Eur J Clin Pharmacol (1991) 40: 287–291
003169709100074Y

European Journal of Clinical Pharmacology

Chronopharmacokinetics of doxorubicin in patients with breast cancer

P. Canal[1, 5], A. Sqalli[2, 3], M. de Forni[1, 3], C. Chevreau[1], A. Pujol[1], R. Bugat[1, 3], H. Roche[1], J. Oustrin[3, 4], and G. Houin[2, 3, 5]

[1] Unités de Pharmacocinétique et de Pharmacologie Cliniques, Centre Claudius Regaud,
[2] Unité de Pharmacocinétique, Hôpital Purpan,
[3] Université Paul Sabatier,
[4] Groupe de Chronobiologie et de Chronopharmacologie, Laboratoire de Biophysique et de Biomathématiques, Faculté de Pharmacie, and
[5] Centre Interdisciplinaire d'Etudes en Pharmacocinétique de Toulouse, Toulouse, France

Received: February 27, 1990/Accepted in revised form: June 7, 1990

Summary. The chronopharmacokinetics of doxorubicin (DOX) has been studied in 18 patients suffering from breast cancer. They received combined chemotherapy, including DOX (50 mg/m^2 as an iv bolus), given at two different times (09.00 h or 21.00 h). The two randomized courses of the protocol were given to each patient at a four week interval.

The total body clearance (CL) of DOX was significantly decreased when the drug was administered at 21.00 h, resulting in a longer elimination half-life and an increase in AUC. The renal clearance of DOX did not differ at the different times of administration, and it appears that the decrease in CL was related to a change in hepatic blood flow. The volume of distribution of the drug was not changed.

Key words: Doxorubicin, breast cancer; chronopharmacokinetics, total body clearance, hepatic clearance, hepatic blood flow

Chronopharmacokinetics refers to rhythmic changes in the bioavailability, metabolism and/or excretion of a medicine. Chronopharmacokinetic effects have been documented for several groups of drugs, such as analgesics and non steroidal anti-inflammatory agents, theophylline, digitalis and propranolol, and drugs used in neurology and psychiatry [Reinberg and Smolensky 1982]. Although the toxicity of at least 20 commonly used anticancer drugs has been shown in animal studies to depend on the time of administration [Levi 1987, Hrushesky et al. 1989, Mormont et al. 1989], until now cisplatin and 5-fluorouracil have been the only anticancer drugs for which a chronopharmacokinetic study has been carried out in patients [Hrushesky et al. 1980, Petit et al. 1988].

Doxorubicin (DOX) is the most active drug in breast cancer, it is commonly given in association with 5FU and cyclophosphamide. Its clinical chronopharmacology was studied by Hrushesky (1985) who showed that DOX, given shortly before the usual awakening time (06.00 h), was better tolerated during the month after treatment than when it was given in the evening (18.00 h).

The aim of the present study was to determine if the time of administration of DOX in patients suffering from breast cancer would modify the pharmacokinetic disposition and metabolism of DOX.

Subjects and methods

18 female patients ranging from 41 to 74 years of age, with a performance status of 3 or better took part in the study, after given written consent to it (Table 1). The study had been approved by the local ethical committee. They were suffering from breast adenocarcinoma and had not previously received chemotherapy, including anthracycline. Five patients had received drugs for adjuvant therapy and 13 others as palliative therapy for bone (8), lung (4), cutaneous (2), pleural (1) or liver (1) metastases. They had a bilirubin < 20 µmol·l^{-1} and a creatinine ranging from 59 to 125 µmol·l^{-1}, and leukocyte and platelet counts greater than 3000 cells per ml and 100.000 cells per ml, respectively.

