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Pharmacokinetics of Adriamycin in Patients with Breast Cancer: Correlation between Pharmacokinetic Parameters and Clinical Short-term Response*

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Abstract—*The pharmacokinetics of adriamycin was evaluated in the plasma of* 12 *patients with breast cancer after injection of an i.v. bolus. The patients were suffering from a locally advanced tumor, were free of metastases and had received no prior treatment. They received a chemotherapy consisting in adriamycin* (50 mg/m^2) *on day* 1, *vincristine* (1 mg/m^2) *on day* 2 *and methotrexate* (6 mg/m^2) *on days* 3, 4 *and* 5. *The response to chemotherapy was assessed as the percentage of reduction of the palpable tumoral mass. Plasma samples were collected at various times after injection of the drug. Adriamycin and its metabolites were extracted using an original column purification technique and were evaluated by high-performance liquid chromatography with fluorometric detection. Pharmacokinetic parameters were calculated with a computer program based upon an algorithm of non-linear function minimization. The three successive half-lives presented little individual variations and were* 4.75 min, 0.822 hr *and* 18.9 hr. *On the other hand, the A, B and C parameters were highly scattered. The total plasma clearance of the parent drug ranged from* 28.3 *to* 98.7 l/hr. *A highly significant correlation was observed between parameter A and the short-term clinical response. Moreover, a mild correlation exists between the half-life of the* 1*st phase and the short-term clinical response. We can therefore assume that the efficacy of the drug may be dependent upon its distribution in the organism. Such a relationship may allow the development of new protocols of chemotherapy in order to obtain an optimal distribution of the drug in every patient.*

INTRODUCTION

ADRIAMYCIN is an antitumoral antibiotic widely used in the treatment of leukemias and solid tumors [1]. A better understanding of its metabolism and elimination in whole organisms, by means of pharmacokinetic studies, should enable a better utilization of that drug, either through an adaptation of doses and regimen to individual patients or through modifications and readjustments of protocols used in phase II and phase III studies. The purpose of such an attempt to monitor the chemotherapy is to try both to increase the efficacy of the treatment and to reduce its toxicity.

Several pharmacokinetic studies have been performed on adriamycin using animal models [2–4] and cancer patients [5–11]. Most of the data were obtained from the estimation of plasma total anthracycline fluorescence [2, 5] or from thin-layer chromatographic analysis of the drug and its metabolites [3, 4, 6, 10, 11]. Numerous high-performance liquid chromatography (HPLC) methods are now available [12–16]. They are more reliable and sensitive than other methods; they are not destructive towards the drugs and can usually be performed rapidly, thus allowing routine serial determinations.

With a reliable analytical method and the mathematical evaluation of pharmacokinetics, it is possible to measure with a good accuracy

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the pharmacokinetic parameters of adriamycin in patients. Large individual variations have already been observed [8] and some of them were attributed to differences in hepatic status [6]. Moreover, it has been routinely observed by physicians that similar doses in similar patients could lead to different efficacies of the treatment [17]. We have tried in this study to compare the individual variations of pharmacokinetic parameters with the individual responses to treatment. This can only be achieved when there is an accurate method of evaluation of the clinical response. Therefore we chose a series of patients with locally advanced breast tumors which could not be surgically removed. A reliable measurement of the palpable tumoral mass can be obtained at various stages of the treatment and can be used as an indication of therapeutic efficacy. In this paper we present the correlations between pharmacokinetic parameters and the short-term efficacy of the drug.

MATERIALS AND METHODS

Patients

Twelve female patients ranging from 35 to 74 years of age, each with a Karnofsky index of more than 8, were included in this study. They were all suffering from breast cancers which were confirmed by convergent clinical, radiological and histological or cytological examinations and classified as adenocarcinomas. These patients had received no previous treatment. The size of the tumor at first examination was evaluated as the product of the two largest diameters of the tumor [18], and ranged between 20 and 120 cm^2. Ten patients presented an axillary nodal involvement on the same side as the tumor. None of them had distant metastasis. They had a bilirubinemia lower than 10 μmol/l and a creatininemia ranging from 41 to 86 μmol/l and were considered free of hepatic or renal failure. According to the TNM classification, these patients were T2 to T4, N0 to N2, M0. Five patients presented local inflammatory signs. The main clinical features are listed in Table 1.

