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Short communication

A pharmacokinetic and pharmacodynamic study of the new anthracycline pirarubicin in breast cancer patients

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Summary. We evaluated the pharmacokinetics of pirarubicin during 16 courses of therapy in 4 patients suffering from breast cancer who were treated with an association of pirarubicin (30–60 mg/m^2 according to the hematologic tolerance to the previous course, the first course being given at a dose of 40 mg/m^2) and continuous infusions of 5-fluorouracil (750 mg/m^2 daily for 5 days). Pirarubicin's pharmacokinetics and metabolism were linear within this dose range; the metabolites identified were pirarubicinol, doxorubicin and doxorubicinol (AUC ratios of metabolite/pirarubicin were 0.6, 0.64 and 0.57 respectively). Pirarubicin's decay from plasma followed a two-compartmental pattern, showing half-lives of 15.6 min and 16.6 h; the total plasma clearance of the drug was 140 l/h^{-1}/m^{-2}, and the total volume of distribution was 2,830 l/m^2. A relationship was observed between some pharmacokinetic parameters and the toxic effects of the drug: the percentage of survival of granulocytes was significantly correlated with the AUC values for doxorubicin and doxorubicinol, whereas that of platelets was significantly correlated with the AUC values for pirarubicin and pirarubicinol. This is the first study to demonstrate a pharmacokinetic/pharmacodynamic relationship for pirarubicin.

Introduction

Pirarubicin (Theprubicine) is a new anthracycline derivative that was originally synthesized in Japan [28] and is characterized by the addition of a tetrahydropyranyl (THP) moiety of the 4′ position of doxorubicin. This molecule exhibits cytotoxic activity that is similar to or higher than that of doxorubicin and produces less cardiac toxicity [4]. From a cellular point of view, it is characterized by enhanced and more rapid uptake in the various models that have been tested in several laboratories [9, 15], and its cross-resistance with doxorubicin is only partial [23]. It has been tested in phase I trials in Japan [16], in France, in Germany [14] and in the United States [18]. Several phase II studies have demonstrated its antitumor activity mainly in breast cancer and lymphoma [10, 26]. The very low cardiac toxicity of this drug, which was expected on the basis of preclinical investigations, was confirmed by the study of Hérait and Bugat [6]. In an early pharmacokinetic study, Miller and Schmidt [13] showed that doxorubicin was a major metabolite of pirarubicin, but the large amounts of this metabolite that were found by these authors were not confirmed in several other studies [11, 12, 18, 22, 27]. Therefore, pirarubicin cannot be considered to be a prodrug of doxorubicin, even if measurable amounts of this compound as well as of two other metabolites are found in the plasma and urine of patients who have been treated with pirarubicin.

An open phase II trial was initiated in France to assess the activity and toxicity of pirarubicin given in association with continuous infusions of 5-fluorouracil to patients exhibiting metastatic breast cancer. The initial dose of pirarubicin was 40 mg/m^2, which was latter individually escalated or reduced in 10 mg/m^2 steps according to the hematological tolerance of the previous course. This original protocol enabled us to study the effect on the pharmacokinetic parameters of a repetition of treatment courses, of a variation in dose between 30 and 60 mg/m^2 in individual patients, and of the association with 5-fluorouracil. Moreover, since the hematologic counts were regularly obtained throughout the study, a pharmacodynamic evaluation of the drug and its metabolites could be carried out so as to relate its toxicity to the pharmacokinetic parameters.

Patients and methods

Of the 38 patients involved in the clinical trial, 4 were entered in the pharmacokinetic study; 16 courses of treatment (5 at 30 mg/m^2, 5 at 40 mg/m^2, 5 at 50 mg/m^2, and 1 at 60 mg/m^2) were analyzed. All patients exhibited evaluable metastatic breast cancer and showed no signs of hepatic or renal dysfunction as assessed by the usual blood tests; no

