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Preben Jakobsen · Bente Sørensen · Lars Bastholt
Mansoor Raza Mirza · Susanne B. Gjedde
Henning T. Mouridsen · Carsten Rose

The pharmacokinetics of high-dose epirubicin and of the cardioprotector ADR-529 given together with cyclophosphamide, 5-fluorouracil, and tamoxifen in metastatic breast-cancer patients

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Abstract A high-pressure liquid chromatographic method for determination of the bisdioxopiperazine derivative ADR-529 (ICRF-187), a compound proven effective in protection against anthracycline-induced cardiotoxicity, has been developed. The limit of quantitation was 5 ng/ml using a narrow-bore 5-μm silica column and UV detection. The method was used for determination of pharmacokinetic profiles of ADR-529 after a 3-weekly i. v. administration of different doses of ADR-529 (600–1000 mg/m^2) together with different doses of epirubicin (E, 60–100 mg/m^2), fixed-dose cyclophosphamide (C, 600 mg/m^2), fixed-dose 5-fluorouracil (F, 600 mg/m^2), and daily administration of tamoxifen (T, 30 mg; CEF-T) in the treatment of patients with metastatic breast cancer. Pharmacokinetic parameters for epirubicin were also determined. The aim of the study was to determine (1) whether the pharmacokinetics of ADR-529 as part of a combination with CEF-T changes with increasing doses of ADR-529 and increasing doses of epirubicin and (2) whether the pharmacokinetics of epirubicin in the same combinations is altered with the administration of increasing doses of ADR-529. A total of 82 patients were included. A crossover study including 16 of the patients showed no significant difference in epirubicin pharmacokinetic parameters when epirubicin was given with or without concomitant administration of ADR-529. Apart from minor changes in the distributional half-lives, the pharmacokinetic parameters of epirubicin were not altered with increasing doses of ADR-529, nor were the pharmacokinetic parameters of ADR-529 itself. Escalating doses of epirubicin did not significantly alter the pharmacokinetic parameters of ADR-529 with the exception of a 30% increase in the terminal half-life and a decrease in total body clearance when the epirubicin dose was raised from 60 to 100 mg/m^2. We conclude that concomitant administration of ADR-529 does not alter the distribution and elimination of epirubicin in doses suitable for preventing the anthracycline-induced cardiotoxicity.

Key words Pharmacokinetics · ADR-529 · Epirubicin · Metastatic breast cancer · High-pressure liquid chromatography

P. Jakobsen (✉)
Institute of Pharmacology, University of Aarhus,
The Bartholin Building, DK-8000 Aarhus, Denmark

B. Sørensen · L. Bastholt · M. R. Mirza · C. Rose
Department of Oncology, Odense University Hospital,
Odense, Denmark

S. B. Gjedde · H. T. Mouridsen
Department of Oncology, Rigshospitalet,
Copenhagen, Denmark

Introduction

Epirubicin is a anthracycline analogue, differing from doxorubicin in the 4′ position where the OH-group has been epimerized [16]. This difference in chemical structure leads to an entirely different pharmacokinetic profile [2–4, 6, 13, 18, 22]. At equimolar doses, a 40% decrease in the AUC (area under the concentration versus time curve) of epirubicin as compared with doxorubicin is observed. This difference probably explains why epirubicin can be given at much higher doses than doxorubicin. At equimolar doses, epirubicin apparently is less cardiotoxic than doxorubicin [20]. However, cardiomyopathy represents one of the major dose-limiting factors to the use of anthracyclines in the treatment of malignant disease.

ADR-529 [ICRF-187; Adria Laboratories, Columbus, Ohio; *d*-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane] has been proven to protect animals from the cardiomyopathy produced by anthracyclines [1, 7–10, 21]. Phase III randomized studies [17, 23, 24, 27] have shown protective effects against chronic doxorubicin-induced cardiac toxicity in patients with advanced breast cancer as well.

The aim of the present study was to determine (1) whether the pharmacokinetics of ADR-529 as part of a combination including epirubicin, cyclophosphamide, 5-fluorouracil, and tamoxifen (CEF-T) changes with increasing doses of ADR-529 and increasing doses of epi-

Table 1 Treatment schedules

Groups	Number of patients	ADR-529 (mg/m²)	Epirubicin (mg/m²
I[a]	16	0	60
II	26	600	60
III	10	600	80
IV	10	600	100
V	10	800	80
VI	10	1000	80
VII	16	1000	100
Subgroups:			
II.1[b]	18	600	60
II.2[b]	8	600	60

[a] Patients participating in the crossover study (see Patients and methods)
[b] Subgroup II.1 received ADR-529 at the first treatment course; subgroup II.2 received ADR-529 at the second course

rubicin and (2) whether the pharmacokinetics of epirubicin in the same combinations is altered with the administration of ADR-529.

