Relationship of Pharmacokinetically-Calculated Volumes of Distribution to the Physiologic Distribution of Liposomal Drugs in Tissues: Implications for the Characterization of Liposomal Formulations

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INTRODUCTION

Liposomes are now an accepted intravenous drug delivery vehicle with the recent approval of formulations to increase the therapeutic index of toxic anti-infective and anti-neoplastic agents (1). The therapeutic benefits bestowed by liposomes are thought to result from their ability to alter the disposition of encapsulated drugs within the body. Since liposomes can be formulated with a wide range of properties that markedly effect their distribution and performance (2), a thorough understanding of the pharmacokinetics and disposition of liposomal formulations is essential for their continued development and effective clinical application.

To date, pharmacokinetic profiles provided to the users of intravenous liposome products have been derived from noncompartmental analysis of total drug concentrations in plasma or blood (3). These methods are clearly appropriate for conventional drugs, but their ability to provide clinically and physiologically relevant disposition parameters for liposomal formulations has not been established. The presence of both liposomal and non-liposomal drug pools, the particulate and potentially heterogeneous nature of liposomes, and the non-linear disposition of some liposomes all suggest that conventionally-calculated pharmacokinetic parameters may be of limited use to the clinician seeking to optimize therapy with liposomal agents.

Drug distribution in the body is conventionally described by the volume of distribution, an intrinsic parameter relating the amount of drug in the body to its plasma concentration. The volume of distribution reflects the rate and extent of drug distribution and binding outside the vascular compartment and is an important determinant of the plasma concentration vs. time curve (4). For anti-infective drugs, the volume of distribution is often cited as an indication of relative tissue distribution. Volumes of distribution reported for two liposomal formulations of Amphotericin B given at 5 mg/kg/day range from 0.1 L/kg (5), suggesting little drug resides outside plasma, to 147 L/kg (6), which implies the amount of drug in the body exceeds the amount actually administered. This 1000-fold difference in volume of distribution suggests tissue distribution should differ markedly, yet actual tissue drug concentrations measured in patients were similar for the two formulations (7,8) Thus, conventional volumes of distribution may not accurately reflect the distribution of liposomal drugs in the body. Since alternative formulations exist for most liposomal drugs, it is important to determine the accuracy and physiologic relevance of reported parameters such as the volume of distribution, and to ask whether parameter differences between formulations are due to actual differences in their disposition or to the methods of calculation employed.

This report critically examines the use of conventional pharmacokinetic analysis for the determination of volumes of distribution for liposome-associated drugs by comparing reported volumes of distribution determined by conventional pharmacokinetic analysis to volumes of distribution determined physiologically, using actual drug concentrations measured in tissues during studies of seven liposomal drugs in four animal species. From these data, the ability of conventionally-calculated volumes of distribution to predict the tissue distribution of liposomal drugs is assessed. Based on the results of this analysis, suggestions are made for the pharmacokinetic characterization of liposome-based drug formulations.

METHODS

Data from eleven studies of liposomal drug disposition, in which both a pharmacokinetic volume of distribution (V_β or V_{SS}) and drug concentrations in tissues were reported (9–17), were retrospectively analyzed to determine the volumes of distribution of the liposomal drugs by physiologic means. The physiologic volume of distribution was defined as the ratio of the amount of drug in the body to the plasma concentration at a given time or over a given time interval. The amount of drug in the body was estimated by summing the amounts of drug in all tissues, including plasma, measured in a given study. The amount of drug in each tissue was the product of the measured tissue concentration and the tissue weight. In cases where they were not reported, tissue weights were estimated from published data for each species (18). For comparison, all volumes are expressed in body weight-normalized units.

RESULTS

Table I compares the apparent volumes of distribution of the seven liposomal drugs, calculated by conventional pharmacokinetic analysis of plasma concentration vs. time profiles, to their physiologic volumes of distribution, calculated from concentrations of drug measured in tissues during these studies. Pharmacokinetic volumes of distribution (29–225 ml/kg) were generally similar to the plasma or blood volumes of these species, indicating little apparent distribution of drug into the extravascular or tissue compartments. In contrast, the physiologic volumes of distribution were markedly larger for

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Table I. Comparison of Pharmacokinetic and Physiologic Volumes of Distribution for Intravenous Liposomal Drugs