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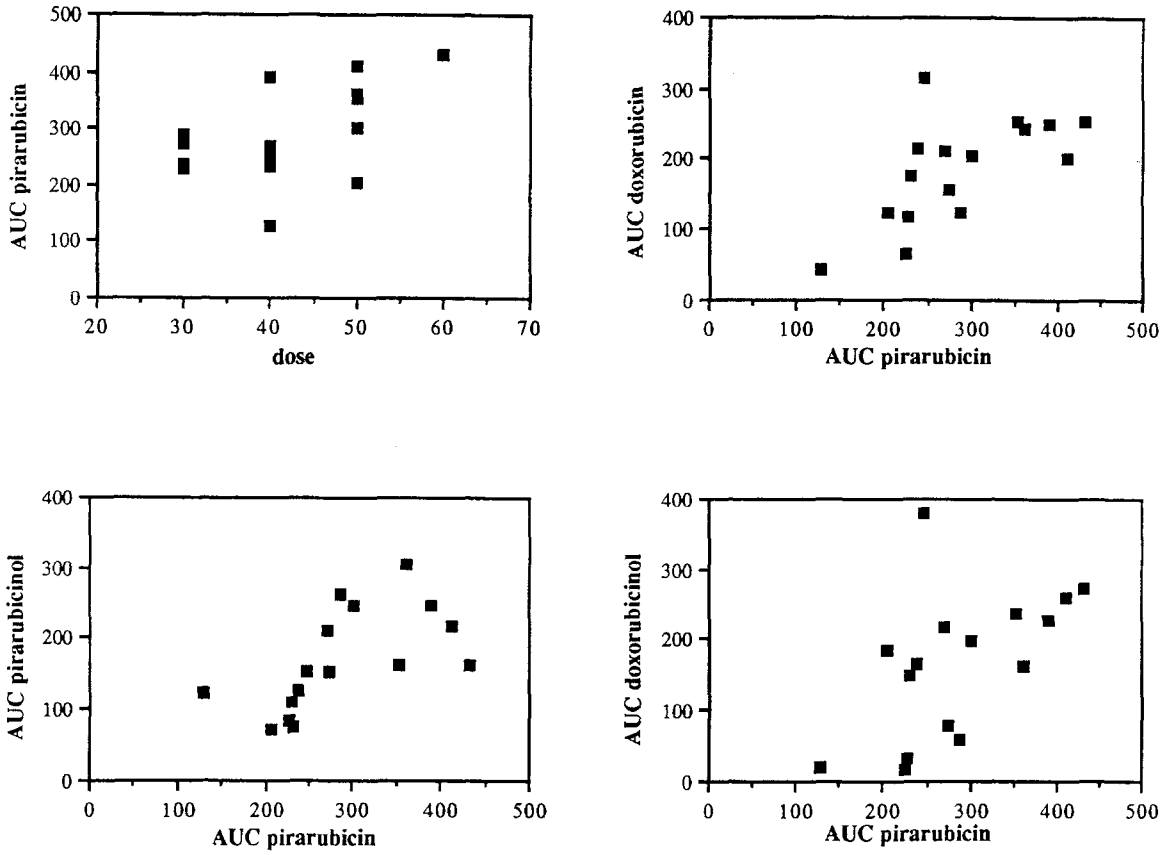

Fig. 1. Relationship between the dose of pirarubicin injected (mg/m^2) and the AUC values for pirarubicin and its metabolites during the 48 h following injection as expressed in ng ml^{-1} h

alteration in these tests was detected during any course of treatment. The clinical results of this phase II study have been published elsewhere [3]. 5-Fluorouracil was given as a continuous infusion at a dose of 750 mg/m^2 daily for 5 days, and pirarubicin was injected as an i. v. bolus within <3 min at an initial dose of 40 mg/m^2. An increase of 10 mg/m^2 (up to 70 mg/m^2) was implemented in subsequent courses if granulocyte and platelet counts had not decreased to $<10^9$/l and $<50\times10^9$/l, respectively; in contrast, a decrease of 10 mg/m^2 was applied if the previous course had produced granulocyte and platelet nadirs of $<0.5\times10^9$/l and $<20\times10^9$/l, respectively. The dose of pirarubicin was maintained for the following course if the hematologic counts fell between the above values. Courses of treatment were separated by 3 weeks, provided that the hematologic counts at that time were $>2\times10^9$/l for granulocytes and $>100\times10^9$/l for platelets.

