D. Nonclinical Absorption, Distribution, Metabolism and Excretion (ADME) Summary

1. Introduction

The pharmacokinetics and metabolism of non-liposomal doxorubicin HCl , e.g., Adriamycin RDF™, have been well characterized. An intravenous bolus injection of doxorubicin HCl in animals and humans produces high plasma concentrations that fall quickly due to rapid and extensive distribution into tissues.[70] Apparent volumes of distribution range from 1400 to 3000 L in humans, reflective of its extensive distribution into tissues. No accumulation in plasma occurs after repeated injections.[71-73] The doxorubicin plasma concentration-time curve in humans is biphasic, with a distribution half-life of 5 to 10 minutes and terminal phase elimination half-life of 30 ± 8 hours.[71,73] A triphasic curve has been described, also with a terminal plasma half-life of approximately 30 hours.[74] Clearance of doxorubicin ranges from 24 to 73 L/hr in humans.[70] Adriamycin pharmacokinetics are similar in animals, with rapid clearance from the plasma, a biexponential plasma concentration-time curve, a high volume of distribution and extensive distribution to the tissues.[75-78] The terminal half-life (24 to 30 hours) and volume of distribution (~65 L/kg) of doxorubicin in the dog, for example, are similar to those reported for humans.[77]

A series of single dose plasma pharmacokinetic studies were conducted in rats, rabbits and dogs, and a multiple dose pharmacokinetic study was conducted in rats to characterize the plasma pharmacokinetics of DOXIL. Tissue levels of doxorubicin and doxorubicinol were determined in single dose and multiple dose studies in rats. Plasma and skin concentrations of doxorubicin were measured in dogs following multiple treatments with DOXIL or Adriamycin. Early plasma pharmacokinetic studies were conducted in mice, rats and dogs with the original formulation, DOXIL 1, which was unbuffered and stored frozen. Later studies were conducted with either of two buffered formulations of DOXIL that differed only in the formulation buffer (DOXIL 2 and DOXIL 3, buffered with 10 mM tromethamine and 10 mM histidine, respectively). The formulation to be marketed is DOXIL 3.

The plasma pharmacokinetics of DOXIL 2 and DOXIL 3 were compared and shown to be equivalent. Therefore, the two formulations will generally be referred to as DOXIL throughout this overview. The pharmacokinetics of DOXIL 1 and DOXIL, in contrast, are not equivalent. Studies conducted with DOXIL 1, which are presented only as supportive information, are included in the NDA nonclinical ADME section.

2. Plasma Pharmacokinetics

Plasma pharmacokinetic studies were conducted following administration of a single dose of DOXIL in rats, rabbits and dogs, and following multiple dose administration in rats. The tissue distribution of DOXIL was studied following single and multiple administration in normal rats and tumor bearing mice.

Table 18, a summary of nonclinical plasma pharmacokinetics studies with DOXIL, is presented at the end of this section.

a. Rat

The plasma pharmacokinetics of four recent clinical lots of DOXIL were compared following intravenous administration of a single dose of 1.0 mg/kg. There was no significant variation in any pharmacokinetic parameter among the clinical lots of DOXIL tested. The plasma concentration-time curve of DOXIL was biexponential with a short first phase and a prolonged second phase that accounted for the majority of the AUC (Fig. 2).

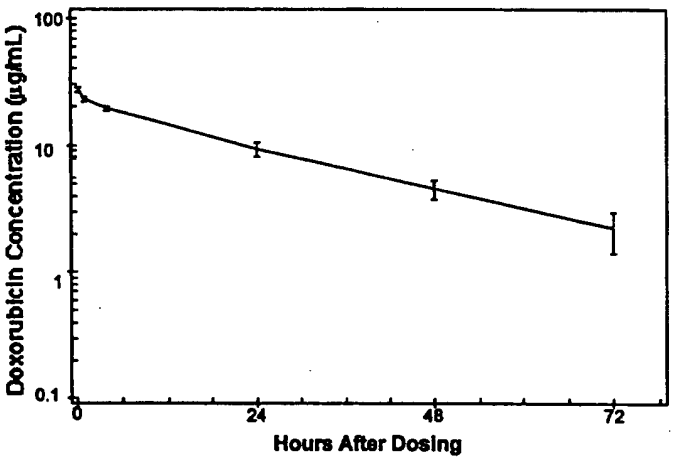

FIGURE 2

Plasma concentration of doxorubicin (µg/mL) in rats treated with DOXIL. Data shown are mean ± s.d. from four clinical lots.