Table 1. Details of the patients

Patient	Age	Metastases	DOX dose mg	Performance status (WHO scale)
BEL.	52	0	100	0–1
DEA	50	pleural	80	2
PON	62	bone	80	0–2
ADE	67	lung-bone	85	3
LAC	60	bone	85	1
DUN	41	0	87	0
ALT	55	bone	89	3
DEB	74	cutaneous-lung	72	1–3
MAU	68	0	82	0
BRU	53	bone	90	2
ROD	41	0	75	0
SAL	54	cutaneous-lung	75	1
GAS	56	bone	75	1
LUE	55	0	80	0–1
LLA	64	bone	80	2–3
CLA	66	bone	73	0–1
FER	55	liver	89	2
GRO	59	lung-bone	84	2

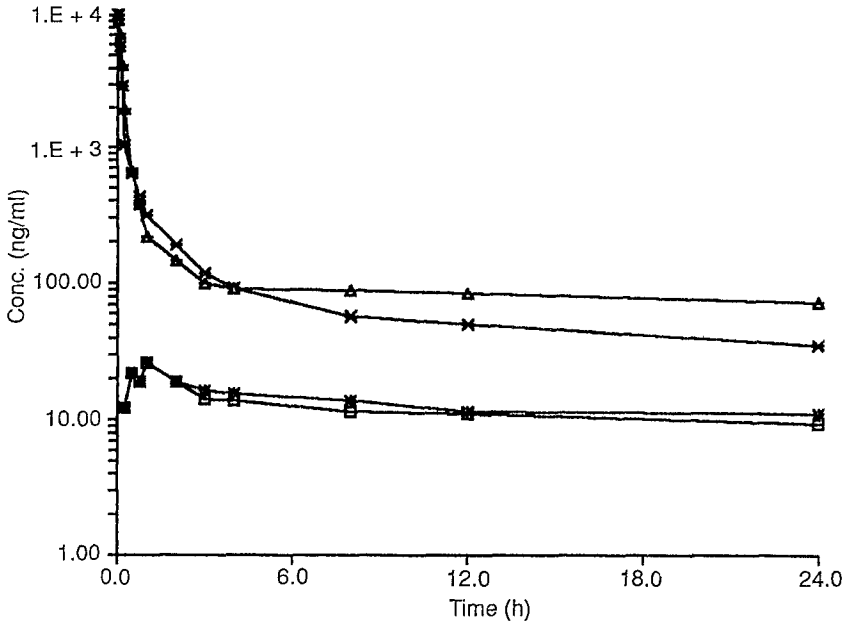

Fig. 1. Plasma concentration-time curves of doxorubicin and doxorubicinol as a function of time. Doxorubicin was administered to the same patient by iv bolus at 09.00 h or at 21.00 h at a 4 week interval, □ DOXOL 09.00 h * DOXOL 21.00 h x DOX 09.00 h △ DOX 21.00 h

The patients received combined chemotherapy including DOX. The circadian treatment schedule was randomly allocated to each patient, the drugs being given at 09.00 h or 21.00 h on one occasion, and the second course was automatically administered at the other time. Each patient was her own control. Nine patients each received the first cycle at 09.00 h or 21.00 h. The order of administration of the other drugs was kept constant.

The chemotherapy regimen consisted of:
5FU (500 mg/m^2) administered in 250 ml isotonic 5% dextrose solution, as a 15 min infusion, at 08.45 h or at 20.45 h.
DOX (50 mg/m^2) administered as an iv bolus over 5 min at 09.00 h or at 21.00 h.
Cyclophosphamide 500 mg/m^2 administered, in 1000 ml isotonic 5% dextrose solution, as a 1 h infusion, at 09.15 h or 21.15 h.
Treatment was repeated at four week intervals.

Blood samples were collected in EDTA tubes ($n = 15$) before, at the end of administration and 2, 5, 15, 30 and 45 min, and 1, 2, 3, 4, 8, 12 and 24 h after the end of the infusion. Blood was immediately centrifuged at 1000 g at 4 °C, and plasma was separated and frozen at −20 °C until analysed. When possible, 24 h urines were also collected, the volume noted and an aliquot was taken after homogenization and frozen at −20 °C until analysed.

Plasma DOX and doxorubicinol (its main metabolite; DOXOL) concentrations were determined by HPLC with fluorescence detection [Sqalli et al. 1989]. In brief, after addition of daunorubicin as internal standard, and 0.05 M borate buffer (pH 9.8) 1.5 ml, plasma 1 ml was extracted with 5 ml chloroform/methanol (4/1). After centrifugation at 1000 g, the organic phase was removed and evaporated to dryness under nitrogen stream. The residue was reconstituted with 200 µl mobile phase and 20 µl or more was injected onto a µBondapak WATERS C18 column eluted by a mobile phase (1 ml/min) of 0.05 N H_3PO_4, acetonitrile, tetrahydrofurane, triethylamine (59.8/35/5/0.2) pH 2.5. The peaks of DOX (retention time 4.8 min), DOXOL (retention time 3.9 min) and daunorubicin (retention time 5.8 min) were detected by fluorescence ($\lambda_{exc.}$ 478 nm; λ_{em} 550 nm). The limit of detection of DOX and DOXOL was 1 ng·ml^{-1}, and the coefficients of variation for within and between day error were 3.7% and 6.9%, respectively.