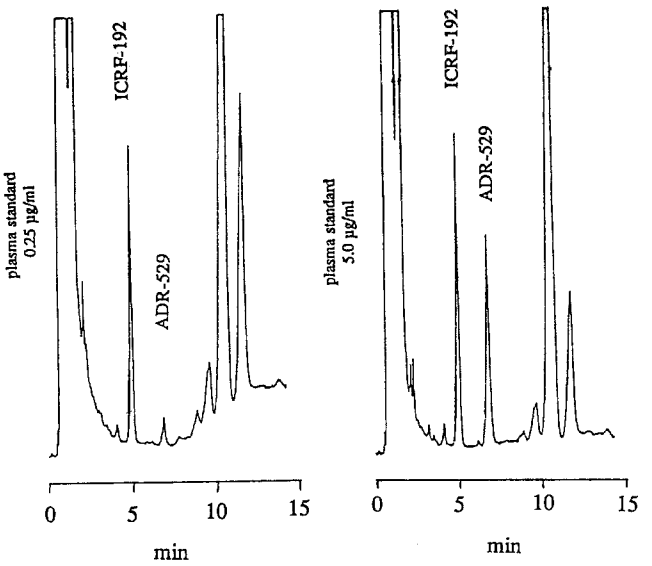

Fig. 1 Typical chromatograms obtained after solid-phase extraction of plasma samples spiked with ADR-529 and the internal standard (*ICRF-192*)

Patients and methods

Patients and blood sampling

A total of 82 patients (aged <70 years, with normal bone marrow function and adequate hepatic and kidney function) with metastatic breast carcinoma were consecutively allocated to receive a 3-weekly combination of ADR-529, CEF (cyclophosphamide, 600 mg/m²; epirubicin; 5-fluorouracil, 600 mg/m²), and tamoxifen (30 mg/day). ADR-529 and epirubicin were given according to the scheme outlined in Table 1.

In all, 16 patients from group II participated in a balanced crossover study. The patients were randomly allocated to receive CEF-T (epirubicin, 60 mg/m²; treatment A, group I) or CEF-T with the addition of ADR-529 (600 mg/m²; treatment B) in the first course of treatment. At the second course the patients were crossed to the alternative treatment. The pharmacokinetic profiles were determined at the first as well as the second treatment course. From the third course onward, all patients received ADR-529 + CEF-T. Cyclophosphamide and 5-fluorouracil were given as i.v. infusions before the administration of ADR-529 and epirubicin. Tamoxifen was given as a single daily oral dose. ADR-529 was given as a 15-min i.v. infusion, which was followed immediately by a 15-min i.v. infusion of epirubicin.

Blood samples were drawn from the arm opposite the infusionsite at –15 min (just before the administration of ADR-529), at time 0 (at the end of the ADR-529 infusion and before epirubicin administration), at 15 min (at the end of the epirubicin infusion), and then at 20, 30, 45, and 60 min and 2, 4, 6, 9, 12, 24, 48, 72, and 96 h. Blood samples (10 ml) were drawn into heparinized ice-cooled glass tubes and immediately centrifuged at 3000 *g* for 8 min. To ensure stability, plasma samples (2 ml) for analysis of ADR-529 were acidified with 0.05 ml 42.5% phosphoric acid. These samples and the remaining plasma for analysis of epirubicin were immediately stored at –20 °C until the shipment to the analytical laboratory, where the samples were stored at –80 °C until analysis.

Analytical methods

Analysis of ADR-529

The high-pressure liquid chromatographic (HPLC) procedure for the determination of ADR-529 used in our laboratory was developed from a method described by Lewis et al. [15]. This method required an electrochemical detector with an $Ag/AgNO_3$ reference electrode that was not available in our laboratory. However, the combination of a mini-bore HPLC column and a UV detector set at 209 nm gave a sensitivity comparable with that obtained using electrochemical detection (5 ng/ml in plasma samples).

Acidified plasma samples obtained from patients before 24 h were diluted with proven drug-free acidified plasma to lie within the working range (below 5 µg/ml), and analysis was carried out on 250 µl plasma; samples with concentrations expected to be below 200 ng/ml (samples obtained from patients at 24–96 h after infusion) were processed using 1-ml plasma samples. To the 250-µl samples were added 500 ng of the internal standard ICRF-192, a compound chemically related to ADR-529 (20 µl 25-µg/ml ICRF-192 in acetonitrile), 125 µl 0.36 *M* trisodium phosphate, and 1.5 ml distilled water; to the 1-ml plasma samples were added 100 ng internal standard (20 µl ICRF-192, 5 µg/ml in acetonitrile) and 1 ml 0.36 *M* trisodium phosphate (pH of the resulting solutions, 7.0–7.4). After centrifugation at 3000 *g* for 10 min, the supernatant was subjected to solid-phase extraction (SPE).