The pharmacokinetic paramaters of DOXIL in rats are shown in Table 11. The low values for clearance and volume of distribution (approximating blood volume) suggest that the disposition of doxorubicin is controlled by its liposome carrier.

TABLE 11
Pharmacokinetics of DOXIL in Rats

Parameter	Mean ± s.d.
k (hr^{-1})	λ_1: 0.95 ± 0.87 λ_2: 0.03 ± 0.01
$T_{1/2}$ (hr)	λ_1: 1.7 ± 1.5 λ_2: 23.6 ± 4.1
$AUC_{0\rightarrow72}$ (μg·hr/mL)	584 ± 51
$AUC_{0\rightarrow\infty}$ (μg·hr/mL)	660 ± 92
CL (mL/hr)	0.38 ± 0.03
V_d (mL)	12.7 ± 1.8

Variations of the standard DOXIL formulation (5.3 mole % MPEG-DSPE, 38.3 mole % cholesterol, approximately 6.3 mole % lysophosphatidylcholine (LPC); mean particle, 100 nm) were tested in order to evaluate the effects of minor variations in liposome composition on DOXIL pharmacokinetics (LTI-30-94-15). The plasma pharmacokinetics of DOXIL varied with alterations in formulation components, including particle size, MPEG-DSPE content, cholesterol content and amount of LPC. In general, as particle size or LPC content increased, plasma concentration fell, clearance and volume of distribution increased and AUC and half-life decreased. DOXIL pharmacokinetics appeared to be independent of the mole % of MPEG-DSPE within the range tested. Neither lower (2 mole %) nor higher (7 mole %) MPEG-DSPE content consistently affected plasma pharmacokinetics. Increasing cholesterol content from 38.3 mole % to 48 mole % also had no effect on DOXIL pharmacokinetics. The degree of change in the plasma pharmacokinetics corresponded to the degree of change from the standard DOXIL formulation. As the change in the formulation from the standard DOXIL formulation became greater, e.g., the particle size became larger or the amount of LPC was raised, the alteration in the pharmacokinetic profile increased. Particle sizes up to approximately 150 nm were pharmacokinetically equivalent to DOXIL. Other studies with formulation variants have provided similar results: DOXIL with mean particle sizes of 100 and 150 nm were comparable in therapeutic effectiveness in a murine C26 colon carcinoma tumor model (LTI-30-94-12). Formulations containing 9 weight % or less LPC showed only minor pharmacokinetic differences from DOXIL. In general, the DOXIL formulation appeared to be relatively resistant to the effects of minor alterations in its components.

Both phases of the biphasic plasma concentration-time curve seen after administration of DOXIL are believed to represent the clearance of liposome-encapsulated doxorubicin from the bloodstream.[42,43] To confirm this supposition, the percentage of liposome-encapsulated doxorubicin was determined in rats following single intravenous administration of DOXIL 1.0 mg/kg (LTI-30-93-27). Plasma samples, collected over 96 hours post-treatment, were treated with Dowex resin to remove non-liposomal doxorubicin, daunosamine-containing metabolites of doxorubicin, such as doxorubicinol, and fraction of the deoxyaglycone metabolites from the plasma.[79] The difference between plasma doxorubicin concentrations measured before and after Dowex treatment represents the fraction of free, i.e., non-liposomal, doxorubicin. In this study, the percentage of free doxorubicin was less than 7% at all time points post-dose, supporting the suggestion that the majority of the doxorubicin in plasma is liposome-encapsulated.

Physiologically based pharmacokinetic (PBPK) modeling suggests that the amount of free doxorubicin in the plasma after the administration of DOXIL may be even lower than the 7% measured. A PBPK model that accurately simulates the plasma concentration-time curve was developed using plasma concentration data from a Phase 1 clinical study in patients (LTI-30-94-16). The model takes into account the rate of leakage of doxorubicin from circulating liposomes, the rate of removal of intact liposomes from the blood and the plasma clearance rate of non-liposomal doxorubicin. Assuming a 24-hour rate of doxorubicin release of 10%, that was higher than measured in human plasma in vitro, the model predicts that the concentration of free doxorubicin will be approximately 1000-fold lower than the concentration of liposome-encapsulated doxorubicin after administration of DOXIL, e.g., 10 ng/mL unencapsulated drug compared to 10 μg/mL encapsulated drug. Thus, the amount of circulating free doxorubicin after DOXIL administration may be much lower than that measured using the Dowex treatment procedure.