For each patient, samples from both cycles of chemotherapy were analyzed at the same time.

The pharmacokinetic parameters were calculated using an open three compartment model with the Siphar computer program. The pharmacokinetic parameters in each patient were compared by two way ANOVA.

Results

Eighteen patients received two cycles of chemotherapy, including DOX, at two times, and at a four week interval. No reduction in dosage or delay in treatment was required for the two schedules of administration. No difference in terms of performance status or biochemical parameters were noticed in individual patients between Cycles 1 and 2.

The plasma concentration-time curve of DOX was well described in all cases by a three exponential model. The DOX and DOXOL profiles in one patient when the drug was administered at 09.00 h or 21.00 h are shown in Fig. 1.

Table 2. Mean pharmacokinetic parameters of doxorubicin (DOX) and doxorubicinol (DOXOL). Doxorubicin was administered by iv bolus either at 09.00 h or at 21.00 h.
CL total body clearance. V_z volume of distribution of the β phase. V_{ss} volume of distribution at steady state. AUC area under the curve. CL_R renal clearance. fe urinary elimination fraction

		CL l·h^{-1}·(m^{-2})	V_z l·(m^{-2})	V_{ss} l·(m^{-2})	$t_{1/2\gamma}$ h	AUC DOX µg·h·l^{-1}	CL_R ml·min^{-1}·m^{-2}	fe %	AUC DOXOL µg·h·l^{-1}	DOXOL/DOX AUC %
09.00 h	Mean	35.3	565	307	12.6	1742	77.8	10.02	317	21.6
	(SD)	(18.4)	(322)	(198)	(6.5)	(646)	(39.2)	(6.93)	(169)	(9.9)
21.00 h	Mean	27.9	682	412	21.7	2503	71.2	9.48	478	26.2
	(SD)	(13.3)	(323)	(223)	(21.7)	(2095)	(29.6)	(4.66)	(355)	(14.1)

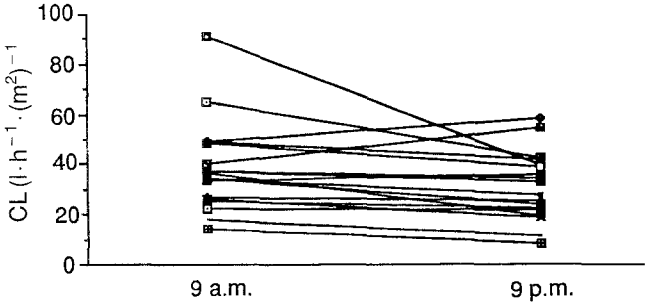

Fig. 2. Comparison of total body clearance of doxorubicin in individual patients as a function of the hour of administration of the drug. Doxorubicin was administered by iv bolus at 09.00 h or at 21.00 h at a 4 week interval

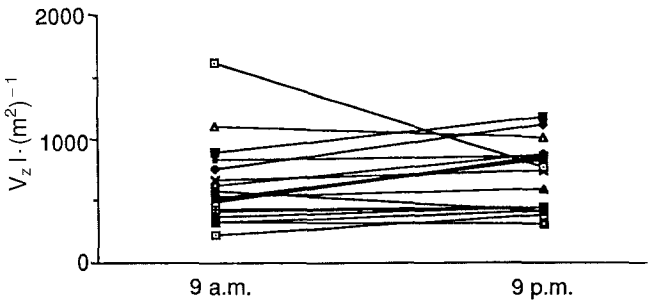

Fig. 3. Comparison of the volume of distribution of the β phase of doxorubicin in individual patients as a function of the hour of administration of the drug. Doxorubicin was administered by iv bolus at 09.00 h or at 21.00 h at a 4 week interval

The main pharmacokinetic parameters of DOX calculated after administration at the two different times are summarized in Table 2.