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and were immediately centrifuged, and the plasma was stored at −20° C until analysis. The samples were obtained at 5, 10, 20 and 40 min and at 1, 2, 4, 8, 12, 24 and 48 h after the end of the injection. Extraction was performed on Sep-Pak cartridges filled with C18-bonded silica according to a technique we described in 1980 for doxorubicin [20] that is now widely used. Chromatography was performed using a Waters apparatus on a column of microbondapak-phenyl, running a solvent mixture containing 1% ammonium formate buffer (pH 4, 66 vol.) and acetonitrile (34 vol.) at a flow rate of 3 ml/min [8]. Detection was achieved using a Perkin-Elmer LS1 spectrofluorometer, with excitation and emission wavelengths being set at 480 and 592 nm, respectively. Quantitation was done using doxorubicin as an internal standard. The AUC value for pirarubicin was calculated by the trapezoidal rule, and the data were then processed through a mathematical multicompartmental open model (APIS [7]) using a non-linear programming method. Metabolites were studied using a model-independent approach, and 0- to 48-h AUC values and elimination half-lives were the only parameters estimated.

Pharmacodynamic studies rely on the estimation of blood cell counts prior to treatment and at the nadir, which generally occurred on day 14. These values can be expressed either as the percentage of change (initial minus nadir/initial) [5] or as the percentage of survival (nadir/initial) [1]. The logarithms of the percentages of survival were tentatively correlated with the AUC values for the drug or its metabolites as proposed by Ratain et al. [19] from analogies with the usual cellular pharmacologic determinations, which plot the logarithm of the percentage of survival of cells as a function of drug exposure [25]. The Pearson coefficient of correlation between these parameters was calculated following linear regression. In one case, no blood cell count was obtained after one course of treatment, and this course was not taken into account in the calculations.

Results

Four fluorescent compounds were consistently found in the plasma of the patients; these were identified as pirarubicin, pirarubicinol, doxorubicin and doxorubicinol. As shown in Fig. 1, the AUC value for pirarubicin was linearly related to the dose ($r = 0.56$, $P < 0.05$) and those for all three metabolites correlated significantly with that for of pirarubicin (AUC pirarubicinol vs AUC pirarubicin: $r = 0.62$, $P < 0.02$; AUC doxorubicin vs AUC pirarubicin: $r = 0.6$, $P < 0.02$; AUC doxorubicinol vs AUC pirarubicin: $r = 0.54$, $P < 0.05$). We can therefore assume that the metabolic transformation of pirarubicin was independent of the delivered dose within the range of 30–60 mg/m^2. The AUC ratios of pirarubicinol/pirarubicin, doxorubi-