The plasma pharmacokinetics of DOXIL 1.0 mg/kg or Adriamycin were compared as part of a single dose tissue disposition study (LTI-30-93-23). The study design was optimized for the collection of tissue disposition data and did not include a time point early enough to fully characterize the first phase of the biphasic plasma concentration-time curve of either DOXIL or Adriamycin. However, significant differences between the two drugs were evident. Concentrations of doxorubicin were approximately 2000-fold higher in the plasma of DOXIL-treated rats compared to Adriamycin-treated rats 30 minutes after treatment. Doxorubicin was not detected in the plasma of Adriamycin-treated rats 4 hours post-dose, compared to approximately 8% of the administered dose that was detectable in the plasma of DOXIL-treated rats 72 hours post-dose. Plasma pharmacokinetic parameters for DOXIL were consistent with those determined in earlier

studies. The pharmacokinetic parameters for DOXIL and Adriamycin in rats are shown in Table 12. DOXIL has exhibited a markedly lower volume of distribution and clearance, and a longer mean residence time (MRT).

TABLE 12
Mean Pharmacokinetic Parameters of DOXIL and Adriamycin in Rats

Parameter	DOXIL	Adriamycin
V_d (mL)	16.5	30,193
CL (mL/hr)	0.4	10,869
MRT (hr)	37.9	2.8
AUC (μg·hr/mL)	605	0.23

No doxorubicinol was detected in the plasma from either DOXIL-treated or Adriamycin-treated animals. This result was not unexpected in the Adriamycin groups owing to the very low concentration of doxorubicin in the plasma. However, the concentration of doxorubicin in the DOXIL group was much higher. Detectable levels of doxorubicinol after DOXIL administration would be expected if a significant portion of the doxorubicin in the plasma were free, i.e., no longer in the liposome and, therefore, available for metabolism. The absence of doxorubicinol in plasma following DOXIL administration suggests that the majority of the doxorubicin measured in the plasma remains encapsulated in the Stealth liposomes, which may account for its reduced relative toxicity compared to Adriamycin.

The plasma pharmacokinetics of DOXIL 3 and DOXIL 2 were compared in male Sprague Dawley rats that received a single intravenous injection of 0.3 mg/kg of either of the two formulations (LTI-30-92-14). DOXIL 2 was buffered at pH 6.5 with 10 mM tromethamine, and DOXIL 3 was buffered at pH 6.3 with 10 mM histidine. The formulations were otherwise identical. Both formulations had biphasic plasma concentration-time curves and exhibited the prolonged plasma residence time characteristic of Stealth formulations (Table 13).

TABLE 13
Mean Pharmacokinetic Parameters of DOXIL 3 and DOXIL 2 in Rats

Parameter	DOXIL 3	DOXIL 2
C_0 (μg/mL)	8.29	8.54
$T_{1/2}$ (hr)	λ_1: 0.94 λ_2: 21.4	λ_1: 1.29 λ_2: 21.9
$AUC_{0\rightarrow 48}$ (μg·hr/mL)	145.8	159.5
$AUC_{0\rightarrow\infty}$ (μg·hr/mL)	184.2	203.0
CL (mL/hr)	0.44	0.38
V_d (mL)	14.6	12.5
MRT (hr)	30.3	31.0

Few differences were observed in peak plasma concentrations of doxorubicin, AUCs, plasma disposition half-lives or any other pharmacokinetic measurement. The two formulations were considered pharmacokinetically equivalent, and, as noted previously, will be referred to simply as DOXIL.

Two additional single dose studies in rats compared the pharmacokinetics of DOXIL to those of DOXIL 1 (LTI-30-92-06, LTI-30-93-06). Both DOXIL and DOXIL 1 exhibited biexponential plasma concentration-time curves with a short initial phase and a prolonged second phase, and both were long-circulating (Fig. 3). The plasma concentration-time curves of each were parallel; however, plasma levels were lower in animals that received the DOXIL 1 formulation. The half-life of the second phase of the concentration-time curve was significantly shorter for DOXIL 1 at all dose levels (mean 21.9 ± 6.2 hours, compared to 27.9 ± 6.4 hours for DOXIL). AUCs were nearly two-fold higher after administration of equivalent doses of DOXIL and DOXIL 1, reflecting the lower clearance rate of DOXIL (0.3 to 0.5 mL/hr), compared to that for DOXIL 1 (0.6 to 0.9 mL/hr). These differences are probably a reflection of the rates of doxorubicin leakage from the liposomes in each of the formulations.

