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Dominique Marchiset-Leca · François-René Leca
Anne Galeani · Alex Noble · Athanassios Iliadis

A limited sampling strategy for the study of pirarubicin pharmacokinetics in humans

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Abstract Pirarubicin (4′-*O*-tetrahydropyranyldoxorubicin, THP-Adriamycin) is a new anthracycline antibiotic that has recently been developed because its reduced cardiac toxicity is associated with an antitumour efficacy similar to that of doxorubicin. Pirarubicin is characterised by strong haematological toxicity, which has been shown to be correlated with pharmacokinetic parameters, especially the area under the time-concentration curve. To obtain routine pharmacokinetic evaluations of pirarubicin for dose monitoring we developed a limited sampling strategy relying on three blood samples taken at the end of the infusion and at 12 and 24 h post-infusion. The characteristics of interindividual variability were assessed on the first courses of treatment performed in 18 patients; the model was then validated on 10 independent first courses of treatment performed in 10 other patients. The main pharmacokinetic parameters (half-lives, total volume of distribution, total plasma clearance) were estimated in the test group by maximum-likelihood estimation using all samples and by Bayesian estimation using three samples. The concordance between the two estimates was correct (the bias and precision for clearance were 2.3% and 12.1%, respectively), which shows that this limited sampling strategy can be used in routine drug monitoring.

Key words Pirarubicin · Limited sampling strategy · Pharmacokinetics · Maximum-likelihood estimation · Bayesian estimation

D. Marchiset-Leca (✉) · F.-R. Leca
Laboratoire de Pharmacocinétique, Centre Hospitalier Départemental de Castelluccio, BP 85, 20177 Ajaccio Cedex, France

D. Marchiset-Leca · A. Iliadis
Laboratoire de Pharmacocinétique, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France

A. Galeani · A. Noble
Service d'Oncologie, Centre Hospitalier Départemental de Castelluccio, 20177 Ajaccio Cedex, France

Introduction

Pirarubicin (4′-*O*-tetrahydropyranyldoxorubicin, THP-Adriamycin) is a new anthracycline antibiotic that has recently been developed, especially in France and Japan, because its reduced cardiac toxicity is associated with an antitumour efficacy similar to that of doxorubicin, the reference drug in the anthracycline family [5]. Early pharmacokinetics (PK) studies have shown that doxorubicin itself is a major metabolite of pirarubicin, but with much lower circulating levels, which precludes the identification of pirarubicin as a prodrug of doxorubicin [18]. Some other metabolites of pirarubicin are pirarubicinol and doxorubicinol, which are found in smaller amounts in human plasma than is doxorubicin and are practically devoid of cytotoxic activity [20].

It has been shown in several PK studies that the terminal half-life ($t_{1/2}$) of pirarubicin in plasma is about 12–24 h [10, 12, 14, 15, 18, 19, 21]; this is much shorter than the $t_{1/2}$ of doxorubicin and doxorubicinol [17], especially considering the protracted $t_{1/2}$ of these two compounds, which has recently been evidenced by a prolonged follow-up of their plasma concentrations using a very sensitive technique [9]. Pirarubicin is characterised by marked haematological toxicity, which calls for careful monitoring of blood cell counts after each course of treatment and governs the administration of increasing doses in subsequent courses. This haematological toxicity has been shown to be correlated with PK parameters, especially the area under the time-concentration curve (AUC): platelet toxicity versus pirarubicin AUC and granulocytopenia versus doxorubicin AUC [19]. It therefore appears that therapeutic monitoring of pirarubicin would be useful for predicting haematological toxicity and would allow the administration of the maximal tolerated doses in every patient. This would optimise the therapeutic effects of the drug, given the highly significant dose-effect

relationship characterising anthracyclines [17]. To take advantage of these PK/pharmacodynamic relationships, one must be capable of estimating the PK parameters very rapidly with a minimum of blood samples. Such a strategy of limited sampling has been developed successfully for other anthracyclines [2, 8, 16] but is not yet available for pirarubicin, despite its potential interest. The use of Bayesian estimation of population PK parameters is of particular interest for such approaches [1]. The aim of our study was therefore to develop a limited sampling strategy by means of Bayesian estimation of pirarubicin PK.

Patients and methods

Patients

For the establishment of the Bayesian procedure, 18 patients of either sex and of different ages were first enrolled in the study as the reference group and received pirarubicin (30–75 mg) as a short infusion (2–10 min). For the validation of the Bayesian estimation, 10 new patients were included in a test group and received pirarubicin under the same conditions (60–75 mg). The main clinical features of these patients are summarised in Table 1.

