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Comparative pharmacokinetics of escalating doses of doxorubicin in patients with metastatic breast cancer

Miguel H. Bronchud[1], Jennifer M. Margison[2], Anthony Howell[1], Michael Lind[1], Samuel B. Lucas[3], and Peter M. Wilkinson[2]

Departments of [1]Medical Oncology and [2]Clinical pharmacology, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, England
[3] Department of Medical Computation, The Medical School, Manchester University, Oxford Road, Manchester, England

Summary. Recombinant human granulocyte colony-stimulating factor (G-CSF) has been shown to reduce neutropenia following cytotoxic therapy, thereby enabling dose escalation to improve the response rate. It is important to know whether drug kinetics change as doses are increased. Doxorubicin was selected because of its broad spectrum of activity and its known efficacy in metastatic breast cancer. Doses of 75, 100, 125 and 150 mg/m^2 were given to 11 patients with metastatic breast cancer by infusion over 30 min. Serum concentrations of parent drug and metabolites were determined during the first 48 h following the infusion by high-performance liquid chromatography (HPLC). The serum concentration vs time curve decayed as a triple exponential function in four patients and as a double exponential function in seven. A four-compartment model, one central and three peripheral, would predict concentrations to within 1 SE of the observed values. Doxorubicinol was the principal metabolite, and doxorubicinone and 7-deoxydoxorubicinone were clearly identified. There was a linear increase in the AUC_∞ with dose. In addition, a small and transient increase in circulating levels of doxorubicinol and other important metabolites was observed 6 h following the administration of doxorubicin, which suggests the existence of an enterohepatic, or other, re-circulation mechanism. We conclude that in the dose range selected the kinetics of doxorubicin are linear and that the increase in toxicities seen with the higher doses of doxorubicin, following the second and third fortnightly administration, may be due to intracellular drug accumulation in tissues.

Introduction

Since its introduction in clinical practice in the early 1970's, doxorubicin has been widely used in the management of a variety of malignancies [5]. It has a very complex metabolism, particularly in the liver, and over 11 metabolites have been identified in human bile [2]. It is reduced to doxorubicinol, which remains an active cytotoxic agent, by ubiquitous cytosolic aldoketoreductase enzymes. The drug binds rapidly to plasma proteins and cell membranes and is concentrated within cells [1]. Using thin-layer chromatography, Benjamin et al. [2] have shown that the plasma disappearance curve of unaltered doxorubicin is tri-phasic, with half-lives of 11.3 min, 3.5 h and 28.1 h. The relatively long terminal elimination half-life reflects doxorubicin distribution into a deep tissue compartment. Some metabolites disappeared from plasma according to a bi-phasic pattern, and the majority of the parent drug and its metabolites appeared to be retained in the body tissues for long periods of time. With modern high-performance liquid chromatographic (HPLC) techniques the kinetics of doxorubicin have been adequately described by a bi-exponential equation, with an initial half-life of 8 min and a terminal half-life of 30 h [11], or by both bi- and tri-exponential equations [17].

Although mathematical models for doxorubicin distribution have generally assumed linear pharmacokinetics [16], other studies have suggested that there might be dose-dependent elimination of doxorubicin [6]. However, in these studies low doses were usually given (17–40 mg/m^2) and drug concentrations were determined with the less sensitive drug-fluorescence assays.

The dose-limiting acute toxicity of doxorubicin is usually myelosuppression, but the advent of recombinant human granulocyte colony-stimulating factor (G-CSF) has recently enabled an up to 4.5-fold increase in dose-intensity in patients with metastatic breast cancer by enhancing the recovery from neutropaenia [4]. Because response rates and acute toxicity appear to be dose- and schedule-dependent [5], we decided to investigate the plasma concentrations of doxorubicin and its metabolites in 11 of these patients.

