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Clinical and Pharmacokinetic Study of 96-h Infusions of Doxorubicin in Advanced Cancer Patients

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Abstract—*A phase I and a pharmacokinetic study of* 96-h *infusions of doxorubicin were performed in order to evaluate the maximum tolerated dose with this schedule of administration. Seventeen patients suffering from a digestive carcinoma were included in the study and a total of* 71 *courses of treatment were performed. The starting dose was* 15 mg/m^2/*day and was increased in* 2.5 mg/m^2/*day increments. The main toxicities observed were neutropenia and mucositis, which became limiting from* 22.5 mg/m^2/*day* (90 mg/m^2 *over a* 96-h *period*); *this dose was therefore defined as the maximal tolerated dose. No objective response to treatment was observed. For further studies, the recommended dose should not exceed* 20 mg/m^2/ *day.*

A plasma plateau concentration of doxorubicin was reached within 24 h. *Despite a constant infusion rate, the plasma concentration of doxorubicin showed transient variations in several patients. However, an average plasma concentration could be evaluated for* 33 *courses of treatment, and this was linearly related to the dose. Doxorubicinol was the only detected metabolite of doxorubicin and its plasma concentration progressively increased throughout infusion. A detailed pharmacokinetic study was performed in* 13 *courses of treatment. The mean plasma clearance of doxorubicin was* 25.2 l/h/m^2 *and the mean terminal half-lives of doxorubicin and doxorubicinol were respectively* 43.6 *and* 66.2 h. *Urinary excretion of doxorubicin plus metabolite was regular from the* 24*th to the* 96*th hour of infusion; however, the proportion of doxorubicinol progressively increased in urine. The protracted half-life of this metabolite probably explains its accumulation during infusion.*

INTRODUCTION

FOR MORE than a decade, doxorubicin has probably been the most prescribed anticancer agent because of its activity in a variety of neoplasms including carcinomas, sarcomas and hematologic malignancies. Severe limitations to its use are particularly due to its considerable toxicity, with both acute and long-term manifestations. Active research is devoted to the development of new anthracyclines, with the aim of discovering drugs devoid of cardiac toxicity. An alternative approach to this hindrance is the search for new modes of administration of doxorubicin, especially through the use of constant infusion pumps. Continuous infusion of anticancer drugs such as cytosine arabinoside or 5-fluorouracil has considerably improved therapeutic results obtained with these drugs [1,2]. In the case of doxorubicin, no general agreement concerning the optimal schedule has emerged until now; some authors recommend dose fractionation and spreading in order to reduce toxicity [3], while others stress the importance of dose intensity in order to achieve good response rates [4]. It clearly appears from the work of Legha *et al.* [5] that 4-day infusions of doxorubicin at a dose of 15 mg/m^2/day significantly reduce the acute toxicity and immediate cardiac damage that occur with standard bolus injections. In another study, Legha *et al.* [6] obtained 13 objective responses in 27 metastatic breast cancer patients treated with 1–4 days continuous infusion of doxorubicin, at a dose of 15 mg/m^2/day. In a recent review on theoretical and clinical aspects on doxorubicin continuous infusion [7], one of us concluded that comparative clinical trials were required to definitively demonstrate which schedule of administration is best.

Before undertaking such clinical trials, it is necessary, however, to establish with accuracy the maximum tolerated dose of doxorubicin when administered continuously. Several phase I studies have been performed for very long-term infusions, ranging from 1 to 6 months [8–10]; these studies have shown a maximum tolerated dose of about 3 mg/m^2/day. However, no phase I study has been published on the more common 4-day infusions, and few patients have been treated with doses higher

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than 15 mg/m^2/day. Moreover, only a few studies have been made on the pharmacokinetic behavior of doxorubicin when administered continuously. The studies of Brenner *et al.* [11] and Speth *et al.* [12] showed that most pharmacokinetic parameters of doxorubicin, such as clearance or elimination half-life, remained unchanged; however, the total volume of distribution of the drug in the body was two times higher after continuous infusion than after bolus injection.

We have undertaken a phase I study of 4-day infusion of doxorubicin in a series of advanced cancer patients. This study also had a phase II component and was disease-oriented in order to examine the efficacy of this mode of administration of doxorubicin in metastatic colon and rectum cancer. This study was run in parallel with a pharmacokinetic study in order to define plasma levels and urine excretion of doxorubicin and metabolites during continuous infusion.

