, the AUCs of the parent drugs and their metabolites were determined by means of the trapezoidal method because of irregular c(t) curves of the metabolites, not allowing calculations according to a pharmacokinetic model. The terminal half-lives of DOX, EPI, and their metabolites were determined from their concentrations in the final plasma samples by least square fitting.

RESULTS

The following results represent summarized data from seven of eight patients studied. All pharmacokinetic data from one patient (J.O.) and $T_{1/2}\gamma$ of second patient (V.R.) were omitted from the calculation of mean values because these data differed <2 standard deviations from the mean.

Pharmacokinetic parameters for the parent drugs, calculated according to the formulas 1 through 5 described in the previous section, are shown in Tables 2 and 3. Besides the calculations with the NONLIN program, the terminal half-life was also calculated by curve-stripping. This procedure allowed a consequent calculation of all final half-lives over an interval of four to 48 hours and may, therefore, provide additional information about interpatient and interdrug variation. The disappearance of both parent drugs was triphasic. The half-lives calculated for DOX were always longer than those calculated for EPI. The mean volume of distribution was larger in the case of DOX when compared with EPI, while the plasma clearance (normalized to 1.74 m^2 body surface area) was slower in the case of

Table 2. Calculated Pharmacokinetic Parameters of DOX in Eight Patients

Patient	PPC* (mol/L $\times 10^6$)	AUC (nmol min/L $\times 10^{-4}$)	Cl_p† (L/h)	V_{dss} (L/kg)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	$t_{1/2\gamma}$‡ (h)
J.O.	14.0§	96.6§	9.83§	6.59§	0.167§	1.49§	48.2§	15.8§
K.U.	11.5	19.7	48.38	31.14	0.062	3.02	48.4	19.9
R.O.	16.0	16.5	53.99	14.05	0.050	0.30	24.1	23.2
S.T.	12.0	9.8	87.08	20.37	0.017	0.07	17.6	24.1
L.A.	14.9	13.0	65.46	14.60	0.046	0.22	21.4	23.1
B.O.	16.5	14.9	60.38	12.29	0.029	0.09	18.7	24.2
B.A.	10.0	8.3	87.46	31.24	0.034	0.17	24.8	22.9
V.R.	24.0	43.0	20.11	44.63	0.047	1.68	157.8§	55.0§
Mean	15.0	17.9	60.40	24.00	0.041	0.79	25.8	22.9
±SD	4.7	11.7	23.40	12.00	0.020	1.13	11.4	1.6

NOTE. Explanation of the calculated parameters in *Pharmacokinetic Analysis* section.
*Peak plasma concentrations normalized to 50 mg/m^2.
†Plasma clearance normalized to 1.74 m^2.
‡Calculations with curve stripping method from four hours onward.
§Omitted for calculation of the mean.

Table 3. Calculated Pharmacokinetic Parameters of EPI in Eight Patients

Patient	PPC* (mol/L $\times 10^6$)	AUC (nmol min/L $\times 10^{-4}$)	Cl_p† (L/h)	V_{dss} (L/kg)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	$t_{1/2\gamma}$‡ (h)
J.O.	5.5§	21.8§	46.00§	18.82§	0.088§	2.96§	27.7§	20.2§
K.U.	12.0	11.7	81.57	10.22	0.051	1.07	16.6	12.2
R.O.	19.5	12.9	68.93	11.95	0.020	0.08	12.9	17.3
S.T.	18.0	8.7	99.01	12.30	0.028	0.37	15.6	15.3
L.A.	8.5	6.7	126.78	23.25	0.035	0.66	17.3	13.3
B.O.	14.5	9.6	95.62	15.93	0.023	0.12	14.0	16.0
B.A.	21.1	8.1	90.46	13.49	0.023	0.22	15.5	16.7
V.R.	31.4	18.2	47.44	16.69	0.029	0.91	32.5§	28.6§
Mean ± SD	17.9 ± 7.4	10.4 ± 4.2	87.10 ± 24.95	14.8 ± 4.3	0.030 ± 0.010	0.49 ± 0.39	15.3 ± 1.6	15.1 ± 2.0

NOTE. Explanation of the calculated parameters in *Pharmacokinetic Analysis* section.
*Peak plasma concentrations normalized to 50 mg/m^2.
†Plasma clearance normalized to 1.74 m^2.
‡Calculations with curve stripping method from four hours onward.
§Omitted for calculation of the mean.

DOX than in the case of EPI. The AUC of DOX was larger than it was in the case of EPI.

DOX and EPI were rapidly metabolized. Immediately after bolus injection all metabolites were detectable. As shown in Tables 2 and 3, peak plasma concentrations (PPC) (normalized to 50 mg/m^2 ranged from 10×10^{-6} mol/L up to 24×10^{-6} mol/L for DOX, and 5.5×10^{-6} mol/L to 32×10^{-6} mol/L for EPI-treated patients. The mean PPC (normalized to 50 mg/m^2) for the glucuronides was $4.7 \pm 3.2 \times 10^{-7}$ mol/L E-glu occurring after 1.2 ± 0.6 hours, and $1.8 \pm 2.3 \times 10^{-7}$ mol/L Eol-glu occurring after 1.9 ± 1.1 hours (Table 4).

Because of the irregular plasma concentration *v* time curves of the metabolites, it was not possible to fit these curves with exponential terms.

Table 4. Peak Plasma Levels of the Glucuronides E-glu and Eol-glu and Time Points of Peak

Patient	E-glu ($\times 10^{-7}$ mol/L)	Time (h)	Eol-glu ($\times 10^{-7}$ mol/L)	Time (h)
J.O.	5.63	2	4.10	6
K.U.	11.00	1	1.35	2
R.O.	5.00	2	0.70	4
S.T.	4.40	1	0.75	1
L.A.	2.40	2	0.95	2
B.O.	7.00	1	1.10	1
B.A.	3.38	0.5	1.16	1
V.R.	5.35	1	0.63	2
Mean ± SD	4.7 ± 3.2	1.2 ± 0.6	1.8 ± 2.3	1.9 ± 1.1

However, it was feasible to calculate the terminal half-lives of the metabolites from 12 hours onward (Table 5). The longest half-life was found in case of Aol and epirubicinol (Eol). The shortest half-life was calculated for 7d-Aon. The half-lives of the two glucuronides were similar to that of their parent drug. Half-lives for Aon and Aolon could not be determined accurately.

Representative plasma decay curves for DOX, EPI, and their metabolites are illustrated in Figs 4 and 5, respectively. AUCs (0 to 48 hours) for the parent drugs and their metabolites, normalized to 50 mg/m^2, calculated with the trapezoidal rule are listed in Table 6. It can be deduced that Aol and 7d-Aolon represent the major metabolites in case of DOX. In case of EPI, E-Glu, the glucuronidated parent drug, is more prominent. The AUC of this metabolite reached nearly twice the value of its parent drug (EPI) and gave rise to a doubling of the total AUC due to EPI compared with DOX. The AUC for Eol was only half of

Table 5. Terminal Half-Lives for the Metabolites of DOX and EPI

Compounds	Dox (h)	EPI (h)
DOX/EPI	28.3 ± 2.8	19.0 ± 2.4
Aol/Eol	32.8 ± 1.7	31.5 ± 6.0
7d-Aolon	16.8 ± 6.3	17.5 ± 4.9
7d-Aon	—	13.9 ± 5.2
Eol-glu	—	18.3 ± 4.0
E-glu	—	18.6 ± 2.1

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