Patients and methods

Patients. A total of 11 patients with metastatic breast carcinoma underwent pharmacokinetic analysis of doxorubicin given at the following doses: 75 mg/m^2 (n = 3 patients), 100 mg/m^2 (n = 3), 125 mg/m^2 (n = 3) and 150 mg/m^2 (n = 2). The median age of the patients at the start of therapy was 51 years (range, 39–61 years), and 9 of them had >2 sites of disease. All patients had a serum bilirubin level of <25 μmol/l and none had received previous anthracyclines. Two patients, who had clinical ascites before therapy, had their ascitic fluid drained prior to drug administration, and none of the subjects were clinically

Offprint requests to: M. H. Bronchud

obese (defined as a body weight of >20% over the ideal). Patients received up to three courses of high-dose doxorubicin at intervals of 2 weeks given with infusions of G-CSF (Amgen, Thousand Oaks, Calif) to reduce the periods of neutropaenia. Informed consent was obtained from each patient. The study protocol was approved by the South Manchester Ethical Committee.

Blood sampling. All patients were studied during their first course of doxorubicin, which was infused via a central vein catheter in 250 ml saline over 30 min to minimise peak levels. Blood samples were drawn before the infusion from a peripheral vein and at 0, 0.167, 0.5, 1, 1.5, 3, 6, 9, 12, 24 and 48 h after the end of the infusion. Samples were centrifuged for 10 min at 500 *g*, after which the serum was separated and stored at −20° C prior to assay.

Analytical method. The method used was essentially that described by Israel et al. [13], with minor modifications, using daunomycin as an internal standard.

Materials. Adriamycin, Adriamycinol, Adriamycinone, daunomycin and 7-deoxyadriamycinone were generously donated by Pharmacia (Upsala, Sweden). All other reagents were of Analar grade.

Extraction procedure. Stored serum was thawed and 0.5 ml, removed. To each sample, 40 µl daunomycin (1 µg/ml) was added, and the solution was precipitated with 1.5 ml ice-cold methanol and then centrifuged at 2,000 *g* for 15 min at 4° C. The supernatant was removed and 4.5 ml chloroform, added. The mixture was then shaken to disperse the chloroform completely and further centrifuged at 650 *g* for 10 min at 20° C. Following centrifugation, the upper lipid layer was discarded and the chloroform solution was passed through phase-separation filter paper into clean tubes to remove any remaining aqueous phase. The chloroform was evaporated to dryness in a Buchler Vortex Evaporator and the residue was resuspended in 100 µl HPLC mobile phase prior to assay. This solution was centrifuged at 11,600 *g* for 10 min at 4° C, and 25-µl aliquots of the resulting supernatant were injected into the HPLC column. The extraction efficiencies from serum were 53% for doxorubicinone, 43% for doxorubicinol, 42% for doxorubicin, 36% for daunomycin and 21% for 7-deoxydoxorubicinone. The coefficients of variation for these extraction efficiencies were: Adriamycin, 7.8%; Adriamycinol, 5.9%; Adriamycinone, 6.4%; and 7-deoxyadriamycinone, 3.3%.

HPLC method. The HPLC assay was carried out using a Waters Associates U6K injector and 6000A pump. Separation of the parent compound, metabolites and internal control was achieved using a 23 cm × 4 mm Technicol (Stockport, UK) ODS Hypersil column (5-µm particle size and 120-Å pore size), which was isocratically eluted with an acetonitrile/ammonium formate buffer (38 : 62, v/v) (pH 4) at a flow rate of 1.5 ml/min. Fluorescence was monitored with a Schoeffel FS970 detector (Cambridge, UK) using an excitation wavelength of 233 nm and a 550-nm cut-off emission filter. Levels of drug and metabolite in patient samples were quantified by comparison with a standard curve using peak height ratios of the drug and metabolites to that of the internal control.