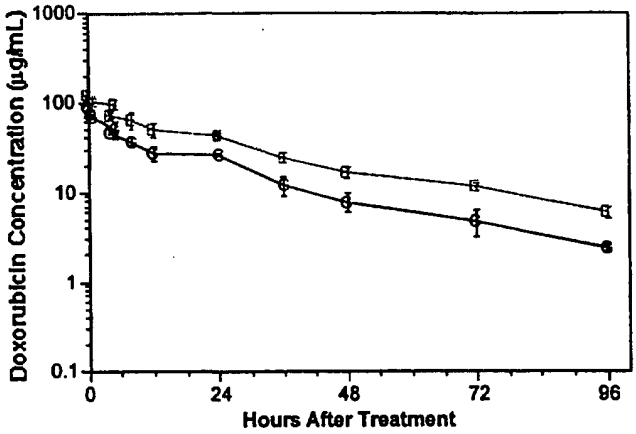

FIGURE 3

Plasma concentration of doxorubicin (µg/mL) in rats that received a single intravenous injection of 4.0 mg/kg DOXIL or DOXIL 1. Upper line: DOXIL; Lower line: DOXIL 1.

The plasma pharmacokinetics of DOXIL after multiple doses were evaluated in a tissue disposition study (LTI-30-93-24). Rats were administered multiple doses of DOXIL 0.25 or 1.0 mg/kg or Adriamycin 1.0 mg/kg. Concentrations of doxorubicin in the plasma of rats treated with DOXIL 1.0 mg/kg were approximately 1000- to 1500-fold higher than those measured in rats that received the same dose of Adriamycin (Table 14).

TABLE 14
Plasma Concentration of Doxorubicin after Treatment with DOXIL or Adriamycin

Treatment[a]	Dose No.	Pre-Dose (µg/mL)	C_o[b] (µg/mL)
DOXIL 0.25 mg/kg	1	--	3.8 ± 0.5
	13	2.3 ± 0.4[c]	7.4 ± 0.6
DOXIL 1.0 mg/kg	1	--	17.4 ± 2.7
	13	6.5 ± 0.9	22.4 ± 3.0
Adriamycin 1.0 mg/kg	1	--	0.012 ± 0.002
	13	0.01 (n = 2)	0.017 ± 0.004

[a] n = 3 or 4 per time point unless otherwise indicated.

[b] Plasma concentration at the first time point post-dose (30 minutes).

[c] Plasma concentration measured 72 hours after the 12th dose.

Plasma concentration in the DOXIL group was dose-dependent, with a four-fold increase in dose producing an equivalent increase in plasma concentration after the first dose. Doxorubicin was detected in the plasma of DOXIL-treated rats prior to the administration of the last dose, indicating that accumulation had occurred, but plasma levels remained approximately dose dependent. Peak plasma levels after the 13th treatment in the low-dose DOXIL group were nearly 90% higher than after the first dose, and peaks after dose 13 in the DOXIL 1.0 mg/kg group were nearly 30% higher than on Day 1, reflecting the accumulation of doxorubicin in the plasma. Plasma pharmacokinetics were consistent with those reported following single administration, although values determined after the final dose reflected the plasma accumulation of doxorubicin. DOXIL was long-circulating, with a longer MRT, a smaller volume of distribution and lower rate of clearance, and a higher AUC than Adriamycin. Plasma half-life, MRT and AUC increased in the DOXIL groups from first to the last dose, while clearance decreased.

b. Rabbits

A study of the comparative plasma pharmacokinetics of DOXIL and Adriamycin after administration of a single 1.5 kg/mg dose was conducted in rabbits (LTI-30-93-28). The plasma concentration data of DOXIL were best fit with a biexponential curve (Fig. 4), which had a short first phase (half-life = 30 minutes) and a prolonged second phase (half-life 22 hours) that accounted for the majority of the AUC.

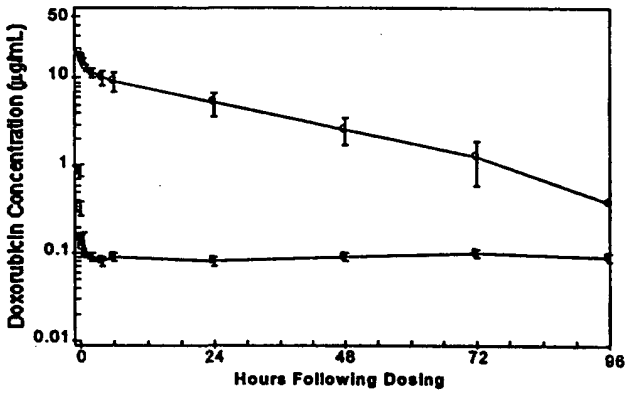

FIGURE 4

Plasma concentration of doxorubicin (µg/mL) in rabbits treated with single intravenous injection of 1.5 mg/kg DOXIL (upper curve) or Adriamycin (lower curve).

