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Pharmacokinetics and Metabolism of Pirarubicin in Advanced Cancer Patients

J. ROBERT, M. DAVID, S. HUET and J. CHAUVERGNE
Fondation Bergonié, 180, rue de Saint-Genès, 33076 Bordeaux cédex, France

Abstract—*We have studied the pharmacokinetics and metabolism of pirarubicin (4'-O-tetrahydropyranyldoxorubicin) in six patients included in an EORTC phase II study. Pirarubicin was injected as an i.v. bolus of* 5 min *on* 3 *consecutive days at a dose of* 20 mg/m^2 *per day. Blood samples were collected at regular times after each injection. Urine was collected over* 12 h *periods for* 3 *days and then over* 24 h *periods. Pirarubicin and metabolites were extracted on Sep-pak cartridges, and analyzed by HPLC with fluorometric detection.*

Unchanged pirarubicin followed three similar plasma concentration curves, which could be fitted by a two-compartment model with successive half-lives of 22.0 min *and* 12.7 h. *Total plasma clearance of the drug was* 90 l/h/m^2 *and total volume of distribution* 1380 l/m^2. *Doxorubicin was the main metabolite in plasma after an injection of pirarubicin; its concentration was lower than that of pirarubicin but progressively increased from day to day and exceeded the level of pirarubicin* 8 h *after the 3rd injection of the drug until the end of the blood sampling. Pirarubicinol and doxorubicinol were also metabolites of pirarubicin in plasma; pirarubicinol followed similar plasma concentration curves during the* 3 *days of treatment whereas doxorubicinol progressively increased from day to day.*

Total urinary excretion represented about 6% *of the dose injected. The same metabolites as in plasma were found in urine. Whereas the total amount of pirarubicin and pirarubicinol was the same in urine during the* 24 h *after each injection, the amounts of doxorubicin and doxorubicinol excreted increased from day to day, so that doxorubicin became progressively the main compound in urine after the end of the treatment.*

The progressive accumulation of pirarubicin metabolites (doxorubicin and doxorubicinol) after the repetitive injections of pirarubicin are probably due to the protracted half-lives of these compounds as compared to that of pirarubicin.

INTRODUCTION

NUMEROUS anthracycline analogs have been synthesized, but only few of them have entered routine clinical use. The pharmacokinetics of these drugs is very different from one compound to another; since animal studies do not provide useful information in the field of clinical pharmacology, it appears necessary to develop a careful pharmacokinetic study of each new drug entering the process of clinical evaluation.

Pirarubicin (4'-*O*-tetrahydropyranyldoxorubicin, THP-doxorubicin, Fig. 1) is a new compound originating from the laboratory of Umezawa [1]. It was selected on the basis of its cytotoxicity against the usual screening systems [2] and its reduced cardiotoxicity in hamsters [3]. Phase I trials were first conducted in Japan and a maximum tolerated dose of about to 60 mg/m^2 was reported. Phase II studies were then undertaken on the basis of 30–60 mg/m^2 every 3 or 4 weeks. Objective remissions were recorded in several cancer types in phase II studies [4–6]. Several accounts of the pharmacokinetics and metabolism of pirarubicin have been presented at meetings [7–10] and a detailed study was published by Miller and Schmidt [11]. However, important discrepancies appear in the results and no agreement exists concerning some important parameters such as half-life or degree of metabolism.

Several phase II trials have been started in Europe. Our comprehensive Cancer Center participates to an EORTC study in which pirarubicin is administered 3 consecutive days every 3 weeks at a dose of 20 mg/m^2 per day. This dose fractionation was chosen because of the very rapid uptake of the drug by tumor cells [12, 13]. The effect of such a schedule of treatment on the plasma levels of the

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Send all correspondence to J. Robert at the above address.

