Rates of Systemic Degradation and Reticuloendothelial System (RES) Uptake of Thermosensitive Liposome Encapsulating Cisplatin in Rats

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Received September 7, 1992; accepted February 5, 1993

The systemic degradation and reticuloendothelial system (RES) uptake of cisplatin (CDDP)-encapsulated thermosensitive liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) (DPPC/DSPC = 9/1, 7/3, and 5/5, w/w) after intravenous administration to rats were examined by measuring the platinum (Pt) levels in the blood and RES (liver and spleen). The blood liposome level profile showed first-order rate elimination for each liposome administration. The elimination rate (K_{el}) was faster when the content of DSPC was lower (K_{el}: 1.3/hr for 9/1-liposomes, 0.7/hr for 7/3-liposomes, 0.5/hr for 5/5-liposomes). On the other hand, the RES liposome level profile showed distribution of liposomes followed by elimination therefrom. The RES level of the liposomes was lower when the content of DSPC was smaller (maximal level: 25% for 9/1-liposomes at 1 hr, 32% for 7/3-liposomes at 1 hr, 37% for 5/5-liposomes at 2 hr). The kinetic analysis demonstrated that the RES uptake rate (K_{res}) was almost the same among the liposomes (0.4/hr), while the systemic degradation rate (K_{deg}; $K_{el} - K_{res}$) became larger as the content of DSPC decreased (0.9/hr for 9/1-liposomes, 0.3/hr for 7/3-liposomes, and 0.1/hr for 5/5-liposomes) and that the RES liposome distribution amount was dependent not only on the K_{res} but also on the K_{deg} and the rate of RES liposome degradation. The K_{deg} for each type of liposome corresponded with the systemic CDDP release rate.

KEY WORDS: cisplatin (CDDP); thermosensitive liposome; intravenous administration; rat; blood level; organ level; systemic elimination; reticuloendothelial system (RES) uptake; systemic degradation; CDDP release.

INTRODUCTION

Combination of thermosensitive liposomes with hyperthermia has been proposed as a useful targeted-tumor drug delivery system (1). In its concept, the liposomes have hyperthermia-dependent phase transition characteristics and therefore release and distribute the encapsulated drug specifically to the heated tumor. In our previous study, we found that administration of thermosensitive large unilamellar liposomes encapsulating cisplatin (CDDP) with hyperthermia increased the tumor CDDP level by 5 times (2,3). However, before such liposomes can be used therapeutically in humans, more must be known about their pharmacokinetics.

When administered intravenously, liposomes generally tend to be taken up by the reticuloendothelial system (RES) existing in the liver and spleen, and even when RES uptake can be avoided, they tend to be decomposed in the systemic circulation (4). Among many reports on the pharmacokinetics of liposomes encapsulating active agents, none afford a quantitative evaluation of the rates of systemic liposome degradation and RES liposome uptake (5–8).

In the present study, therefore, we evaluated the systemic degradation rate and the RES uptake rate by examining the levels of liposome-encapsulated CDDP in the blood and RES (liver and spleen) when administering CDDP-encapsulated liposomes prepared in different heat-sensitive formulations to rats.

THEORETICAL

Clearance Kinetics for Liposomes

The systemic elimination of liposomes after intravenous administration can be explained by RES uptake of liposomes and systemic degradation of liposomes (Fig. 1). The administered liposomes are immediately mixed with the systemic blood. During circulation, some of the liposomes are degraded therein, while others are taken up by the RES when they pass through the RES compartment. Lacking in extravasation ability, however, liposomes will not be eliminated via distribution into other tissues or organs except liver and spleen (4).

According to a clearance theory (9,10), the average rate of systemic liposome elimination, K_{el}, can be expressed as

$$K_{el} = [100 - B_{lip}(t)]/AUC_b(t) \quad (1)$$

where $B_{lip}(t)$ and $AUC_b(t)$ refer to the blood liposome level at time t and the area under the curve (AUC) of blood liposome level between time 0 and time t, respectively.