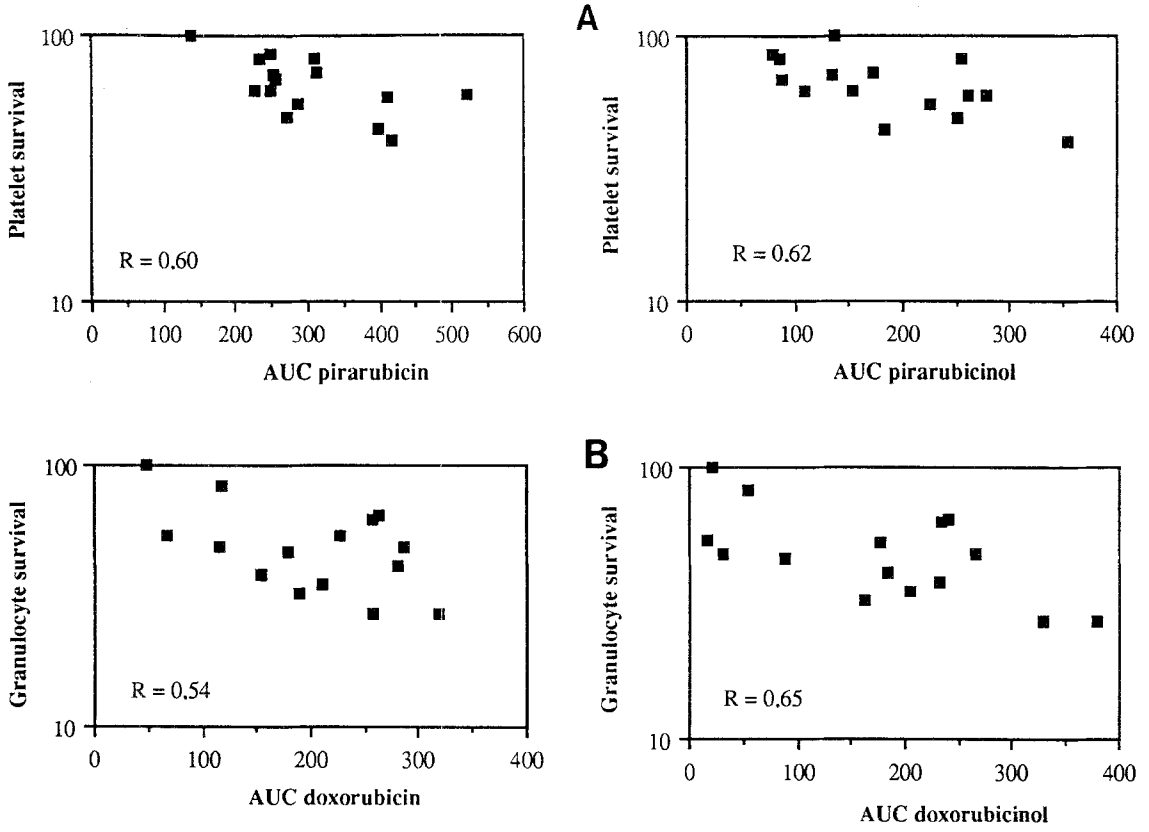

Fig. 2. A Relationship between the AUC values for pirarubicin and pirarubicinol (ng ml^{-1} h) and platelet survival. **B** Relationship between the AUC values for doxorubicin and doxorubicinol (ng ml^{-1} h) and granulocyte survival

cin/pirarubicin and doxorubicinol/pirarubicin were 0.6±0.21, 0.64±0.24 and 0.57±0.36, respectively. For the compartmental analysis of pirarubicin pharmacokinetics, the mathematical process used gave better results for a two-compartment model than for a three-compartment model in all courses; the use of a three-compartment model even yielded divergent estimates in 4 of 16 courses. Table 1 shows the model-dependent parameters of the pharmacokinetics of pirarubicin. All of the parameters were independent of the dose received, except for the elimination half-life, which tended to increase as a function of dose, the correlation between the parameters lying at the limit of significance ($r = 0.51$, $P < 0.05$).

Two hematologic parameters were tentatively related to the AUC values for pirarubicin and its metabolites so as to define the pharmacokinetic/pharmacodynamic relationships. The granulocyte survival was more closely related to the AUC values for doxorubicin and doxorubicinol ($r = -0.54$ and $r = -0.65$, $P < 0.05$ and $P < 0.01$, respectively) than to those for pirarubicin and pirarubicinol ($r = -0.4$ and $r = -0.01$, not significant). In contrast, the platelet survival was more significantly correlated with the AUC values for pirarubicin and pirarubicinol ($r = -0.60$ and $r = -0.62$, $P < 0.02$) than with those for doxorubicin and doxorubicinol ($r = -0.37$ and $r = -0.36$, respectively, not significant). Figure 2 shows two examples of significant results.

Table 1. Pharmacokinetic parameters of pirarubicin in the patients investigated in the present study

Parameter	Dose (number of courses)				Overall
	30 mg/m² (5)	40 mg/m² (5)	50 mg/m² (5)	60 mg/m² (1)	
AUC (ng ml^{-1} h)	260 ± 35	268 ± 98	380 ± 103	517	–
$t_{1/2\alpha}$ (min)	16.2± 5.7	23.5± 8.5	22.2± 12.1	3.6	16 ± 9.8
$t_{1/2\beta}$ (h)	13.8± 2	15.5± 0.8	20.6± 5.4	16.7	16.6± 4.2
Total plasma clearance (l h^{-1} m^{-2})	117 ± 15	169 ± 71	140 ± 39	116	140 ± 48
Total volume of distribution (l/m²)	2,014 ±335	3,132 ±1,319	3.478 ±1,701	2,140	2,830 ±1,300