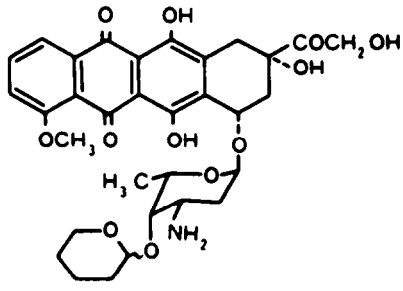

Fig. 1. Structure of pirarubicin.

drug and its metabolites was however not considered; unexpected drug kinetics may appear when changing this schedule. We have shown recently that repetitive injections of idarubicin led to a progressive increase of plasma concentration of idarubicinol, due to the protracted half-life of this compound [14]. Our objectives were to investigate the pharmacokinetics and metabolism of pirarubicin in patients treated with repetitive doses of this drug.

PATIENTS AND METHODS

Patients

Six patients, one man and five women, entered the study after informed consent had been obtained. The median age was 55 (range 34–69) years and the median Karnofsky performance status was 80 (range 70–100). All patients had pathological confirmation of cancer, progressive and measurable malignancies. The tumor types included two tumors of the head and neck, two ovarian carcinomas, one uterine cervix carcinoma and one endometrial carcinoma. All patients had received prior surgery and chemotherapy with a median of 9 (range 3–26) courses, and five patients had received prior radiotherapy. Four patients were pretreated with doxorubicin (range 125–320 mg/m^2). No patient had prior heart disease or serious echo or electrocardiogram alteration. Eligibility criteria included WBC above 3000/μl, thrombocytes above 100,000/μl; bilirubin and creatinine values had to be normal. Prior to the treatment with pirarubicin, no cytostatic agent had been given for at least four weeks, and no radiotherapy had been administered for at least six weeks.

Pirarubicin injections (20 mg/m^2 per day) lasted for 3–5 min and blood samples were obtained after each daily injection at the following times: 5, 10, 20, 40 min; 1, 2, 4, 8, 12 and 24 h. Samples were also obtained 48 and 72 h after the last injection. Blood was collected in EDTA-coated tubes and immediately centifuged. Plasma was kept at −30°C until analysis. Urines were collected in 12 h fractions during the 3 days of treatment and in 24 h fractions during the 2 days thereafter. They were filtered through gauze when necessary and frozen until analysis.

Drug analysis

Drugs and standards were provided by Laboratoire Roger-Bellon. All chemicals were analytical grade and were used without further purification. A known amount of daunorubicin was added to the plasma samples as an internal standard. Extraction of pirarubicin and its metabolites was performed on Sep-pak C-18 chromatographic cartridges, as already published for doxorubicin [15]. This technique yielded a 75% recovery of the drug in the extract and the same recovery was obtained for the metabolites and the internal standard. High-performance liquid chromatography was performed on a Microbondapak C-18 column (Waters Associates, Millipore). The mobile phase consisted in a mixture of 0.1% ammonium formate buffer, pH 4.0 and acetonitrile (66/34 by volume), with a flow rate of 3 ml/min [16]. A Perkin–Elmer LS1 spectrofluorometer with an excitation wavelength of 480 nm and an emission wavelength of 592 nm was used for detection. Under these conditions, complete separation of all the fluorescent peaks present in the extracts of plasma or urine was achieved in about 7 min. Identification of the peaks was made by comparison of the retention times with those of known standards. The lower detection limit of this method was about 1 ng.

Mathematical analysis of the data

The data concerning unchanged pirarubicin were fitted to a sum of exponential curves thanks to a non-linear programming method already presented [17] and based upon the equation giving the plasma concentration $C(t)$ as a function of time t

$$C(t) = \frac{D}{T} \sum_{i=1}^{i=n} A_i(e^{\alpha_i T} - 1)e^{-\alpha_i t}$$

where D is the dose injected, T the duration of the injection, A_i and α_i the parameters of each exponential curve and n the number of compartments. Computations with $n = 2$ and $n = 3$ were always performed and compared. Several pharmacokinetic parameters were calculated from the data, especially the total plasma clearance (Pl.Cl), the total volume of distribution V_d (ss) and the mean residence time of the drug in the body (MRT), with the usual pharmacokinetic equations [18]. The areas under the curve from 0 to 24 h (AUC 0–24) were calculated using the trapezoidal rule.

The data concerning the metabolites were treated with a model-independent approach; elimination half-lives were calculated by linear regression and AUC 0–24 were computed using the trapezoidal rule.

