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Pharmacokinetics and metabolism of iodo-doxorubicin and doxorubicin in humans

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Summary. The pharmacokinetics of doxorubicin (DOX), iodo-doxorubicin (I-DOX) and their metabolites in plasma has been examined in five patients each receiving 50 mg/m^2 of both anthracyclines as a bolus injection.

Terminal half-life, mean residence time (MRT), peak plasma concentration C_{max}, and area under the curve (AUC) appeared smaller for I-DOX, whereas its plasma clearance (CL_P) and volume of distribution at steady state (V_{ss}) were larger than for DOX.

The major metabolite of I-DOX was iodo-doxorubicinol (I-AOL) followed by doxorubicinol aglycone (AOLON). The AUC of I-AOL was 6-times larger than that of its counterpart AOL, which is the major metabolite of DOX. AOLON generated after I-DOX administration is a further important metabolite, as its AUC was 10-times larger than that of AOLON generated from DOX.

The other aglycones, such as doxorubicin aglycone (AON) and the 7-deoxy-aglycones were only minor metabolites after either I-DOX or DOX injection. The ratio $AUC_{I\text{-}AOL/AOL}/AUC_{I\text{-}DOX/DOX}$ was 27 in the case of I-DOX and 0.4 after DOX.

The terminal half-lives of the cytostatic metabolites I-AOL and AOL were similar, although a longer MRT for AOL was calculated. Both metabolites had much longer MRTs than their parent drugs. The MRTs of the aglycones AOLON and AON were greater than those of the 7-deoxy-aglycones after both I-DOX and DOX.

Approximately 6% DOX and less than 1% I-DOX were excreted by the kidneys during the initial 48 h. About 5% of I-DOX was excreted via the kidneys as I-AOL. Aglycones were not detected in significant amounts.

The plasma concentrations of all compounds measured were highest during the first few minutes after administration of I-DOX and DOX. The I-AOL concentration was comparable to that of I-DOX immediately after the injection, due to very rapid metabolism within the central compartment (vascular space) by the aldo-keto reductase system in the erythrocytes. The plasma concentration-time curves of (7d)-aglycones showed a second peak between 2 and 9 h after injection, suggesting enterohepatic circulation of metabolites lacking the daunosamine sugar moiety.

Key words: Anthracyclines, cancer patients; iodo-doxorubicin, doxorubicin, pharmacokinetics, metabolism

A variety of anthracyclines has been synthesized and investigated in order to identify compounds superior to doxorubicin (DOX) in terms of broader anticancer activity or reduced toxicity, or both [1]. DOX is one of the most effective anticancer drugs available to date. Major types of tumours, however, such as lung, colorectal and pancreatic cancer, and malignant melanoma have an intrinsic resistance to anthracyclines. The development of drug resistance during anticancer therapy, which is associated with the expression of a P-glycoprotein in tumour cell membranes [2], which functions as an active efflux pump, requires a search for non-cross-resistant anthracyclines.

Modification at the 4′-position of the daunosamine sugar resulted in the introduction of epirubicin, the 4′-epimer of DOX. This minimal structural modification had a great impact on pharmacokinetics, metabolism and toxicity, although the spectrum of anticancer activity remains similar or identical to that of DOX [3, 4]. It was recently shown that changes at the adjacent 3′- or 4′-position of the sugar can also cause activity in multi drug resistant cell lines [5].

4′-deoxy-4′-iodo-doxorubicin (I-DOX, iodo-doxorubicin; Fig. 1) is a DOX analogue modified at that critical 4′-position by substitution with iodine. It was investigated in Phase I studies [6, 7]. In preclinical studies, I-DOX has been shown to be more potent and less cardiotoxic than DOX, and to be active in certain tumour cell lines resistant to DOX [8, 9]. Introduction of the electronegative iodine at the 4′-position reduces the basicity of the neighbouring aminogroup at the 3′-position. At physiological pH I-DOX is more than 95% unprotonated and I-DOX is more lipophilic than DOX. Human metabolism of DOX involves carbonyl reduction by aldo-keto reductase, the major enzymatic conversion [10], as well as reductive glycosidic and hydrolytic glycosidic cleavage. The molecular