Data represent mean values ± SD

Table 2. Comparison of the data in the literature on the pharmacokinetic parameters of pirarubicin

Author	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	Total plasma clearance ($l\ h^{-1}\ m^{-2}$)	Total volume of distribution (l/m^2)
Majima et al. [12]	0.78±0.06	0.245±0.177	5.11±2.7	238[a] ±227	576[a]± 252
Miller and Schmidt [13]	1.4 ±0.3	0.317±0.047	13 ±1.6	115 ± 11	2,124 ± 221
Robert et al. [22]	3.09±0.4	0.717±0.05	16 ±1.5	90.4± 5.2	1,379 ± 145
Raber et al. [18]	5.6 ±0.9	1.4 ±0.3	19.3 ±2.1	68[a] ± 25	212[a]± 56
Mader et al. [11]	1.48±0.36	0.42 ±0.1	11.3 ±1.7	86 ± 24	–
Scridhar et al. [27]	2.5 ±0.85	0.427±0.108	23.6 ±7.6	204 ± 39.3	3,504 ± 644
Present study	–	0.266±0.163	16.6 ±4.2	140 ± 48	2,830 ±1,300

Data represent mean values ± SD as presented in the various papers

[a] Values were recalculated from data expressed per kilogram, assuming a mean weight of 40 kg/m^2 body surface area

In contrast, none of the model-dependent parameters of pirarubicin pharmacokinetics was significantly related to the effects of the drug.

Discussion

There has been no agreement concerning the relative importance of doxorubicin as a metabolite of pirarubicin. After the AUC ratio of 2.1 for doxorubicin/pirarubicin was presented by Miller and Schmidt [13], other authors reduced this estimate to 0.43 [22], 0.4 [11] or even less [18, 27]. The differences might have resulted from poor extraction conditions used by Miller and Schmidt [13], resulting in pirarubicin degradation, or from a preexisting contamination of the clinical pirarubicin formulation with doxorubicin in the early studies.

We prefer the use of a two-compartment model over that of a three-compartment model on the basis of the lower standard deviation/estimate ratio that we obtain. Other authors prefer a three-compartment model [18, 22, 27]. In any case, we found good agreement of our findings with those published in the literature for the half-lives of piririubicin as well as for the total plasma clearance and the total volume of distribution, which were both very high as compared with those of doxorubicin [2, 21]. This may have been due to the enhanced and rapid uptake of pirarubicin that has been emphasized by several authors [9, 15, 23]. Table 2 presents a summary of the values reported in the literature on the main pharmacokinetic parameters of pirarubicin. It is noteworthy that the association of 5-fluorouracil with pirarubicin had no detectable effect on pirarubicin's pharmacokinetics and metabolism.

The present study represents the first pharmacodynamic evaluation of pirarubicin in relation to pharmacokinetic data. Only very few data have been reported on anthracyclines using this recent concept [19]. It should be noted that both pirarubicin and its 13-dihydro derivative exert a more significant effect on platelet counts than on granulocyte counts and that doxorubicin and its 13-dihydro derivative are more clearly related to granulocyte survival. This might indicate a difference in the toxic properties of the two compounds; on the other hand, it might indicate that platelets are more sensitive to the drug that exhibits a high plasma peak concentration (pirarubicin) than to that which displays a plateau all along the time curve (doxorubicin). Of course, other explanations are also possible, especially the lack of accuracy of statistical tests when a relatively low number of courses are involved ($n = 15$). These relationships between the AUC values for pirarubicinol and doxorubicinol and the blood cell counts do not indicate that the metabolites are active against bone marrow, but rather they are probably attributable to the very strict dependence of the value for the 13-dihydro derivative on that of its parent compound ($r = 0.9$ between doxorubicin and doxorubicinol AUCs and $r = 0.65$ between pirarubicin and pirarubicinol values; $P < 0.0001$ and $P < 0.01$, respectively). It is well known that doxorubicinol has a very weak cytotoxic effect [17, 24]. As nothing is presently known about the cytotoxicity of pirarubicinol, such studies are warranted.

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