The SPE columns used were Analytichem Bond Elut C-18 reversed-phase material contained in 3-ml syringes. By means of gentle suction the columns, two in series, were preconditioned with 15 ml pure methanol followed by 20 ml distilled water, with care being taken that the columns did not dry out. The sample (2 ml) was then slowly passed through the columns. The columns were rinsed with 20 ml water, after which the columns were sucked completely dry. Then, 3 ml *n*-hexane was passed through. The *n*-hexane was discarded and the columns were sucked dry. Absorbed drugs were then eluted with 3 ml acetonitrile. The extract was evaporated to dryness at 40 °C under a gentle stream of nitrogen and the residue was dissolved in 200 µl of the chromatographic eluent, of which 50 µl was chromatographed.

The HPLC system consisted of a narrow-bore stainless-steel column (25 cm × 2 mm inside diameter) slurry-packed with Spherisorb 5µ-silica, a Shimadzu LC-9A pump, an LCD Analytical Spectromonitor variable-wavelength detector set at a wavelength of 209 nm, and a Spark-Holland Promis II automatic injector. HPLC spectra were recorded on a Shimadzu C-R3A automatic integrator/recorder. The chromatographic eluent, acetonitrile/0.3 *M* phosphoric acid (93/7, v/v), was pumped through the system at a flow rate of 0.7 ml/min. Retention times were 5.2 min for the internal standard and 7.1 min for ADR-529. A large peak eluting 0.7 min before the ADR-529 peak was observed in

one patient receiving epirubicin (100 mg/m^2), CF-T, and ADR-529 (1000 mg/m^2). This peak, the appearance of which could not be explained, interfered with the determination of the late (small) ADR-529 concentrations. Apart from this, no other interference from the patients' plasma samples was observed. Figure 1 shows chromatograms obtained from two calibration samples.

Two sets of standard curves were constructed, one for high-range samples (5–0.2 μg/ml) using 250-μl calibration samples and one for low-range samples (200–10 ng/ml) using 1-ml calibration samples. Standard curves were constructed from weighted linear regression analysis of the peak height ratio expressed as a percentage of the internal standard versus known calibration standards. The square of regression coefficients were always between 0.99 and 1.0. The intercept of the regression line with the X-axis was in no case more than 10% of the lowest calibration sample. Recoveries of ADR-529 and internal standard were more than 90%. Within-day coefficients of variation (CV) were determined from 1-ml plasma samples containing 10, 50, and 100 ng/ml and from 0.25-ml plasma samples containing 0.25, 1, and 5 μg/ml. At 10 ng/ml, CV were 15%; at the other concentration levels they were about 5%.

Analysis of epirubicin/epirubicinol

Epirubicin and epirubicinol concentrations in plasma were determined by HPLC using a method described by Jakobsen et al. [14]. In brief, 0.5-ml plasma samples containing doxorubicin (100 ng/ml) as the internal standard were extracted with 5 ml chloroform : isopropanol (4 : 1, v/v). After centrifugation at 3000 *g* for 5 min, the organic phase was separated and evaporated to dryness under nitrogen at 40 °C. The residue was redissolved in 100 μl of the chromatographic eluent, of which 25 μl was analyzed in an HPLC system consisting of a stainless-steel column (12.5 cm × 4 mm inside diameter) packed with Nucleosil 100-5 C18 (Machery-Nagel, Düren, Germany). The eluent consisted of 30% acetonitrile in 10 m*M* ammonium formate buffer adjusted to a pH of 4.0. The flow rate was 1.5 ml/min. The chromatographic peaks were detected with a Hitachi F-1000 fluorescence spectrophotometer using an excitation wavelength of 480 nm and an emission wavelength of 560 nm. The HPLC peaks were recorded on an HP 3394A automatic recorder/integrator. Retention times were 3.5, 4.5, and 5.6 min for epirubicinol, internal standard, and epirubicin, respectively. The minimal detectable concentration was 1 ng/ml for both compounds.