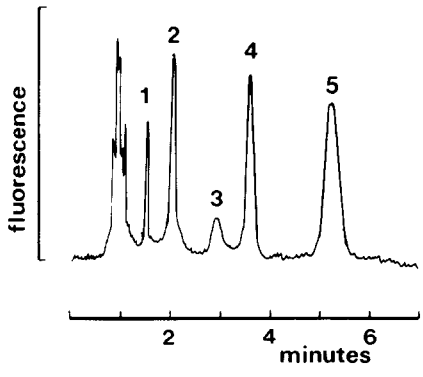

Fig. 2. Typical high-performance liquid chromatogram of a plasma sample taken 2 h after i.v. administration of 20 mg/m² pirarubicin. Peaks were identified as follows: 1. doxorubicinol; 2. doxorubicin; 3. pirarubicinol; 4. daunorubicin (internal standard); 5. pirarubicin.

The mean values obtained for pharmacokinetic parameters were compared using Student's *t*-test.

RESULTS

A maximum of four peaks were observed in plasma or urine extracts after an injection of pirarubicin. They were identified, in the decreasing polarity order, as doxorubicinol, doxorubicin, 13-dihydropirarubicin (pirarubicinol) and pirarubicin. The internal standard, daunorucibin, migrated between pirarubicinol and pirarubicin. A typical chromatogram is presented in Fig. 2.

For unchanged pirarubicin, a two compartment model fitted the experimental data better than a three-compartment one: with the latter, the standard deviation of the estimate given by the computer was generally higher than the estimate itself. The three daily plasma time curves were quite similar and no significant difference could be detected between the pharmacokinetic parameters obtained on day 1, 2 or 3, when calculated in the same manner. Figure 3 presents the mean plasma concentrations of pirarubicin and its metabolites in the six patients studied. The successive mean half-lives of unchanged pirarubicin in plasma were 22.0 ± 1.9 min and 12.7 ± 0.8 h (mean ± S.E.M.). When computed with three compartments, the mean successive half-lives were 3.09 ± 0.40 min, 0.723 ± 0.062 h and 16.0 ± 1.5 h. Table 1 presents the main pharmacokinetic parameters of the drug.

The main metabolite in plasma was doxorubicin; its level was low after the 1st injection, but it progressively increased from day to day (Fig. 3); it became similar to the parent compound 24 h after the first injection, 12 h after the second one and 8 h after the last one, and was maintained at higher levels than pirarubicin until the end of blood sampling. The AUC ratios doxorubicin/pirarubicin, measured during the 24 h following each injection, increased from day to day (Table 2).

Other identified metabolites of pirarubicin in plasma were pirarubicinol and doxorubicinol. Both had very low levels in plasma (see Fig. 3). Pirarubicinol followed three similar time curves, whereas doxorubicinol increased from day to day as doxorubicin did (Table 2). It is worth noting that in two patients pirarubicinol remained undetectable in plasma all along the study.