Considerable interpatient variability in the pharmacokinetic parameters CL ($P < 0.01$) and V_z ($P < 0.05$) was established by the two-way ANOVA (Table 3).

No modification in the pharmacokinetic parameters was found depending on the order of the administration.

Comparison of the parameters obtained after DOX administration to the same patient at 09.00 h or 21.00 h by two way ANOVA showed that the total body clearance (CL) of DOX was significantly decreased ($P < 0.05$) when DOX was administered at 21.00 h. The mean percentage decrease, which was found in 15 of the 18 patients, was 47.9 (40.8)%. In the other three patients there was a small increase or no change in CL (Fig. 2). The volume of distribution of the β phase (Fig. 3) and the volume of distribution at steady state did not differ as a function of the time of administration. The decrease in CL led to a longer elimination half-life ($t_{1/2\,\gamma}$ $P < 0.05$; mean percentage increase 78.5%) in all cases (Fig. 4), and significantly greater exposure (AUC) of the patient to the drug at 21.00 h ($P < 0.05$; increase in 15 cases +49.1 (42)%) and no change in the other three cases (Fig. 5).

In 8 patients it was possible to calculate the renal clearance of DOX in the two cycles of chemotherapy, and no significant difference between the morning and the evening was observed. This elimination pathway represented only about 10% of the administered dose (9.74 (5.68)%).

When comparing the AUC of DOXOL in individual patients treated at 09.00 h or 21.00 h, a significant difference ($P < 0.05$) was found in 13 cases, in whom the AUC was higher at 21.00 h than at 09.00 h (Fig. 6). The mean percentage increase was 62.4 (48.5)%. The DOXOL/DOX AUC ratio was not significantly different in the two courses of chemotherapy.

Table 3. Pharmacokinetic parameters of doxorubicin and doxorubicinol, its main metabolite, in individual patients after iv administration of doxorubicin at different times of day, at a two cycles of chemotherapy four week interval

		First cycle at 09.00 h (C1) second cycle at 21.00 h (C2)							First cycle at 21.00 h (C1) second cycle at 09.00 h (C2)				
		CL	$t_{1/2\gamma}$	AUC	V_z	Doxol AUC			CL	$t_{1/2\gamma}$	AUC	V_z	Doxol AUC
GRO	C1	22.7	8	2.198	263	0.198	FER	C1	18.6	9.55	2.692	256	0.459
	C2	19.5	12.76	2.559	360	0.171		C2	19.5	9.49	2.558	268	0.422
LLA	C1	31.2	8.3	1.55	374	0.135	CLA	C1	8.2	28.4	6.106	335	1.596
	C2	24.4	10.8	2.045	381	0.149		C2	15.2	7.46	3.285	164	0.653
LUE	C1	37.2	11.41	1.344	612	0.106	SAL	C1	20.6	13.15	2.424	391	0.202
	C2	51.3	18.79	0.974	692	0.115		C2	31	7.97	1.614	356	0.143
GAS	C1	23.36	9.28	2.099	313	0.275	RO	C1	5.36	106.2	9.33	821	0.421
	C2	15.72	17.32	3.181	393	0.477		C2	11.0	34.9	4.44	567	0.280
ALT	C1	46.3	6.79	1.01	462	0.194	BRU	C1	16.22	15.28	3.083	358	0.456
	C2	38.47	14.27	1.27	792	0.536		C2	33.28	10.9	1.503	523	0.238
DUN	C1	45.8	11.63	1.04	769	0.480	MAU	C1	29.53	19.34	1.61	811	0.440
	C2	36.09	15.85	1.33	812	0.490		C2	34.2	8.79	1.38	438	0.353
LAC	C1	30.8	10.54	1.51	443	0.437	DEB	C1	22.15	17.27	2.1	543	1.120
	C2	33.12	17.41	1.42	781	0.425		C2	24.1	14.54	2.01	475	0.692
PON	C1	88.17	12.55	0.542	1550	0.165	BEL	C1	39.75	16.6	1.189	950	0.340
	C2	36.79	13.6	1.33	715	0.284		C2	61.65	11.8	0.78	1050	0.335
ADE	C1	34.55	16.5	1.425	706	0.285	DEA	C1	55.06	14.1	0.89	1118	0.473
	C2	31.7	29.3	1.53	1064	0.319		C2	46.17	11.8	0.078	838	0.373