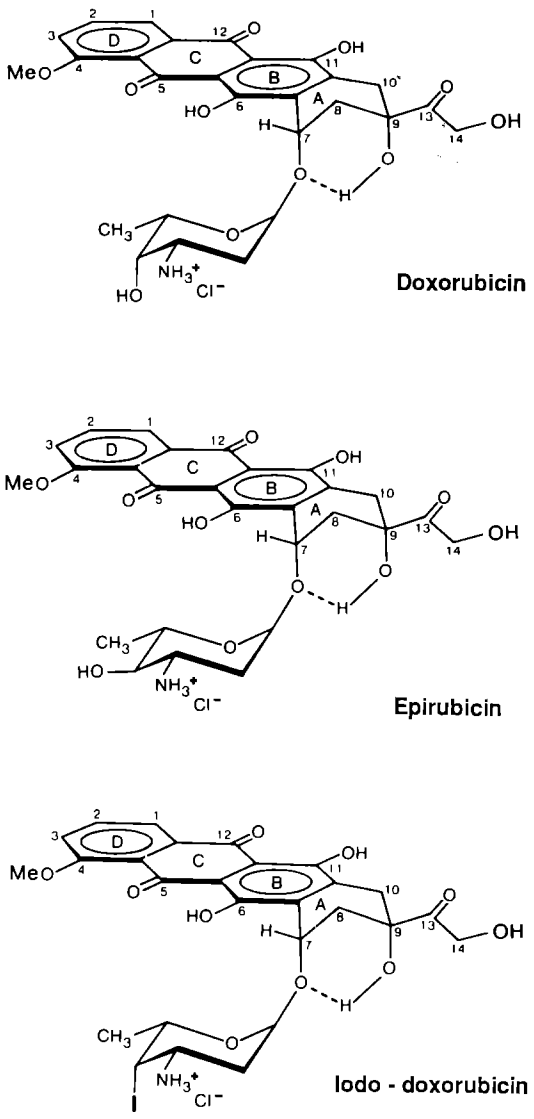

Fig. 1. Chemical structure of DOX, EPI, and I-DOX

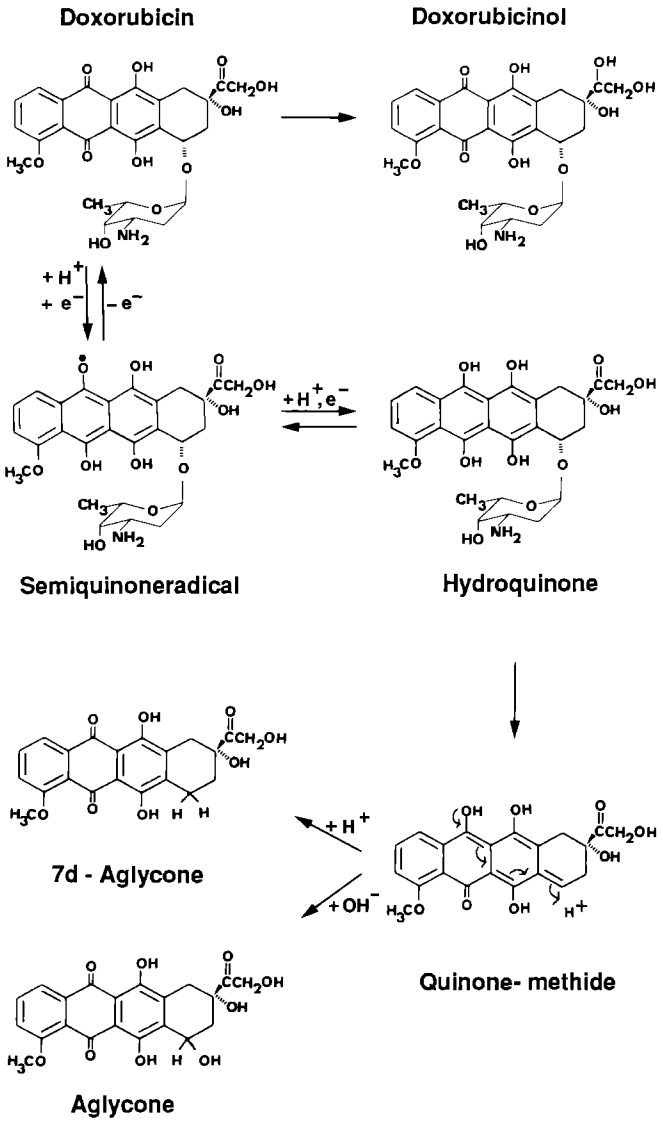

Fig. 2. Metabolism of DOX to AOL and the aglycones. The metabolism of I-DOX is very similar

