Cancer Chemother. Pharmacol. 3, 125–131 (1979)

Cancer Chemotherapy and Pharmacology

Mathematical Model for Adriamycin (Doxorubicin) Pharmacokinetics*

S. D. Reich[1], F. Steinberg[1], N. R. Bachur[2], C. E. Riggs[2], Jr., R. Goebel[3], and M. Berman[3]

[1] Northwestern University,
Superior Street and Fairbanks Court, Chicago, Illinois 60611, USA

[2] Baltimore Cancer Research Center,
22 South Greene Street, Baltimore, Maryland 21201, USA

[3] National Cancer Institute,
Bethesda, Maryland 20014, USA

Summary. *Adriamycin (doxorubicin), an active antineoplastic drug, is rapidly distributed across cell membranes and is concentrated within cells. Binding to protein and to tissue readily occurs. The drug is metabolized to both fluorescent and nonfluorescent compounds, the liver being the main organ of biotransformation and elimination. A multicompartment, open model that accounts for these processes has been derived. The model assumes an initial volume of distribution of 60% of body weight and includes two peripheral adriamycin compartments and a subsystem for adriamycinol, a major metabolite. Plasma and urine concentrations of adriamycin and adriamycinol were determined for four patients treated with adriamycin (60 mg/m²), and these concentrations were used to calculate rate constants for the model. Concentrations were measured by fluorescence assay after thin-layer chromatographic separation of parent compound and metabolites. Differential equations were solved by the SAAM computer program. Evaluation of adriamcinol pharmacokinetics suggests that the previously reported high concentrations of adriamycinol immediately after IV infusion of adriamycin are an artifact of the fluorescence method and that observed plasma concentrations of adriamycinol are the sum of adriamycinol concentrations and approximately 10% of the adriamycin concentrations. Corrected peak plasma concentrations of adriamycinol occur 2–12 h after infusion of adriamycin.*

Introduction

Since the toxicity of the antineoplastic drug adriamycin (doxorubicin) appears to correlate to some degre with plasma concentrations [4], a mathematical model to predict plasma concentrations of drug and metabolites might be helpful in sorting out relationships between the pharmacokinetics of the drug and adverse effects. As a preliminary step toward relating the dose of drug, schedule of administration, drug distribution, biotransformation, and elimination with toxicity, a compartmentalized model has been developed to describe plasma concentrations of adriamycin. The model includes a three-compartment adriamycin system linked to a two-compartment adriamycinol subsystem. Both systems have pathways for metabolism and for elimination by renal and nonrenal mechanisms.

The basis for modeling plasma concentrations of adriamycin and metabolites is that response rates and acute toxicity are dose- and schedule-dependent [11]. A cardiomyopathy that can lead to a fatal, congestive heart failure syndrome limits the number of courses of therapy that can be given to a patient [21]. The limit imposed by this is usually 550 mg/m² total cumulative dose in patients who receive single, IV injections every 3 weeks. The incidence of cardiotoxicity is related not only to the cumulative dose, but also to the schedule of drug administration, since weekly, low doses of adriamycin seem to cause less cardiotoxicity [26].

Ideally, any model for adriamycin would have to be compatible with what is known about adriamycin pharmacology. Adriamycin is an anthracycline antibiotic, which acts primarily by intercalation with DNA, which interferes with DNA and DNA-directed RNA synthesis [12]. The drug is rapidly distributed across cell membranes and is concentrated within cells [19]. Binding to plasma proteins, cell membranes, and other tissue components readily occurs [1]. An aldo-keto reductase requiring NADPH as co-factor resides in mammalian cell cytosol and reduces adriamycin to adriamycinol, which is cytotoxic [2]. A reductive glycosidase, also requiring NADPH as co-factor is associated with the microsomal enzyme fraction of cells, especially liver and

* The content of this paper was presented in part at the American Society for Clinical Pharmacology and Therapeutics Meeting, April, 1978, Atlanta, Georgia

Reprint requests should be addressed to: S. D. Reich at his present address, i.e., Bristol Laboratories, P. O. Box 657, Syracuse, New York 13201, USA

