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Pharmacokinetics of Weekly Low Dose Doxorubicin

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Abstract—*Weekly low dose doxorubicin (WLD-dox) is an interesting alternative to the classical 3 week schedule because of its reduced cardiotoxicity, the major dose-related side-effect. This study characterized the pharmacokinetic behaviour of WLD-dox with particular attention to the effect of treatment duration on the variability of individual pharmacokinetic parameters. Twenty-eight patients with advanced breast cancer were treated by WLD-dox (12 mg/m² week). Individual pharmacokinetic analyses were performed at the first injection and every month thereafter; residual drug levels were measured every week before injection. Dox and its main metabolite doxol were measured by HPLC and fluorescence. Pharmacokinetic data were available for 51 cycles. The mean concentration–time profile for 25 patients with normal liver function tests fitted well with a two-compartment model:* CO_{ext} (nM) = 2905 ± 1834; $t_{1/2}\alpha$ = 0.08 ± 0.03 h; $t_{1/2}\beta$ = 10.4 ± 3.6 h; *clearance* (l/h) = 55.4 ± 24.8; V_d (l) = 809 ± 434. *The findings concur with those for classical 3-week dox schedule (45 mg/m²) analysed in six patients as controls. Exceptions were the initial extrapolated concentration and area under curves which were reduced for WLD-dox according to the dose. Dox and/or doxol overexposure was patent in all three patients with elevated pre-treatment serum bilirubin. During treatment, up to 48 WLD-dox administrations, no significant trends were noted for 10 patients in the evolution of initial extrapolated concentration, terminal half-life, total body clearance or the proportion of doxol formed. Residual drug levels were controlled up to 40 WLD-dox administrations (135 samples); in half the cases they comprised between 1 and 20 nM without particular sign of increasing along the treatment course. In this case WLD-dox can be assimilated to a continuous exposure to low drug levels with intermittent pulses thus representing an original pharmacological profile for dox.*

INTRODUCTION

ON THE basis of biological [1] and clinical evidence [2–5], weekly low dose doxorubicin (WLD-dox) (10–12 mg/m²) can be considered an attractive alternative to the classical 3-week schedule owing to its reduced cardiotoxicity, the major dox-related side-effect. In most non-randomized studies, the response rates with WLD-dox have been comparable to those reported with the 3-week protocol [2–4], although the efficacy of WLD-dox has been questioned [6].

Recently, however, a unique prospective randomized study concluded that use of WLD-dox for advanced breast cancer is as effective as a combination of vincristine, dox and cyclophosphamide administered every 3rd week and is considerably less toxic [7]. The use of WLD-dox has been proposed as a means of reducing the maximum initial plasma concentration, which is assumed to be directly related to the severity of myocardial injury [8]. This concept has been logically extended to the intrinsically less cardiotoxic dox analogue, 4′-epi-adriamycin, epirubicin [9]. A cellular and pharmacokinetic study was recently conducted using such a protocol for 12 patients [10]. As stressed by Smith [11] pharmacokinetic data are not yet available for a WLD-dox schedule: such information seems justified not only to validate the initial rationale behind the use of WLD-dox itself (degree of peak reduction, possible accumulation of the parent compound and/or metabolite), but also to complete our knowledge about dox pharmacokinetics (effect of repeated treatment on pharmacokinetic parameters, on the intensity of drug metabolism in doxorubicinol, doxol). The present paper describes a pharmacokinetic analysis of 51 courses of WLD-dox (12 mg/m²) administered to 28 pati-

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ents with advanced breast cancer. Six patients treated by 45 mg/m^2 every 3 weeks were included as controls to allow kinetic comparisons.