structures of the metabolites are shown in Fig. 2. The main metabolite doxorubicinol (AOL) maintains anticancer activity, whereas the aglycones are not active. The pharmacokinetics and metabolism of I-DOX in mice are known. Carbonyl reduction was the most important enzymatic conversion according to an HPLC assay, but several unknown peaks were described [11]. A pharmacokinetic study of DOX and I-DOX in patients with advanced cancer was carried out to describe the pharmacokinetic behaviour of the drugs and their metabolites.

Patients and methods

Treatment schedule

I-DOX and DOX were obtained as sterile lyophilized powders (10 mg or 50 mg/vial, Farmitalia Carlo Erba GmbH, Freiburg, FRG). The drugs were reconstituted with 10 ml, and 50 ml, respectively, of sterile water. The prescribed dose was administered within 1 min through a central venous catheter. Pharmacokinetic studies were performed in patients receiving. I-DOX within a running Phase I study. DOX was administered to patients with breast cancer and of unknown primary tumours if they were not eligible for treatment according to protocols (Phases II and III).

Ten patients were included in the study after informed consent had been obtained. All patients had advanced disease requiring treatment with anthracyclines in the case of DOX, or had advanced disease still progressing after different polychemotherapeutic procedures and therefore eligible for new drugs being tested in Phase I studies in the case of I-DOX. During the 48 h sampling period no other cytostatic agent was administered.

Patient characteristics and baseline laboratory values are summarized in Tables 1 a and b for the I-DOX and DOX treated patients, respectively. All patients had a total bilirubin less than 1.1 mg · 100 ml^{-1}, and serum creatinine was normal in all except two patients.

Blood and urine samples

Blood samples 10 ml were obtained – 1, 0, 5, 10, 15, 30, 60 min and 2, 4, 6, 9, 12, 24, (30), 36, (42), 48 h after bolus injection. Blood was collected in heparinized polypropylene tubes (75 IU NH_4 heparin;

Table 1 a, b. Details of patients treated with
a) I-DOX

Pt	Age y	Prim. Tumor	Met. Lesion	Crea	Bili	AP	γ-GT	SGOT	SGPT	LDH	TP	Alb
				mg%				$U \cdot l^{-1}$			$g \cdot l^{-1}$	
1	53	lung	bone	0.8	0.6	105	77	4	4	102	64	35
2	73	adeno kidney	lung LN, cutan.	1.9	0.5	85	10	7	5	217	67	38
3	67	adeno kidney	lung LN	2.9	0.4	91	8	7	4	312	62	32
4	60	ovar	perit	1.0	0.3	82	13	9	6	192	66	42
5	58	lung	liver brain	1.4	0.3	111	127	22	38	369	58	33
References values:				0.6 – 1.4	0.2 – 1.0	40 – 90	6 – 28	3 – 18	3 – 22	40 – 240	60 – 80	35 – 50

b) DOX

Pt	Age y	Prim. Tumor	Met. Lesion	Crea	Bili	AP	γ-GT	SGOT	SGPT	LDH	TP	Alb
				mg%				$U \cdot l^{-1}$			$g \cdot l^{-1}$	
1	53	breast	liver	1.4	1.1	335	198	40	52	243	70	40
2	55	breast	liver bones	0.7	0.9	80	90	52	59	598	64	35
3	45	breast	bones	0.7	0.8	140	10	8	8	210	70	36
4	57	adeno	lungs bones	0.6	0.5	90	7	10	9	201	75	37
5	58	adeno	lungs pleura	0.5	0.8	110	35	5	9	205	60	34
Reference values:				0.6 – 1.4	0.2 – 1.0	40 – 90	6 – 28	3 – 18	3 – 22	40 – 240	60 – 80	35 – 50

Plasma-monovette, Sahrstedt, FRG) and centrifuged within a few minutes at room temperature, at 2000 g for 10 min. The plasma was transferred to Eppendorf reaction tubes (1.5 ml vol.).