Since surgical removal of the tumor was considered to be impossible, these patients received a combination therapy consisting of adriamycin (50 mg/m^2) on day 1, vincristine (1 mg/m^2) on day 2 and methotrexate (6 mg/m^2) on days 3, 4 and 5. Adriamycin was injected as in i.v. bolus during about 3 min. In this chemotherapy protocol five similar courses were performed at three-weekly intervals [19] before any local specific treatment. The pharmacokinetics of adriamycin was only studied during the first course of chemotherapy. Since the two first courses of chemotherapy were given in our Institute, the palpable tumoral mass was measured before the first and just before the second course of treatment. The percentage of reduction of the size of the tumor was taken as a short-term sign of efficacy and is referred to below as the 'short-term clinical response'. Both evaluations in the same patient were always made by the same physician without him knowing the results of the pharmacokinetic study. Eleven of the 12 patients also had tumor measured at the end of the five courses of chemotherapy.

Analysis of adriamycin and its metabolites

The blood samples from the patients were collected on EDTA-coated vacutainer tubes at various times after the end of the injection of the drug (10 min, 20 min, 40 min, 1 hr, 2 hr, 4 hr, 6 hr or 8 hr, 12 or 16 hr and 24 hr). The 24 hr sample was collected before the injection of vincristine. Blood was immediately centrifuged and two samples of plasma were taken and frozen until extraction and analysis were performed. An internal standard was added to the plasma before extraction. We used daunorubicin or 4'-epiadriamycin as internal standard. Both of them migrate in our chromatographic system in a region where we failed to detect any adriamycin metabolite.

Extraction was carried out on C-18 Sep-pak cartridges (Waters Associates, Milford, MA). The detailed procedure is described elsewhere [20]. In short, the plasma was run through the open mini-columns after they had been equilibrated with methanol and 0.05 M phosphate buffer. After washing the Sep-pak with the same solution, anthracyclines were eluted with chloroform/methanol mixture. This eluate was evaporated, reconstituted in a small volume of the HPLC solvent and centrifuged. An aliquot of the clear supernatant was then injected in the liquid chromatograph. We have shown that such an extraction procedure yields quantitative recoveries of all the anthracyclines and metabolites tested.

Analysis of the extracts were performed on a Waters Associates high-performance liquid chromatograph. The conditions were similar to those presented by Israel *et al.* [12], with minor modifications. Separation was achieved on a microbondapak-phenyl column (30 × 0.4 cm) with an isocratic solvent mixture (0.1% formate buffer, pH 4/acetonitrile, 68/32, v/v). The solvent flow was 3 ml/min. The peaks were detected on a Schoeffel model SF 970 fluorometer with excitation wavelength of 254 nm and an emis-

Table 1. Clinical features of the patients entering the study

Patient	Age	Tumor size before treatment (cm^2)	TNM status	Dose injected (mg)	Percentage tumor regression after 1 course	Percentage tumor regression after 5 courses
BAD	71	36	T4 N0 M0	65	40	85
BAV	39	30	T3 N1 M0	80	30	70
BLC	63	20	T3 N1 M0	75	80	95
BOU	51	90	T3 N1 M0	75	50	50
DEL	55	49	T4 N2 M0	80	80	90
FOU	31	12	T2 N1 M0	80	30	95
JAI	73	25	T4 N1 M0	75	70	95
PIL	34	80	T4 N2 M0	75	20	50
RIC	42	27	T3 N1 M0	80	10	10
ROB	35	120	T4 NI M0	75	10	n.d.
TAC	74	22	T4 N1 M0	70	50	90
VAL	51	20	T2 N1 M0	75	40	80

sion cut-off filter at 580 nm. Recording and integration of the peaks allowed the quantification of the fluorescence detection.