If the liposomes taken up by the RES are not redistributed to the systemic blood, the average rate of RES liposome uptake, K_{res}, can be expressed as

$$K_{res} = CR_{lip}(t)/AUC_b(t) \quad (2)$$

where $CR_{lip}(t)$ refers to the percentage of cumulative dose taken up by the RES after time t. K_{res} can also be expressed as a function of RES extraction ratio (E_{res}) and blood-flow rate at the RES (Q_{res}):

$$K_{res} = Q_{res} E_{res}/V_b \quad (3)$$

where V_b refers to the total volume of the blood in the body. If liposomes taken up by the RES are not eliminated from the RES (RES is a "deadend"), $CR_{lip}(t)$ can be represented by RES liposome level at time t, $R_{lip}(t)$, and K_{res} can be calculated by using $R_{lip}(t)$. However, if the RES liposome elimination cannot be neglected, K_{res} calculated using $R_{lip}(t)$ is smaller than the actual one. Yet the obtained K_{res} can afford us a useful index for the intrinsic RES uptake rate.

Upon the assumption that systemic liposome elimination rate is determined by the rates of the RES uptake and systemic liposome degradation, K_{el} is the sum of K_{res} and the systemic liposome degradation rate (K_{deg}), and therefore, we can estimate K_{deg} from K_{el} and K_{res}:

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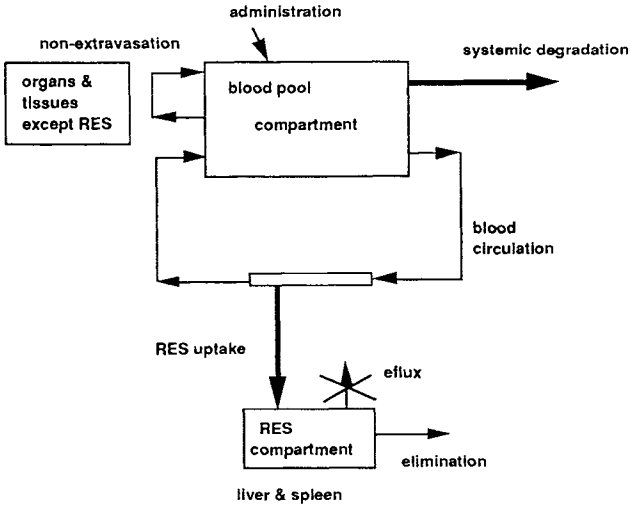

Fig. 1. Elimination of liposomes after intravenous administration.

$$K_{deg} = K_{el} - K_{res} \quad (4)$$

Systemic liposome degradation results in the release of the encapsulated drug to the systemic blood. Therefore, we can also evaluate the systemic liposome degradation rate with the rate of drug release from liposomes in the systemic blood. By assuming that liposomes distributed in the RES do not release the encapsulated drug back into the systemic circulation and applying a convolution equation to the systemic release of drug from liposomes, the blood level of the released drug (free drug) can be expressed as

$$B_{free}(t) = \int \mathrm{In}(X)\, G(t - x)dx \quad (5)$$

where $B_{free}(t)$ refers to the level of the released drug in the blood at time t after liposome administration, $G(t)$ refers to a multiexponential function describing the level of the free drug at time t after solution administration, and $\mathrm{In}(X)$ refers to an input rate function at time t and is equal to the release rate (fraction/unit time). Then, using the same deconvolution method as that reported (11), we can estimate the cumulative percentage of dose released after time t.

MATERIALS AND METHODS

Liposome Characteristics

Three types of liposomes encapsulating CDDP were prepared using different heat-sensitive lipid compositions [dipalmitoylphosphatidylcholine (DPPC)/distearoylphosphatidylcholine (DSPC) = 9/1, 7/3, and 5/5 (w/w)]. The preparation method was the same as that reported previously (reverse-phase evaporation vesicle method) (2). The unencapsulated CDDP was removed by dialysis against saline.