Urine samples were collected in black polypropylene containers. After 6 hours the volume was measured and a small sample (1 ml) was transferred to an Eppendorf reaction tube. All samples were stored at −20°C until analysis. After thawing, all samples were centrifuged at 4°C for 5 min, at 4000 g, to remove clotted material.

High-performance liquid chromatography analysis

The HPLC assay for detection and quantification of the two parent drugs and their metabolites was recently developed by Mross et al. [12]. Briefly, the chromatographic system consists of a solvent delivery system L-6000, an autosampler 655 A-40, a fluorescence spectrophotometer F-1000 and a chromato-integrator D-2000 with a double-disk drive for data storage (Merck, Darmstadt, FRG). The HPLC column used was packed with reversed phase material Microspher 3 µm, C-18, 200 × 4.6 mm (Chromsep, Chrompack, Frankfurt, FRG) connected to a guard column. The emission wave length was 580 nm and the excitation wave length 480 nm. An isocratic eluent was used consisting of 0.02 M NaH_2PO_4 pH3 and acetonitrile (3/2 v/v) at a flow rate of 0.8 $ml \cdot min^{-1}$.

DOX, I-DOX and their metabolites were extracted from human plasma using Bond Elut C-18 columns, (ICT, Frankfurt, FRG) pretreated with 5 ml methanol and 5 ml distilled water. Plasma/ml was introduced onto the extraction column, subsequently purged with 4 ml buffer (0.02 M NaH_2PO_4 pH3), dried with a flow of air, and eluted with 4 ml chloroform/methanol (1/1 v/v). The eluate was evaporated to dryness at 50°C under a stream of pure nitrogen. The residue was redissolved in 100 µl buffer, vortexed and centrifuged (1 min, 15000 g, 20°C), and 50 µl was injected onto the analytical HPLC column. The internal standard was daunorubicin, which was added to all plasma samples prior to extraction. All samples were prepared in duplicate. A full calibration line was included in each patient series. All samples up to 4 h were diluted with heparinized blank plasma in various degrees so that all the assays could be run at the highest sensitivity level without any change in the detector settings.

Pharmacokinetic analysis

Each set of concentration-time C(t) values of DOX, I-DOX, AOL and I-AOL was fitted to the appropriate polyexponential equation using the program JANA (Statistical Consultants, Inc., Lexington, USA). Based on these initial estimates, it was decided to describe the results of DOX and I-DOX according to a three compartment model, because the r^2 of the JANA leastsquares fit was always better than 0.99.

The final pharmacokinetic calculations were done by MedUSA, a software package specifically designed to our specifications. The Medical Usage of Scientific Algorithms (MedUSA) program version 1.5 was developed by Dr. P. Varkonyi and is distributed by SCIAN Software, Inc., Toronto, Canada.

All fitting followed the tri-exponential equation:

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

The pharmacokinetic parameters were calculated using the following equations:

$$(2)\ C_{max} = A + B + C\ (\mu mol)$$

$$(3)\ V = D/C_{max}\ (l)$$

$$(4)\ V_{ss} = D \times AUMC/BW \times AUC^2\ (l \cdot kg^{-1})$$

$$(5)\ CL_P = D/AUC\ (l \cdot h^{-1})$$

(6) AUC: The area under the curve was calculated from the experimental data by the trapezoidal rule and the terminal elimination ($\mu mol \cdot l^{-1} \times h$).

Table 2a, b. Pharmacokinetics
a) of I-DOX

No.	Dose	C_{max}	V	V_{ss}	CL_P	AUC	$t_{1/2\alpha}$	$t_{1/2\beta}$	$t_{1/2\gamma}$	MRT
1	152	1.36	113	10.9	739	0.18	0.013	0.24	1.6	1.1
2	122	1.43	85	30.2	505	0.26	0.051	0.27	17.8	3.9
3	145	1.08	134	28.6	748	0.19	0.022	0.39	5.6	2.5
4	114	0.75	153	30.1	576	0.23	0.074	2.82	12.7	3.5
5	160	1.72	93	7.5	597	0.22	0.021	0.21	2.2	1.0