0344-5704/79/0003/0125/$ 01.40

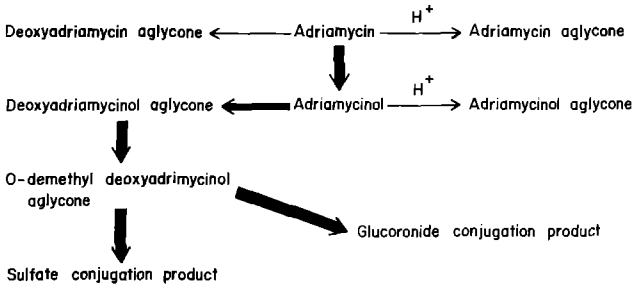

Fig. 1. Metabolic pathways of adriamycin biotransformation. *Heavy arrows* show major pathways. Acid-catalyzed route is shown by $\xrightarrow{H^+}$. All other routes are enzyme-medicated. (Redrawn from reference 22)

kidney cells [3]. This enzyme cleaves the sugar moiety, daunosamine, from the anthracycline ring structure to form an aglycone that cannot intercalate with DNA [12]. There is limited evidence that aglycone formation contributes to cardiotoxicity [20]. Parent drug, adriamycinol, the various aglycones, and other products are conjugated by the liver and excreted in bile [1]. Important metabolic pathways of adriamycin biotransformation are shown in Figure 1. A small amount of drug and metabolites is excreted into urine, although renal dysfunction does not appear to influence response or toxicity in the clinical situation.

Adriamycin is cytotoxic during all phases of the cell cycle [18] so its effect can be related to active drug exposure, or area under the concentration-versus-time curve (CXT) [17]. Clinical studies have shown that toxicity is increased with liver dysfunction and pharmacokinetic studies have shown that plasma concentrations of both parent drug and metabolites are increased, so that CXT is increased [4]. When appropriate dose reductions are made, the incidence and severity of toxicity is decreased, and CXT approaches that of patients with normal liver function.

Early pharmacokinetic studies suggested that a two-compartment model could describe the kinetics of adriamycin [5, 6]. However, later studies showed the triphasic nature of the log concentration-versus-time curve [4, 7, 23]. Rapid metabolism was thought to occur, since approximately 20% of the drug appeared in plasma as metabolites by 5 min after a bolus injection of adriamycin [5]. Current evaluation of adriamycinol pharmacokinetics with the aid of computer modeling suggests that the concentrations of adriamycinol are too high and are due to an artifact of the fluorescence assay used to measure concentrations.

Methods

An operational approach to modeling was taken for this study. The fewest compartments necessary to fit the data were used, since with more compartments there is an increase in the number of degrees of freedom for the system, which leads to greater uncertainty of the model. Not enough information is acquired from urine and plasma sampling to justify a physiologic approach to modeling [15].

Four patients with advanced neoplasms were studied at the Baltimore Cancer Research Center after giving their informed consent. These patients had normal liver and renal function prior to therapy as assessed by routine clinical tests. The standard dose of 60 mg/m^2 body surface area was dissolved in sterile water and injected IV over 5 min. Blood samples were obtained at several points in time, the first sample being drawn from the opposite arm at 5 min from the end of injection. Urine samples from patients were collected quantitatively. Blood and urine samples were processed as previously described [5, 7] but with slight modification of the fluorescence assay.

Total fluorescence adriamycin equivalents (μM equivalents) in plasma were measured after acidified ethanol extraction. This technique measures the combined concentration of adriamycin and all fluorescent metabolites in plasma. Pretreatment plasma and urine samples were assayed to correct for endogenous fluorescence. Extraction efficiency was established at 98%—99% by adding known amounts of adriamycin to pretreatment plasma and applying the extraction procedure. Reference standard curves for adriamycin extraction were linear from 0.01 μM to 12.5 μM.

Recovery of native adriamycin and adriamycinol from plasma was accompoished by chloroform-isopropanol (C : I) extraction. Two volumes of C : I (1 : 1, vol : vol) solution were added to one volume of sonicated plasma in a glass test tube. The tube was stoppered and vortexed for 30 s to form a gel. Saturating amounts of ammonium sulfate were added to the gel and the tube was vortexed for an additional 10–15 s. The salted gel was centrifuged for 20 min at 48,000 *g*. The upper, organic phase was isolated, evaporated to dryness under a nitrogen jet, and redissolved in 50 µl pure chloroform. The extracts were spotted onto thin-layer chromatography (TLC) plates (Silica Gel H, E. Merck) and developed sequentially in ascending fashion in ethyl acetate, Solvent I ($CHCl_3$: MeOH : HAc : H_2O, 80 : 20 : 14 : 6) and Solvent II ($CHCl_3$: MeOH : H_2O, 100 : 2 : 5). Adriamycin and metabolites were identified on TLC plates by their orange-red fluorescence under 235.7 nm light. Red fluorescent bands and the interposed areas of nonspecific fluorescence were individually scraped from TLC plates, and the compounds were eluted from the silica gel with 95% isopropanol/0.6N HCl. Fluorescence of the eluates was determined at an emission wavelength of 585 nm with excitation wavelength of 470 nm