The amount of CDDP encapsulated in liposomes was determined by measuring the concentration of platinum (Pt) using an atomic absorption spectrometer (flameless, F7000, Hitachi) and the concentration of liposomal lipids (DPPC and DSPC) was determined by HPLC [μBondasphere, 5μ-C4-100A, Waters; methyl alcohol/0.1 M KH_2PO_4 = 9/1 (v/v); refraction index analyzer, Showadenko] (Table I). The average size for each type of liposome was approximately 0.2 μm (N4 submicron particle analyzer, Coulter Electronics).

Table I. The Liposomal Characteristics of CDDP-Encapsulated Thermosensitive Liposomes

Liposomes	Composition (w/w)	CDDP content (μg/mL)	Lipid content (mg/mL)
9/1	DPPC/DSPC = 9/1	202	28.8
7/3	DPPC/DSPC = 7/3	194	26.7
5/5	DPPC/DSPC = 5/5	224	26.1

Blood- and Organ-Level Experiments

SD-JCL rats (male, 8 weeks old, weighing about 300 g) were given a single bolus intravenous dose of liposomes (2 mg CDDP/kg) via the femoral vein. The blood samples (n = 3) were periodically taken from the tail vein. The blood total Pt (encapsulated Pt + free Pt) levels were determined by measuring the Pt concentration using an atomic absorption spectrometer. Prior to the assay, the blood sample was solubilized with methylbenzethonium hydroxide. The blood free-Pt levels were determined by forming an adduct of Pt with diethyldithiocarbamate and measuring its concentration using HPLC [Zorbax CN; heptane/isopropyl alcohol = 9/1 (v/v); UV 254 nm] (3). Prior to the assay, the blood sample was diluted with 10 vol of 5% glucose and centrifuged, and the liposome-encapsulated Pt in the supernatant was separated using a filter (Centrisart; molecular weight cutoff, 20,000; Sartorius) (3). To express the blood level as a percentage of the administered dose, the content of the whole blood in the body was assumed to be 8% (12).

In order to examine the organ liposome distribution, rats (n = 3) given an intravenous dose of liposomes were sacrificed at appropriate time intervals by exsanguination from the abdominal aorta, and the liver, spleen, left kidney, and lung were removed. Each organ sample was homogenized with 10 vol of water and solubilized with methylbenzethonium hydroxide and the total Pt concentration in the solubilized sample was measured using an atomic absorption spectrometer. To express the liver- and spleen-Pt levels, the amount of Pt in the blood contained by these organs was subtracted by assuming that the blood contents in these organs were 7% (12).

RESULTS AND DISCUSSION

Systemic Clearance of Free CDDP After Solution Administration

When administered as a solution, CDDP is rapidly eliminated from the systemic circulation (13). In the present study, similar results were obtained (Fig. 2). The free Pt was eliminated rapidly at a first-order rate from the blood (elimination half-life, about 10 min). Some portion of the Pt in the blood existed as Pt bound with the plasma protein (13) and the total Pt (free Pt + bound Pt) resulted in slower elimination as compared with the free Pt (Fig. 1). However, the elimination of the total Pt was much more rapid as compared with the total Pt after liposome administration (Fig. 3A).

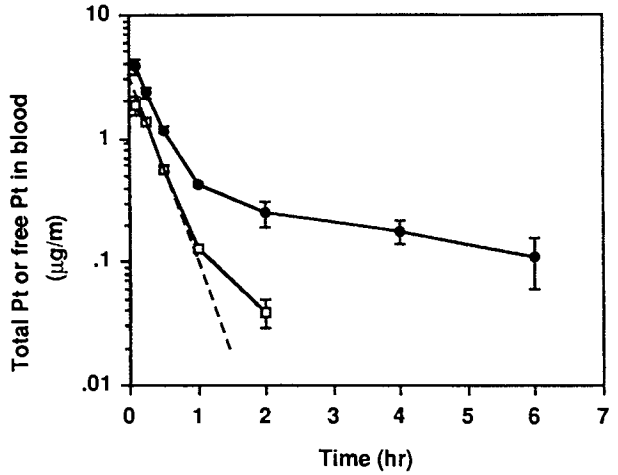

Fig. 2. The levels of total-Pt (free CDDP + bound CDDP; ●) and free-Pt (free CDDP; □) in the blood after administration of a CDDP solution to rats (dose: 2 mg CDDP/kg). The dashed line shows the result of curve fitting with a mono-exponential function [13.2exp(−3.44t)].

