ABSTRACT

The plasma pharmacokinetics of Adriamycin and adriamycinol following a 15-min infusion of 75 mg/sq m of Adriamycin were studied in ten patients previously untreated with Adriamycin. The disappearance kinetics of Adriamycin could adequately be described by a biexponential equation with an initial half-life of 8-min and a terminal half-life of 30 hr. The major drug exposure (area under the concentration-time curve) occurs during the terminal phase where drug concentrations are generally less than $10^{-7}$ M (0.05 μg/ml). An improvement in the high-performance liquid chromatography sensitivity facilitated the determination of the terminal phase. The plasma kinetics of adriamycinol, the major and only known active metabolite of Adriamycin, show a rapid initial increase in plasma concentration followed by a slow decline which parallels that of Adriamycin during the terminal phase. The relative drug exposure of adriamycinol to Adriamycin was approximately 50%.

The relationship between the measured plasma drug levels and free drug available for distribution into tissues was studied by comparing the plasma binding characteristics of Adriamycin and adriamycinol. A constant 20 to 25% of the total plasma concentrations of both Adriamycin and adriamycinol was freely diffusible over the whole range of observed concentrations, 20 nm to 2 μM. Thus, the free drug exposure (area under the concentration-time curve) of tumor and host tissues in vivo can be determined from these plasma measurements, since the free drug exposures in plasma and in extracellular fluid are equivalent. These results can also serve as a guide for the design of clinically relevant in vitro studies of Adriamycin and adriamycinol.

The pharmacokinetic parameters determined in this study have been used to simulate plasma concentration-time courses for a variety of Adriamycin treatment schedules. Alternatives are suggested which reduce peak plasma Adriamycin concentration while antitumor area under the concentration-time curve is maintained.

INTRODUCTION

Adriamycin is an antitumor agent used in the standard chemotherapy regimen of most hematological and many solid tumors (8). Adriamycin is commonly administered clinically as a bolus dose of 60 to 90 mg/sq m every 3 weeks (7). The plasma kinetics of the drug following bolus administration exhibit a rapid initial decline followed by a slow decline in plasma concentration which has been ascribed to the ability of tissues to rapidly accumulate the drug intracellularly followed by a slow release of the drug from tissue stores as plasma levels decline due to drug elimination (25). A physiological model of Adriamycin pharmacokinetics based solely on flow limitation for tissue uptake and first-order elimination by the liver has simulated the actual experimental data in rabbits and humans reasonably well (9, 17). Compartmental modeling has also been used to describe the disposition of Adriamycin in plasma. Initially, a biphasic loss of the drug was proposed based on total fluorescence or radioactivity assay methods (5, 12). The use of assay methods specific for Adriamycin led to the inclusion of an intermediate phase showing a triphasic loss of the drug (4, 6, 24), although no compelling pharmacokinetic or pharmacodynamic justification for the third phase has been proposed.

Although many studies of the plasma pharmacokinetics of Adriamycin have been undertaken, certain deficiencies make the interpretation of the pharmacokinetic data or its relevance to the clinical use of Adriamycin difficult to understand: (a) The plasma kinetics of Adriamycin have been fairly well studied for time periods of up to 24 hr following a bolus injection; however, the terminal half-life of Adriamycin is about 30 hr, which requires sampling for several days for adequate characterization. (b) Metabolism of Adriamycin requires assay techniques which separate parent drug and metabolites and allow sensitive quantitation of Adriamycin and other metabolites of interest. The presence of active metabolites can obscure the evaluation of either the therapy or toxicity of the drug unless the activity of the metabolite is evaluated. This general difficulty has been compounded in the case of Adriamycin, since much of the original metabolite data was subsequently ascribed to artifacts from the analytical methodology (23). (c) Although it is thought that only free drug is available to produce drug effects, prior studies have not quantitated the binding of either Adriamycin or adriamycinol over the range of observed concentrations.

The primary purpose of this study is to provide a complete description of the plasma pharmacokinetics of Adriamycin and adriamycinol. The relevance of the pharmacokinetic parameters to the in vivo and in vitro efficacy and toxicity of the drug is discussed. Pharmacokinetic and pharmacodynamic principles are applied to practical issues of Adriamycin scheduling and cardiotoxicity.