The plasma concentration-time curve after Adriamycin administration was also biphasic, as previously reported in the rabbit by other investigators.[78] The Adriamycin curve was characterized by a rapid distribution phase (half-life = 2 minutes) and a longer terminal phase (half-life 4.0 hours), which is believed to represent the circulation of a low concentration of protein-bound doxorubicin and the slow elimination of doxorubicin and its metabolites from the tissues. The elimination half-life determined in this study was shorter than the approximately 35 hours half-life reported in the literature, probably because the relatively high lower limit of quantitation (LLOQ) of the spectrofluorometric assay used (70 ng/mL) prevented the consistent quantitation of plasma levels of doxorubicin later than two hours after treatment. Doxorubicin clearance was decreased in DOXIL-treated rabbits compared to Adriamycin animals (6.0 vs. 2536 mL/hr), volume of distribution was significantly smaller (176 vs. 13,651 mL) and AUC was significantly higher (368 vs. 0.8 μg·hr/mL). The large volume of distribution of Adriamycin is consistent with its rapid and extensive distribution to the tissues.[70] Doxorubicin is distributed more slowly to the tissues after DOXIL administration (LTI-30-93-23).

c. Dog

The pharmacokinetics of DOXIL and DOXIL 1 were compared in dogs that received a single intravenous injection of 1.5 mg/kg of DOXIL or DOXIL 1 (LTI-30-93-07). The plasma concentration-time curves of the two DOXIL formulations were parallel, and both formulations were long circulating. Maximum plasma concentrations of doxorubicin did not differ between the two formulations (27.7 and 29.0 μg/mL for DOXIL and DOXIL 1, respectively). Plasma levels of doxorubicin were significantly lower in dogs given DOXIL 1 only at the later time points. Both formulations had biexponential plasma concentration-time curves, with a short initial phase followed by a prolonged second phase (Fig. 5).

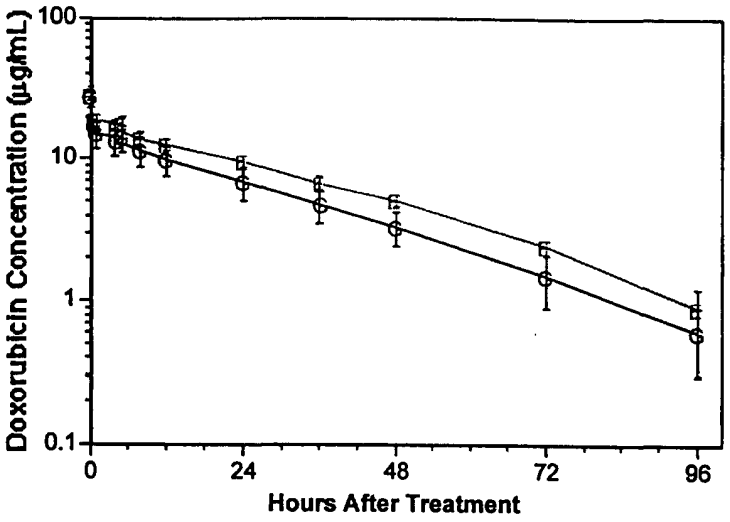

FIGURE 5

Plasma concentration of doxorubicin (µg/mL) in dogs that received a single intravenous injection of 1.5 mg/kg DOXIL (upper, dashed curve) or DOXIL 1. (lower, solid curve).

However, the second phase (λ_2) of the plasma concentration-time curve was significantly longer in animals that received DOXIL (25.9 vs. 21.6 hours for DOXIL 1), as was MRT (37.2 vs. 30.7 hours). The AUC for DOXIL was approximately 40% higher than the AUC for an equivalent dose of DOXIL 1 (656 vs. 463 µg·hr/mL), reflecting the significantly lower clearance rate of the DOXIL formulation (15.5 mL/hr vs. 24.0 mL/hr clearance of DOXIL 1).

Plasma concentrations of doxorubicin were determined pre-dose in beagle dogs that received multiple doses of DOXIL 0.25, 0.75 or 1.0 mg/kg or Adriamycin 1.0 mg/kg (LTI-30-94-18). Plasma was collected prior to each dose for determination of doxorubicin concentration. Plasma levels of doxorubicin and doxorubicinol were below the assay LLOQ (5 ng/mL) in all animals from the three DOXIL treatment groups and the Adriamycin treatment group. These data demonstrate that no accumulation of doxorubicin occurred when DOXIL was dosed once every three weeks in dogs.