Systemic Clearance of Encapsulated CDDP After Liposome Administration

The levels of the total-Pt (liposome-encapsulated CDDP + free CDDP + bound CDDP) and the free Pt (free CDDP) after liposome administration are shown in Fig. 3A. In each liposome administration, the total Pt levels were much higher than the corresponding free Pt levels so that they were approximately equal to the levels of encapsulated CDDP. The percentages of dose remaining as encapsulated CDDP (liposomes) in the whole-body blood after administration are shown in Fig. 3B. Liposomes were eliminated gradually from the systemic circulation at a first order rate (4- and 6-hr levels for 9/1-liposomes do not represent the liposome levels but the blood-associated Pt levels). The liposome level at 6 min after administration indicated that almost all of the administered dose remained in the blood at this time.

K_{el}'s calculated using Eq. (1) and various time point data for each liposome administration are shown in Table II. They were almost constant with time and similar to the rates estimated from the slope of the blood level curve (9/1-liposomes, 1.3/hr; 7/3-liposomes, 0.7/hr; 5/5-liposomes, 0.5/hr).

Generally, reentering of liposomes into the systemic circulation after RES distribution results in a biexponential blood drug level curve. However, this characteristic was not obtained with the present liposomes.

RES Uptake of Encapsulated CDDP After Liposome Administration

The levels of total Pt in various organs after liposome administration were shown in comparison with solution administration in Fig. 4. The total Pt levels in the liver and spleen after liposome administration were much higher than those after solution administration, indicating that the Pt levels in these organs after liposome administration were nearly equal to the encapsulated Pt levels. They were also higher

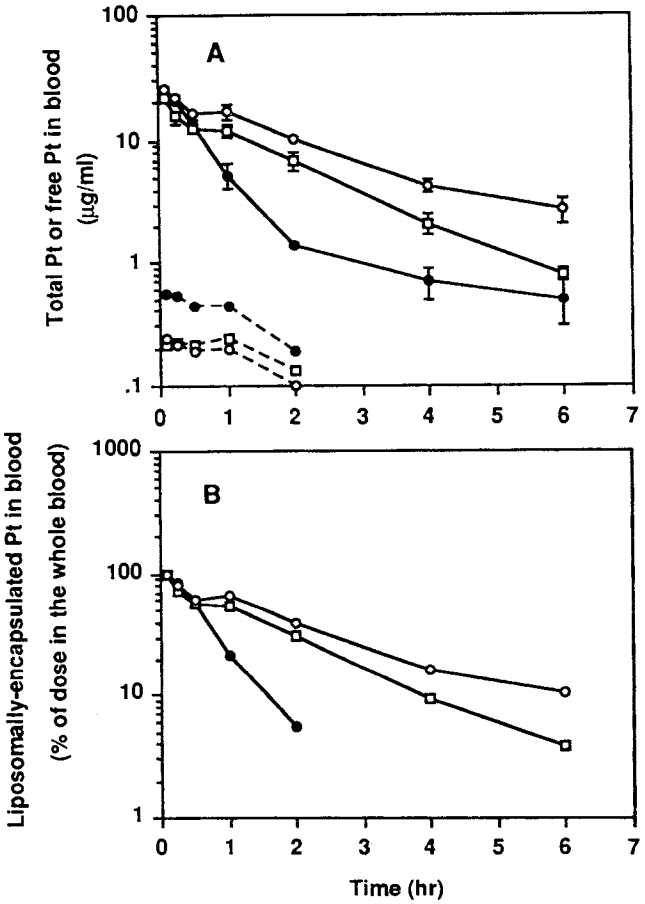

Fig. 3. The levels of total-Pt (liposome-encapsulated CDDP + free CDDP + bound CDDP; solid line) and free-Pt (free CDDP; dashed line) (A) and the levels of encapsulated CDDP (B) in the blood after administration of CDDP-encapsulated thermosensitive liposomes to rats (dose: 2 mg CDDP/kg). 9/1-liposomes (●); 7/3-liposomes (□); 5/5-liposome (○).