MATERIALS AND METHODS

Patients

Twenty-eight patients with advanced breast cancer (all females; mean age 67.5 years, range 41–85) were treated with WLD-dox (12 mg/m^2). There were 42% of patients with primary tumour only; 10% with primary and distant metastases; 38% with multifocal metastases and 10% with isolated metastases (liver and bone). There was non-concomitant irradiation. This was the only cytostatic treatment given during the study period. The cumulative dose of dox per patient was (mean, range) 372 mg, 100–600 mg. Five patients had previously been treated by a chemotherapy regimen including anthracyclines, the total cumulative dose of dox, including WLD-dox was (mean, range) 640 mg, 160–1011 mg; two patients had received a chemotherapy protocol without anthracyclines. Twenty-five patients had normal pretreatment serum bilirubin (5–17 μmol/l) and three abnormal levels (119, 372, 461 μmol/l). The control group was six patients treated by the classical 3-week dox schedule at 45 mg/m^2. Liver function tests were normal for all six patients.

Treatment

WLD-dox was administered every week by a 5 min infusion through a venous catheter. Treatment was continued until disease progression or toxicity was observed. A haemogram including a platelet count was obtained systematically before each weekly injection of dox; cardiac function was evaluated before treatment by physical examination, electrocardiogram and radionuclide ventriculography. Chemical, radiologic and biological evaluations were performed every 2 months.

Pharmacokinetics analyses

A complete pharmacokinetic profile was obtained at the time of the first injection in 25 patients and once a month thereafter in 10 of them, i.e. at the 5th, 9th, 13th injections. Blood samples were taken 5, 20, 40 min and 1, 2, 4, 8 and 24 h after injection. As all patients were treated on an outpatient basis, it was not possible to obtain blood samples any longer after the 24 h time point. In addition, a weekly blood sample was taken just before each injection to determine the residual drug level. Blood was collected in EDTA tubes and immediately centrifuged. Plasma was stored at −20°C until analysed (within 2 weeks). Dox and doxol were separated and quantified by HPLC. Extraction was performed on Sep-Pak C_{18} cartridges (Millipore, Waters) as previously described [12] with slight modifications: cartridges were conditioned by successive elutions with 2 ml methanol and 5 ml phosphate buffer (Na_2 HPO_4, 0.05 M; NaH_2PO_4, 0.05 M; 2:1). One millilitre of plasma (patient or plasma for standard curve) spiked with 50 μl of internal standard (daunorubicin, 1 nmol/ml) was passed through the cartridge, followed by 1 ml of phosphate buffer (discarded). Drug material was eluted by 3 ml of methanol in previously siliconated tubes. After drying (N_2, 40°C), the residue was diluted in 250 μl of HPLC buffer, centrifuged for 10 min at 4°C, and injected into the HPLC system. Analysis was performed on an HPLC column μ Bondapack phenyl 30 × 0.4 (Millipore-Waters) with a CH_3CN formiate (33.5/66.5) buffer, pH 4, at a flow rate of 2.5 ml/min. Fluorescent detection was performed with a spectrofluorimeter (Kontron SFM 25) at λ_{ex} = 470 nm and λ_{em} = 600 nm.

Mathematical analysis

Concentration–time data were best fitted to a two exponential equation using a pharmacokinetics program based on the classical least squares procedure with a weight as $1/y$ (Siphar-Base, Simed, Creteil, France). The main pharmacokinetic parameters were thus computed: initial extrapolated concentration = CO_{ext}; half lives for the distribution and elimination phases = $t_{1/2}\alpha$ and $t_{1/2}\beta$; area under curve extrapolated to infinity ($AUC_{0-\infty}$ = $AUC_{0-24\,h}$, trapezoidal rule, + $C_{24\,h}/\beta$ = AUC; total body clearance = Cl = dose/AUC; volume of distribution (β phase) = V_d = Cl/β).