Pharmacokinetic analysis

Plasma decay curve generated for ADR-529 were fitted to a two-exponential function and those generated for epirubicin, to a three-exponential function (according to the Akaike information criterion) [28] by means of a computer program (GraphPad InPlot, Graphpad, Software, San Diego, Calif., USA) for weighted iterativ nonlinear least-squares analysis using a personal computer:

$$\text{Conc} = A' \times \exp(-\alpha \times t) + B' \times \exp(-\beta \times t)[+ C' \times \exp(-\gamma \times t)],$$

where A',B', and (C') represent the intercepts with the ordinate; α, β, and γ are rate constants; and t is the time from the end of the infusion. When T is the time of constant infusion, A', B', and C' are given by:

$$A' = A \times (1-e^{-\alpha \times T})/\alpha \times T,\ B' = B \times (1-e^{-\beta \times T})/\beta \times T, \text{ and } C' = C \times (1-e^{-\gamma \times T})/\gamma \times T.$$

The half-lives, AUC, early clearance (Cl_{ea}), total plasma clearance (Cl_{tot}), mean residence time (MRT), and steady-state volume of distribution (Vd_{ss}) were calculated from:

$t_{1/2\alpha} = 1n2/\alpha$, $t_{1/2\beta} = 1n2/\beta$, $t_{1/2\gamma} = 1n2/\gamma$, $AUC = A/\alpha + B/\beta + C/\gamma$, $Cl_{ea} = Dose \times \alpha/A$, $Cl_{tot} = Dose/AUC$, $MRT = (A/\alpha^2 + B/\beta^2 + C/\gamma^2)/AUC$, and $Vd_{ss} = Dose \times MRT/AUC$.

For the metabolite epirubicinol, the terminal half-life was determined by linear regression analysis of the logarithms of three to four late experimental data. The AUC for the metabolite was calculated by the trapezoidal method and extrapolated to infinity using the formula:

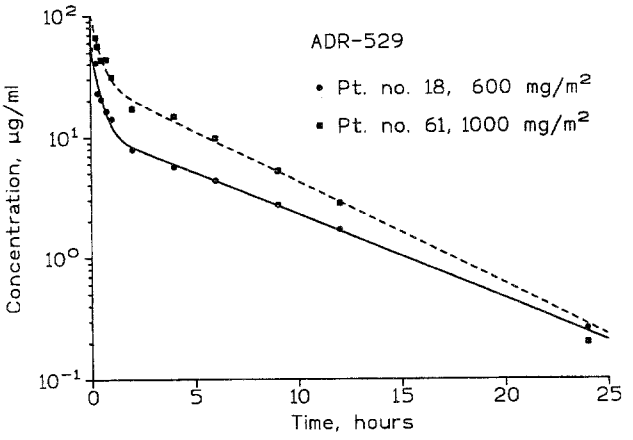

Fig. 2 Plasma pharmacokinetics of ADR-529 after intravenous administration of two different doses to two patients (*Lines* Plasma concentration-time curves calculated from determined pharmacokinetic parameters)

$$AUC = AUC_{trap\ 0\text{-}term} + c(term) \times t_{1/2elim}/1n2,$$

where *c(term)* is the concentration at the latest time point at which the metabolite was detectable.

Statistical evaluation

Group comparison was performed with the StatGraphics program package (Statistical Graphics Corporation, Rockville, Md., USA) according to the following nonparametric statistical tests: the Wilcoxon signed-rank test, the Mann-Whitney rank-sum test, and the Kruskal-Wallis one-way analysis by ranks test. Furthermore, one-way analysis of variance (ANOVA) was performed on log-transformed data.

Results

If possible, curve fittings were performed on all patients. Examples are shown in Figs. 2 and 3. A two-exponential

Fig. 3 Plasma pharmacokinetics of epirubicin after intravenous administration of two different doses to two patients (*Lines* Plasma concentration-time curves calculated from determined pharmacokinetic parameters)

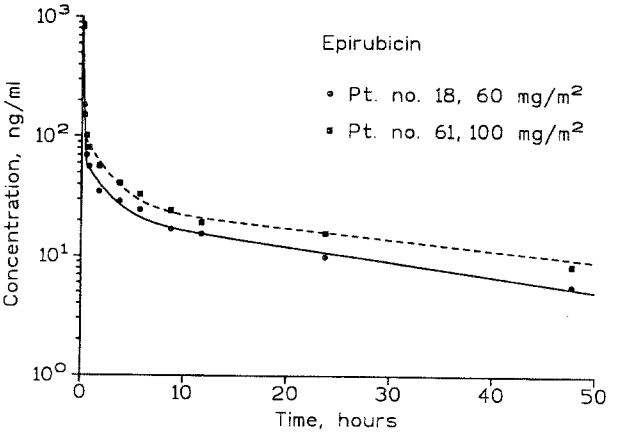

Table 2 Pharmacokinetic data obtained for ADR-529 at the first and second treatment courses