PATIENT INCLUSION AND TREATMENT

Included in this study were patients suffering from a metastatic evolutive carcinoma of the digestive tract with one or more evaluable targets; patients with brain metastases were excluded. All patients gave their informed consent to the treatment. They had to be under 70, with a quality of life (WHO grading) lower than 3. None had received any previous treatment except one patient who had received two courses of intraarterial 5-fluorouracil. An isotopic ventricular ejection fraction higher than 50% was required for inclusion. Biological criteria for inclusion were: granulocytes >2000/mm^3, platelets >120,000/mm^3, bilirubin <35 μmol/l, creatinine <135 μmol/l.

Doxorubicin was administered as an i.v. infusion of 96 h through a central catheter connected to an electric syringe or an external pump device. The drug solution was changed each 24 h. The first dose level was 15 mg/m^2/day for 11 patients, and 20 mg/m^2/day for six patients; dose increments were 2.5 mg/m^2/day if permitted by hematological status. Courses were repeated at 4-week intervals. For clinical evaluation, each patient received at least two courses of treatment; the treatment was continued if a stable disease was obtained, but was interrupted if progression of the disease or major toxic effects occurred. An isotopic evaluation of cardiac function was made every two courses.

MATERIAL AND METHODS

Blood samples were obtained in 13 patients for a total of 38 courses of treatment. In 25 cases, a daily sampling was performed for 5 days, i.e. at the following times after the beginning of infusion: 24, 48, 72, 96 and 120 h. In 13 other cases, a more detailed analysis was performed, and the samplings were made during infusion, at 4, 12, 24, 36, 48, 60, 72, 84 and 96 h, and after the end of the infusion, at 98, 100, 108, 112, 120, 132 and 144 h. Urine was collected in 24 h periods from the 1st day of infusion to the last day after its arrest.

Blood was collected in EDTA-containing tubes and immediately centrifuged. Plasma was frozen at −20°C until analysis. Extraction was performed, after adding a known amount of daunorubicin as an internal standard, on chromatographic cartridges containing C18-bonded silica (Sep-pak, Waters Associates) as already described [13]. Analysis was done by high-performance liquid chromatography using fluorometric detection. The column used was microbondapak-phenyl (Waters Associates) and the mobile phase was a mixture of 0.1% ammonium formate, pH 4.0 and acetonitrile (66/35) at a flow rate of 3 ml/min as described by Israel *et al.* [14]. Detection was achieved with a Perkin–Elmer spectrofluorometer (model LS1) with excitation and emission wavelengths respectively set at 480 and 592 nm.

Results were processed without mathematical modeling. For each dose level, the mean plasma levels of drug and metabolite were calculated at each time after the beginning of infusion. For 33 courses in 12 patients, it was also possible to evaluate a plateau level of doxorubicin obtained during a given infusion. For 13 courses of treatment in nine patients, it was possible to evaluate the area under the curve (AUC) of doxorubicin and doxorubicinol, and therefore the total plasma clearance of doxorubicin (dose/AUC); the terminal half-lives of doxorubicin and doxorubicinol after the infusion were evaluated by linear regression. Cumulative urinary excretion of drug and metabolite was studied during infusion; the daily percentage of the total dose excreted as well as the relative proportions of doxorubicin and doxorubicinol were evaluated.

RESULTS

Phase I study

Seventeen patients were included, with a sex ratio (M/F) of 9/8 and a median age of 56 (range: 43–70). Ten had a colon cancer and seven a rectal cancer, all histologically proven. The measurable metastases were 11 hepatic, five pulmonary and one cutaneous metastases.

Seventy-one courses of treatment were performed; 16 at a dose of 15 mg/m^2/day; 13 at 17.5 mg/m^2/day; 29 at 20 mg/m^2/day; 11 at 22.5 mg/m^2/day and two at 25 mg/m^2/day. The number of courses per patient were as follows: 1: 11 courses; 1: 8 courses; 2: 6 courses; 1: 5 courses; 4: 4 courses; 3: 3 courses; 5: 2 courses. The median cumulative dose was 314 mg/m^2 (range: 130–820 mg/m^2).

Toxicity of WHO grade >3 is shown in Table 1. Nausea, vomiting and alopecia did not apparently

Table 1. *Toxicity of* 96-h *infusions of doxorubicin*

Dose level (mg/m²/day)	Number of patients	Number of courses	Nausea/ vomiting	Alopecia	Leucopenia	Thrombopenia	Infection	Mucositis
15	11	16	1	5	1	0	0	0
17.5	10	13	1	3	1	0	1	0
20	13	29	1	4	4	0	1	7
22.5	6	11	1	2	2	1	0	5
25	2	2	0	1	2	0	1	1

The table indicates the number of courses which caused a WHO grade ≥3 toxicity.