Extraction efficiency for the C : I method averaged 70% based on extraction of known amounts of adriamycin from plasma. However, the range of efficiency extended from 40%–80%, necessitating a normalization of C : I extract values. This was accomplished by converting adriamycin and adriamycinol fluorescence to percentages, and referring them to the total equivalent concentration obtained by the acidified alcohol technique. The fluorescence of all silica gel eluates for a sample were summed, and the percentage of total fluorescence contributed by adriamycin and adriamycinol was calculated. Multiplying the calculated percentages by the total concentrations, (μM equivalents) yielded the concentrations of these two drugs.

Total fluorescent adriamycin μM equivalents and adriamycin and adriamycinol concentrations were measured in urine by C : I extraction. One volume of C : I solution was added to 5 vol. urine. The solution was vortexed and saturating quantities of ammonium sulfate were added; the solution was vortexed again, and then centrifuged for 20 min at 48,000 *g*. The highly colored, upper, organic phase was isolated. A small aliquot of this concentrated extract was added to 95% isopropanol/0.6N HCl, and fluorescence at 585 nm determined with excitation set at 470 nm. Appropriate standards were similarly read. Aliquots (20 µl) of the extracts were immediately

applied to TLC plates and developed in ascending fashion in Solvent I. Orange-red fluorescent bands and interposed nonspecific, fluorescent areas were identified and quantified as in the case of plasma. The concentrations of adriamycin and adriamycinol were multiplied by the corresponding urine volume to yield the number of micromoles of drug excreted per time period. Extraction efficiency of the C : I method for urine was about 100%.

Differential equations for the model were solved with the Simulation, Analysis, and Modeling (SAAM) program [8], implemented on a Control Data Corporation CDC 6600 computer. The numerical method employed in the program was a Runge-Kutter approximation. Best-fit parameter values were determined by the program. The program allowed for 'time interrupts' such that a 5-min infusion could be simulated. Statistical constraints of the program allowed for weighting of determined concentrations with respect to the inverse of the variance, which was assigned a value of 20%.

Development of the Model

Since the importance of pharmacokinetic modeling does not lie with the mathematics but rather with an increased understanding of the pharmacology of the drug, the explanation of how the adriamycin model was developed is presented with little attention to the mathematical manipulations involved. The usefulness and reliability of the SAAM computer program has been documented [8–10].

Adriamycin concentrations in plasma were plotted on a $\log_{10}$ scale against a linear time scale. The curves obtained for the four patients suggested, as other studies have in the past [4, 7, 23], that a three-compartment model would describe adriamycin kinetics. However, there are 13 possible three-compartment, linear mammallary disposition models [24]. Since the data consisted of only plasma and urine concentration determinations, there was insufficient information to determine which model would best describe the kinetics. The standard three-compartment, open model consisting of a central and two peripheral compartments with an exit route from the central compartment was therefore chosen. Graphic, curve-stripping techniques [14] were used to obtain initial estimates of the various rate parameters of the adriamycin system and the volume of distribution (V_d) of adriamycin. The SAAM computer program was then used to find the parameters for the model that best fit the adriamycin plasma data for each of the four patients.

It became apparent that the parameters and the volume of distribution of the drug were interdependent. Since it was known that adriamycin rapidly crossed cell membranes and was concentrated within cells, it was felt that the initial distribution of drug into extravascular fluid, including intracellular fluid, from the intravascular space was so rapid that this distribution phase was not apparent from the plasma disappearance curve. It was assumed therefore that the initial V_d was total body water, which was arbitrarily fixed at 60% of body weight.