Two sampling protocols were used for this PK study:

1. Protocol P1 – eight blood samples were obtained as follows: before the infusion, at the end of the infusion and at 0.26, 1, 2, 6, 12 and 24 h after the end of the infusion (patients 1–6, 8 and 15–18 in the reference group patients 23, 26 and 28 in the test group)

2. Protocol P2 – ten blood samples were obtained as follows: before the infusion, at the end of the infusion and at 1, 2, 4, 10, 24, 48, 72 and 120 h post-infusion (patients 7 and 9–14 in the reference group patients 19–22, 24, 25 and 27 in the test group). This protocol was used to monitor cisplatin, which was associated with pirarubicin in the corresponding patients; it differed from protocol P1 mainly in that very late samples were available, which could provide a bias in estimating the terminal $t_{1/2}$.

Blood samples were collected into lithium heparin-coated tubes with a gel separator and immediately centrifuged, and the plasma was kept frozen at − 30°C until analysis.

Extraction and assay of pirarubicin and its metabolites

Pirarubicin was extracted from plasma by a liquid-liquid extraction procedure as described elsewhere [11]. Briefly, we added the following successively to 1 ml plasma: 3 ml 0.1 *M* K_2HPO_4 (pH 9), 3 ml acetonitrile and 10 ml chloroform. The mixture was stirred mechanically for 40 min and centrifuged for 30 min at 3500 rpm, and the organic phase was evaporated to dryness under a stream of nitrogen. The dry residue was reconstituted in methanol for injection onto the liquid chromatograph.

High-performance liquid chromatography was conducted as previously described [11] on a Lichrocart Supersher RP8 column (4 µm; 250 × 4 mm; Merck). The eluent was pumped by a quaternary pump (Waters model 600E) and was a mixture of acetonitrile/methanol/50 m*M* ammonium formate buffer (pH 4; 30/25/45, by vol.) with a flow rate of 0.7 ml/min. Detection was performed with a spectrofluorometer (Hitachi model F1000), with excitation and emission wavelengths being set at 480 and 560 nm, respectively. Zorubicin, obtained from Roger Bellon Laboratory, was used as an internal standard. The detection limit was 200 pg/ml.

Table 1 Characteristics of the patients included in the study; patients 1–18 form the reference group and patients 19–28, the test group. (*CTX* Cyclophosphamide, *CDDP* cisplatin, *VB* vinblastine, *5FU* 5-fluorouracil, *MTX* methotrexate, *ca.* carcinoma, *BSA* body surface area)

Patient number	Sex (M/F)	Age (years)	Disease	Co-medication	BSA (m^2)	Dose (mg)
1	F	66	Breast ca.	CTX, 5FU	1.6	75
2	F	71	Breast ca.	CTX, 5FU	1.6	60
3	F	32	Breast ca.	CTX, 5FU	1.5	50
4	F	38	Breast ca.	CTX, 5FU	1.7	75
5	F	69	Breast ca.	CTX, 5FU	1.8	50
6	F	71	Myeloma	Vindesine	1.4	60
7	M	62	Neuroblastoma	CDDP, etoposide	1.8	75
8	M	68	Bladder ca.	None	1.8	75
9	F	68	Ovarian ca.	CDDP, CTX	1.6	30
10	M	56	Bladder ca.	CDDP, VB, MTX	1.9	50
11	F	68	Ovarian ca.	CDDP, ifosfamide	1.4	50
12	M	61	Bladder ca.	CDDP, VB, MTX	1.8	50
13	F	54	Ovarian ca.	CDDP	1.7	65
14	F	57	Ovarian ca.	CDDP, CTX	1.8	70
15	M	74	Lymphoma	CTX, mesna	1.75	75
16	F	63	Breast ca.	CTX, 5FU	1.5	50
17	F	49	Lymphoma	CTX, VB	1.7	50
18	F	71	Breast ca.	CTX, 5FU	1.8	50
19	M	64	Bladder ca.	CDDP, VB, MTX	1.8	50
20	F	72	Ovarian ca.	CDDP, CTX	1.4	50
21	M	57	Bladder ca.	CDDP, VB, MTX	1.9	75
22	M	71	Bladder ca.	CDDP, VB, MTX	1.8	75
23	F	68	Breast ca.	CTX, 5FU	1.6	60
24	F	58	Ovarian ca.	CDDP, CTX	1.5	75
25	M	59	Bladder ca.	CDDP, VB, MTX	1.9	75
26	F	79	Breast ca.	CTX, 5FU	1.5	50
27	M	68	Bladder ca.	CDDP, VB, MTX	1.7	75
28	F	61	Breast ca.	CTX, 5FU	1.6	75