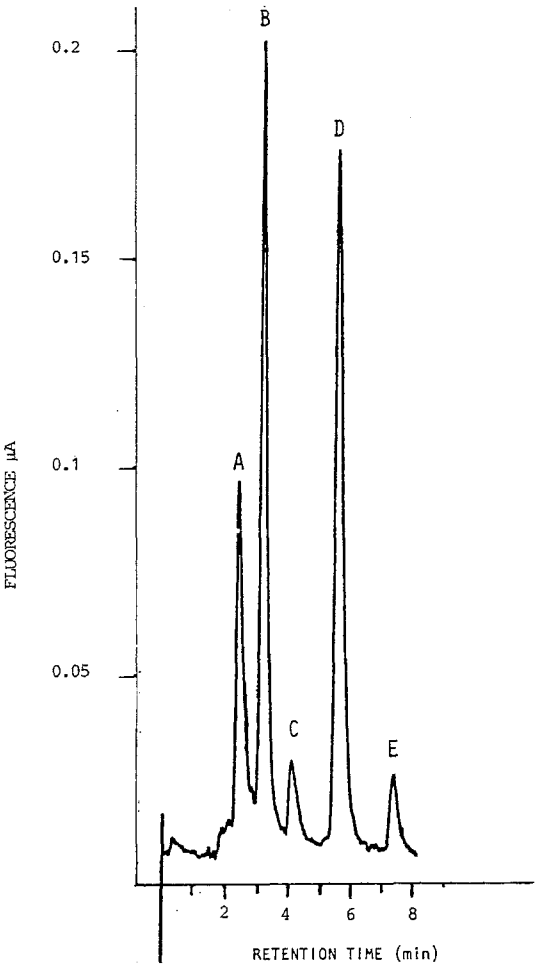

Fig. 1. Representative chromatogram from one patient treated with 75 mg/m^2 doxorubicin. Parent drug, internal standard (daunomycin) and main metabolites are clearly identified. Typical HPLC elution profile of serum sample from a patient 60 min after treatment with doxorubicin 75 mg/m^2. *A*, doxorubicinol; *B, doxorubicin; C*, doxorubicinone; *D*, daunomycin (internal control); *E*, 7-deoxydoxorubicinone

Pharmacokinetic analysis. The use of Nelder and Mead non-linear optimization [3], with the numerical methods used for the solution of differential equations of the pharmacokinetic model, was not found to be effective. Montecarlo methods for non-linear optimization [8] were therefore used and were found to be much more robust. The values for the different volumes of distribution (Vd 1–4) and for the inter-compartmental rate constants (k_{1-4}) and the four elimination rate constants (k_{el-4}) of a four-compartment model were derived from this computer program. Double and triple exponential decay curves were fitted by a MODFIT computer program [15] based upon a steepest-descent algorithm. The total area under the time-drug concentration curve (AUC_∞) was calculated using the trapezoidal rule from time 0 to 48 h + C/k_{el}, where C represents the concentration at the final data point. It has been suggested that the AUC_∞ can be underestimated when a drug is given by i. v. infusion, if the infusion period is ignored and the post-infusion data are analysed as if they derived from a bolus injection. The charts by Freedman and Workman [9] were used to determine the approximate

Table 1. The main pharmacokinetic parameters of high-dose doxorubicin. Mean values and standard errors

Dose (mg/m^2)	Pts	Peak Conc. (μM)	Terminal $^1/_2$ life (hours)	Clearance (ml/min)	Doxorubicin AUC (μMhl^{-1})	Doxorubicinol AUC (μMhl^{-1})
75	3	3.5±0.62	30.7±1.2	1173± 45	3.06±0.18	2.14±0.54
100	3	4.8±1.01	27.9±2.5	1482±216	3.43±0.45	2.11±0.52
125	3	5.5±1.4	37.8±9.0	1502±123	4.20±0.21	3.03±0.39
150	2	6.7±2.3	29.1±3.4	1574±152	4.47±0.21	3.46±0.18

underestimation of AUC_∞ values. Clearance (Cl) was calculated from the equation $Cl = Dose/AUC_\infty$ and half-life values ($t_{1/2}$) were determined from the equations $t_{1/2}\alpha = 0.693/\alpha$, $t_{1/2\beta} = 0.693/\beta$, $t_{1/2\gamma} = 0.693/\gamma$.