Subgroup	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	MRT (h)	Cl_{tot} (ml min^{-1} m^{-2})	Vd_{ss} (l/m^2)
II.1 (mean ± SD)	22.4 ± 13.4	2.88 ± 1.02	3.20 ± 0.88	122 ± 33	22.5 ± 5.8
II.2 (mean ± SD)	28.4 ± 20.0	2.88 ± 0.79	3.06 ± 0.88	123 ± 37	20.9 ± 3.3
Statistics: Mann-Whitney					
P value	0.80	0.72	0.80	0.98	0.49

function satisfactorily described the ADR-529 kinetics, whereas a three-exponential function was necessary in describing the epirubicin kinetics. Estimates of the individual asymptotic standard errors in determination of the α- and β-phases for ADR-529 were within the ranges of 7%–54% (mean, 29%) and 1%–18% (mean, 5%), respectively, whereas estimates of the asymptotic standard errors in determination of the α-, β-, and γ-phases for epirubicin were within the ranges of 3%–22% (mean, 12%), 13%–80% (mean, 39%), and 5%–31% (mean, 20%), respectively.

For various reasons, pharmacokinetic analysis of ADR-529 could not be performed in one patient from group III and one patient from group VII; pharmacokinetic analysis of epirubicin could not be performed in one patient from group II, two patients from group III, and seven patients from group VII. In the seven patients from group VII the blood samples were drawn from the same arm in which the drugs were infused. In these patients, fittings of ADR-529 plasma concentration-time curves were performed after omission of the concentration at 15 min. No significant difference could be detected between the calculated pharmacokinetic parameters from the aforementioned seven patients and those from the remaining nine patients in this group. However, the epirubicin concentrations detected in the early samples (from 20 min to 2 h) from the seven patients were extremely different from those measured in the other patients in the group. Thus, these seven patients were omitted from the pharmacokinetic evaluation of epirubicin.

A test for significant differences (Mann-Whitney rank-sum test) between the ADR-529 pharmacokinetic parameters of the 18 patients (subgroup II.1) treated with ADR-529 plus epirubicin at the first course of treatment and those of the 8 patients (subgroup II.2) treated with ADR-529 plus

Table 3 Statistical analysis of the pharmacokinetic parameters of ADR-529

Treatment group	Statistics	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	MRT (h)	Cl_{tot} (ml min^{-1} m^{-2})	Vd_{ss} (l/m^2)
II	Arithmetic mean	24.3	2.88	3.16	122	22.0
n = 26	± SEM	3.1	0.19	0.16	7	1.0
	Geometric mean	20.3	2.74	3.03	118	21.4
	95% conf. intervals	16.5–24.8	2.45–3.05	2.72–3.38	106–130	19.4–23.6
III	Arithmetic mean	16.8	2.51	2.96	145	25.0
n = 9	± SEM	2.1	0.15	0.19	18	3.0
	Geometric mean	15.7	2.47	2.91	137	23.9
	95% conf. intervals	11.2–22.2	2.05–2.98	2.42–3.49	115–163	20.2–28.2
IV	Arithmetic mean	16.2	2.12	2.45	160	23.4
n = 10	± SEM	1.5	0.16	0.13	9	1.8
	Geometric mean	15.4	2.07	2.42	158	22.9
	95% conf. intervals	11.1–21.4	1.73–2.47	2.03–2.88	134–186	19.5–26.8
V	Arithmetic mean	19.3	2.36	2.60	143	21.8
n = 10	± SEM	2.0	0.27	0.22	6	1.3
	Geometric mean	18.1	2.27	2.52	141	21.4
	95% conf. intervals	13.1–25.1	1.90–2.70	2.12–3.00	120–167	18.2–25.1
VI	Arithmetic mean	19.9	2.50	3.10	128	22.3
n = 10	± SEM	3.2	0.22	0.25	12	1.3
	Geometric mean	18.0	2.46	2.97	123	21.9
	95% conf. intervals	13.0–25.0	2.06–2.93	2.50–3.54	104–145	18.7–25.7
VII	Arithmetic mean	21.1	2.79	2.91	125	20.8
n = 15	± SEM	3.2	0.19	0.24	9	2.5
	Geometric mean	17.7	2.69	2.76	120	19.9
	95% conf. intervals	13.6–22.9	2.34–3.10	2.40–3.16	105–137	17.5–22.5
ANOVA log transformation:						
Groups II, III, IV	*P* value	0.25	0.041*	0.07	0.02*	0.57
Groups VI, VII	*P* value	0.93	0.40	0.56	0.80	0.38
All groups	*P* value	0.72	0.10	0.23	0.045*	0.59