Since the maximum concentration of adriamycinol appeared as 10%–20% of the total drug fluorescence in the first sample, which was taken at 5 min postinfusion, the model was required to represent extremely rapid biotransformation. Yet, because the third phase of the plasma disappearance curve had a long half-life, the adriamycin to adriamycinol conversion could not be represented by a direct pathway from the central compartment unless at least one of the adriamycin peripheral compartments acted as a large adriamycin reservoir. An

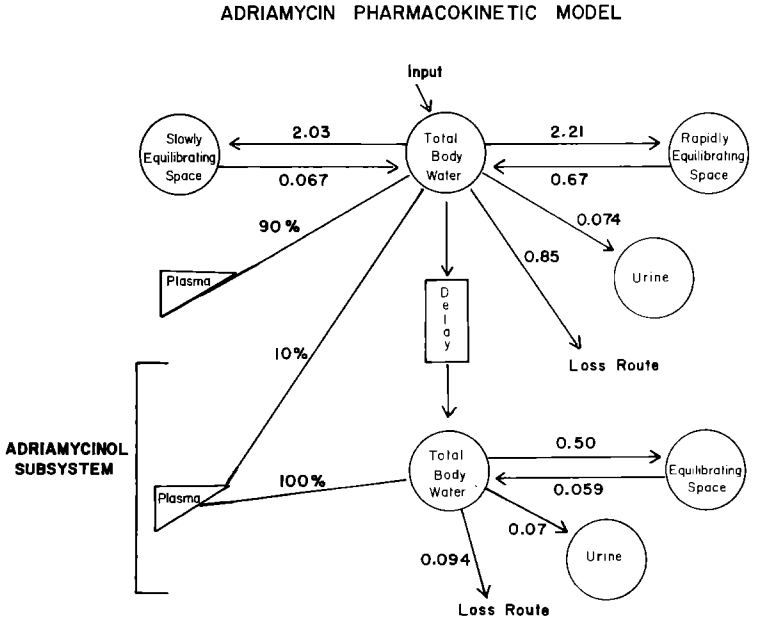

Fig. 2. Compartmental model for adriamycin. *Arrows* represent drug transfer between compartments (*circles*). Numbers associated with *arrows* are average rate constants (h^{-1}). The *triangles* are operational units representing plasma sampling as a linear function of material in total body water. The numbers associated with the *lines* to the *triangles* show the portion of material sampled from the corresponding compartment. The *input function* represents a zero-order infusion of drug over 5 min. The *rectangle* is an operational unit that causes a delay in drug transfer. The rate constant to the delay compartment is 0.087 h^{-1}