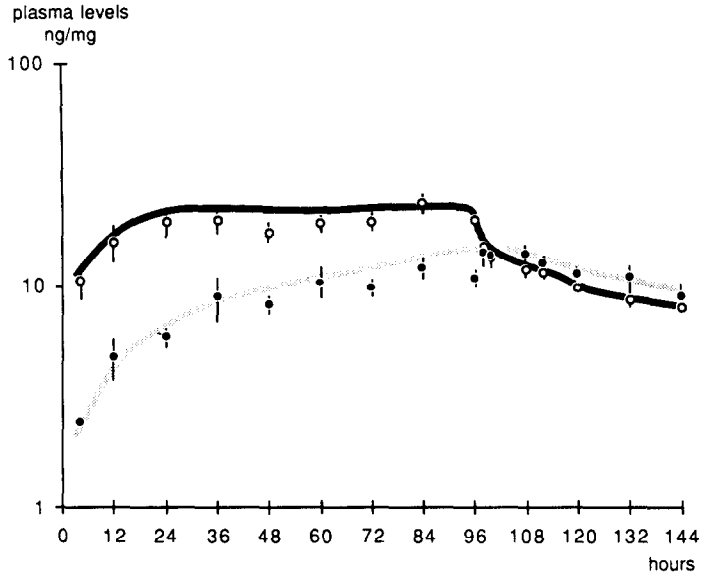

Fig. 1. *Time-course of doxorubicin* (—○—) *and doxorubicinol* (--●--) *plasma concentrations for the dose level* 15 mg/m²/*day. Values are means* ± *S.E.M. of* 4–17 *measurements according to the sampling protocol.*

depend on the dose received. Thrombopenia was never remarkable, even at doses higher than 20 mg/m²/day; in contrast, leucopenia and mucositis were the limiting toxicities at 20 mg/m²/day and above; from that level, mucositis became progressively more severe with four recorded cases of WHO grade 4 stomatitis, one at 20 mg/m²/day, two at 22.5 mg/m²/day and one at 25 mg/m²/day. The median leukocyte nadirs at those dose levels were respectively 2.4, 1.7 and 0.2 × 10^3/mm³. Infections were infrequent despite the neutropenia. A 20% decrease in the ventricular ejection fraction was observed in two patients for cumulated doses of 400 and 570 mg/m². No lethal toxicity was observed.

No objective response to treatment was noticed. A stable disease was observed for five patients and a progression for 12 patients.

Pharmacokinetic study

For each dose, the mean plasma levels of doxorubicin and doxorubicinol were evaluated at each blood sampling time; Fig. 1 shows the curve obtained at a dose level of 15 mg/m² per day. Doxorubicin concentrations reached a plateau by the 24th hour of infusion, whereas doxorubicinol did not do so before the end of the infusion: its level progressively increased to the doxorubicin level, exceeded it at the end of infusion, and was thereafter higher than the doxorubicin level.

For 33 infusions in 12 patients, it was possible to evaluate a doxorubicin plateau concentration between 24 and 96 h by averaging the plasma levels obtained throughout the course of infusion (Table 2). It is worth noting that sharp variations of doxorubicin levels were noticed in several patients, at times corresponding to a change of the infusion vial; doxorubicinol levels did not present such great variations. The mean plateau value was calculated for each dose level and is presented in Fig. 2. A linear relationship between the mean plateau level and the dose from 15 to 22.5 mg/m²/day ($r = 0.98$) was observed. A dose level of 25 mg/m²/day was reached in only one patient.

From the 13 courses in nine patients for which a complete blood sampling was obtained, during and

Table 2. Plateau levels of doxorubicin during the 33 courses of treatment evaluable for drug analysis

Patient	Plasma plateau level (ng/ml) at a dose level of:				
	15 mg/m²	17.5 mg/m²	20 mg/m²	22.5 mg/m²	25 mg/m²
1	25.3	15.0	26.7		
	14.1		23.4		
	16.0		21.0		
2	16.8	22.7	26.0	28.1	
				28.2	
				34.9	
3	16.5				
4	17.9	23.8			
5		26.4			
		23.6			
		26.0			
6	17.8				
7	17.3	18.7			
8	26.3	21.2	21.3		
9	19.9	21.4			
10	22.0				
	11.9				
11			21.7	26.5	
12				18.8	42.1
Mean	18.5	22.1	23.4	27.3	
± S.D.	4.3	3.6	2.5	5.7	