* Differences detected ($P \leq 0.05$)

Table 4 Individual pharmacokinetic parameters of epirubicin obtained for treatments A (epirubicin, 60 mg/m^2) and B (epirubicin, 60 mg/m^2; ADR-529, 600 mg/m^2) in the crossover study

Treatment	Sequence	Patient number	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	MRT (h)	Cl_{tot} (ml min^{-1} m^{-2})	Cl_{ea} (ml min^{-1} m^{-2})	Vd_{ss} (l/m^2)	AUC_{EOL}/ AUC_{EPI}
A	AB	1	2.07	1.69	28.2	10.1	480	794	291	0.12
		4	3.75	1.03	14.8	13.6	1332	4677	1088	0.65
		6	3.20	2.10	24.3	15.2	374	652	341	0.28
		7	3.92	1.44	19.3	12.2	726	1428	532	0.43
		9	2.83	1.00	24.1	20.9	446	1254	559	0.43
		11	5.47	2.57	13.3	7.9	2911	5496	1386	1.34
		12	3.55	1.47	23.9	19.5	1039	2907	1219	0.42
		15	2.91	1.36	26.7	19.1	511	1119	584	0.35
	BA	2	4.62	0.87	24.8	21.4	655	2078	839	0.44
		3	5.07	6.30	31.8	24.9	608	1246	910	0.29
		5	3.17	1.65	20.4	14.8	665	1465	590	0.44
		8	5.78	4.08	26.0	9.5	772	1503	441	0.69
		10	4.29	1.22	27.5	23.6	539	1491	762	0.31
		13	4.38	1.93	27.7	27.8	594	2819	991	1.28
		14	4.24	1.19	29.2	31.4	888	4873	1671	0.28
		16	3.55	1.19	27.0	21.6	163	404	211	0
Mean			3.92	1.94	24.3	18.3	796	2138	776	0.42
± SD			0.97	1.36	4.9	6.6	607	1535	401	0.27
B	AB	1	3.25	1.47	36.5	23.3	788	1564	1103	0
		4	1.35	0.21	19.7	7.1	283	398	121	0.27
		6	3.44	1.93	23.2	20.3	507	1648	617	0.40
		7	2.28	2.77	22.6	8.4	451	642	226	0.09
		9	2.60	1.69	35.5	35.9	575	2748	1238	0.65
		11	5.01	2.57	27.3	16.6	633	1335	631	0.36
		12	5.13	1.82	25.9	19.7	893	2393	1053	0.42
		15	3.44	2.48	20.6	18.2	976	2959	1067	0.23
	BA	2	3.96	1.33	32.5	20.4	498	1101	609	0.40
		3	3.17	0.83	17.8	12.9	691	1591	533	0.34
		5	5.07	2.17	26.3	18.5	492	1082	548	0.38
		8	4.00	2.67	48.8	39.2	608	1849	1428	0.45
		10	3.41	2.39	21.7	15.9	798	2096	764	0.52
		13	4.08	1.47	30.9	30.0	372	1674	669	0.79
		14	2.67	0.67	22.6	14.4	630	1230	546	0.18
		16	4.52	1.78	26.7	16.3	482	1193	472	0.40
Mean			3.59	1.77	27.4	19.8	605	1594	727	0.37
± SD			1.03	0.72	7.7	8.5	182	678	348	0.19
Statistics: Wilcoxon P value			0.352	0.712	0.224	0.897	0.518	0.587	0.737	0.193

epirubicin at the second course was performed. Table 2 illustrates the mean values ± SD for half-lives, total plasma clearance, MRT, and Vd_{ss} obtained for the two subgroups. No statistically significant difference ($P \leq 0.05$) among the subgroups was detected.

In Table 3, the arithmetic mean values ± SEM and the geometric mean values (with 95% confidence intervals) obtained for the ADR-529 pharmacokinetic parameters are listed together with the P values derived from ANOVA. All the grouped pharmacokinetic parameters were tested for deviations from normal distributions. As a marginal skew was observed in nearly all the groups, all data were log-transformed. After transformation, the grouped data did not significantly deviate from normality. The log-transformed data from groups II, III, and IV (the groups treated with ADR-529, 600 mg/m^2, and with escalating doses of epirubicin, 60/80/100 mg/m^2) and groups VI and VII (the groups treated with ADR-529, 1000 mg/m^2, and with escalating doses of epirubicin, 80–100 mg/m^2) were compared. ANOVA was performed on all groups together as well. Significant ($P \leq 0.05$) differences were found in the β-half-life and the total plasma clearance of ADR-529 among groups II, III, and IV. Multiple-range tests using the Scheffe or Tukey methods suggest that escalation of the concomitantly given epirubicin dose from 60 to 100 mg/m^2 decrease the β-half-life of ADR-529 and consequently increases the total plasma clearance.