Drug [liposome type]	Species dose[a]	Tissues measured	Plasma half-life	Vd, ml/kg	Physiologic Vd, ml/kg	Ref.
Amphotericin B[b] [rigid, cholesterol containing SUV]	Rat 30 × 3 mg/kg	Li, Sp, Ki, Lu	9.6 hr	97	1172 (0–24 hr)	9
Amphotericin B[b] [rigid, cholesterol containing SUV]	Rat 91 × 4 mg/kg	Li, Sp, Ki, Lu	10.4 hr	78	1836 (0–24 hr)	10
Amphotericin B [rigid, cholesterol containing SUV]	Dog 30 × 1 mg/kg	Li, Sp, Ki, Lu	6.0 hr	77	12180 (24 hr)	11
Amikacin [rigid, cholesterol containing SUV]	Rat 1 × 50 mg/kg	Li, Sp, Ki, Lu, Bm, Mu, Sk, Ht, Br, Te	24.5 hr	75	51 (24 hr) 103 (48 hr) 128 (72 hr) 591 (120 hr) 3456 (168 hr)	12
Mitoxantrone [rigid, cholesterol containing LUV]	Mouse 1 × 10 mg/kg	Li, Sp, Ki, Lu	12 hr	29	56 (1 hr) 81 (4 hr) 849 (24 hr) 2078 (72 hr)	13
Mitoxantrone [rigid, sterically stabilized LUV, cholesterol]	Mouse 1 × 10 mg/kg	Li, Sp, Ki, Lu	N/D	29	51 (1 hr) 61 (4 hr) 131 (24 hr) 1823 (72 hr)	13
Cisplatin [sterically stabilized, cholesterol, 110 nm]	Mouse 1 × 3 mg/kg	Li, Sp, Ki	16 hr[c]	110	97 (8 hr) 102 (24 hr) 109 (48 hr) 136 (96 hr) 728 (168 hr)	14
Daunorubicin [rigid, cholesterol containing SUV]	Mouse 1 × 20 mg/kg	Li, Sp, Ki	4.0 hr	57	120 (0–48 hr)	15
^{67}Ga-deferoxamine [cholesterol, sterically stabilized, 100 nm]	Rat 1 × 75 μmol/kg	Li, Sp, Lu	16 hr	38	67 (24 hr)	16
^{67}Ga-deferoxamine [cholesterol, sterically stabilized, 380 nm]	Rat 1 × 75 μmol/kg	Li, Sp, Lu	8.0 hr	74	560 (24 hr)	16
Nystatin [fluid, MLV]	Rabbit 15 × 4 mg/kg	Li, Sp, Ki, Lu, Bm, Mu	1.5 hr	225	128 (0.5 hr)	17

Note: Vd, volume of distribution from pharmacokinetic analysis; Physiologic Vd, volume of distribution determined as the ratio of amount of drug in all tissues to the plasma concentration; Li, liver; Sp, spleen; Ki, kidneys; Lu, lungs; Bm, bone marrow; Mu, skeletal muscle; Sk, skin; Ht, heart; Br, brain; Te, testes; LUV, Large Unilamellar Lipsomes; SUV, Small Unilamellar Lipsomes; MLV, Large Multilamellar Liposomes; N/D, insufficient data for determination.

[a] Number of daily doses and size of each dose are indicated.

[b] For these studies, values shown are the average of separate determinations for male and female animals.

[c] Non-linear disposition, value shown is average half-life over 0–96 hr period.

most formulations, reaching values between 2 and 12 L/kg for liposomal formulations of amphotericin B, amikacin and mitoxantrone. The physiologic volumes of distribution show that these liposomes achieved far more extensive tissue distribution than indicated by their pharmacokinetic volumes of distribution. Since drug concentrations were not measured in all tissues of the body, the values in Table I underestimate the physiologic volume of distribution to some extent. Thus, differences between physiologic and pharmacokinetic volumes of distribution are likely to be even larger than these data suggest.

In the studies in which tissue distribution was measured at multiple time points, physiologic volumes of distribution consistently increased over time, for as long as one week after dosing with liposomal drugs (Figure 1). This is in contrast to conventional drugs, for which tissue/plasma concentration ratios become constant after the initial distribution phase, resulting in a constant apparent volume of distribution during elimination (19). This is illustrated in Figure 2A, where concentrations in plasma and extracellular fluid decline in parallel from 2 hr after intravenous dosing with a conventional drug formulation.