b) DOX

No.	Dose	C_{max}	V	V_{ss}	CL_P	AUC	$t_{1/2\alpha}$	$t_{1/2\beta}$	$t_{1/2\gamma}$	MRT
1	147	12.9	11.3	18.4	48.3	3.1	0.076	2.72	36.7	24.6
2	146	11.7	12.5	18.1	56.1	2.8	0.063	1.74	25.5	19.9
3	138	10.7	12.9	17.3	73.3	1.8	0.047	0.37	23.4	17.6
4	138	14.9	9.3	9.5	55.2	2.7	0.048	0.29	22.1	11.8
5	172	15.4	11.2	13.7	56.1	2.6	0.050	0.65	24.8	17.2

Dose µmol
C_{max} (peak plasma concentration) µmol
V (volume of distribution) l
V_{ss} (Vd at steady state) $l \cdot kg^{-1}$
CL_P (plasma clearance normalized to 1.74 m^2) $l \cdot h^{-1}$
$t_{1/2}$ (distribution, intermediate, elimination half life) h
MRT (mean residence time) h

(7) AUMC: The area under the drug concentration-time versus time plot to infinity was calculated from time × concentration values by the trapezoidal rule and the terminal elimination.

(8) MRT = AUMC/AUC (h)

(9) $t_{1/2} = \ln 2 \cdot k$ ($k = \alpha, \beta, \gamma$) (h)

The abbreviations used are:

C_{max}	peak plasma concentration,
V	volume of distribution,
V_{ss}	volume of distribution at steady state,
CL_P	plasma clearance,
D	dose,
BW	body weight,
AUC	area under the c(t) curve,
AUMC	area under the c × c(t) curve,
MRT	mean residence time,
$t_{1/2}$	half-life.

Results

Summarized data from 5 patients treated either with I-DOX or DOX are presented. Pharmacokinetic parameters for the parent drugs, calculated according to the Formulae 1–9 in the previous section, are shown in Tables 2a and 2b. The disappearance of both parent drugs was triphasic. The half-life of DOX was always longer than at of I-DOX. The mean volume of distribution of I-DOX ten-times higher of than DOX, whereas their volumes of distribution at steady state were not significantly different. The plasma clearance of I-DOX, normalized to 1.74 m^2 body surface area, was 10-fold higher than that of DOX. The AUC of DOX was larger than that of I-DOX. For ease of comparison, the mean values ± SD of all estimated pharmacokinetic parameters of DOX and I-DOX are summarized in Table 3.

DOX and I-DOX were rapidly metabolized and immediately after the bolus injection all metabolites were detectable. As shown in Tables 2a and 2b, peak plasma concentrations ranged from 10.7 to 15.4 µmol DOX and from 0.75 to 1.72 µmol I-DOX. The conversion from DOX to AOL and from I-DOX to I-AOL were different. Immediately after bolus injection I-DOX was metabolized to I-AOL, the levels of detectable I-AOL being as high or even higher than those of the parent drug. The conversion from DOX to AOL was less rapid.

The pharmacokinetics of I-AOL best fibled a two exponential equation. Because of the irregular plasma concentration-time curves of the other metabolites (including AOL) it was not possible to fit exponential terms to those curves. It was feasible, however, to calculate the terminal half-lives of the metabolites from 4 h onwards (Table 4a) by least squares fitting. The longest half-lives were found for AOLON and AON in case of DOX, but the accuracy of the determination was poor (high standard deviation), because of the very low concentrations of those two metabolites. In case of I-DOX, the longest calculated half-life was for 7d-AON, but again the accuracy of the calculation was low. The half-lives of AOL and I-AOL were similar and much longer than those of the parent drugs. The mean residence times of all compounds are shown in Table 4b. All calculated MRTs were shorter in case of I-DOX and its metabolites than of DOX and its metabolites. The most striking difference found was in the parent drugs.

Representative plasma decay curves for DOX, I-DOX and their metabolites are illustrated in Figs. 3a and b, respectively. AUCs of the parent drugs and their metabolites, calculated by the trapezoidal rule, are listed in Table 4c. It can be deduced that AOL is the major metabolite in case of DOX. In case of I-DOX, I-AOL and AOLON, the aglycone of I-AOL, are the major metabolites. The AUCs of those two metabolites reached almost 25-times and 10-times, respectively, the value of their parent drug and led to doubling of the total AUC of I-DOX compared to DOX. The AUC for AOL was only one-fifth of that of the corresponding I-DOX metabolite.