compared to the other organs (kidney and lung), indicating that the liver- and spleen-Pt distribution resulted from the RES uptake of liposomes. The RES (liver + spleen) level for each liposome administration was maximal at 1 or 2 hr and thereafter decreased gradually with time. The maximal level was larger with liposomes prepared using larger amount of DSPC (25% at 1 hr for 9/1-liposomes, 32% at 1 hr for 7/3-liposomes, 37% at 2 hr for 5/5-liposome).

Rates of RES Uptake and Systemic Degradation

At early times when RES liposome elimination is assumed to be neglected, we can estimate K_{res} theoretically, using Eq. (2) and RES levels (Table II). In all types of liposomes, K_{res}'s calculated using the 30-min and 1-hr RES levels were the same (approximately 0.4/hr), while K_{res}'s calculated using the RES level at later times when the RES liposome elimination cannot be neglected were lower. Thus K_{res} of 0.4/hr may be an adequate representation of the RES uptake rate for all liposomes. The value accounts for approximately 1% uptake at a single pass through the RES [$E_{res} = K_{res}\, V_b/Q_{res}$ = 0.4(/hr) × 24 (mL)/1060 (mL/hr) = 0.009] (11). One may assume that the RES liposome elimination

Table II. The Systemic Elimination Rate (K_{el}), RES Uptake Rate (K_{res}), and Systemic Degradation Rate (K_{deg}) for Liposome-Encapsulated CDDP After Administration of CDDP-Encapsulating Thermosensitive Liposomes to Rats

Time (hr)	9/1-liposomes			7/3-liposomes			5/5-liposomes		
	K_{el} (/hr)	K_{res} (/hr)	K_{deg} (/hr)	K_{el} (/hr)	K_{res} (/hr)	K_{deg} (/hr)	K_{el} (/hr)	K_{res} (/hr)	K_{deg} (/hr)
0.5	1.31	0.43	0.88	0.65	0.39	0.26	0.48	0.40	0.08
1	1.30	0.42	0.88	0.68	0.47	0.21	0.47	0.42	0.05
2	1.28	0.25[b]		0.62	0.27[b]		0.48	0.29[b]	
4	1.28[a]			0.60			0.46		
6	1.28[a]			0.59			0.42		
Expected value	1.3	0.4	0.9	0.7	0.4	0.3	0.5	0.4	0.01

[a] Blood liposome level at this time point was assumed to be zero.
[b] Elimination of liposomes from RES could not be neglected.