3. Tissue Distribution

The tissue distribution of DOXIL has been studied in tumor-bearing mice and non-tumor-bearing rats and dogs. Table 19, located at the end of this section, presents a summary of the nonclinical tissue distribution studies conducted with DOXIL.

a. Tumor-bearing Mouse

Tissue doxorubicin levels were measured in tumor-bearing mice (Balb/c mice with C26 colon carcinoma) following a single intravenous injection of DOXIL 6 mg/kg or Adriamycin (LTI-30-94-11). Blood, heart, kidney, liver, spleen and tumor tissue were collected and analyzed for doxorubicin concentration. Peak tissue concentrations were measured at the first time point in most tissue in the Adriamycin treatment group. At one hour after treatment, doxorubicin concentrations were higher in heart, liver, spleen and kidneys in Adriamycin-treated mice, and slightly higher in tumor tissue in DOXIL-treated mice. Levels continued to rise in the liver, spleen and tumor in the DOXIL group, reaching a peak 24 hours after treatment. Peak levels in the tissues, including the heart, were lower in DOXIL-treated mice. Peaks also tended to occur later and tissue levels remained elevated longer after treatment in the DOXIL group. In contrast, with the exception of spleen, tissue levels decreased rapidly in the Adriamycin animals and were at or below the assay LLOQ by 96 to 120 hours after treatment. Because of the persistence of doxorubicin in the tissues of DOXIL-treated mice, tissue AUCs were consistently higher compared to tissue AUCs in Adriamycin-treated mice (Table 15).

TABLE 15
Tissue AUCs in C26 Tumor-Bearing Mice
Treated with DOXIL or Adriamycin

Tissue	DOXIL AUC (μg·hr/g)	Adriamycin AUC (μg·hr/g)
C26 Tumor	596.0	84.9
Heart	473.6	125.2
Kidney	799.9	339.4
Liver	775.6	239.3
Spleen	1982.5	1075.1

This difference is particularly evident in the C26 tumor, in which the AUC in DOXIL-treated mice was approximately seven-fold higher than the tumor AUC in mice that received an equal dose of Adriamycin. Tumor doxorubicin levels in the DOXIL group reached 7.2 ± 0.8 μg/g at 24 hours after treatment and decreased slowly with time, declining to 1.4 ± 0.6 μg/g at 120 hours post-dose. In contrast, doxorubicin concentration in tumors of Adriamycin-treated mice peaked at 2.0 ± 0.7 μg/g at 4 hours post-dose and decreased relatively rapidly to approximately 0.2 ± 0.2 μg/g at 120 hours after treatment (below the assay LLOQ). The administration of doxorubicin as DOXIL results in significantly higher tumor levels of drug compared to treatment with the same dose of Adriamycin. High peak levels of doxorubicin are correlated with increased risk of cardiotoxicity and high AUC values are believed to be optimal for anti-tumor efficacy. The low peak heart levels and higher tumor AUC observed following single administration of DOXIL suggests DOXIL may be both safer and more efficacious than non-liposomal doxorubicin.

Tissue distribution of doxorubicin was measured using laser confocal fluorescence microscopy in female Swiss nude mice implanted subcutaneously with explants of the prostatic carcinoma PC-3. Thirty days after tumor implantation, mice received a single intravenous injection of DOXIL 0.9 mg/kg or Adriamycin (LTI-30-93-15). PC-3 tumor, liver and kidney tissue were analyzed for doxorubicin. Doxorubicin concentration in all three tissues was higher after DOXIL treatment than after Adriamycin treatment. One hour after injection the doxorubicin concentration of tumor tissue was nearly three-fold higher in DOXIL-treated animals than in Adriamycin animals, and, by 24 hours after treatment, tumor drug levels were nearly 80-fold higher in DOXIL animals. Compared to Adriamycin-treated animals, doxorubicin levels persisted in tumors of DOXIL-treated animals over one week (Fig. 6). AUC values calculated for doxorubicin concentration in the tumor gave relative values of 36.5 μg·hr/g and 919 μg·hr/g in the Adriamycin and DOXIL animals, respectively, representing a 25-fold increase in the drug concentration in the tumor.