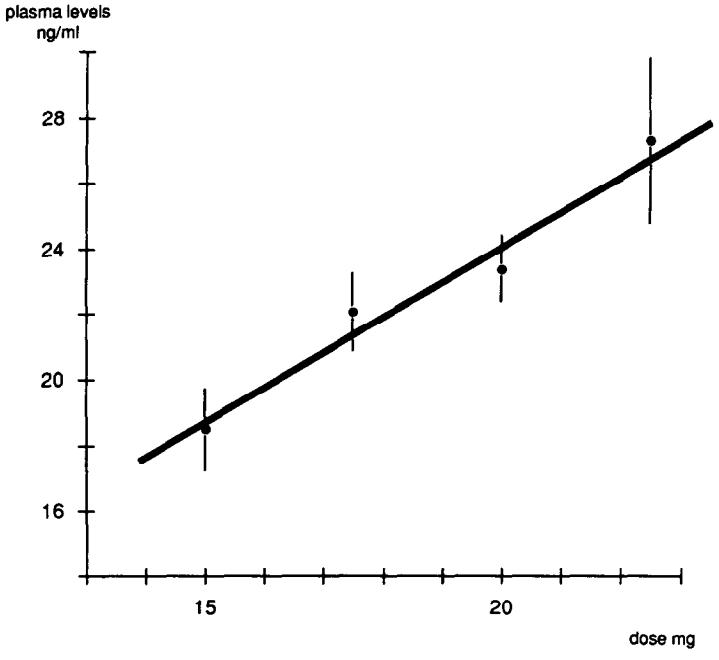

Fig. 2. Relationship between dose level and plateau concentration (r = 0.98). *Value are means ± S.E.M. of 5–12 plateau concentrations.*

Table 3. Pharmacokinetic parameters of doxorubicin and its metabolite doxorubicinol during the 13 courses analyzed

Patient	Dose	Total plasma clearance	Terminal half-lives		AUC ratio doxorubicinol/ doxorubicin
			Doxorubicin	Doxorubicinol	
1	20	19.6	n.e	n.e.	0.32
2	22.5	19.8	40.6	83.6	0.52
	22.5	20.5	41.5	71.0	0.57
5	17.5	18.6	41.0	40.1	0.27
7	15	23.7	n.e.	n.e.	0.63
	17.5	31.7	51.1	71.9	0.93
8	15	19.6	52.2	n.e.	0.42
	17.5	31.5	43.6	n.e.	0.70
9	15	27.5	37.7	n.e.	0.42
10	15	19.5	47.5	62.1	0.40
11	20	25.9	34.8	77.2	0.66
12	22.5	44.5	50.4	75.1	0.62
	25	25.7	39.5	48.2	0.59
Mean		25.2	43.6	66.2	0.54
± S.D.		7.4	5.8	15.0	0.18

Pharmacokinetic parameters were evaluated as described in the text. n.e. = non evaluable.

Table 4. Cumulative urinary excretion of doxorubicin plus doxorubicinol as a function of the dose administered

Dose administered (mg/m^2/day)	15 (15)	17.5 (8)	20 (6)	22.5 (6)
Percentage of the dose recovered in urine ± S.E.M.	9.38 ± 0.69	11.02 ± 0.92	12.00 ± 0.77	14.95 ± 1.44

The number of courses studied is indicated in parentheses.

after infusion, it was possible to evaluate total plasma clearance as well as terminal half-life of doxorubicin and doxorubicinol (Table 3). Mean total plasma clearance was 25.2 ± 7.4 l/h/m^2 and terminal half-lives of doxorubicin and doxorubinol were respectively 42.6 and 66.2 h.

Cumulative urinary excretion was 9–12% of the administered dose for doses of 15, 17.5 and 20 mg/m^2/day (Table 4); it was significantly higher ($P < 0.05$) for a dose of 22.5 mg/m^2/day, which suggests that a saturation phenomenon occurred. Urinary excretion of the drug plus metabolite was regular from the 2nd day to the end of infusion (Table 5), but the relative proportion of doxorubicinol in urine progressively increased during and after infusion (Table 5). The dose administered had no influence on these parameters.