MATERIALS AND METHODS

Clinical Characteristics. Ten patients undergoing single-agent Adriamycin therapy for metastatic breast cancer, metastatic soft-tissue sarcoma, or nodular lymphomas, who had not been treated previously with Adriamycin, were studied during their first course of Adriamycin therapy. Adriamycin was administered at a dose of 75 mg/sq m through a fresh i.v. infusion line over 15 min. As part of a study on cardioprotection,
some patients were randomly selected to receive 5.6 g/sq m of NAC\textsuperscript{2} p.o. 1 hr before the dose of Adriamycin. All 10 patients had prior surgical resections, but only one patient had prior chemotherapy (with streptozotocin). Liver function tests were within the normal range for all patients at the time of study.

**Sample Analysis.** Blood samples were obtained at 0-, 0.08-, 0.17-, 0.5-, 1-, 3-, 6-, and then 24-hr increments following administration of the drug for at least 3 days. Blood samples were generally collected from a vein on the opposite arm from the infusion. If necessary, samples were taken from the same vein used for infusion, after extensive flushing. No major differences were observed between patients sampled by the 2 methods. The most likely error would be an overestimate in the early samples. Since most of the drug exposure is in the later phases, such an error would not be critical.

The blood was collected in glass tubes containing EDTA and were immediately placed on ice to prevent metabolism by the cellular blood components. The blood was centrifuged at 600 \textbf{x} g for 10 min to obtain plasma which was frozen at \(-40^\circ\text{C}\) until analyzed.

Each plasma sample was analyzed for Adriamycin and adriamycinol by means of a HPLC with fluorescence detection capable of separating and yielding sensitive and specific quantitation of each compound. Samples were prepared for analysis by adding 1 ml of 0.1 M sodium borate buffer (pH 9.8) and 50 ng daunomycin as an internal standard to 1 ml of the plasma sample and extracting the drug into 17 ml of chloroform:methanol (4:1, v/v) in a 50-ml glass tube. The organic layer was transferred to a conical glass tube and evaporated to dryness under nitrogen at room temperature. The residue was redissolved in 150 \mu l methanol, and an aliquot was injected into the HPLC. Exposure of the samples to light was limited as much as possible. Standards containing Adriamycin (0 to 200 ng/ml), adriamycinol, and Adriamycin aglycone in plasma were processed in an identical fashion and used for quantitation.

The HPLC system used was essentially the reverse-phase system described by Israel et al. \cite{18} with one modification. An excitation wavelength of 228 nm rather than 482 nm was used which yielded an approximately 10-fold increase in sensitivity for each compound. No evidence of interfering fluorescent peaks was observed in "pre"-dose samples.

Quantitation of drug concentrations was achieved by measuring the peak height ratios of drug:internal standard for the plasma standards and obtaining a least-squares fit of peak height versus concentration. The concentrations of Adriamycin and adriamycinol in the plasma samples were then obtained from the peak height ratios of the samples.

The recovery of Adriamycin, adriamycinol, and the internal standard in the assay procedure was approximately 70% within the concentration range of the standards. The detection limit of the assay (signal of 5 times the average noise) was experimentally determined to be 2 \textmu M for Adriamycin and adriamycinol using 1 ml of plasma. The within-day precision (C.V.) of the assay (\(n = 5\)) at 1 \textmu M was 7.1% for Adriamycin and 8.2% for adriamycin; at 10 \textmu M, it was 11.2% for Adriamycin and 18.6% for adriamycinol.

**Pharmacokinetic Analysis.** The Adriamycin plasma concentration-time data were fitted to a biexponential equation:

\[
C_d(t) = A \text{exp}(-\alpha t) + B \text{exp}(-\beta t)
\]

where \(C_d(t)\) is the drug concentration at time, \(t\), after an i.v. dose of the drug. \(A\) and \(B\) are constants, and \(\alpha\) and \(\beta\) are the apparent first-order elimination rate constants. The data fits were performed on MLAB, a nonlinear fitting program, using a 1/concentration squared weighting function \(20\). The areas under the curve \((C \times t)\) of the initial and terminal phases for Adriamycin were calculated from the ratios of \(A/\alpha\) and \(B/\beta\).

The total AUC for Adriamycin and adriamycinol were also calculated based on the trapezoidal rule from zero to the last measured time point and then by first-order extrapolation to infinite time using the experimentally determined half-life value for Adriamycin. The extrapolation averaged 14% of the total area for Adriamycin and 20% for adriamycinol. The percentage of drug exposure of adriamycinol in relation to Adriamycin was calculated from the ratio of the total AUC of adriamycinol to Adriamycin.