RESULTS

Time–concentration profiles in plasma during the first course of WLD-dox for 25 patients with normal liver function tests are shown in Fig. 1 for dox and Fig. 2 for doxol. Table 1 summarizes the main pharmacokinetics parameters values characterizing respectively WLD-dox at first course for patients with normal and those with abnormal pretreatment hepatic function tests and the control group. Marked interpatient variability was observed for all parameters. The presence of doxol was significant. This metabolite appears rapidly in plasma (Fig. 2) and is cleared more slowly than dox (23.7 ± 11.8 h vs. 10.4 ± 3.6 h, $P < 0.001$, Table 1). Drug overexposure (AUC) was patent for all three patients with abnormal bilirubin levels; it was due to dox itself for two patients and doxol for one patient. The main pharmacokinetic parameters of dox and doxol were not significantly different in the control group (45 mg/m^2). There was a significant difference for initial extrapolated concentration, AUCdox and AUCdoxol.

Ten patients were evaluable concerning the evolution of individual dox pharmacokinetics during treatment (between four and 48 WLD-dox injec-

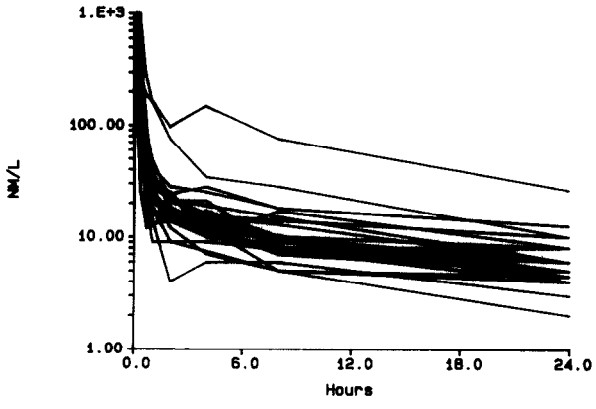

Fig. 1. Time–concentration profile of dox for all patients with normal bilirubin levels at the first WLD-dox cycle.

tions). Figure 3 shows the individual variations in CO_{ext}, $t_{1/2}\beta$, Cl, and the proportion of doxol formed with respect to the total drug (dox plus doxol). Only Cl tended to decrease with repeated treatment, this being particularly evident for the two patients followed on the longest basis. The other parameters were not particularly modified and remained within the range of interpatient variability. Statistical analyses based on ANOVA and regression analyses did not disclose significant trends in any of these parameters. Figure 4 illustrates residual plasma levels of dox and doxol during treatment up to 40 repeat WLD-dox injections. Concentrations were above the limit of detection (1 nM) in approximately half of the cases for dox (69/135) and dox (68/135). Plasma levels of dox and doxol at the day 7 of successive weekly injections were ranged between 1 and 20 nM and did not show any particular elevation with prolonged treatment. Dox was measurable in pleural fluid from two patients under treatment by WLD-dox: the respective concentrations were 7 nm (4 weeks of treatment, 3 days after the last WLD-dox dose) and 22 nM (3 weeks of treatment, 2 days after the last WLD-dox dose); in these samples, levels of doxol were below 1 nM.

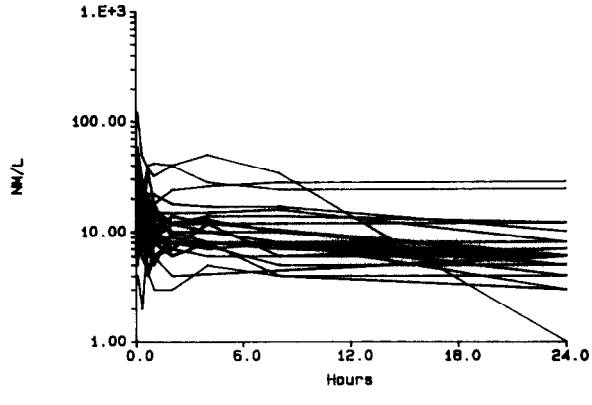

Fig. 2. Time–concentration profile of doxol for all patients with normal liver function test at the first WLD-dox cycle.