Results

Figure 1 shows a representative chromatogram of doxorubicin and its major metabolites. The serum concentration vs time curve decayed as a triple exponential function in four patients and could be described by the equation $Ct = Ae^{-\alpha\tau} + Be^{-\beta\tau} + Ce^{-\gamma\tau}$. but in the remaining seven patients it decayed as a double exponential function expressed by $Ct = Ae^{-\alpha\tau} + Be^{-\beta\tau}$. This difference was probably not dependent on the dose of doxorubicin since the triple exponential decay was observed in one patient in each of the groups treated with 75 and 100 mg/m^2 and in two patients at 125 mg/m^2 but was not seen at 150 mg/m^2. The median short, intermediate and long half-lives were 5.5 min (range, 4.5–6.7 min), 1.8 h (range, 0.8–3.9 h) and 3.4 days (range 1.2–3.8 days). In the seven patients whose pharmacokinetic profiles were bi-phasic, the median short and long half-lives were 5.6 min (range 4.4–11 min), and 24 h (range 18.3–32 h), respectively. The main pharmacokinetic, model-independent parameters are shown in Table 1. Doxorubicinol was the principal metabolite (Fig. 2). A small and transient increase in circulating levels of doxorubicinol and other major metabolites was observed in 9/11 patients some 6 h following the administration of the parent drug.

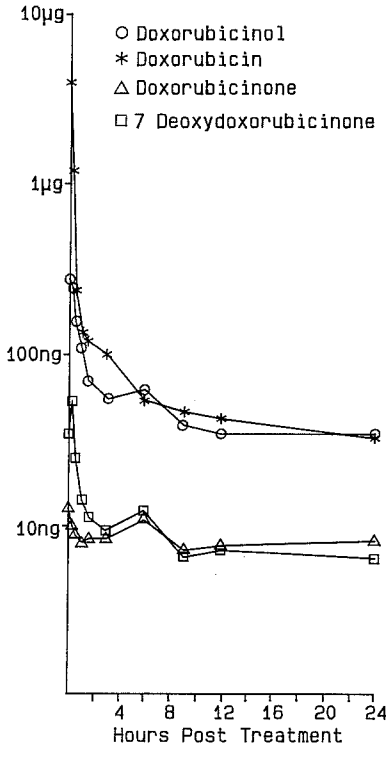

Fig. 2. Plasma decay curves in one patient treated with 100 mg/m^2 doxorubicin, showing a transient increase in the concentrations of all metabolites at 6 h post-infusion

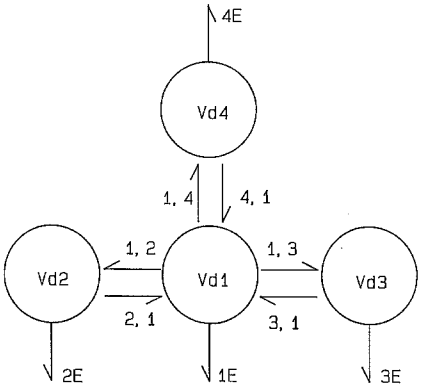

Fig. 3. Four-compartment model of doxorubicin distribution. The four compartments drawn have the following volumes of distribution: Vd1, 87 l; Vd2, 89 l; Vd3, 37 l; Vd4, 103 l. The intercompartmental rate constants have the following values: $k_{1,2}$, 217; $k_{2,1}$, 8.4; $k_{1,3}$, 295; $k_{3,1}$, 22; $k_{1,4}$, 4.6; $k_{4,1}$, 8.2. The elimination rate constants are as follows: k_{e1} (1E), 0.4; k_{e2} (2E), 0.2; k_{e3} (3E), 5.8; k_{e4} (4E), 0.9

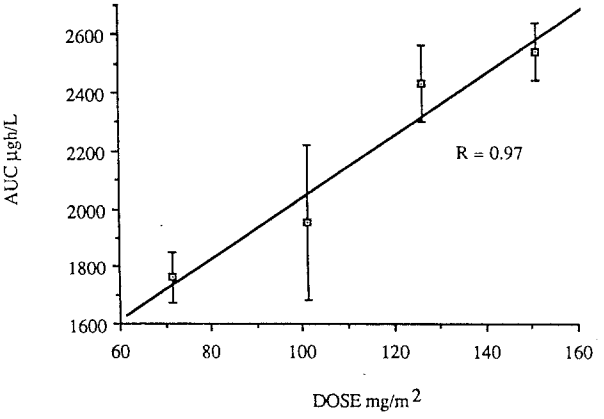

Fig. 4. Doxorubicin dose vs AUC_∞; the mean and standard error of 3 values up to 125 mg/m^2 and 2 values at 150 mg/m^2 are represented