DISCUSSION

The phase I study showed a maximum tolerated dose at 22.5 mg/m^2/day when doxorubicin was used on the basis of 96-h continuous infusion in non-pretreated patients. At this dose level, neutropenia and mucositis were the limiting toxicities. No clinical cardiac toxicity was observed during the study. It is worth noting that this maximum tolerated dose per course of treatment (90 mg/m^2) was about the same as that obtained by bolus injection, which is generally considered to be 75–90 mg/m^2 [15]. A study has even shown the possibility of administering 120 mg/m^2 as a bolus with appropriate supportive care [16]. Spreading the usual doxorubicin dosage over 96 h did not, therefore, offer the possibility of increasing the amount of drug administered per course of treatment. An acute toxcity similar to that observed with bolus injections is shown by our study. However, our data do not point to immediate cardiac damage, which was shown to be greatly reduced in the study of Legha *et al.* [6].

The treatment protocol did not provide any objective response in tumors generally resistant to anthracyclines. Achieving a response rate was not the main

Table 5. Urinary excretion of doxorubicin and doxorubicinol as a function of time

	0–24	24–48	48–72	72–96	96–120
Daily percentage of excretion ± S.E.M.	14.1 ± 0.9	22.7 ± 1.6	24.4 ± 1.1	25.4 ± 1.4	13.3 ± 0.9
Ratio doxorubicinol/doxorubicin M ± S.E.M.	0.224 ± 0.020	0.290* ± 0.023	0.351** ± 0.021	0.424*** ± 0.037	0.673*** ±0.059

Significance of the results: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

goal of this study; the only conclusion that can be made is that this mode of administration does not overcome primary drug resistance to anthracyclines.

Only a few kinetic studies have been performed during continuous administration of doxorubicin. Brenner *et al.* [11] have compared the parameters obtained after the administration of 60 mg/m^2 of doxorubicin by bolus i.v. injections and continuous infusions over 6.5 days. Half-lives and total AUCs of the drug and metabolite were similar, but the total volume of distribution of the drug at steady state was significantly higher after long-term infusion than after a bolus injection, thus suggesting better tissue fixation. We observed several sharp variations of doxorubicin plasma concentration during infusion. The phenomenon might be attributed to changes in the infusion flow rate occurring especially during the change of infusion vial. However, circadian variations of doxorubicin pharmacokinetic parameters during continuous infusion cannot be excluded and should be considered in future studies. It was observed in this study that the plateau concentration of doxorubicin was reached at the 24th hour. Theoretically, a plateau would be obtained in more than three half-lives, i.e. 4–5 days after the beginning of infusion, since the elimination half-life of doxorubicin is about 30–40 h. Speth *et al.* [12] have compared bolus injections of doxorubicin to 96-h infusions at a dose of 9 mg/m^2/day. They have also observed similar total plasma clearance and elimination half-life. Both in our study and in that of Speth *et al.* [12], the plateau was rapidly attained.

The plateau concentrations observed for each dose level were about the values predicted by the theory (plateau concentration = perfusion rate/total plasma clearance). For instance, at a dose level of 15 mg/m^2/day, the perfusion rate was 0.625 mg/h; with a total plasma clearance of 30 l/h/m^2 (deduced from our previous experience [17]), we obtain a theoretical plateau concentration of 20.8 ng/ml, in comparison with the observed value (18.5 ± 4.3 ng/ml). In the studies of Brenner *et al.* [11] and Speth *et al.* [12], the plateau concentration was around 15 ng/ml for a perfusion rate of 0.375 mg/h/m^2, which is consistent with a clearance of about 25 l/h/m^2. It is worth noting that Speth *et al.* [12] observed a total plasma clearance of 24–28 l/h/m^2, whereas Brenner *et al.* [11] obtained higher values 35–38 l/h/m^2).

The metabolism of doxorubicin to doxorubicinol was similar in our study and those of Brenner *et al.* [11] and Speth *et al.* [12]; in all cases, the plasma level of this metabolite progressively increased until the end of infusion. This accumulation is due to the protracted half-life of the metabolite; the progressive increase of the relative proportion of doxorubicinol in urine must also be due to the same factor. Some studies have shown that doxorubicinol could be more cardiotoxic than doxorubicin itself [18], although this fact seems to have no consequence on clinical cardiac tolerance, as our study and that of Legha *et al.* [6] have shown.

This phase I trial of doxorubicin 96-h infusion has defined a maximum tolerated dose of 22.5 mg/m^2/day (90 mg/m^2 over the 96-h period); it opens the way for a comparative phase II study on usually doxorubicin-sensitive tumors such as soft tissue sarcomas. Such a study will be undertaken in our Institutes with doses not exceeding 20 mg/m^2/day.

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