DISCUSSION

A comparison of pharmacokinetic and physiologic volumes of distribution has shown that pharmacokinetically-calculated volumes may not accurately predict the amount of drug in the body after administration of liposomal formulations. Most of the liposomes examined had higher and more prolonged distribution of drug into tissues than suggested by their pharmacokinetic volumes of distribution. Volumes of distribution determined by pharmacokinetic analysis were similar to the plasma or blood volume, suggesting that this volume of distribution mainly reflects the amount of drug in the circulating liposomes, rather then the extent of drug distribution to tissues. The observation that physiologic volumes of distribution continued to increase over time, even during the apparent terminal elimination phase in plasma, demonstrates that liposomes do not exhibit the behavior assumed in

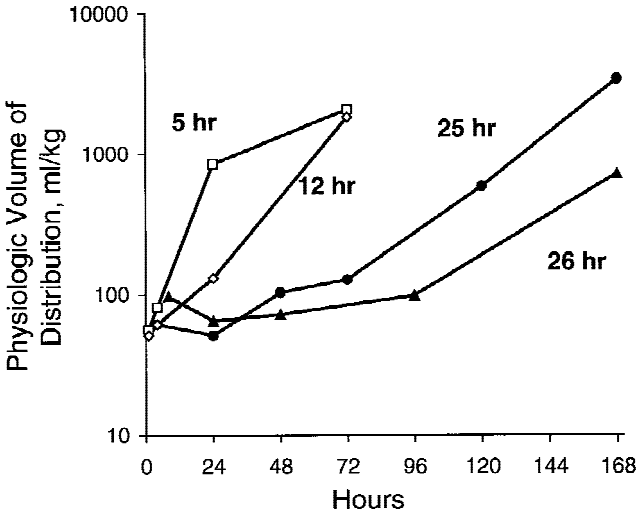

Fig. 1. Physiologic volumes of distribution for liposomal drugs increase over time after administration. Physiologic volumes of distribution were calculated from concentrations measured in plasma and tissues at each time point as described in the text for formulations of Mitoxantrone in rigid LUVs (open squares, ref. 13), Mitoxantrone in sterically stabilized rigid LUVs (open diamonds, ref. 13), amikacin in rigid SUVs (filled circles, ref. 12), and cisplatin in sterically stabilized SUVs (filled triangles, ref. 14). Annotations indicate the principal half-life of each liposomal formulation over the time course of the studies.

the conventional calculation of V_β, that plasma and tissue levels decline in parallel during the terminal (post-distributional) elimination phase (4,19). The failure of tissue concentrations to reach a post-distributional equilibrium with plasma implies that liposome uptake into tissues does not constitute a distributional process in which the liposomes can freely return to plasma, but is more characteristic of elimination, the one-way transfer of drug out of the central compartment. This explanation is consistent with the observation that maximum concentrations in some tissues are not reached for 3 or more days after the administration of liposomal drugs (12–14). Thus, the fact that liposomes can greatly increase and prolong drug levels in tissues is not reflected in conventionally-calculated volumes of distribution, but is apparent in physiologic volumes of distribution determined from measured concentrations in tissue. Since the therapeutic advantages of liposomes are related to their altered disposition, pharmacokinetically-calculated volumes of distribution appear to be of limited use in characterizing liposomal formulations.

Physiologic volumes of distribution varied between the different liposomal formulations examined. Some of this variability may be due to the different rates at which these liposomes leave the plasma compartment. As shown in Figure 1, the physiologic volumes of distribution for all liposomal drugs approximate the plasma volume at time zero, when no drug has yet entered the tissues. Tissue uptake of liposomes leads to increased tissue drug concentrations, and the physiologic volume of distribution rises, more rapidly for liposomes with short plasma residence times, more slowly for "long-circulating" liposomes. The fact that not all studies measured tissue concentrations at the same time points may account for some of the differences observed in their physiologic volumes. Other factors that could reduce the apparent physiologic volume of distribution include distribution to unmeasured tissues, instability or rapid elimination of the drug in tissues or leakage of the drug from liposomes.

This analysis of liposomal distribution also has implications for other liposome pharmacokinetic parameters, such as clearance. For example, the pharmacokinetic profiles of many liposomal formulations are multiexponential, a profile typically observed for non-liposomal drugs which undergo reversible distribution to extravascular compartments. The calculation of conventional pharmacokinetic parameters for drugs with multiexponential disposition assumes that clearance is constant, and that changes in plasma half-life observed after intravenous dosing result from a volume of distribution that increases during the distribution phase(s), and then remains relatively constant during the elimination phase (Figure 2A, ref. 19). For purposes of parameter calculation, liposomal formulations have been assumed to behave similarly. Since the assumption of post-distributional equilibrium is not met by liposome formulations, it is necessary to consider an alternative explanation when multiexponential plasma profiles are observed for liposomes: that the changing half-life results from changing clearance rather than changing volume of distribution. The assumption that circulating liposomes are largely confined to the vascular space (a relatively constant volume from which the liposomes are cleared) is reasonable since some liposomes do exhibit monoexponential disposition with a volume of distribution equal to that of the plasma compartment (16), and even small liposomes (< 100 nm diameter) do not readily enter the extracellular fluid space, as demonstrated in the rat air pouch model (Fig. 2B). The hypothesis of changing clearance is consistent with reports that liposomal disposition involves non-linear, saturable processes (7,11,20), and may demonstrate convex terminal elimination (14,21). Thus, the multiexponential profiles observed for many liposomes may arise from changes in clearance over time. These changes could result from saturable clearance mechanisms, opsonin depletion, liposomal heterogeneity (i.e., larger liposomes are cleared more rapidly than small liposomes), variable drug leakage or changes in liposome properties over time (20,21).