The pharmacokinetic parameters of epirubicin obtained in individual patients participating in the balanced crossover study are listed in Table 4. Mean values ± SD are given together with P values derived from the Wilcoxon signed-rank test. No significant difference in the pharmacokinetic parameters of epirubicin was detected between patients receiving epirubicin (together with cyclophosphamide, 5-fluorouracil, and tamoxifen), without (treatment A) versus with (treatment B) concomitant administration of

Table 5 Statistical analysis of the pharmacokinetic parameters of epirubicin

Treatment group	Statistics	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	MRT (h)	Cl_{ea} (ml min^{-1} m^{-2})	Cl_{tot} (ml min^{-1} m^{-2})	Vd_{ss} (l/m^2)
I	Arithmetic mean	3.92	1.94	24.3	18.3	2137	794	776
$n = 16$	± SEM	0.24	0.34	1.2	1.6	384	152	100
	Geometric mean	3.8	1.7	23.7	17.1	1670	654	671
	95% conf. intervals	3.4–4.3	1.3–2.2	20.5–27.4	13.6–21.5	1278–2183	542–791	507–888
II	Arithmetic mean	3.45	1.65	26.8	20.4	1875	636	796
$n = 25$	± SEM	0.19	0.15	1.5	1.5	186	34	81
	Geometric mean	3.3	1.4	25.8	18.7	1661	612	687
	95% conf. intervals	3.0–3.7	1.2–1.8	22.9–28.9	15.6–22.5	1341–2059	526–712	549–860
III	Arithmetic mean	3.78	2.09	27.6	21.4	1637	611	768
$n = 8$	± SEM	0.22	0.32	3.4	3.1	124	32	122
	Geometric mean	3.7	1.9	26.0	19.7	1599	605	714
	95% conf. intervals	3.1–4.4	1.3–2.8	21.2–31.9	14.3–27.2	1095–2335	463–790	549–860
IV	Arithmetic mean	2.96	1.94	26.1	16.1	1314	623	601
$n = 9$	± SEM	0.16	0.23	1.7	1.4	110	42	66
	Geometric mean	2.9	1.8	25.5	15.5	1270	610	568
	95% conf. intervals	2.5–3.5	1.3–2.6	21.0–31.0	11.4–21.0	888–1814	474–785	391–824
V	Arithmetic mean	3.41	1.57	27.4	21.2	1839	660	786
$n = 10$	± SEM	0.25	0.26	3.0	2.8	270	68	83
	Geometric mean	3.3	1.4	25.9	19.7	1270	628	743
	95% conf. intervals	2.8–3.9	1.0–1.9	21.5–31.1	14.8–26.3	1183–2330	495–798	522–1059
VI	Arithmetic mean	2.81	1.85	25.1	16.0	1403	619	602
$n = 10$	± SEM	0.17	0.16	2.0	2.4	225	60	106
	Geometric mean	2.8	1.8	24.1	13.2	1225	589	468
	95% conf. intervals	2.4–3.2	1.2–2.5	20.1–29.0	9.9–17.7	873–1718	464–748	328–667
VII	Arithmetic mean	3.62	1.29	22.5	19.0	2391	766	928
$n = 9$	± SEM	0.37	0.25	3.2	3.0	350	117	135
	Geometric mean	3.5	1.16	21.8	17.8	2086	716	763
	95% conf. intervals	3.0–4.2	0.8–2.1	17.9–26.4	13.1–24.1	1459–2981	556–921	525–1108
ANOVA log transformation:								
Groups I, II	*P* value	0.15	0.34	0.35	0.51	0.64	0.98	0.90
Groups III, V, VI	*P* value	0.021*	0.31	0.87	0.23	0.88	0.30	0.19
Groups IV, VII	*P* value	0.14	0.049*	0.95	0.21	0.13	0.14	0.09
All groups	*P* value	0.037*	0.38	0.42	0.37	0.94	0.46	0.41

* Differences detected ($P \leq 0.05$)

ADR-529. The statistical test for sequence difference showed no significant difference.