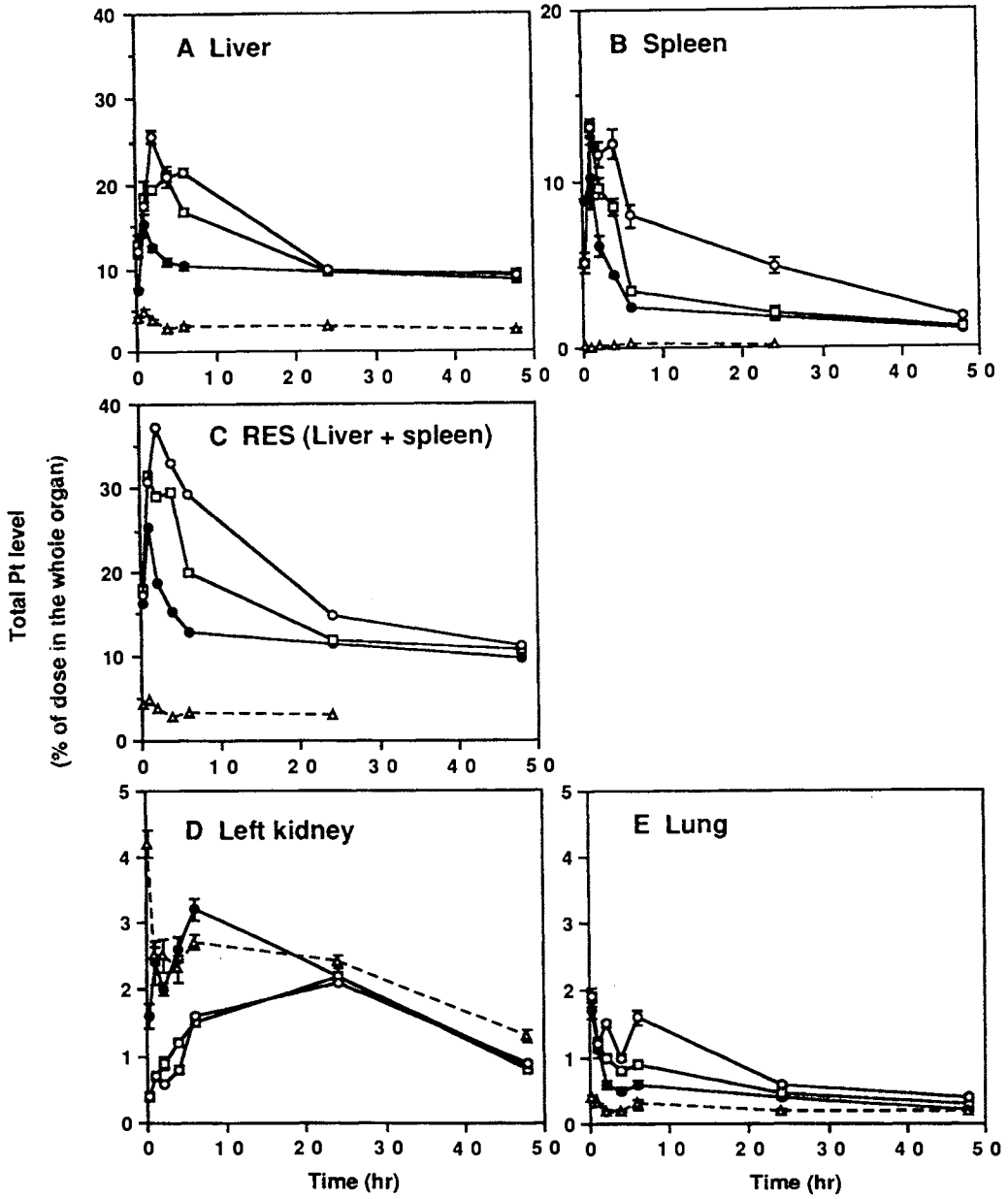

Fig. 4. The levels of total-Pt in the liver (A), spleen (B), RES (liver + spleen; C), kidney (D), and lung (E) after administration of CDDP-encapsulated thermosensitive liposomes (solid line) and a CDDP solution (dashed line) to rats (dose: 2 mg CDDP/kg). 9/1-liposomes (●); 7/3-liposomes (□); 5/5-liposome (○).

cannot be neglected at early times and therefore K_{res} is larger than such an estimate. However, this possibility is small by considering that K_{res} value does not exceed the value of K_{el}.

The percentage of K_{res} to K_{el} (approximately 30% for 9/1-liposomes, 60% for 7/3-liposomes, and 80% for 5/5-liposomes) represents a hypothetical RES level as that obtained at infinity by assuming liposomes taken up by the RES are not eliminated from the RES. The time profile of the difference between this hypothetical infinity level and the actual RES Pt level demonstrates biphasic or triphasic RES liposome elimination (data not shown). The early phase elimination (relatively fast elimination during the first 4 hr post-administration) suggests that the elimination rate was faster in the liposomes containing smaller amount of DSPC. It also suggests the partial lysis of liposomes during their incorporation into the phagocytic cells in the RES.

Using Eq. (4) and assuming K_{res} = 0.4/hr, we can estimate the systemic liposome degradation rate, K_{deg}. The K_{deg}'s for 9/1-liposomes, 7/3-liposomes, and 5/5-liposomes were 0.88, 0.23, and 0.06/hr, respectively (Table II). The rate became smaller as the content of DSPC increased. This result appears realistic in light of a general consideration that a lamellar membrane constructed with the larger amount of DSPC was more stable in the blood (4).