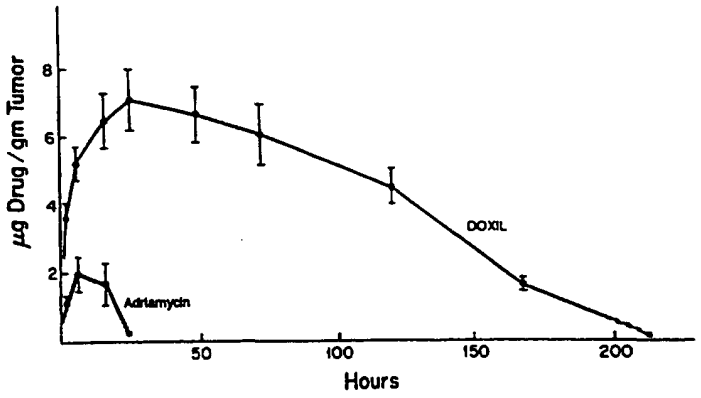

FIGURE 6

Concentration of doxorubicin (µg/g tissue) in human prostatic carcinoma xenografts in nude mice treated with 0.9 mg/kg DOXIL (upper curve) or 0.9 mg/kg Adriamycin (lower curve).

b. Rat

The tissue distribution of DOXIL 1.0 mg/kg or Adriamycin was compared in tumor-free rats (LTI-30-93-23). Doxorubicin and doxorubicinol concentration were measured in tissues and plasma, and doxorubicin concentration was measured in urine. Plasma results have been discussed previously. The peak tissue concentration of doxorubicin in most tissues, including the heart and kidney, was lower after DOXIL administration compared to Adriamycin (Table 16). Both the heart and the kidneys are known target organs of doxorubicin in the rat. Peak tissue doxorubicin concentrations were measured in tissues of Adriamycin animals 30 minutes post-dose, but not until 24 hours post-dose in the DOXIL group.

Doxorubicin persisted in the tissues of DOXIL-treated animals, and surpassed the concentrations in the tissues of Adriamycin-treated animals by 24 hours after treatment. Tissue AUCs were consistently higher in the DOXIL group, reflecting the slower elimination of doxorubicin from the tissues. Although doxorubicinol was detected in tissues from both DOXIL-treated and Adriamycin-treated rats, it was generally below the

assay LLOQ and could not be quantitated. The metabolite was detected more frequently in animals that received Adriamycin, primarily in the liver and kidneys.

TABLE 16
Peak Doxorubicin Concentrations in Tissues of Rats that Received a Single Dose of DOXIL or Adriamycin[a]

Tissue	DOXIL[a] (µg/g tissue)	Adriamycin[b] (µg/g tissue)	% Difference[c]
Bone Marrow	1.97 ± 1.63	1.34 ± 1.23	+ 47
Duodenum	2.47 ± 0.93	1.61 ± 0.12	+ 53
Heart	1.67 ± 0.55	2.21 ± 0.17	- 24
Kidney	1.71 ± 0.11	3.61 ± 0.40	- 53
Liver	1.27 ± 0.11	1.67 ± 0.16	- 24
Lungs	1.59 ± 0.33	2.71 ± 0.49	- 41
Skin	0.46 ± 1.1	0.46 ± 0.06	0
Spleen	7.18 ± 1.41	2.56 ± 0.33	+ 180
Stomach	0.94 ± 0.19	1.47 ± 0.18	- 36

[a] Peak concentration was measured 24 hours post-dose in the DOXIL 1.0 mg/kg group, except in skin, which peaked at 48 hours post-dose.

[b] Peak concentration was measured 30 minutes post-dose in the Adriamycin group, except in skin, which peaked at 4 hours post-dose.

[c] $\% \text{ Difference} = \frac{[DOX]_{DOXIL} - [DOX]_{Adriamycin}}{[DOX]_{Adriamycin}} \times 100$

The tissue disposition of doxorubicin after repeated dosing was determined in tumor-free rats that received DOXIL 0.25 or 1.0 mg/kg or Adriamycin 1.0 mg/kg every third day for a total of 13 treatments (LTI-30-93-24). Peak tissue doxorubicin concentrations were measured at the first time point (one day) after dosing in all treatment groups and were highest in the DOXIL 1.0 mg/kg group. Tissue levels were comparable in the DOXIL 0.25 mg/kg and Adriamycin 1.0 mg/kg groups, with the exception of kidney, lung and stomach, which had significantly higher levels in Adriamycin-treated animals. Tissue levels of doxorubicin were approximately dose proportional in the DOXIL 0.25 and 1.0 mg/kg groups.