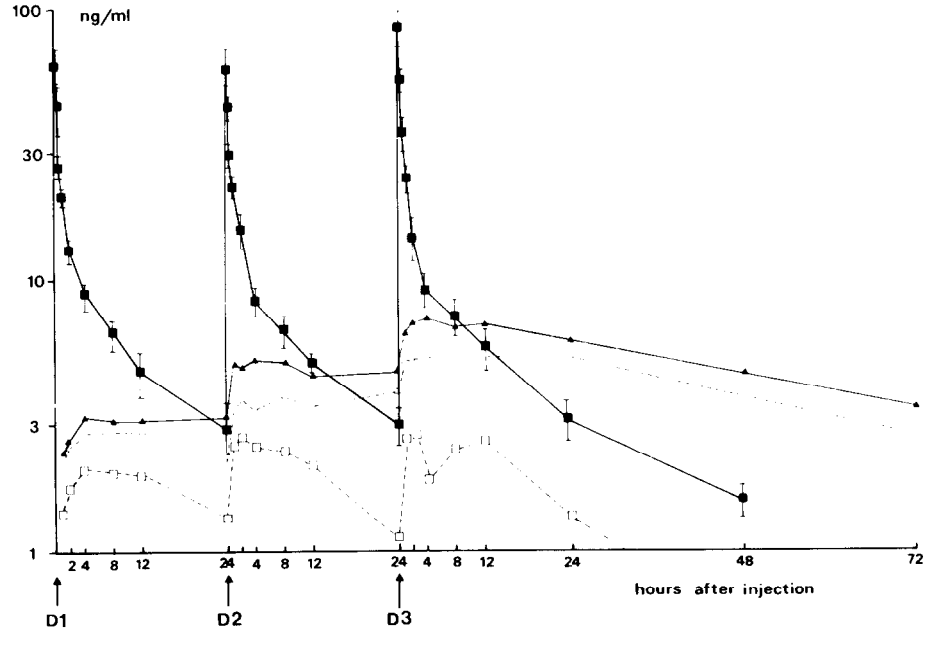

Fig. 3. Concentration of pirarubicin and its metabolites in plasma of patients receiving three successive i.v. boluses of 20 mg/m² pirarubicin. Points are means (± S.E.M. in the case of unchanged pirarubicin) of values obtained in six patients. —■— : Pirarubicin; ---□--- : pirarubicinol; —▲— : doxorubicin; ---△--- : doxorubicinol.

Table 1. Pharmacokinetic parameters of unchanged pirarubicin in plasma after three daily bolus injections in six patients

	Day 1	Day 2	Day 3
$A \times 10^3$ (/l)	3.10 ± 1.12	2.64 ± 0.25	4.13 ± 1.25
$B \times 10^1$ (/l)	3.48 ± 0.43	3.57 ± 0.35	4.16 ± 0.71
α (h)	2.10 ± 0.51	2.15 ± 0.25	2.36 ± 0.27
$\beta \times 10^2$ (/h)	5.56 ± 0.49	5.38 ± 0.52	6.51 ± 0.63
Pl.Cl(l/h/m^2)	92.9 ± 9.8	87.3 ± 7.5	91.1 ± 9.5
V_d (l/m^2)	1468 ± 244	1462 ± 266	1208 ± 243
MRT (h)	15.7 ± 1.8	16.6 ± 1.9	12.9 ± 1.7

Results are given as means ± S.E.M.

Table 2. Pharmacokinetic parameters concerning pirarubicin metabolites

	Doxorubicin	Doxorubicinol	Pirarubicinol
AUC ratio metabolite/drug			
1st day	0.428 ± 0.052	0.359 ± 0.055	0.223 ± 0.104
2nd day	0.627 ± 0.057	0.466 ± 0.025	0.243 ± 0.101
3rd day	0.780 ± 0.092	0.633 ± 0.085	0.270 ± 0.118
Elimination half-life (h)	44.1	38.8	16.2

Cumulative urinary excretion of pirarubicin and its fluorescent metabolites accounted for 5.93 ± 0.90% of the total dose injected over the 5 days of the study. The amount of products excreted in urine increased from the first day to the third day and decreased thereafter. A typical profile of urinary excretion in a patient is presented in Fig. 4. The amount of pirarubicin and pirarubicinol excreted daily reamined almost constant, while the amount of doxorubicin and doxorubicinol excreted progressively increased from day to day during and after the treatment; the proportion of doxorubicin among the fluorescent compounds was 17 ± 5% during the 12 h following the first injection and 43 ± 6% in the 24–48 h period following the last injection.

DISCUSSION

The results we obtained for unchanged pirarubicin can be compared to those obtained by other workers. In the study of Miller and Schmidt [11], successive half-lives of 1.4 min, 19 min and 13 h were observed when the kinetics was analyzed with a three-compartment model; our values are in complete agreement with these values; however, the total plasma clearance and volume of distribution are 30% lower in our study than in the study of Miller and Schmidt. The various other preliminary reports on pirarubicin pharmacokinetics [7–9] are also in favor of a very short half-life in the first phase of distribution of the drug; this has been attributed to the rapid uptake of this drug by cells and tissues. In contrast, the elimination half-lives range from 5 to 33 h in these reports.