Rate of Encapsulated-CDDP Release into Systemic Blood

The free CDDP levels in the blood (Fig. 3A) suggested that the drug release from liposomes in the systemic circulation was responsible for the systemic liposome degradation. Using Eq. (5) [$G(t)$ = 13.2 exp(−3.44t): curve-fitting result in Fig. 2] and the blood free drug levels at early time points when the drug release from the RES back into the systemic circulation is assumed to be neglected, we can estimate the release rate (Fig. 5). The result showed that in all liposomes, the release amounts obtained as such were somewhat larger but corresponded fairly well with those calculated using K_{deg}.

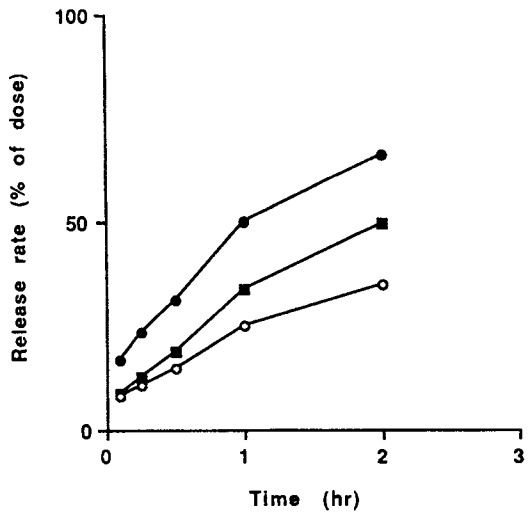

Fig. 5. The time-dependent rate of systemic CDDP release from liposomes calculated using Eq. (5) and the blood free-Pt levels at early times in Fig. 3A. 9/1-liposomes (●); 7/3-liposomes (■); 5/5-liposome (○).

CONCLUSION

Measuring the levels of liposome-encapsulated CDDP in the blood and RES and applying clearance kinetics to these data, we could evaluate the rates of systemic elimination (K_{el}), RES-uptake (K_{res}), and systemic degradation (K_{deg}) for the liposomes. K_{el}'s for 9/1-liposomes, 7/3-liposomes, and 5/5-liposomes were approximately 1.3, 0.7, and 0.5/hr, respectively. K_{res}'s for all three liposomes were 0.4/hr. K_{deg} for 9/1-liposomes, 7/3-liposomes, and 5/5-liposomes were 0.9, 0.2, and 0.1/hr, respectively. The systemic degradation accounted for a larger part of the systemic elimination than the RES uptake clearance did particularly with the liposomes prepared using smaller amount of DSPC. The RES was not a "dead end" with regard to the present liposomes; the liposomes distributed in the RES were eliminated therein gradually. The described pharmacokinetics will offer useful information when investigating factors that control the efficacy of thermosensitive liposome-based CDDP delivery.

NOMENCLATURE

K_{el}	Systemic elimination rate for encapsulated drug (liposomes) (L/hr)
K_{res}	RES-uptake rate for encapsulated drug (liposomes) (L/hr)
K_{deg}	Systemic-degradation rate for liposomes (L/hr)
$B_{lip}(t)$	Blood liposome level at time t (% of dose)
$AUC_b(t)$	Area under the curve of blood liposome level between time 0 and time t (% of dose hr)
$CR_{lip}(t)$	Percentage of cumulative dose taken up by the RES after time t (% of dose)
$R_{lip}(t)$	RES liposome level at time t (% of dose)
Q_{res}	Blood-flow rate at the RES (mL/hr)
E_{res}	RES-extraction ratio
V_b	Total volume of the blood in the body (mL)
$B_{free}(t)$	Blood free-drug level at time t after liposome administration (μg/mL)
In(t)	Input rate function at time t (fraction/hr)
G(t)	Multiexponential function describing blood free-drug level at time t after solution administration (μg/mL)

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