The doxorubicin concentration of the tissues decreased significantly after the final treatment in all three groups. By 31 days after the last dose, the doxorubicin

concentration in the tissues of rats that received DOXIL 1.0 mg/kg was below the assay LLOQ, with the exception of the duodenum, kidney and spleen, which retained small amounts of doxorubicin (0.47 ± 0.20, 0.33 ± 0.21 and 0.45 ± 0.15 µg/g, respectively). Tissue levels of doxorubicin were no longer measurable three days after the last dose in the DOXIL 0.25 mg/kg and Adriamycin treatment groups, except in the spleen, which had measurable levels of doxorubicin until ten days after the final treatment (Day 47).

Although the tissue pharmacokinetics of doxorubicin could not be determined in this study owing to the rapid decline in the tissue concentration of the drug with time, the rate of elimination of drug from the tissues of Adriamycin-treated animals appeared to be faster than in animals treated with DOXIL. Tissue doxorubicin levels were higher after 13 doses than after a single dose (Table 17), but remained approximately dose proportional in the two DOXIL-treatment groups. Thus, repeated administration of DOXIL apparently did not result in saturation of the tissues.

TABLE 17
Ratio of Tissue Concentrations of Doxorubicin After One or Thirteen Doses of 1 mg/kg DOXIL or Adriamycin

Tissue	DOXIL 1.0 mg/kg Ratio[a]	Adriamycin 1.0 mg/kg Ratio[a]
Bone Marrow	3.0	1.5
Duodenum	2.2	1.5
Heart	0.8	0.9
Kidney	2.5	1.7
Liver	1.3	1.0
Lungs	2.1	1.3
Skin	6.3	1.2
Spleen	2.1	1.4
Stomach	1.8	1.7

[a] Ratio of doxorubicin concentration in the tissues 24 hours after 13 doses (LTI-30-93-24) to concentration 24 hours after one dose (LTI-30-93-23) of DOXIL or Adriamycin.

c. Dogs

The concentration of doxorubicin and doxorubicinol was determined in skin lesions seen in DOXIL-treated dogs from a multiple dose study in which the dogs received DOXIL 0.25, 0.75 or 1.0 mg/kg, or Adriamycin 1.0 mg/kg once every three weeks for a total of

ten treatments (LTI-30-94-18). Only one dog developed skin lesions in the low dose DOXIL group, and no lesions were seen in animals treated with Adriamycin. Doxorubicin was detected in all lesions collected one week after the final dose. Although the concentration varied greatly among dogs within the dose groups, doxorubicin levels were dose-dependent and generally higher in lesions than in adjacent unaffected skin. Samples from the extremities tended to have higher concentrations than those collected from more central areas of the body. Lesion and skin concentrations of doxorubicin were five- to ten-fold lower by five weeks post-treatment, and the differences seen between lesions and normal skin and between distal and proximal sites were no longer evident. Doxorubicinol levels were low, dose-dependent and also quite variable. No dependency on lesion site was seen, and concentrations in lesions and normal skin were approximately equivalent. The variability of the doxorubicin concentration measured was likely due to the developmental stage of the lesions collected, i.e., newly formed or in the process of healing, since doxorubicin concentration is known to decrease as lesions heal (LTI-30-93-23).

4. Metabolism

No metabolism studies have been conducted with DOXIL, nor has doxorubicinol been administered directly. Following administration, doxorubicin is rapidly metabolized. Doxorubicinol is the most prominent doxorubicin metabolite in plasma and urine and results from the reduction of the C-13 carbonyl function in the C-side chain to give the hydrophilic 13-hydroxyl metabolite. Concentrations of doxorubicinol in plasma are typically 40 to 60% of doxorubicin concentrations, reflecting the extensive metabolism of the parent compound.[80,81] Another major route of metabolism is the reductive cleavage of the daunosamine sugar moiety to yield the poorly water soluble aglycones doxorubicin- one and 7-deoxydoxorubicinone.[82] Doxorubicinol, like doxorubicin, has antitumor activity, but is only about one-tenth as cytotoxic as doxorubicin.[83,84] The aglycone metabolites do not have antitumor activity.[85] Doxorubicin and its metabolites are primarily excreted in the bile, with much of the administered dose excreted in the first pass.[82,86,87] Over seven days, cumulative fecal excretion ranges from 24 to 45%, compared to an average of 5% excreted in the urine.[82,88]

5. Excretion

Urinary recovery of doxorubicin in rats administered DOXIL 1.0 mg/kg (2.8%) was lower than urinary recovery following administration of Adriamycin 1.0 mg/kg (8.4%) (LTI-30-93-23). The lower urinary recovery after DOXIL treatment may have been due to the slower clearance of DOXIL from the bloodstream. Doxorubicinol could not be quantitated in the urine due to the presence of a co-eluting interfering peak also present in