The aglycones, especially 7d-AOLON, behaved irregularly in terms of their plasma concentration versus time curves. The highest concentrations were found immediately after injection, followed by a rapid decrease. A second peak was always detected 4 to 16 h after drug administration. The cumulative urinary excretion (0–48 h) is listed in Table 5. After DOX the parent drug and AOL

Table 3. Comparison of the summarized pharmacokinetic data of I-DOX and DOX, ( ) = standard deviation (SD)

	DOX		I-DOX	
Dose [µmol]	148.0	(14.0)	139.0	(20.0)
PPC [µmol]	13.1	(2.0)	1.3	(0.4)
V [l]	11.4	(1.4)	116.0	(26.0)
V_{ss} [$l \cdot kg^{-1}$]	15.4	(3.8)	19.5	(10.0)
CL_P [$l \cdot h^{-1}$]	58.0	(9.3)	633.0	(106)
AUC [µmol $\cdot l^{-1} \times h$]	2.6	(0.5)	0.2	(0.03)
$t_{1/2\alpha}$ [h]	0.06	(0.01)	0.04	(0.02)
$t_{1/2\beta}$ [h]	1.2	(1.0)	0.9	(1.1)
$t_{1/2\gamma}$ [h]	26.5	(5.8)	7.9	(7.0)
MRT [h]	18.2	(4.6)	2.4	(1.4)

Table 4a. Terminal half-lives of I-DOX, DOX, and their metabolites

COMPOUNDS	DOX (h)	I-DOX (h)
DOX/I-DOX	26.5 (5.8)	7.9 (7.0)
AOL/I-AOL	28.2 (7.4)	24.0 (15.0)
AOLONE	83.0 (82.0)	16.0 (7.0)
7d-AOLONE	19.5 (9.9)	23.0 (10.0)
AON	114.0 (99.0)	14.0 (4.1)
7d-AON	30.4 (12.2)	66.1 (82.3)

b) their mean residence times

COMPOUNDS	DOX (h)	I-DOX (h)
DOX/I-DOX	18.2 (4.6)	2.4 (1.4)
AOL/I-AOL	42.0 (9.0)	29.6 (12.6)
AOLONE	122.0 (115)	60.5 (51.8)
7d-AOLONE	24.5 (11.2)	20.6 (11.0)
AON	105.0 (58.1)	24.7 (13.8)
7d-AON	33.7 (10.5)	16.6 (7.9)

c) their areas under the C(t) curves

COMPOUNDS	DOX [$\mu mol \cdot l^{-1} \cdot h$]	I-DOX
DOX/I-DOX	2.6 (0.5)	0.2 (0.03)
AOL/I-AOL	1.2 (0.5)	5.5 (1.8)
AOLONE	0.2 (0.2)	2.1 (1.7)
7d-AOLONE	0.3 (0.2)	0.5 (0.4)
AON	0.3 (0.3)	0.4 (0.4)
7d-AON	0.2 (0.1)	0.1 (0.1)
Total AUC	4.8	8.8

Table 5. Cumulative urinary excretion of I-DOX, DOX, and their metabolites in % of the amount of drug administered

COMPOUND	I-DOX	DOX
I-DOX/DOX	0.4 (0.2)	5.8 (1.4)
I-AOL/AOL	4.7 (3.5)	1.1 (0.5)
Total	5.1	6.9

were detectable in urine, as were I-DOX and I-AOL after I-DOX. The urinary excretion of DOX was 6% whereas less than 1% I-DOX was found. Comparable differences were found for AOL and I-AOL. The excretion of AOL was about 1% of the total drug, whereas about 5% I-AOL was excreted.