The volume of distribution \((V_d)\) and total body clearance \((C_{\text{LR}})\) for Adriamycin were calculated by means of noncompartmental techniques:

\[
C_{\text{LR}} = \frac{\text{Dose}}{\text{AUC}}
\]

where the area under the moment curve \((\text{AUMC})\) was calculated using a published method \cite{3}.

**RESULTS**

**Pharmacological Studies.** Following the 15-min infusion of Adriamycin, the Adriamycin plasma concentration exhibited an initial rapid decline from approximately 5 to 0.1 \mu M within 1 hr. This initial phase was followed by a slower decrease in concentration which was fairly log-linear beyond 6 hr (Chart 1). A rapid

![Chart 1. Measured Adriamycin and adriamycinol plasma concentration-time patterns following a 15-min infusion of Adriamycin 75 mg/sq m to 10 patients.](chart1.png)

\[
\text{Adriamycin (A)}
\]

\[
\text{Adriamycinol (O)}
\]

\[
\text{Mean ± SD (n=10)}
\]
increase in adriamycin plasma concentration was observed in the first hr followed by a decline in concentration which generally paralleled that of Adriamycin. At the retention time for Adriamycin aglycone, only small fluorescent peaks were observed corresponding to concentrations of generally less than 10 nm (based on the aglycone standard).

The concentration-time data for Adriamycin for each patient were analyzed separately as described in "Materials and Methods" to obtain estimates of the pharmacokinetic parameters. Table 1 shows the relative contribution of each phase to the total AUC (C x t) and the estimates obtained for Adriamycin when the data were grouped by treatment category (Adriamycin alone versus Adriamycin plus NAC). No significant differences in the values of each group were observed. Using the data from all the patients studied, the pharmacokinetics of Adriamycin could best be fit to a biexponential equation of the form:

\[ C_p(t) = 4147 \exp(-5.39t) + 82 \exp(-0.229t) \]

where \( C_p(t) \) is the nm Adriamycin concentration, and time, \( t \), is in hr.

Table 2 shows the total body clearance and steady-state volume of distribution of Adriamycin and the AUC of adriamycinol and Adriamycin for the 2 groups. Similar values were obtained; thus, the plasma pharmacokinetics of Adriamycin and adriamycinol appear to be unaffected by administration of NAC. The \( V_{ss} \) of 25 liters/kg compares favorably with the tissue:plasma partition coefficients determined in a rabbit study (17).

**Binding Studies.** For nonradioactive drug (\( n = 12 \)), Adriamycin was 74 ± 1.7% (S.D.) bound, and adriamycinol was 76 ± 1.4% bound. The percentage bound was independent of plasma concentration over the range observed in this study, 20 nm to 2 \( \mu \)m. There was no difference in Adriamycin binding for plasma samples from patients receiving NAC versus patients not receiving NAC.

At 1 \( \mu \)m, the [\(^{14}C\)]adriamycin was found to be 82 ± 0.7% bound by radioactivity measurements and 75 ± 2.7% bound by the HPLC method. More than 95% of the total radioactivity initially present was recoverable in the plasma and buffer solutions at equilibrium. For both radioactive and nonradioactive studies, binding of the drug to the dialysis apparatus was corrected for by sampling both the plasma and buffer sides of the dialysis membrane.

**DISCUSSION**

The major conclusions drawn from the analysis of the pharmacokinetic data for Adriamycin are: (a) the bulk of the drug exposure (C \( \times \) T) occurs during the terminal phase; (b) the free drug exposure is directly proportional to the total drug exposure in plasma; and (c) the free drug exposure of adriamycinol is approximately one-half of that for Adriamycin.

A biexponential model for Adriamycin appears to be sufficient to describe the major features of both the pharmacokinetic profile and the pharmacological activity of Adriamycin. The initial phase consists of a very rapid decline in plasma Adriamycin concentrations. Initial concentrations are 50-fold higher than those observed at the start of the terminal phase. Despite the relative magnitude of the concentrations observed during the initial phase, the terminal phase provides 75% of the total drug exposure and maintains cytotoxic concentrations for several days, since the \( t_{1/2} \) is 30 hr. The biphasic curve can be used to examine the 2 patterns of Adriamycin therapeutic and toxic effects which have been observed: those due to high peak concentrations, and those related to total drug exposure.