Table 1. Main pharmacokinetic parameters

Patient groups	Pharmacokinetic parameters* dox Co_{ext}(nM)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	AUC (nM/l:h)	Cl (l/h)	V_d (l)	$t_{1/2}$ (h)	doxol AUC (nM/l h)	R†
WLD-dox patients with *normal* liver function tests (12 mg/m²)									
Mean	2905	0.075	10.4	861	55.4	809	23.7	465	37.8
(S.D.)	(1834)	(0.034)	(3.6)	(598)	(24.8)	(434)	(11.8)	(333)	(12.9)
WLD-dox patients with *abnormal* liver function tests (12 mg/m²)									
BER	3033	0.070	9.4	692	53.5	726	152	2917	81
DUR	17,714	0.068	14.8	2921	12.7	271	42	1796	37
NEL	1073	0.085	77.5	3315	11.1	1248	89	2517	23
Controls $n = 6$ (45 mg/m²)									
Mean	17,600	0.095	12.8	3957	46.7	934	31	2719	42.3
(S.D.)	(19,131)	(0.059)	(5.0)	(2183)	(21.6)	(579)	(22.5)	(1439)	(17.7)
Statistical comparison‡ with WLD-dox patients with *normal* liver function tests	$t = 3.98$ $P < 0.001$	NS	NS	$t = 6.44$ $P < 0.001$	NS	NS	NS	$t = 6.57$ $P < 0.001$	NS

* See Materials and Methods for determination of parameters

† $\frac{\text{AUCdoxol}}{\text{AUCdox} + \text{AUCdoxol}} \times 100$.

‡ Student's t test (double sided) for comparison of means.

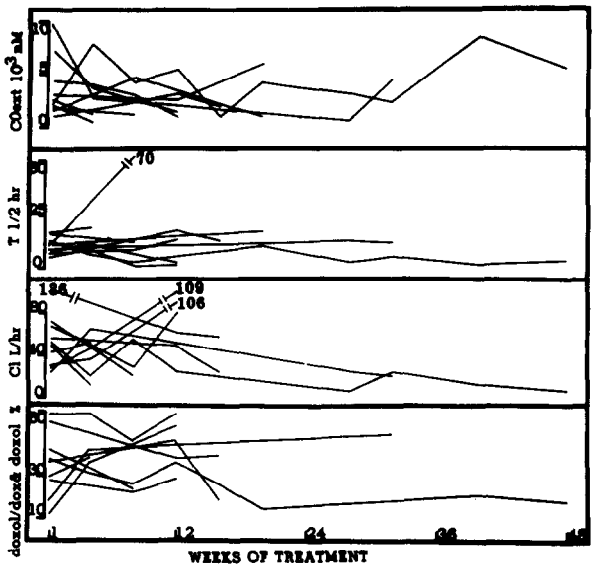

Fig. 3. Evolution of main pharmacokinetic parameters of dox from cycle to cycle (patient with normal liver function test).

DISCUSSION

This study was designed to examine the pharmacokinetic behaviour of dox administered on the basis of repeated WLD injections (12 mg/m^2) to advanced breast cancer patients. Obtaining such information seems justified because WLD-dox has been claimed to be an appropriate means of significantly reducing drug-related side-effects, and particularly the incidence of cardiac injuries [1–4], without any loss in antitumoural activity, as stressed by the majority of investigators [2–5]. A recent prospective randomized study comparing WLD-dox to VAC (vincristine, dox, cyclophosphamide) given over 3 weeks confirmed this view [7]. Our clinical experience indicated that WLD-dox could be administered to a total cumulative dose greater than 900 mg/m^2 without any clinically evident cardiac toxicity [5]. The dox concentration–time profile of this study population was well described by a two compartment model. In parallel, other investigators have claimed that a biphasic kinetic profile could adequately fit the data for both low doses of dox (15 mg/m^2 [13]) and higher doses [16].