Discussion

I-DOX and DOX differ at the 4′-position of the daunosamine sugar. In case of I-DOX an iodine has replaced the hydroxy group at that position (see Fig. 1). This significantly increases the lipophilic character of the drug, as evidenced by the partition coefficients of I-DOX and DOX – 31.4 and 0.52 [13]. Furthermore, the electronegative iodine atom reduces the basicity of the neighbouring amino group at the 3′-position, lowering the pK_a from 8.2 for DOX to 6.4 for I-DOX. Consequently, I-DOX is almost unionized (98%) at physiological pH whereas DOX is ionized (90%). Higher lipophilicity combined with an uncharged molecule are features of drugs which can cross

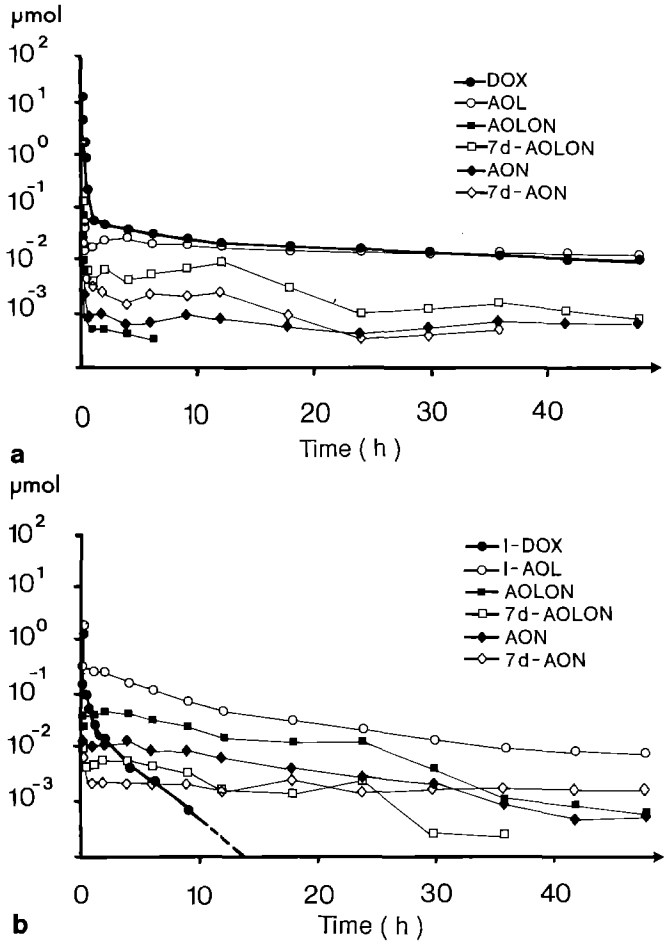

Fig. 3a, b. Pharmacokinetics (c(t) for **a**) DOX and its metabolites; and **b**) I-DOX and its metabolites

cell membranes very easily and very fast. More rapid uptake and a higher intracellular content of I-DOX in comparison to DOX has been found [8].

The structural modification and different physicochemical properties of I-DOX appear to be critical, as the pharmacokinetics and metabolism of the two drugs differ in several aspects. The present study was a comparison of the pharmacokinetics of I-DOX and DOX in ten patients with advanced cancer. The plasma elimination of I-DOX and DOX appeared to be triphasic after an intravenous bolus, which is consistent with previously published data in the case of DOX [3]. The pharmacokinetics of I-DOX in mice were best described by biphasic elimination [10]. The half-life of distribution was extremely short both for I-DOX and DOX, while in all patients the half-life of terminal elimination of DOX was longer than that of I-DOX. The same holds true for the mean residence time, which was eight-times shorter for I-DOX than DOX.

Both drugs appeared to be distributed into a deep tissue compartment, as can be seen from the size of the apparent volume of distribution at steady state. The very high values for V_{ss} reflect the great affinity of both drugs for enzymes, proteins and/or lipids. Less than 0.4% of either drug is detectable in the plasma, almost the entire amount injected being distributed in organs or metabolized [14].

The mean residence times of I-AOL and AOL were longer than those of the parent drugs (respectively 10-times and 2-times), and were even longer in the case of the 4 aglycones. Exact determination of the half-lives for the aglycones was hampered by irregularities in the C(t)-curves. The decision to use the last 10 values of the C(t) curve was quite arbitrary. The MRT is more independent. It is known that AOL is cytotoxic and may contribute to myelotoxicity and cardiotoxicity [15]. I-AOL produces cytotoxicity in sensitive tumor cells comparable to that of its parent drug, but it is much less active in DOX-resistant cell lines when compared to I-DOX [16].