When compared to the reference anthracycline, doxorubicin, it clearly appears that pirarubicin is characterized by rapid distribution and elimination phases, and therefore by high total plasma clearance and volume of distribution. For instance, we observed recently for doxorubicin a total plasma clearance of 30 l/h/m^2 and a volume distribution of 800 l/m^2 [19]. The values we obtained for pirarubicin (90 l/h/m^2 and 1400 l/m^2) suggest that pirarubicin is cleared from plasma and fixed in tissues to a larger extent than doxorubicin.

In the different reports already cited, important discrepancies are noted concerning the metabolism of pirarubicin. In their study, Miller and Schmidt [11] observed high levels of doxorubicin in plasma and urine of patients treated with pirarubicin, and the plasma AUC ratio doxorubicin/pirarubicin were always higher than 1. In contrast, two other presen-

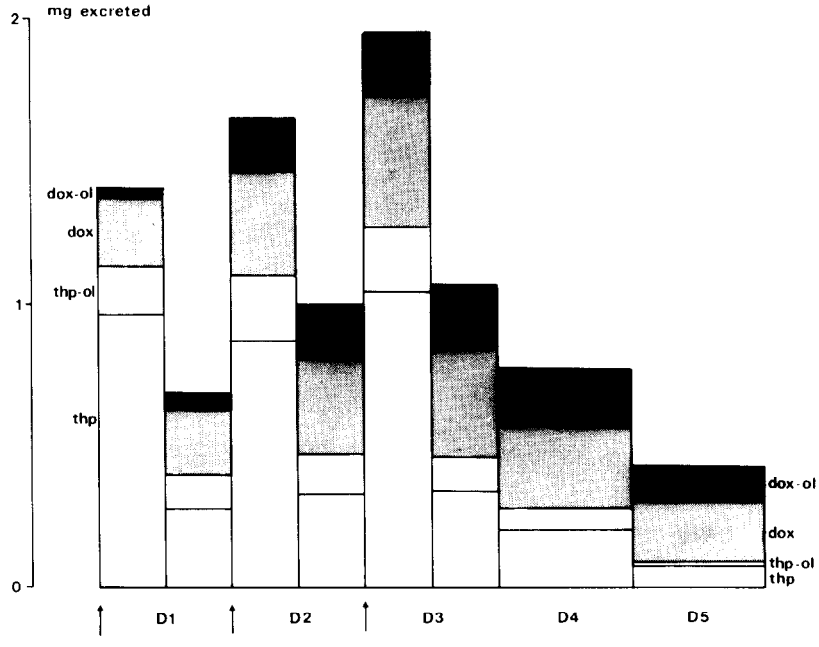

Fig. 4. Typical representation of urinary excretion of pirarubicin and metabolites in a patient. Columns are proportional to the quantities excreted; from bottom to top: pirarubcin (thp); pirarubicinol (thp-ol); doxorubicin (dox); doxorubicinol (dox-ol).

tations [7, 10] reported smaller amounts of doxorubicin after a treatment with pirarubicin. In our study, we also found low amounts of doxorubicin and doxorubicinol during the first day after injection, but these levels progressively increased and became important. We have verified that the amount of doxorubicin we found in our samples did not come from a spontaneous degradation of pirarubicin; we have also verified that it could not come only from preexisting doxorubicin in the pirarubicin clinical formulation, which is less than 1%. We observed that the levels of doxorubicin and doxorubicinol in plasma increased from day to day and exceeded those of pirarubicin 8–12 h after the third injection of the drug. This is probably due to the protracted half-life of these compounds in comparison to those of pirarubicin and pirarubicinol. A similar accumulation of doxorubicin and doxorubicinol is exhibited by the urinary patterns of excretion: whereas the amounts of pirarubicin and pirarubicinol excreted each day was similar, the amount of doxorubicin and doxorubicinol progressively increased.

The repetitive administration of pirarubicin is therefore followed by the progressive accumulation of metabolites in plasma and urine. At the end of the course of treatment, the main therapeutic species circulating in the body is doxorubicin and not pirarubicin. If pirarubicin has original properties in terms of antitumor spectrum and in terms of reduced cardiac toxicity, it could be prejudicial to lose this originality because of the accumulation of its metabolite doxorubicin.

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