Calculations

The method of estimation of the pharmacokinetic parameters was based on the fitting of a compartmental model to the observed plasma concentrations. We have used a weighted least squares method to evaluate these parameters. This method consists in the minimization of the following expression:

$$J = \sum_{i=1}^{m} Wi\ [Yi - y(t_i)]^2,$$

where Yi are the experimental values, $y(t_i)$ the values generated from the model and Wi the weights defined as the reciprocals of the squares of the uncertainties of the measurements [21].

$$Wi = \frac{1}{\sigma_i^2}.$$

This kind of weighting is a statistical one and it can be obtained by expressing the variance σ^2 as a function of the concentration Y. This function is a linear one in this case (unpublished results):

$$\sigma = kY.$$

Consequently,

$$J = \frac{1}{k^2} J' = \frac{1}{k^2} \sum_{i=1}^{m} \frac{1}{Yi^2} [Yi - y(t_i)]^2.$$

The minimization of J' has been done by using an iterative algorithm based on the non-linear programming principle; this is the algorithm of the variable metric method of Goldfarb [22], adapted to the case of noised observations.

The model used was either a two-compartment one or a three-compartment one, taking into account the duration T of the perfusion (3 min) for a total dose injected D. The concentration at each time t is calculated as follows:

$$y(t) = \frac{D}{T}\left[\frac{A}{\alpha}(1 - e^{-\alpha t}) + \frac{B}{\beta}(1 - e^{-\beta t}) + \frac{C}{\gamma}(1 - e^{-\gamma t})\right]$$

when $t < T$, and

$$y(t) = \frac{D}{T}\left[\frac{A}{\alpha}(e^{\alpha T} - 1)e^{-\alpha t} + \frac{B}{\beta}(e^{\beta T} - 1)\, e^{-\beta t} + \frac{C}{\gamma}(e^{\gamma T} - 1)\, e^{-\gamma t}\right].$$

when $t \geq T$.

In some cases the values obtained from the three-compartment model were not consistent (negative values, divergence of the algorithm). In this case a two-compartment model was used ($B = 0$). This could be due to inadequate blood sampling times during the β phase. In these cases only the α and the γ phases were accurately estimated. A, B and C, and α, β and γ are the macroconstants of the system. These parameters allow the determination of the total plasmatic clearance of the drug:

$$\mathrm{Cl} = \frac{D}{\mathrm{AuC}},$$

where AuC is the area under the curve which is given by the equation:

$$\mathrm{AuC} = D\left(\frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma}\right).$$

Parameters α, β and γ also allow the calculation of the three successive half-lives of the drug in plasma:

$$t_{1/2} = \frac{\ln 2}{\alpha}, \frac{\ln 2}{\beta}, \frac{\ln 2}{\gamma}.$$

An example of the time courses of adriamycin and its main metabolite plasma concentration is presented in Fig. 1.

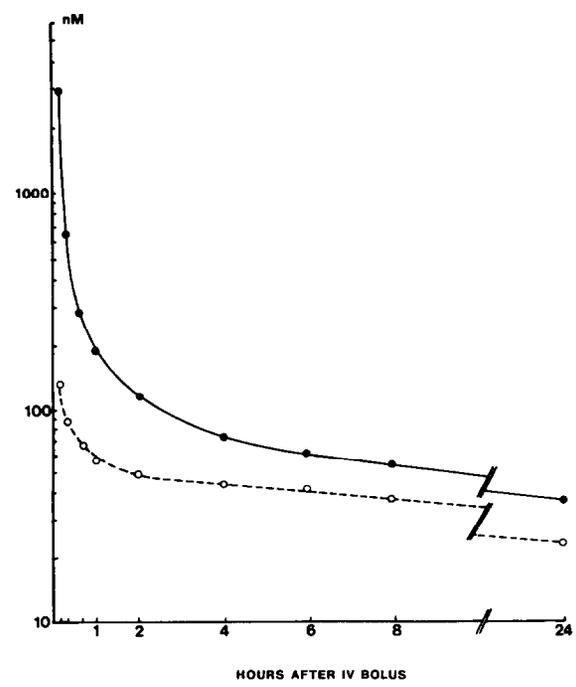

Fig. 1. Plasma time course of adriamycin (—●—) and adriamycinol (----○---) in one of the patients studied (TAC).