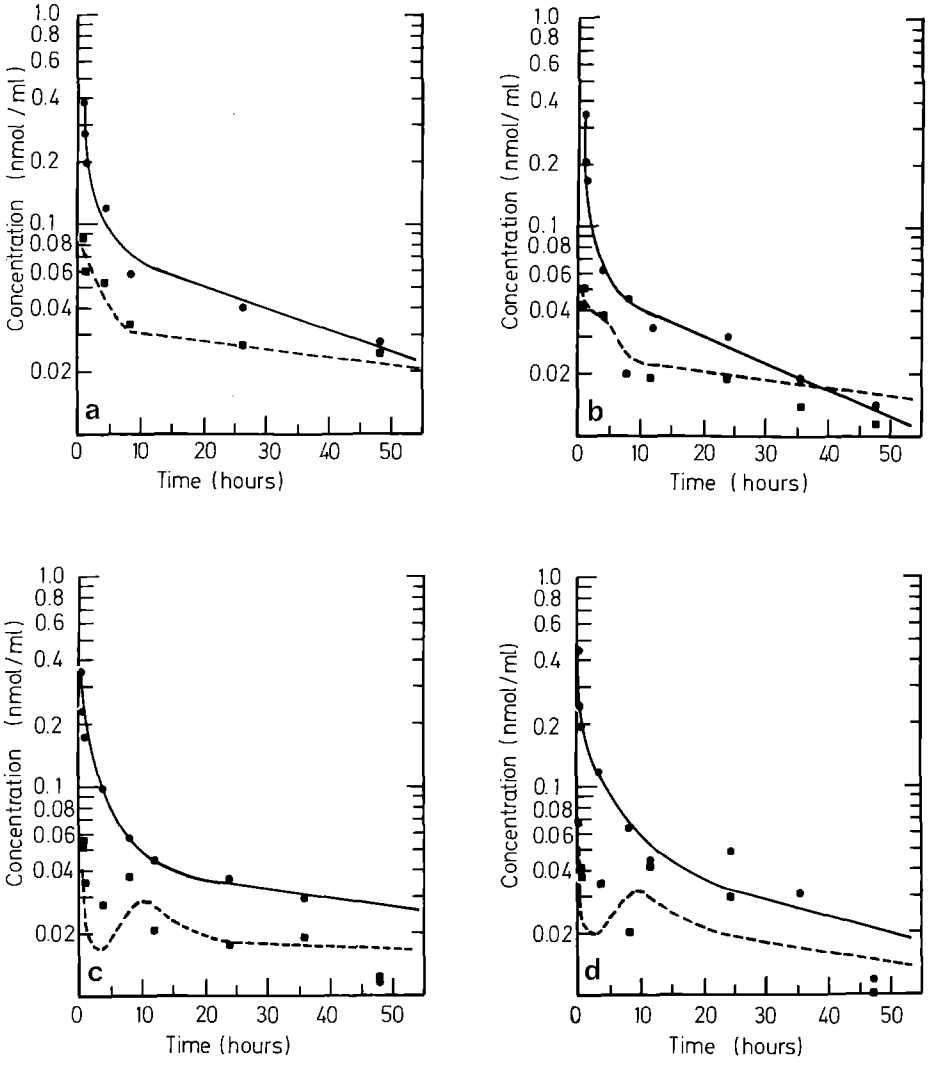

Fig. 3. a–d Predicted (*lines*) and observed (*points*) plasma concentrations of adriamycin (●) and adriamycinol (■) in four different patients who received adriamycin (60 mg/m²) as a 5-min infusion. Patient 1 (**a**) received 195 μmol, patient 2 (**b**), 177 μmol, patient 3 (**c**) 195 μmol, and patient 4 (**d**) 150 μmol

equilibrium between the two central compartments was unacceptable since there was no reason to believe that the enzymatic conversion of adriamycin to adriamycinol was reversible in vivo. Furthermore, it was known from in vitro studies with purified aldo-keto reductase that the conversion was not rapid [13].

On theoretical grounds it seemed easier to accept the proposition that at 5 min after the infusion, adriamycinol plasma concentrations were negligible. This meant that either clinical-grade adriamycin contained large amounts of adriamycinol or that our assay methods were measuring 10%–20% of the adriamycin as adriamycinol. According to TLC, clinical-grade adriamycin had about 10% impurities, but only a small proportion of these impurities was due to adriamycinol. It was assumed therefore that the plasma concentration of adriamycin was inaccurately measured by fluorescence assay, and that measured values represented about 90% of the true concentration. The missing 10% was added to the actual plasma adriamycinol concentration to give measured adriamycinol levels.

A delay compartment was added to the model to account for the time necessary for intracellular transport and metabolic processes needed for biotransformation of adriamycin to adriamycinol. It was also found that a two-compartment adriamycinol subsystem would describe adriamycinol kinetics adequately. Since urine excretion accounted for so little of the total adriamycin