In addition to its own pharmacological activity, Adriamycin is also metabolized to one known active metabolite, adriamycinol. The plasma kinetics of both compounds were determined in an attempt to determine the relative importance of these 2 chemical species to the pharmacological activity observed after Adriamycin administration. These studies showed a relative drug exposure for total plasma adriamycinol of approximately one-half of the value for total Adriamycin. Since equivalent plasma binding was found for Adriamycin and adriamycinol, the free drug exposure ratio is the same as for total drug exposure. Studies of the relative cytotoxicity of Adriamycin and adriamycinol have shown that adriamycinol is less active than Adriamycin (22), at least for 1-hr exposures. Presumably, these short-term results are also applicable to longer exposures since, as discussed below, cytotoxicity of Adriamycin is not directly time dependent (16). Thus, for patients with normal hepatic function, Adriamycin appears to be the principal species responsible for therapeutic effect. The role of adriamycinol may be more important in patients with liver dysfunction.
for Adriamycin has recently been studied under conditions relevant to Adriamycin pharmacology (16). Exposure of human colon tumor cells to Adriamycin concentrations of 10, 100, and 1000 nM for either 2 or 24 hr demonstrated a good correlation between the C × T value and the log of the surviving fraction.

The observed correlation of in vitro tumor cell survival and C × T suggests that the antitumor effects of Adriamycin are dose dependent, but schedule independent. Clinical studies of the antitumor effects of Adriamycin have supported this conclusion. It has been reported that Adriamycin is equally effective regardless of the administration schedule used, namely, weekly or triweekly bolus or continuous infusion (11, 21).

The importance of dose and schedule should also be viewed from the perspective of limitations imposed by host tissue toxicity. Both cardiotoxicity and drug-induced emesis have been reported to be schedule-dependent and may be more associated with the peak drug concentrations than with total drug exposure. These latter side effects have been lessened by an infusion rather than as a bolus, without any concomitant changes in the tumor efficacy or bone marrow depression (21). Earlier studies (8) suggest that bone marrow depression may be schedule dependent.

Even this limited information about Adriamycin pharmacodynamics can permit the more effective use of pharmacokinetics towards a goal of optimal chemotherapy. Although the 2- to 5-day continuous infusion schedule may be superior to bolus administration with respect to cardiotoxicity, it possesses drawbacks in terms of practical implementation, especially in the setting of a large outpatient practice. The need for central venous catheter placement, a pump, and additional patient monitoring would make routine use of these protocols difficult.

One practical application of pharmacokinetics is to predict concentration-time profiles for alternate dosing strategies. Using the pharmacokinetic data from Table 1 and standard formulas for schedule adjustment (15), we have explored a variety of delivery options and summarized the results in Chart 2. Dividing a single large dose into several smaller doses is one method of reducing peak concentrations. For example, the peak plasma Adriamycin concentration can be reduced 5-fold by simply splitting the single 75 mg/sq m dose into 5 daily doses of 15 mg/sq m.

Lengthening the infusion time also lowers the peak plasma concentration. The peak concentration observed after a 15-min infusion, such as that used in this study, is already 40% lower than the peak concentration predicted for a 15-sec rapid i.v.
push. An additional 6-fold reduction in peak concentration can be achieved by lengthening the infusion time from 15 min to 2 hr, which could still be administered peripherally. The combination of dividing the single dose into 5 daily doses and prolonging each infusion from 15 to 120 min provides an alternate schedule which produces a peak concentration 30-fold lower than that observed in the current study.

No simple combination of divided doses and constant infusions actually produces a constant plasma concentration. For the alternate schedule, there is some daily increase and decrease in concentration, while the continuous infusion protocol only reaches steady state by the end of a 120-hr period. Both the intermittent 2-hr schedule and the 120-hr continuous infusion schedule provide comparable concentrations for most of the 5-day period. For a 48-hr infusion, which has also been tested (21), the simulated plasma concentration rises throughout the infusion period. This behavior is expected for a drug with a controlling $t_{1/2}$ of 30 hr. Whether this alternate schedule can reduce the incidence of cardiotoxic side effects to a level achievable by 2- to 5-day constant infusions will have to be experimentally verified. The scheme, however, illustrates the value of pharmacokinetics as a tool which can be used to assist in the search for optimal delivery methods.

ACKNOWLEDGMENTS

We thank Dr. Ian Kerr and Dr. Luca Gianni for helpful discussions and Raymond Klecker for analytical assistance.

REFERENCES


Clinical Pharmacokinetics of Adriamycin
Plasma Pharmacokinetics of Adriamycin and Adriamycinol: Implications for the Design of *in Vitro* Experiments and Treatment Protocols

Raymond F. Greene, Jerry M. Collins, Jean F. Jenkins, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/7/3417

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.