Analysis of the data

Using maximum-likelihood estimation (MLE), all the time-concentration curves of pirarubicin were fitted to an open two-compartment model. It describes the concentration, $y(t)$, as a function of time t by the sum of two exponential terms involving coefficients A_1 and A_2 and exponents a_1 and a_2 as follows:

$$y(t) = \frac{D}{T} \sum_{i=1}^{2} \frac{A_i}{\alpha_i} \left[e^{-\alpha_i (t-T) u(t-T)} - e^{-\alpha_i t} \right],$$

where D and T are the total amount of drug infused and the infusion duration, respectively, and $u(t - T)$ is the step function, which is equal to 1 when $t > T$ and is equal to 0 elsewhere.

We first analysed the data of the reference group obtained from P1 and P2 protocols (11 and 7 courses, respectively) so as to estimate the potential bias related to the difference in sampling protocols. This was done using the Mann-Whitney test. If no significant difference between P1 and P2 protocols appeared, population characteristics were evaluated by the standard two-stage method compiling all these data. This technique consists of estimating the individual PK parameters obtained in the 18 patients and then computing the mean parameters; the covariance matrix, which expresses the individual dispersion; and the coefficient of variation of the residual error [7]. The stabilisation of the interindividual variability in the reference group was checked by means of an index expressing the extent of dispersion for multivariate data, i.e. the amount of information. The index is computed directly from the covariance matrix and it measures the entropy or the uncertainty associated with a statistical distribution [6].

The ten courses from the test group were used to evaluate the maximum a posteriori (MAP) Bayesian estimation associated with the limited sampling protocol. For these courses, the MLE values obtained using all the samples were taken as reference values. The MAP used a limited sampling based on the following three samples: end of the infusion and 12 and 24 h post-infusion. These sampling times were selected for practical and ethical reasons (the least discomfort to both the patient and the nurse [4]). Analysis of MLE and MAP estimates was conducted on the relevant PK parameters, i.e. total plasma clearance (CL), total volume of distribution at steady state (V_{dss}) and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$), which were derived from the model parameters $\{A_1, A_2, a_1, a_2\}$. These PK parameters were further analysed since they are those most useful for the evaluation of distribution and elimination mechanisms.

The performance of the MAP was assessed through an analysis of differences between MAP and MLE estimates. The absolute bias and precision for each parameter were computed respectively as the mean and the standard deviation of these differences. The relative expression of bias and precision was obtained by reducing the absolute bias and precision to the mean value for each parameter. To check statistically the significance of biases, we used the Wilcoxon non-parametric test.

All statistical calculations were performed using the BMDP software package [3] and the tests were assessed with a significance level of 0.05. All calculations on MLE, MAP and population characteristics were performed on a PCD 3Bsx Siemens Nixdorf computer using APIS software [7].

Results

The Mann-Whitney test did not reveal any significant difference between the parameters of patients receiving the P1 versus P2 protocols for any of the PK parameters considered, even for the a_2 parameter that expresses the terminal $t_{1/2}$ ($P = 0.238$, 0.389, 0.297 and 0.364 for A_1, A_2, a_1 and a_2, respectively). The patients receiving the P1 and P2 sampling protocols will thus not be further considered separately.

The courses of the reference group were used to establish the population characteristics required as prior knowledge in MAP. Figure 1 presents the stabilisation of the amount of information, calculated for an increasing number of patients twice randomly ordered. It appears that beyond 16 patients, the two curves generated for the amount of information are maintained at a constant level; the addition of other patients is not likely to improve the estimation of individual dispersion. Therefore, the 18 courses included in the reference group are sufficient to express reliably the interindividual PK dispersion. Table 2 presents the mean parameters, the covariance matrix and the coefficient of variation of residual variability computed on the reference group. The value of this last parameter (18%) means that the two-compartment model correctly describes the PK data of pirarubicin.

Figure 2 shows for all the available data (eight in this course) a typical fitting of the simulated time-concentration curve based on the MAP estimates. The