The data fitted both a three- and a four-compartment model to within 1 SE of the observed values. The four-compartment model is shown in Fig. 3. Input occurs through a "false" central compartment, with a large volume of distribution (Vd1) due to rapid equilibration during the infusion period, and three peripheral compartments (Vd2–Vd4) with individual exit routes (1 E–4 E). there was a linear increase in AUC_∞ with dose (Fig. 4). Using the charts by Freedman and Workman [9] to determine the approximate underestimation of AUC_∞ in the seven patients whose pharmacokinetic profiles were bi-exponential, we found that their AUCs had been underestimated by between 10% and 20% in all cases. Blood samples were taken for pharmacokinetic analysis from one patient at 125 mg/m^2 following her first and third doses of doxorubicin. Doxorubicinol levels following her last dose of doxorubicin were increased by 187% when compared with the levels following her first dose, and qualitatively similar yet quantitatively minor increases were also found for 7-deoxydoxorubicinone but not for doxorubicinone or doxorubicin (data not shown).

Discussion

As G-CSF has been shown to be tolerated well by patients and to enable dose escalations of doxorubicin to improve response rates to chemotherapy [4], more studies using this growth factor in combination with other cytotoxic agents are likely to follow. Therefore, it is important to know whether drug kinetics change as the doses of cytotoxic agent increase. The present study shows that in the dose range selected the kinetics of doxorubicin were linear. The fact that AUC_∞ values, when corrected for the infusion period, had been underestimated by between 10% and 20% in all patients did not change the linearity of the relationship between dose and AUC_∞

Other authors have reported bi- or tri-exponential equations describing the pharmacokinetic profiles for doxorubicin in patients, and the selection of a two- or three-compartment model appears to be dependent on the number and timing of blood samples, the duration of the i.v. infusion and the methods used for assaying the drug [7]. A ten-compartment physiological model using data from normal rabbits has also been proposed [12]. We found that our data fitted both a three- and a four-compartment model to within 1 SE of the observed values. Because a three-compartment model has previously been described by others [16], the four-compartment model is described in this paper.

Input occurs into a central compartment with a large volume of distribution (Vd1 = 87 l) and very rapid intercompartmental rate constants ($k_{1,2}$ and $k_{1,3}$). This pattern is consistent with an initially rapid distribution of doxorubicin (during and immediately after the infusion period) into tissues highly perfused with blood, followed by distribution into other tissues. Our model predicts two shallow compartments (Vd2 and Vd3) and a deeper compartment with slow equilibration rates. It is tempting to speculate that the two shallow compartments mainly reflect the liver, lungs, spleen, lymph nodes and tumour tissue. The liver is the main site of metabolism of doxorubicin, and tumour tissue and lymph nodes are known to have the second highest tissue concentrations of doxorubicin in patients [14]. All patients in this study had considerable tumour burden as assessed both clinically and radiologically [4]. The deeper compartment could represent skin, muscle, adipose tissue, bone marrow and connective tissue. However, we cannot equate pharmacokinetic compartments with physiological or anatomical entities because our data consisted of plasma concentration determinations only, rather than direct tissue determinations, and the pharmacokinetic model is theoretical.

Similarly, to suggest what the elimination rate constants mean would involve conjecture, but k_{e3} could possibly represent hepato-biliary elimination and the others, local biotransformation or metabolism. The fact that we observed a transient increase in circulating levels of several metabolites some 6 h following administration suggests the existence of an entero-hepatic, or other, re-circulation mechanism. Data from one patient would also indicate that the repeated administration of doxorubicin changes the metabolism of this drug. Similar changes in the kinetics of doxorubicin during successive courses of treatment have been reported by others [10].