The metabolism of the two drugs was very similar. The major enzymatic step in human metabolism of both drugs involved carbonyl reduction by aldo-keto reductase [10], but there were considerable differences. The AUC of I-AOL was 25-fold larger than that of I-DOX, and the AUC of AOL was half that of DOX. I-AOL is detectable in plasma within seconds, reaching levels similar to or higher than those of the parent drug. Therefore, the enzymatic conversion must be extremely rapid and takes place within the vascular space. I-DOX can be rapidly transformed into 13-OH-I-DOX (I-AOL) by human red blood cells. Looking at the ratios of $AUC_{met}/AUC_{parent\ drug}$ of different anthracyclines, it is clear that the ease of crossing membranes, which depends on the physico-chemical properties of the molecules (pK_a, lipophilicity etc.), bears on this phenomenon. In the case of EPI, the EOL/EPI ratio is 0.2, for AOL/DOX it is 0.35, in case of DNRol/DNR it is 3, for IDAol/IDA it is 3, and in the case of I-AOL/I-DOX it is 27 [3, 17]. These results are in accordance with the fact that the second important metabolite of I-DOX is AOLON, which can only be generated via AOL by reductive or hydrolytic cleavage of the daunosaminesugar moiety. The ratios $AUC_{AOL}/AUC_{AOLON} + AUC_{7d\text{-}Aolon}$ and $AUC_{I\text{-}AOL}/AUC_{AOLON} + AUC_{7d\text{-}Aolon}$ were identical.

Search for polar metabolites was unproductive and the treatment of plasma samples with sulphatase and glucuronidase had no effect, suggesting that there was no glucuronidation or sulphatation of I-DOX or DOX. Nevertheless, after administration of I-DOX to mice several unknown metabolites were recently described [1]. There were eleven peaks, only three of which could be identified (I-DOX, I-AOL and AON). It appears possible that the other three aglycones were included in the group of unknown peaks. In the present study unknown peaks were not seen in some cases, apart from an invariable peak between I-AOL and 7d-AON. The gut microflora are known to be very active in reductive reactions [18]. Ingested compounds which are poorly absorbed from the gut will stand the greatest change of undergoing metabolism through the intestinal flora, although a large number of compounds gain entry to the gut via biliary secretion, as in the case of the anthracyclines. Reductive dehalogenation would give the anthracycline 4′-deoxy-doxorubicin (ESO). During the present study ESO was not included in the metabolite mixture for construction of the calibration lines, but ESO has a retention time very near the unknown peak, suggesting that dehalogenation may well have taken place. The Phase I study is still running and pharmacokinetic evaluation will be done at higher drug levels ($75\ mg \cdot m^{-2}$ and $90\ mg \cdot m^{-2}$). Special attention will be given to this problem. The reductive and hydrolytic capacity of the gut affords a good explanation for the re-entry of apolar metabolites (expecially the aglycones). I-DOX and DOX, I-AOL and AOL are excreted by biliary excretion and can undergo deglycosidation and reduction in the intestine, thereby promoting reabsorption and enterohepatic cycling of the aglycones. This may explain why the 7d-aglycones always showed a second peak in the plasma C(t) curves, a phenomenon which was recently described for EPI and DOX [3] too.

I-DOX pharmacokinetics and metabolism have interesting aspects when compared to DOX. In preclinical studies I-DOX was described as more potent than DOX [19], as having greater toxicity to haemopoietic progenitor and blood cells [20], less cardiotoxicity [21], lack of cross resistance in DOX-resistant cell lines [9], and a similar or identical mechanism of action to DOX [22]. Its greater potency could only be measured in cell culture systems, because of their highly artificial environment and the very special pharmacokinetics and metabolism of I-DOX were not taken into account. The MTD for I-DOX was not reached at $50\ mg \cdot m^{-2}$, $75\ mg \cdot m^{-2}$ has been given to man and one more escalation is likely to be necessary to reach the MTD. These values show that the MTD is very similar to that of DOX and greater potency has been not observed [23]. This special pharmacokinetic behaviour of I-DOX was not predicted by animal studies [11], a fact well known from EPI, too [24].

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