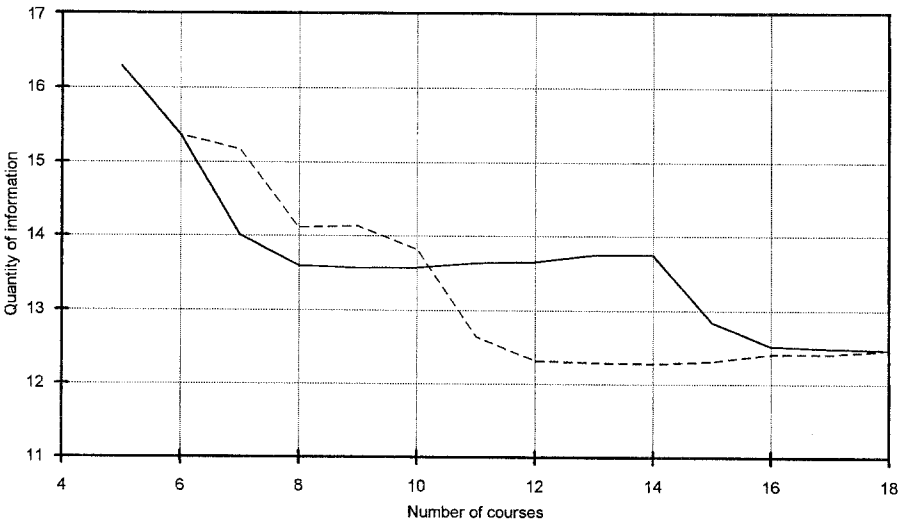

Fig. 1 Amount of information plotted as a function of the number of courses taken into consideration

Table 2 Population PK parameters evaluated for the patients of the reference group (*CV* coefficient of variation)

		A_1 (l^{-1})	A_2 (l^{-1})	a_1 (h^{-1})	a_2 (h^{-1})
Mean vector		2.872E-3	3.031E-4	3.607	7.366E.2
Covariance matrix	A_1 (l^{-1})	8.790E-6	–	–	–
	A_2 (l^{-1})	2.079E-8	1.876E-8	–	–
	a_1 (h^{-1})	6.045E-3	8.234E-5	7.547	–
	a_2 (h^{-1})	– 1.159E-5	1.784E-6	2.259E-2	1.194E-3
CV of residual error		17.89%			

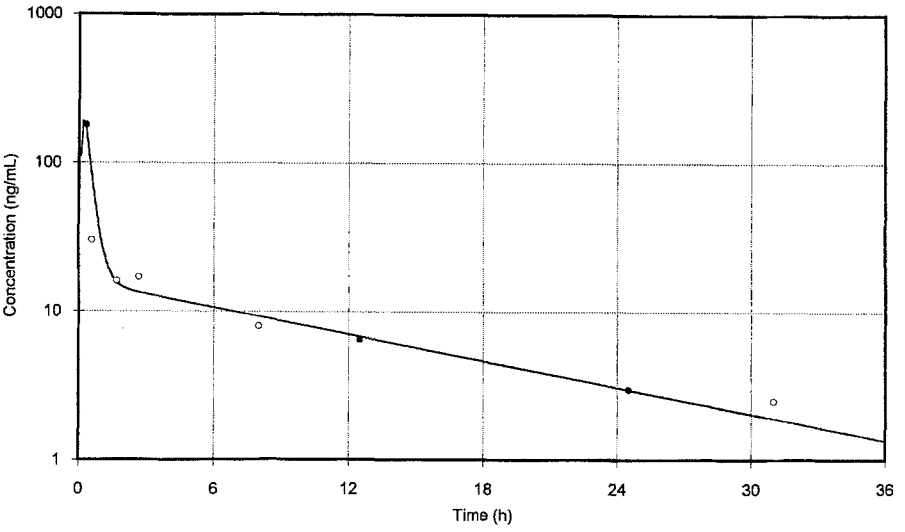

Fig. 2 The model fitting all available observations (○, full sampling protocol) for a given patient, using MAP estimates obtained from the limited sampling protocol (●) with three individual data

Table 3 PK parameters evaluated for the patients of the test group (*min* Minimal value, *max* maximal value)

Patient number	MLE				MAP			
	CL (l/h)	V_{dss} (l)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	CL (l/h)	V_{dss} (l)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)
19	107	1695	0.38	12.5	123	2030	0.21	12.7
20	109	1714	0.19	11.7	101	2160	0.27	16.2
21	130	2859	0.34	17.8	157	3302	0.25	17.5
22	264	4069	0.57	18.0	282	4209	0.27	15.8
23	150	1384	0.08	7.6	145	1824	0.13	11.9
24	210	3672	0.24	14.7	220	3711	0.25	14.3
25	136	2412	0.39	14.4	143	2862	0.27	16.1
26	78	466	0.31	9.2	91	675	0.46	10.2
27	400	5255	0.43	12.3	351	4729	0.35	15.1
28	172	2651	0.40	12.6	185	2584	0.25	11.0
Min	78	466	0.08	7.6	91	675	0.13	10.2
Max	400	5255	0.57	18.0	351	4729	0.46	17.5
Mean	176	2618	0.33	13.1	180	2809	0.27	14.1
SD	96	1418	0.14	3.3	83	1216	0.09	2.5
CV	54.5	54.2	42.4	25.2	46.1	43.3	33.3	17.7