Finally, the two higher doses of doxorubicin (125 and 150 mg/m^2) led to higher response rates, but also to a marked increase in epithelial toxicities, particularly following the third dose at 125 mg/m^2 and the second at 150 mg/m^2 [4]. This toxicity was most marked in epithelial areas with high turnover rates (such as oral and vaginal mucosa and the palms and soles), and epithelial regeneration occurred within 2 weeks after the end of chemotherapy. At 125 mg m^2, recovery from neutropenia following the third cycle of chemotherapy was also slower than that following the first two cycles. Since this increase in toxicity was not due to non-linear kinetics, it could have been related to the large tissue capacities for doxorubicin and the prolonged elimination half-lives which are known to predispose to accumulation. As suggested by previous studies in man and rat, this intra-cellular accumulation of drug in tissues may be responsible for the greater toxicity of treatment schedules with short intervals between consecutive doses [18].

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References

1. Bachur NR (1975) Adriamycin (NSC-123127) pharmacology. Cancer Chemother Rep 6 (3): 103–108
2. Benjamin RS, Riggs CE, Bachur NR (1977) Plasma pharmacokinetics of Adriamycin and its metabolites in humans with normal hepatic and renal function. Cancer Res 41: 1416–1420
3. Box MJ, Davies D, Swann WH (1969) Non-linear optimization techniques. ICI monograph 5. Oliver and Boyd, Edinburgh, p 22
4. Bronchud MH, Howell A, Crowther D, Hopwood P, Souza L, Dexter TM (1989) The use of granulocyte-stimulating factor to increase the intensity of treatment with doxorubicin in patients with advanced breast and ovarian cancer. Br J Cancer 60: 121–125
5. Carter SK (1975) Adriamycin – a review. J Natl Cancer Inst 55: 1265–1274

6. Creasey WA, McIntosh LS, Brescia T, Odujinrin O, Aspnes GT, Murray E, Marsh JC (1976) Clinical effects and pharmacokinetics of different dosage schedules of Adriamycin. Cancer Res 36: 216–221
7. Crom WR (1986) Doxorubicin. In: Taylor WS, Caviness MHD (eds) A textbook for the clinical applications of therapeutic drug monitoring. Abbott Laboratories, Irving, Texas, pp 421–429
8. Durran JH (1970) Montecarlo method. In: Statistics and probability. Cambridge University Press, pp 8–9
9. Freedman LS, Workman P (1988) When can the infusion period be safely ignored in the estimation of pharmacokinetic parameters of drugs in humans? Cancer Chemother Pharmacol 22: 95–103
10. Gil P, Favre R, Durand A, Iliadis A, Cano JP, Carcassone Y (1983) Time dependency of Adriamycin and Adriamycinol kinetics. Cancer Chemother Pharmacol 10: 120–124
11. Greene RF, Collins JM, Jenkins JF, Speyer JL, Myers CE (1983) Plasma pharmacokinetics of Adriamycin and Adriamycinol: implications for the design of in vitro experiments and treatment protocols. Cancer Res 43: 3417–3421
12. Harris PA, Gross JF (1975) Preliminary pharmacokinetic model for Adriamycin. Cancer Chemother Rep 59: 819–825
13. Israel M, Pegg WJ, Wilkinson PM, Garnick MB (1978) Liquid chromatographic analysis of Adriamycin and metabolites in biological fluids. J Liquid Chromatogr 1: 795–809
14. Lee YN, Chan KK, Harris PA, Cohen JL (1980) Distribution of Adriamycin in cancer patients: tissue uptakes, plasma concentration after i. v. and hepatic i. a. administration. Cancer 45: 2231–2239
15. McIntosh JEA, McIntosh RP (1980) MODFIT: a general model-fitting program. In: Mathematical modelling and computers in endocrinology. Springer, Berlin, pp 250–260
16. Reich SD, Steinberg F, Bachur NR, Riggs CE, Goebel R Jr, Berman M (1979) Mathematical model for Adriamycin (doxorubicin) pharmacokinetics. Cancer Chemother Pharmacol 3: 125–131
17. Rodvold KA, Rushing DA, Tewksbury DA (1988) Doxorubicin clearance in the obese. J Clin Oncol 6: 1321–1327
18. Wilkinson PM, Mawer GE (1974) The persistence of Adriamycin in man and rat. Br J Clin Pharmacol 1: 241–247

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