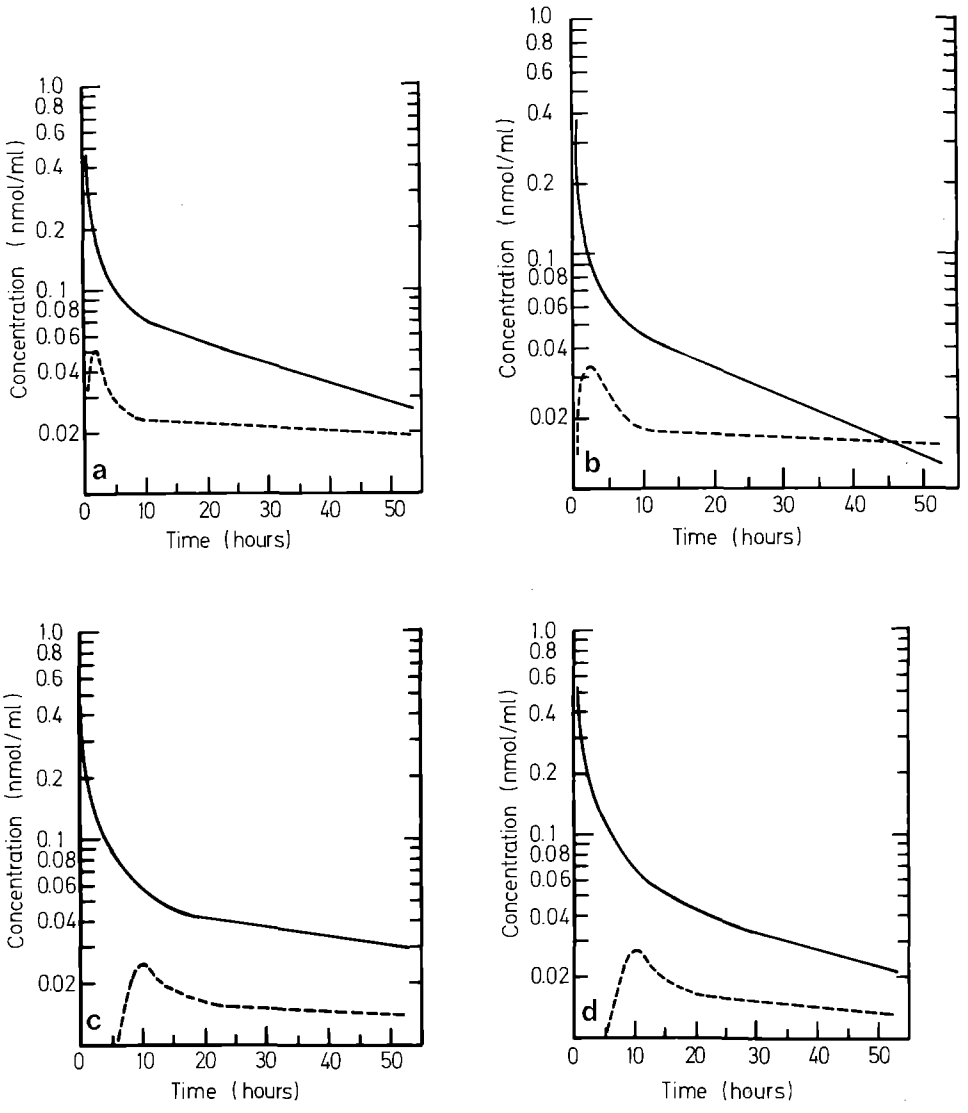

Fig. 4. a–d Predicted plasma concentration of adriamycinol (*broken line*) corrected for the adriamycin contribution to the fluorescence assay of adriamycinol in patients 1–4. Predicted plasma concentration of adriamycin (*solid line*) is shown as a reference

dose, for the sake of simplicity no corrections were made for the presumed assay inaccuracies of urine levels. The proposed model is shown in Figure 2.

Table 1. Average deviation of predicted from observed values (%) for concentrations of adriamycin ([ADR]) and adriamycinol ([ADR–OL]) in plasma (P) and urine (U)

Patient	$[ADR]_P$	$[ADR–OL]_P$	$[ADR]_U$	$[ADR–OL]_U$
1	8.7	14.4	4.3	12.2
2	5.8	17.8	7.2	24.8
3	17.1	59.4	6.8	26.3
4	17.5	42.5	2.5	28.2

Results

Predicted and observed concentrations for the four patients are shown in Figure 3. Average rate constants for the model determined from these patients are given in Figure 2. The standard deviation of these rate constants ranged from 25%–75%. Standard deviations of more than 50% were seen with the loss route and urinary excretion rate constants in the adriamycinol subsystem. The delay compartment was adjusted for individual patients. The delay caused by this compartment was 0.45, 1.33, 8, and 8 h for patients 1 thru 4, respectively. Corrected concentrations for adriamycinol based on computer-generated values are shown in Figure 4. The average deviation of predicted from observed values is shown in Table 1.

Since the compartments of the model represent averages of various processes within the patient, physiological significance of the model is difficult to assign. Since the central compartment takes into account total body water, the peripheral compartments represent drug that is being concentrated intracellularly. Uptake of adriamycin is rapid for both peripheral compartments in the adriamycin system, but one equilibrates less rapidly than the other. The two peripheral compartments dominate the shape of the concentration curves for the first 12 h. The rapidly equilibrating compartment exerts its greatest effect on the curve in the 0–2 h range, whereas for the more slowly equilibrating compartment the strongest effect on the shape of the curve occurs in the 2–8 h range. The long elimination half-life for adriamycin is due to the relatively small elimination rate constants and the reservoir effect of the peripheral compartments. The peripheral compartment of the adriamycinol subsystem is fairly rapidly equilibrating and has a plasma equivalent volume of 10%. This peripheral compartment, like those of the adriamycin system, represents drug at intracellular concentrations higher than plasma.

The delay from the adriamycin system to the adriamycinol central compartment varies from patient to patient, and presumably is due to interpatient variation in enzyme concentration or kinetics. The intercompartment delay constant was approximated by imposing a series of compartments between the central compartments of adriamycin and adriamycinol.