validation of this estimation was done in the courses of the test group. The relevant PK parameters obtained by MLE and by MAP based on the limited sampling protocol are presented in Table 3. Noteworthy is the wide dispersion of CL and V_{dss} reference values (about 54%), indicating that they are highly dispersed parameters to be followed using a reduced sampling protocol. It is also noteworthy that the dispersion of parameters estimated by MAP is lower than that of parameters estimated by MLE. This phenomenon is due to the role played by the prior term in MAP; it attracts estimates towards the prior mean and, consequently, reduces the dispersion.

The performance of MAP with respect to MLE is summarised in Table 4. In this table, positive biases indicate parameter over-estimation by MAP. We noted moderate bias values (biases did not exceed 10% except for the $t_{1/2\alpha}$) and the Wilcoxon test did not reveal

any significant difference between MAP and MLE estimates ($P = 0.17$, 0.11, 0.17 and 0.28 for CL, V_{dss}, $t_{1/2\alpha}$ and $t_{1/2\beta}$, respectively). The precision of MAP is satisfactory (i.e. the relative precision is less than the dispersion reported in Table 3) for all parameters except $t_{1/2\alpha}$. It is noteworthy that MAP was capable of discriminating patients with extreme CL and V_{dss} values (patient 26 had the lowest and patient 27, the highest values). It can be concluded that even with few individual samples, MAP estimation identifies CL, V_{dss}, and $t_{1/2\beta}$ parameters closely to the reference values.

Discussion

Two- and three-compartment models have been used by various authors to describe pirarubicin PK [10, 12, 14, 15, 18, 19, 21]. In the studies reporting a three-exponential decay, the rate of the first elimination $t_{1/2}$ is so rapid that too many early plasma samples would be required for routine clinical pharmacology; in addition, taking into account the very early distribution phase would not alter the estimation of CL.

The PK parameters we obtained by MLE on the 28 available courses are in the range of the values reported in the literature (Table 5). In comparison with the reference anthracycline doxorubicin, pirarubicin is characterised by a shorter terminal $t_{1/2}$, a much higher CL and a much larger V_{dss} [17]. This is probably due to the differences in cellular pharmacology; a very high extent of tissue uptake is especially characteristic of pirarubicin [13]. Our estimation of the PK parameters of pirarubicin was not biased by the reduced sampling protocol used, probably due to the relatively short $t_{1/2}$ of this drug, which allows a reliable estimation even when late samples are lacking (P1 protocol).

Table 4 Absolute and relative bias and precision for PK parameters estimated by MLE and MAP; relative values are given in parentheses

	Bias	Precision
CL (l/h)	4.2 (2.3%)	21.4 (12.1%)
V_{dss} (l)	191 (7.3%)	313 (11.9%)
$t_{1/2\alpha}$ (h)	− 0.062 (− 18.6%)	0.13 (40.4%)
$t_{1/2\beta}$ (h)	1.00 (7.5%)	2.31 (17.7%)

The choice of the three sampling times for MAP was dictated by ethical and practical considerations. MAP predictive performances could be improved in a optimised protocol by seeking the most informative sampling times. This calculation may be performed on the basis of the D-optimality theory [8], ensuring precise estimation of PK parameters. The validation of MAP versus MLE showed that relevant PK parameters were predicted with good precision and without bias from three plasma samples; this opens the possibility of rapid identification of pirarubicin PKs, especially for the establishment of pharmacokinetic/pharmacodynamic relationships and their use in routine monitoring of this drug.

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Table 5 Comparison of the pirarubicin PK data in the literature

	CL (l/h/m^2)	V_{dss} (l/m^2)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)
Miller and Schmidt [12]	115 ± 11	2124 ± 221	0.317 ± 0.047	13.0 ± 1.6
Raber et al. [15]	–	–	1.4 ± 0.3	19.3 ± 2.1
Robert et al. [18]	90.4 ± 5.2	1379 ± 145	0.717 ± 0.05	16.0 ± 1.5
Robert et al. [19]	140 ± 48	2830 ± 1300	0.266 ± 0.163	16.6 ± 4.2
Sridhar et al. [21]	204 ± 39	3504 ± 644	0.427 ± 0.108	23.6 ± 7.6
Present study[a]	118 ± 54[b]	1497 ± 734[b]	0.32 ± 0.17	11.6 ± 3.8

[a] Values were obtained by compiling the 28 MLE estimates (reference and test groups)
[b] Estimated parameters reduced according to the patients' body surface area

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