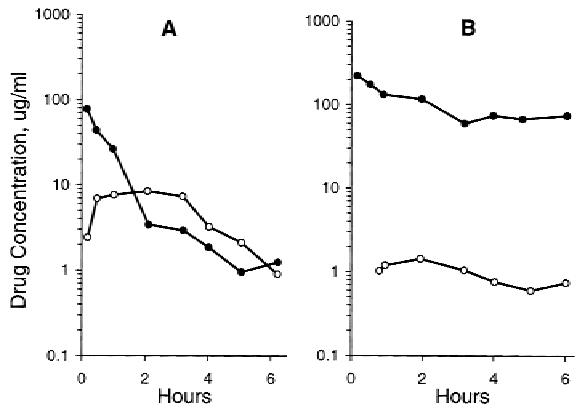

Fig. 2. Concentrations of total amikacin in plasma (filled circled) and extracellular fluid (open circles) after injection of 10 mg/kg conventional amikacin (A) or liposomal amikacin (B) in rats with sterile dorsal air pouches. Extracellular drug concentrations were measured in the air pouch fluid. Adapted from R.M. Fielding, et al., Pharm. Res. 14: S-329 (1997).

CONCLUSIONS

The inability of pharmacokinetically-calculated volumes of distribution to predict the distribution of liposomal drugs in the body, and the possibility that liposomal clearance changes over time, suggest that neither of these conventional parameters accurately describes the disposition of liposomal drugs. Alternatives to conventional pharmacokinetic analysis may improve the characterization of liposome formulations.

Due to the variable nature of V_β and V_{SS} calculations, it is suggested that the initial volume of distribution (termed V_0, V_c, or V_1), calculated as the ratio of dose to time zero concentration in plasma, be the only pharmacokinetic volume reported for liposomal drugs. V_0 is useful in predicting C_{max}, and should approximate the plasma volume for most formulations. The observation of higher values would indicate rapid drug leakage or the presence of unencapsulated drug in the liposomal formulation (22).

To adequately characterize the disposition of liposome formulations, the extent and timecourse of drug distribution in tissues should be directly measured, since it can not be implied from conventional volumes of distribution or plasma concentration vs. time curves. Since even physiologic volumes of distribution appear to change over time, ADME studies of liposomal drugs should include drug concentration measurements in the major tissues and sites of action at multiple time points after a clinically-relevant regimen to demonstrate tissue exposure profiles and compare them between formulations. While such studies are not possible in humans, careful preclinical evaluation of liposome disposition coupled with available clinical data should provide a more accurate prediction of human tissue disposition than can be obtained by conventional pharmacokinetic analysis of liposome formulations.

In addition, plasma profiles of liposomal drugs should be characterized in terms of the number of phases, their half-lives and relative areas. For conventional drugs, these are related to the rate and extent of distribution to tissue compartments, but for liposomes with changing clearance, the area of the initial phase(s) may be related to the fraction of drug that is rapidly cleared. Since formulations with similar reported half-lives may have different AUCs, liposomes claiming to be "long-circulating" should indicate the fraction of their AUC observed during the longest half-life. Where possible, concentrations of non-liposomal drug should be measured so that the contribution of both free and liposomal drug pools to drug effects can be assessed. When liposomal formulations exhibit multiexponential disposition, the hypothesis that clearance changes over time should be evaluated, and clearances calculated as Dose/AUC should be reported as time-averaged values. An alternative approach would be to estimate instantaneous clearances as $CL_t = (V_C * \ln 2)/t_{1/2}$, where $t_{1/2}$ is the half-life and CL_t the clearance at time t. This assumes that tissue uptake of liposomes is a clearance rather than a distribution process. Instantaneous clearance may be more relevant to physiologic processes such as blood flow and intrinsic clearance than are time-averaged clearances.

Another, more empirical, approach to liposome characterization and comparison would be to emphasize directly observable parameters (Cmax, Tmax, and AUC in plasma and tissues) rather than the calculated parameters Vd and CL, which appear to have little physiologic relevance for liposomes. Future studies are needed to establish the most clinically and physiologically relevant disposition parameters for liposomes, that can be used to compare and predict the relationships between dose regimen, pharmacokinetics in plasma and tissues, and therapeutic effects.

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