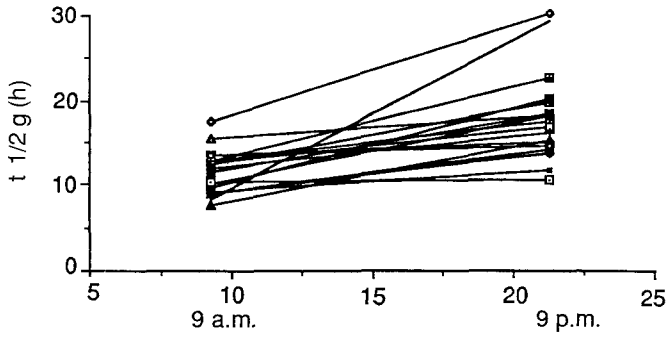

Fig. 4. Comparison of the elimination half-life of doxorubicin in individual patients as a function of the hour of administration of the drug. Doxorubicin was administered by iv bolus at 09.00 h or at 21.00 h at a 4 week interval

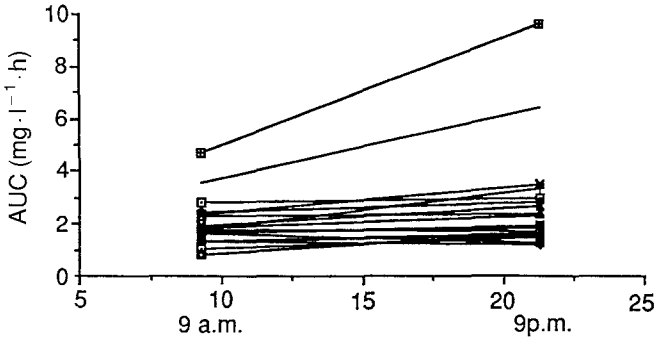

Fig. 5. Comparison of the AUC of doxorubicin in individual patients as a function of the hour of administration of the drug. Doxorubicin was administered by iv bolus at 09.00 h or at 21.00 h at a 4 week interval

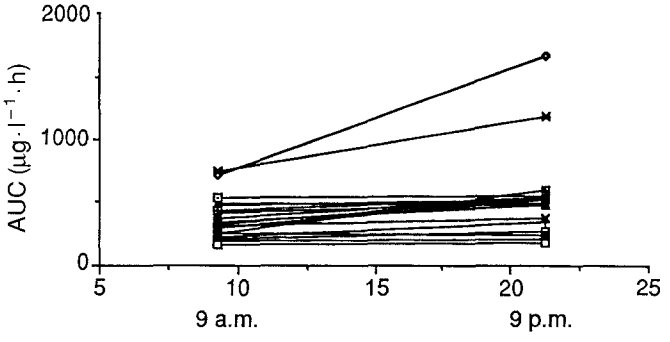

Fig. 6. Comparison of the AUC of doxorubicinol, the main metabolite of doxorubicin, in individual patients as a function of the hour of administration of doxorubicin, which was given by iv bolus at 09.00 h or at 21.00 h at a 4 week interval

Discussion

Doxorubicin is an antineoplastic drug that has been in common clinical use for more than a decade. Its clinical effects and pharmacokinetics are well known and have recently been reviewed [Speth et al. 1988]. The pharmacokinetics of DOX in breast cancer patients has well been documented by Robert et al. (1982). The present results agree with the published values: the plasma concentration curve can be well described by a triexponential model, with an elimination half-life between 27 and 50 h, and the pharmacokinetics is characterized by a high total body clearance, ranging between 28.3 and 98.7 $l \cdot h^{-1}$, and a volume of distribution of the β phase of 391 to 1281 l [Robert et al. 1985].

Larger inter and intra-patient variations in DOX pharmacokinetics may be related to some extent to individual differences in metabolism, toxicity or in time-dependent variations. Several authors [Piazza et al. 1980; Gil et al. 1983] have shown an increase in CL in patients who have received successive courses of treatment. Robert et al. (1983) also found dose-dependency of early phase-pharmacokinetics within a 6 h interval. Comparison of the pharmacokinetic parameters obtained here in each patient during two cycles of chemotherapy by two way ANOVA did not reveal any time-dependency of the pharmacokinetics of DOX.