The model approximates the contributions of the various processes of elimination of adriamycin. Hepatic clearance of adriamycin and metabolites is the principal route of elimination from patients. Unspecified loss from the model of adriamycin accounts for about 84% of the administered dose. Renal elimination accounts for another 7%, which is close to that found in other studies [7, 23]. Metabolism to adriamycinol is estimated by the model to be 9% of the administered dose.

Discussion

Mathematical modeling of the kinetics of drug absorption, distribution, biotransformation, and elimination can, and should, be a tool for the clinician to use. As Wagner has aptly pointed out, pharmacokinetic models may be used for (1) estimation of appropriate loading doses, (2) prediction of appropriate dosage regimens, (3) prediction of proper dosage adjustment under certain pathologic conditions, (4) aiding in the diagnosis of disease, (5) correlation of structure and activity, (6) prediction of toxicity in overdose cases, and (7) prediction of the effects of perturbation of a system [25]. Another item can be added to the list, namely (8) resolution of inconsistencies in theory and experimental results.

The process of developing a pharmacokinetic model for adriamycin led to the realization that previous reports of adriamycinol kinetics were probably incorrect. The present study relied on techniques for drug assay that were considered state-of-the-art at the time. However, since the model predicted that 10% of the adriamycin was being measured as adriamycinol, the problem of artifactual metabolite generation in plasma as a consequence of the C : I extraction procedure was studied. Experiments to define and quantify fluorescent artifacts were performed by extracting chromatographically pure adriamycin from normal plasma by the C : I method. An artifact that had chromatographic migration similar to that of adriamycinol was identified. Its concentration, determined from several experiments, could be as high as 10% of the adriamycin concentration, and averaged 8%. In addition, at high concentrations of adriamycin, trailing of the drug into the adriamycin band on the TLC plates led to the false elevation of the measured adriamycinol concentration. The aglycone bands were also affected and artifact production was as high as 15% of the adriamycin concentration and averaged 10%.

Since the above assays were performed, newer techniques using high-pressure liquid chromatography have become available [16]. Preliminary studies on plasma obtained from patients who had received adriamycin 5–10 min before show that after C : I extraction no appreciable amount of adriamycinol is detectable (S. D. Averbuch, personal communication, 1977).

Because the problems with the assay procedure have been elucidated, it is no longer necessary to assume almost instantaneous metabolism of adriamycin to adriamycinol. This assumption was necessary to explain the high concentrations of adriamycinol immediately following an IV injection of adriamycin. With the artifact component eliminated from the adriamycinol concentrations, it appears that peak concentrations of adriamycinol are reached 2–12 h after the infusion of adriamycin. Some degree of interpatient variability occurs, as might be expected for an enzyme-mediated process.

The proposed pharmacokinetic model describes adriamycin and adriamycinol kinetics for the four patients in the study. However, the ability to predict concentrations in the clinical situation has not been shown. Because of the problems with assay procedures as described above, new studies are not planned until an accurate and precise assay procedure suitable for routine clinical use can be developed.

The basic structure of the model, namely a three-compartment, open adriamycin system with a two-compartment open system for adriamycinol and a delay compartment connecting the two central compartments, will probably be a good starting point once the newer

assay techniques are developed. If these techniques can add to the information on various metabolites, the model can be modified to include other metabolic pathways. It is hoped that mathematical modeling of adriamycin and metabolites will lead to prediction of drug concentrations in patients with normal and abnormal liver or renal function. Potentially, the clinician will be able to use these predictions to optimize therapy for patients with cancer.

Acknowledgements: SDR was supported in part by a Faculty Development Award in Clinical Pharmacology (1977) from the Pharmaceutical Manufacturers Association Foundation. FS was supported in part by a Fellowship Award made by the American Cancer Society, Illinois Division (1977).