Table 5 shows the arithmetic mean values ± SEM and the geometric mean values (with 95% confidence intervals) obtained for the grouped pharmacokinetic parameters of epirubicin. As a slight skew in distribution was also observed for these data, the following comparisons (ANOVA) were performed on log-transformed data: groups I and II, fixed epirubicin dose, 60 mg/m^2, with escalating doses of ADR-529, 0–600 mg/m^2; groups III, V, and VI, fixed epirubicin dose, 80 mg/m^2, with escalating doses of ADR-529, 600/800/1000 mg/m^2; and groups IV and VII, fixed epirubicin dose, 100 mg/m^2, with escalating doses of ADR-529, 600–1000 mg/m^2. All groups together were compared as well. Significant differences were found with respect to the early distributional half-life, $t_{1/2\alpha}$, ($P = 0.021$) in comparing groups III, V, and VI. Multiple-range tests (Scheffe and Tukey) suggest that at an epirubicin dose of 80 mg/m^2, escalation of the ADR-529 dose from 600 to 800 to 1000 mg/m^2 produces a slight decrease in the mean primary-disposition distribution half-life, $t_{1/2\alpha}$, of epirubicin. No significant effect was detected on the early clearance (Cl_{ea}). A marginally significant ($P = 0.049$) decrease in the secondary distributional half-life, $t_{1/2\beta}$, was found when the ADR-529 dose was escalated from 600 to 1000 mg/m^2 in patients receiving epirubicin at 100 mg/m^2. No significant difference in the terminal half-life ($t_{1/2\gamma}$), MRT, total plasma clearance (CL_{tot}), or Vd_{ss} was observed.

Discussion

Plasma concentration-time curves generated for ADR-529 fitted well with biexponential decay curves, and the calculated pharmacokinetic parameters are in close agreement with those reported earlier [11, 12]. The pharmacokinetic parameters of ADR-529 are independent of prior treatment with CEF-T and, with the exception of the terminal half-life ($t_{1/2\beta}$) and the total plasma clearance (Cl_{tot}), are independent of concomitant treatment with CEF-T. The parameters $t_{1/2\beta}$ and Cl_{tot} are independent of the ADR-529 dose given but

seem to change with increasing doses of epirubicin. When the epirubicin dose is increased from 60 to 100 mg/m^2, the terminal half-life of ADR-529 is decreased and the total plasma clearance of ADR-529 is increased by about 30%. This change in half-life and total clearance might be due to the induction of liver microsomal metabolizing enzymes caused by the administration of high doses of epirubicin. To clarify this interaction, more investigations are needed.

Epirubicin plasma concentration-time curves fitted well with triexponential decay curves. The crossover study concerning the influence of ADR-529 on the pharmacokinetic parameters of epirubicin shows that concomitant administration of 600 mg/m^2 ADR-529 does not influence the pharmacokinetic parameters obtained after the administration of epirubicin (60 mg/m^2).

The only significant differences found in epirubicin pharmacokinetic parameters concerned minor changes observed in the primary ($t_{1/2\alpha}$) and secondary ($t_{1/2\beta}$) distributional half-lives when increasing doses of ADR-529 were given. Following the administration of 80 mg/m^2 epirubicin, $t_{1/2\alpha}$ decreases from 3.8 ($\pm$0.2 SEM) to 2.8 ($\pm$0.2 SEM) min when the ADR-529 dose is increased from 600 to 1000 mg/m^2. Changes in $t_{1/2\beta}$ and early clearance (Cl_{ea}) as well as all other pharmacokinetic parameters were not found among these treatment groups. However, a significant change in $t_{1/2\beta}$ was detected between the two groups (IV and VII) in which the patients were treated with epirubicin, 100 mg/m^2, and escalating doses of ADR-529, 600–1000 mg/m^2. This observed change might be due to the relatively large experimental errors involved in determining the individual β-values (SE, 13%–80%). No change in the pharmacokinetic parameters of doxorubicin (50 mg/m^2) were found in a double blind crossover study [19] when this anthracycline was given with and without the concomitant administration of ADR-529 (500 mg/m^2). Hochster et al. [11] have reported no change in the pharmacokinetic parameters of doxorubicin (60 mg/m^2) when it is given with escalating doses of ADR-529 (60–900 mg/m^2).

Originally ADR-529 was introduced as a new antitumor agent [5]. An important question is whether ADR-529 has any impact, positive or negative, on the efficacy of concomitant cytotoxic therapy. Neither animal experiments [26] nor previously published clinical data [24, 25] have shown any negative impact on the efficacy. Ongoing phase III trials will further clarify this question. If a negative impact should emerge, one possible explanation could be an alteration of the pharmacokinetic profile of the cytotoxic drugs. The present study shows that such alterations do not appear when ADR-529 is given together with epirubicin in the treatment of women with metastatic breast cancer.

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