RESULTS

The clinical short-term responses ranged between 10 and 80% of tumoral regression, thus indicating large individual variations. No correlation was found between these responses and the size of the tumor or the local extension (Table 1).

The main pharmacokinetic parameters are presented for each patient in Table 2. The parameters α, β and γ presented little individual variations, and half-lives were 4.75 ± 1.72 min, 0.822 ± 0.338 hr and 18.9 ± 7.6 hr. On the other hand, the parameters A, B and C presented very important individual variations, ranging respectively from 15.7×10^{-3} to 130×10^{-3}/l, from 0.420×10^{-3} to 6.712×10^{-3}/l and from 0.277×10^{-3} to 0.979×10^{-3}/l. The total plasmatic clearance could vary greatly, in a 1:4 ratio for extreme values.

A mild correlation was observed between the short-term clinical responses and the half-life of the first phase. However, a highly significant correlation was found between this short-term clinical response and the parameter $A (P < 0.001)$ (Fig. 2).

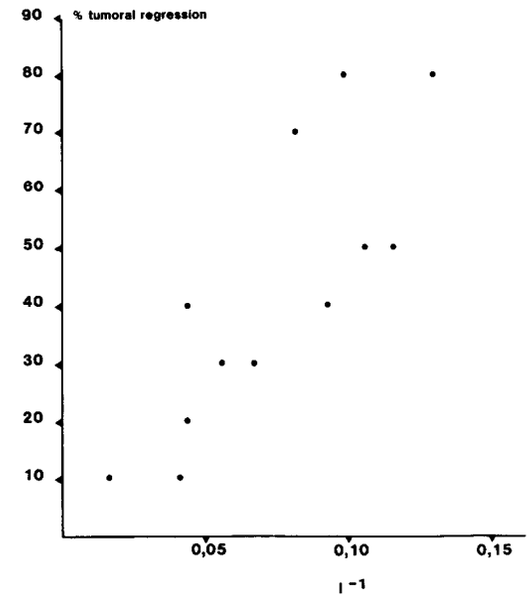

Fig. 2. Correlation between parameter A and the short-term response of the tumor; $r = 0.820$, $P < 0.001$.

The metabolization of adriamycin was similar in all the patients studied. Adriamycinol was the only detectable metabolite and no aglycone was detected in the plasma of these patients. On account of the low levels reached, it was impossible to calculate accurately the pharmacokinetic parameters of adriamycinol in every patient.

DISCUSSION

The metabolization of adriamycin in the human body has been shown to lead to various metabolites, the main one of which is adriamycinol; several aglycones are formed by the liver and are then conjugated to form more soluble compounds (glycurono- and sulfo-conjugates) [23]. It has been previously shown that adriamycin aglycones could be detected in the plasma of patients treated with this drug [5, 8]. However, we were unable to detect any other metabolite than adriamycinol, although the aglycones could be extracted by our procedure [20], as well as adriamycin itself. Several authors, working now with HPLC methods, have failed to find any aglycone in patients' plasma [12, 14]. It has been suggested by Israel *et al.* [24] that the acidic solvents used in TCL could lead to artificial hydrolysis of the parent compound into aglycones.

Although several authors have described the adriamycin plasma time course in cancer patients, few of them calculated the kinetic

Table 2. Pharmacokinetic parameters obtained from the mathematical modeling of analytical data

Patient	α hr^{-1}	β hr^{-1}	γ hr^{-1}	A $l^{-1} \times 10^3$	B $l^{-1} \times 10^3$	C $l^{-1} \times 10^3$	Clearance l/hr
BAD	5.62	—	0.0524	93.5	—	0.977	28.3
BAV	9.96	—	0.0248	67.1	—	0.446	40.5
BLC	12.45	0.807	0.0497	130.3	6.58	0.932	26.8
BOU	10.08	2.179	0.0334	106.4	6.07	0.365	41.2
DEL	13.21	0.566	0.0403	99.3	1.25	0.408	50.4
FOU	9.77	1.016	0.0196	55.7	0.779	0.495	31.5
JAI	11.49	0.771	0.0348	82.0	1.043	0.519	42.8
PIL	11.47	0.520	0.0286	43.7	0.415	0.337	61.1
RIC	7.91	0.914	0.0325	40.9	0.873	0.277	68.2
ROB	5.09	—	0.0545	15.7	—	0.385	98.7
TAC	12.76	1.374	0.0570	116.3	2.924	0.640	44.5
VAL	6.36	—	0.0804	44.0	—	0.839	57.6