References

1. Bachur, N. R.: Adriamycin (NSC-123127) pharmacology. Cancer Chemother. Rep. **6,** (3) 103–108 (1975)
2. Bachur, N. R.: Cytoplasmic aldo-keto reductase: A class of drug-metabolizing enzymes. Science **193,** 595–597 (1976)
3. Bachur, N. R., Gee, M.: Microsomal reductive glycosidase. J. Pharmacol. Exp. Ther. **197,** 681–686 (1976)
4. Bachur, N. R., Riggs, C. E., Jr., Langone, J. J., Van Vunakis, H., Levine, L.: Plasma adriamycin and daunorubicin levels by fluorescence and radioimmunoassay. Clin. Pharmacol. Ther. **21,** 70–77 (1977)
5. Benjamin, R. S., Riggs, C. E., Jr., Bachur, N. R.: Pharmacokinetics and metabolism of adriamycin in man. Clin. Pharmacol. Ther. **14,** 592–600 (1973)
6. Benjamin, R. S., Wiernik, R. H., Bachur, N. R.: Adriamycin chemotherapy – efficacy, safety and pharmacologic basis of an intermittent single high-dosage schedule. Cancer **33,** 19–27 (1974)
7. Benjamin, R. S., Riggs, C. E., Jr., Bachur, N. R.: Plasma pharmacokinetics of adriamycin and its metabolites in humans with normal hepatic and renal function. Cancer Res. **37,** 1416–1420 (1977)
8. Berman, M., Weiss, M. F.: User's manual for SAAM (Public Health Service Publication no. 1703). Bethesda, Md.: National Institutes of Health 1967
9. Berman, M., Shahn, E., Weiss, M. F.: A routine fitting of kinetic data to models: A mathematical formalism for digital computers. Biophys. J. **2,** 275–288 (1962)
10. Berman, M., Weiss, M. F., Shahn, E.: Some formal approaches to the analysis of kinetic data in terms of linear compartmental systems. Biophys. J. **2,** 289–316 (1962)
11. Carter, S. K.: Adriamycin – A review. J. Natl. Cancer Inst. **55,** 1265–1274 (1975)
12. DiMarco, A.: Adriamycin (NSC-123127: Mode and mechanism of action. Cancer Chemother. Rep. **6** (3), 91–106 (1975)
13. Felsted, R. L., Gee, M., Bachur, N. R.: Rat liver daunorubicin reductase. J. Biol. Chem. **249,** 3672–3679 (1974)
14. Gibaldi, M., Perrier, D.: Pharmacokinetics. New York: Marcel Dekker 1975
15. Harris, P. A., Gross, J. F.: Preliminary pharmacokinetic model for adriamycin (NSC-123127). Cancer Chemother. Rep. **59,** 819–825 (1975)
16. Israel, M., Wilkinson, P. M., Pegg, W. J., Frei, E., III: Hepatobiliary metabolism and excretion of adriamycin and *N*-trifluoroacetyl-adriamycin-14-valerate in the rat. Cancer Res. **38,** 365–370 (1978)
17. Jusko, W. J.: Pharmacodynamics of chemotherapeutic effects: Dose-time-response relationships for phase-nonspecific agents. J. Pharmacol. Sci. **60,** 892–895 (1971)
18. Kim, S. H., Kim, J. H.: Lethal effect of adriamycin on the division cycle of HeLa cells. Cancer Res. **32,** 323–325 (1972)
19. Meriwether, W. D., Bachur, N. R.: Inhibition of DNA and RNA metabolism by daunorubicin and adriamycin in L1210 mouse leukemia. Cancer Res. **32,** 1137–1142 (1972)
20. Mhatre, R., Herman, E., Huidobro, A., Waravdekar, V.: The possible relationship between metabolism and cardiac toxicity of daunomycin and related compounds. J. Pharmacol. Exp. Ther. **178,** 216–222 (1971)
21. Minow, R. A., Benjamin, R. S., Gottlieb, J. A.: Adriamycin (NSC-123127) cardiomyopathy – An overview with determination of risk factors. Cancer Chemother. Rep. **6** (3), 195–201 (1975)
22. Reich, S. D.: Clinical correlations of adriamycin pharmacology. Pharmacol. Ther. (C) **2,** 239–249 (1978)
23. Riggs, C. E., Jr., Benjamin, R. S., Serpick, A. A., Bachur, N. R.: Biliary disposition of adriamycin. Clin. Pharmacol. Ther. **22,** 234–241 (1977)
24. Wagner, J. G.: Do you need a pharmacokinetic model and, if so, which one? J. Pharmacokinet. Biopharm. **3,** 457–477 (1975)
25. Wagner, J. G.: Fundamentals of clinical pharmacokinetics. Hamilton, Ill.: Drug Intelligence 1975
26. Weiss, A. J., Manthel, R. W.: Experience with the use of adriamycin in combination with other anticancer agents using a weekly schedule, with particular reference to lack of cardiac toxicity. Cancer **40,** 2046–2052 (1977)

Received September 29, 1978/Accepted April 16, 1979