The results have shown a difference in CL in each patient related to the time of DOX administration. The CL is the sum of the renal and hepatic clearance, which, in turn, represents metabolic clearance CL_M and biliary clearance. Analysis of the urinary elimination of DOX (both in terms of renal clearance and total amount excreted) showed that this route of elimination was not modified by the hour of DOX administration. Consequently, it seems that it was the hepatic clearance which was modified by the hour of administration.

DOX is rapidly metabolized by the liver into the active, hydrophilic 13-hydroxy-metabolite doxorubicinol [Takanashi and Bachur 1976; Bachur et al. 1976]. Variations in metabolism have been noted and the DOXOL/DOX AUC ratio has been reported to range between 0.3 and 0.9 [Speth et al. 1988]. Theoretically, this ratio is dependent upon three factors as shown in Equ. 1: CL, the elimination clearance of the metabolite CL_M and the fraction of drug converted to metabolite fm (Rowland and Tozer, 1980).

$$\frac{\text{AUC DOXOL}}{\text{AUC DOX}} = \frac{\text{fmCL}}{CL_M} \qquad \text{(equation 1)}$$

This ratio was not modified here by the time of administration. Since CL decreased, one possible explanation is that fm was increased during the night in the same proportion as CL was decreased. As it is recognized [Radzialowski and Bousquet 1968] that hepatic enzymatic activity increases during the nocturnal period of activity of rodents, which corresponds to the daylight period in man, this hypothesis seems unlikely to be true.

Or, CL_M might be decreased in the same conditions as CL. The decrease in CL_M would correspond to the biliary elimination of DOXOL, which is its major route of elimination, as for DOX [Ballet et al. 1987]. This mechanism could well explain the lack of change in the ratio.

Finally, it has been shown that DOX has the intermediate extraction ratio of 0.5 [Garnick et al. 1978]. Consequently, its hepatic clearance is a function of the hepatic blood flow and the protein binding ratio [Wilkinson 1987]. Some authors [Greene et al. 1983, Celio et al. 1983] have demonstrated that 50 to 85% of plasma DOX is bound to proteins. There is a large amplitude circadian rhythm in plasma proteins, with a peak at 16.00 h and a trough at 04.00 h [Touitou et al. 1979]. The present study has demonstrated that the volumes of distribution (β phase and at steady state) were not modified by the hour of DOX administration, or consequently by the protein binding ratio.

In rats, Labrecque et al. (1988) have demonstrated in rodents that hepatic blood flow was greater in the dark phase than in light phase. As the dark phase in rats can be compared to the light phase in man, it could be suggested that the hepatic blood flow would be decreased during the night in man, which could explain the observed decrease in the CL of DOX. In addition, there are many data demonstrating that the half life of drugs may be increased during the night in man, e.g. indomethacin [Clench et al. 1981], amidopyrine [Shively et al. 1981], and clorazepate [Aymard and Soulairac 1979].

There are few reports on the relationship between the pharmacokinetic and pharmacodynamic parameters of DOX. Robert et al. (1982) showed a weak correlation between the half-life of the first pharmacokinetic phase and the short-term clinical response. More recently, Ackland et al. (1989) have established a pharmacodynamic relationship between nadir WBC and the temporally related steady-state plasma DOX concentration after long-term continuous infusion of DOX. Steady state concentration is only a function of the clearance of a product, so a change in DOX clearance could be related to a difference in its haematological toxicity. Hrushesky (1985) has shown in 247 courses of doxorubicin therapy administered either at 06.00 h (115) or at 18.00 h (132) that dose reduction (due to haematological toxicity) was three-times more frequent when DOX was administered at 18.00 h. Those results are supported by the present pharmacokinetic data, which show that the clearance of DOX was reduced during the night, consequently increasing exposure of normal and tumour tissues to the drug.

Acknowledgements. The help and the cooperation of the nursing staff of the "Unité de Pharmacologie Clinique" is greatly appreciated. The work was supported by a grant from the "Comités Départementaux (Région Midi-Pyrénées) de la Ligue Nationale de Lutte Contre le Cancer".

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Dr. P. Canal
Centre Claudius Regaud
20–24, rue du Pont Saint-Pierre
31052 Toulouse Cedex, France