parameters of the system. Benjamin *et al.* [25] described in 1973 a biphasic model with half-lives of 1.1 hr and 16.7 hr, and then in 1977 a triphasic plasma disappearance curve with half-lives of 12 ± 9 min, 3.3 ± 2.2 hr and 29.6 ± 13.5 hr, the second phase lasting from 2 to 12 hr after drug administration. Chan *et al.* [9] presented the results obtained from 23 patients, with mean half-lives of 9.2 min, 1.51 hr and 25.9 hr. Ehninger *et al.* [8] gave only two half-lives of 9.0 ± 1.1 min and 35 ± 11 hr for adriamycin. An early phase study by Piazza *et al.* [11] allowed the calculation of the two first half-lives of adriamycin (estimated as the total plasma fluorescence): 4.88 ± 1.28 min and 2.95 ± 0.93 hr. In our study we were lead to use either a three-compartment model or a two-compartment one as a function of the quality of the fitting of the calculated curves with the experimental data. The half-lives we found were the same magnitude as those already presented. The half-life of the second phase was, however, lower in our study than in the other ones. This may be due to a difference in mathematical analysis of the curve and to a difference in blood sample timing. The variability of the half-lives is as low in our study as in other ones. This contrasts with the high degree of variability of the parameters *A*, *B* and *C*. This is also evident from the data presented by other authors [5, 8, 11].

Total plasma clearance can be calculated from the data presented by several authors. Benjamin *et al.* [5] obtained a clearance of 640 ml/min/m^2, while Wilkinson [26] reports a value of 140 ml/min. Ehninger *et al.* [8] obtained values ranging from 68 to 1667 ml/min/m^2, which gives a much larger individual variation than the one we observed (267–1051 ml/min/m^2). The data of Piazza *et al.* [11] cannot be compared to ours since the last phase of adriamycin kinetics was not studied.

The existence of a correlation between pharmacokinetic parameters and the clinical response had never been observed before for adriamycin. Such a relationship may be of great interest for the management of individual doses and protocols. We were able to study a small but homogenous and well-defined series of patients. The tumor response was always objectively assessed for similar breast cancers and lymph nodes with clinical and radiological examinations. All patients were free of metastasis and had a good performance status, with normal hepatic and renal functions. We observed a correlation between pharmacokinetic parameters and tumoral responses. This correlation was significant only for short-term response, after one course of chemotherapy; this correlation, however, was not significant for the tumor regression observed at the end of the five courses of chemotherapy, mainly because 8 out of 11 patients achieved 70% or more tumor regressions. Therefore it appears that most patients reached a substantial response after the end of the five courses, as already observed [19]. It was thus necessary to look at short-term responses, which were much more scattered, but which do not correlate the long-term response. It must also be underlined that the kinetic study avoids drug interactions, but that the response evaluation cannot, since vincristine was given on day 2 and methotrexate on days 3, 4 and 5. The combination chemotherapy could therefore have important effects on the clinical response without any linkage to adriamycin pharmacokinetics. In view of the highly significant correlation ($r = 0.820$, $P < 0.001$) between *A* and the short-term response, the intervention of the drugs associated to adriamycin may be of little importance.

In view of the results obtained, some proposals can be made towards modifying the adriamycin treatment protocols. We are trying to develop, on the basis of our pharmacokinetic studies, individual approaches of the treatments. Our results demonstrate that only the first phase of the kinetics is well-correlated with the short-term responses of the tumors. We can therefore propose an individual adaptation of the therapeutic doses calculated from the parameters defined by a test-dose administered to each patient before the treatment. If optimal tumoral responses can be obtained after a few injections (1 or 2), the local treatment (radiotherapy ± surgery) could be performed long before the end of the five courses of treatment, as is now the case according to our protocol. Our purpose would not be to obtain larger tumoral regressions than those obtained at present time, but to obtain them faster.

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