Although important interpatient variability does exist in WLD-dox kinetic parameters, the initial dox peak value (CO_{ext}) appears reduced in proportion to the dose reduction when comparing to control group (Table 1). The toxicologic benefit of using WLD-dox might be explained at least partly by a peak attenuation phenomenon. The values of other main pharmacokinetic parameters ($t_{1/2}\alpha$, $t_{1/2}\beta$, Cl, V_d) were comparable and did concur with those published for dox at conventional doses (30–60 mg/m^2) [14–16]. This suggests that dox kinetics are not dose-dependent. This agrees with results of Eksborg *et al.* [17] showing that the pharmacokinetics of dox are linear between 20 and 60 mg/m^2. In contrast, Boston and Phillips [13] reached opposite conclusions for dox doses ranging from 15 to 60 mg/m^2.

Long term injections of WLD-dox administered to these patients gave the opportunity to examine the effect of repeat treatment with short intervals on the stability of dox pharmacokinetic parameters. For a drug with such a quantitative metabolism this particular point merited attention. Data collected for CO_{ext}, Cl, $t_{1/2}\beta$ and the doxol/dox + doxol ratio did not reveal any marked changes during the course of treatment up to 48 successive injections. Only the total body clearance tended to decrease after 12 or more WLD-dox injections (Fig. 3). Similarly, Speth *et al.* [10] studying weekly 20 mg epirubicin did not observe any significant difference in pharmacokinetic parameters during however a shorter period of follow-up (weeks 1, 4 and 8). In addition residual dox and doxol levels were not affected by repeated weekly injections (Fig. 4). It is important for clinicians to know that individual WLD-dox kinetics remain globally unchanged even after 6 months to 1 year of constant weekly injections.

The importance of liver status in dox pharmacokinetics remains controversial and has been much debated [18]. Three of our study patients with important elevations of blood bilirubin exhibited marked overexposure to dox or doxol. A prolonged terminal half-life has been reported in patients with elevated bilirubin concentrations treated by weekly epirubicin [10]. Gundersen *et al.* [7] found that the

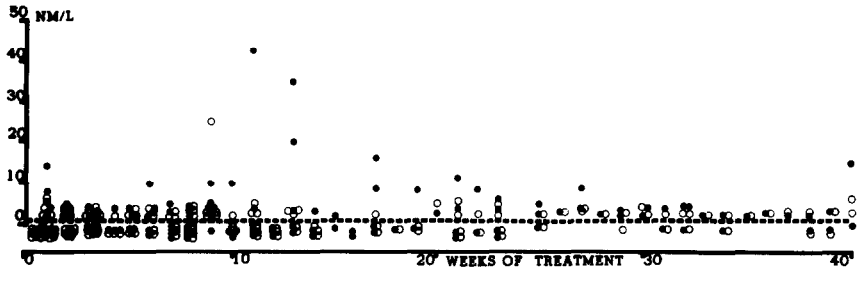

Fig. 4. Dox (full circles) and doxol (open circles) levels at day 7 after successive WLD-dox injections. Horizontal dashed line: limit of detection, 1 nM/l.

few patients exhibiting toxicity after WLD-dox were those with extensive liver metastases. We did not observe this association in our experience. With this well-tolerated low dose dox regimen we feel, however, that pretreatment bilirubinaemia remains important in both patient selection and dose determination.

Approximately 50% of the levels at day 7 of successive weekly injections were between 1 and 20 nM (Fig. 4). In these cases, WLD-dox could be assimilated to continuous treatment using basal low drug concentrations with intermittent pulses. A recent study has demonstrated that the antileukaemic effect of dox was not directly related to the peak plasma concentration [19]. *In vitro* data [20] have shown that the cytostatic effect of dox is the product of the extracellular drug concentration (0.5–3 μg/ml) and the exposure time (0–240 min). Further complementary experimental studies are needed to check whether prolonged exposure to low dox concentration associated to intermittent bolus dox may play a specific pharmacological role during WLD-dox treatment. This could be the basis of a rational optimization of this rather empirical but nevertheless clinically relevant dox administration schedule.

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