Heat-Induced Drug Release Rate and Maximal Targeting Index of Thermosensitive Liposome in Tumor-Bearing Mice

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To evaluate the rate of drug release at the tumor and maximal drug targeting after administration of thermosensitive liposomes with hyperthermia, a theoretical and experimental method was derived assessing the fraction of drug released from liposomes in a single pass through the heated tumor, F, and the drug targeting index when drug release occurs completely in response to heat ($F = 1$), DTI_{max}. The F and DTI_{max} were evaluated for four types of liposomes (LUV-1 and LUV-2, thermosensitive large unilamellar liposomes; LUV-3, a nonthermosensitive large unilamellar liposome; and SUV-1, a thermosensitive small unilamellar liposome) using reported data on blood liposome levels and tumor drug levels after the liposomes were administered to tumor bearing mice. DTI_{max} values for LUV-1 and SUV-1 were approximately 6, while the value for LUV-2 with a relatively large systemic clearance was only 2.3. The F values for LUV-1, LUV-2, and SUV-1 with hyperthermia were 0.71, 1.17, and 0.34, respectively, whereas the values for these liposomes without hyperthermia and for LUV-3 with or without hyperthermia were nearly zero. These results confirm earlier findings that LUV-1 and LUV-2 release CDDP almost completely at the heated tumor and that the large DTI value obtained in LUV-1 (DTI = 4.6) was due to its high heat sensitivity and its small systemic clearance.

KEY WORDS: thermosensitive liposome; cisplatin; hyperthermia; blood-drug level; tumor-drug level; targeting index; drug release.

INTRODUCTION

In the absence of selective activity confined to tumors, many antitumor drugs cause undesirable side effects. The use of a thermosensitive liposome which is designed to release drug at a targeted tumor in response to hyperthermia (HT) can enhance the drug efficacy and decrease systemic toxicity (1–5). Among several reports on the theoretical basis of drug targeting (6–9), none afford a quantitative evaluation of the site-specific drug release or the upper limit of the targeting efficiency.

The purpose of the present study is to derive a theoretical and experimental method to estimate the fraction of drug released at the heated tumor when liposomes pass concurrently with blood through heated tumor (F) and the upper limit of drug targeting index assuming complete drug release (DTI_{max}) and to evaluate the F and DTI_{max} for thermosensitive liposomes containing cisplatin (CDDP) using the reported data when the liposomes were administered to tumor-bearing mice (4).

THEORETICAL

Pharmacokinetic Concept Behind HT-Combined Thermosensitive Liposome Delivery

The pharmacokinetics of the liposome-encapsulated drug and the free drug after intravenous administration of a liposome can be described using an anatomical tissue-perfusion model which is commonly used in the physiological pharmacokinetic model analysis (10–12) (Fig. 1). The administered liposome first enters the systemic circulation. Upon repeated circulation, it is degraded in the systemic blood or taken up by the reticuloendothelial system (RES) (13). However, little is taken up by the tumor and the normal tissue (organ) (14). Therefore, although a certain amount of the drug encapsulated in liposomes is taken up by the RES, the residual amount is eliminated from the systemic blood via drug release from the circulating liposomes. As a result, the free drug is distributed in the tumor or in the normal tissue (organ). The elimination kinetics are the same as those after administration of the drug as a solution.

If a liposome is thermosensitive and its administration is followed by tumor heating, drug release also occurs from the liposomes at or adjacent to the tumor when the liposomes travel concurrently with the blood through the heated tumor, and the released drug is distributed in the tumor, resulting in a high drug concentration in the tumor. This is the concept behind the HT-combined thermosensitive liposome delivery system (4).

Drug Targeting Index (DTI) as a Function of the Blood Drug Concentration

According to the theory derived from the physiological pharmacokinetic model analysis (11,12), the distribution of drug in the tumor after administration of a solution in the case that the tumor is assumed to be a noneliminating tissue can be expressed by the following differential equation:

$$V_T \, dT_{drug}/dt = Q\,(B_{in} - B_{out}) = Q\,(B_{drug} - T_{drug}/K) \quad (1)$$

where V_T, T_{drug}, Q, B_{in}, B_{out}, and K refer to the volume of the tumor, the concentration of drug in the tumor, the blood flow rate, the concentration of drug in the arterial blood which is equal to the concentration of drug in the systemic blood (B_{drug}), the concentration of drug in the venous outflow (effluent) blood from the tumor compartment, and the ratio of tissue concentration to the venous outflow blood concentration, respectively (Fig. 2).

The integration of Eq. (1) with respect to time, t, from zero to infinity gives

$$AUC(T_{drug}, sol) = K\,AUC(B_{drug}, sol) \quad (2)$$

where $AUC(T_{drug}, sol)$ and $AUC(B_{drug}, sol)$ refer to the area under the curve (AUC) of the concentration of drug in the tumor after solution administration and the AUC of the concentration of drug in the systemic blood after solution administration, respectively.

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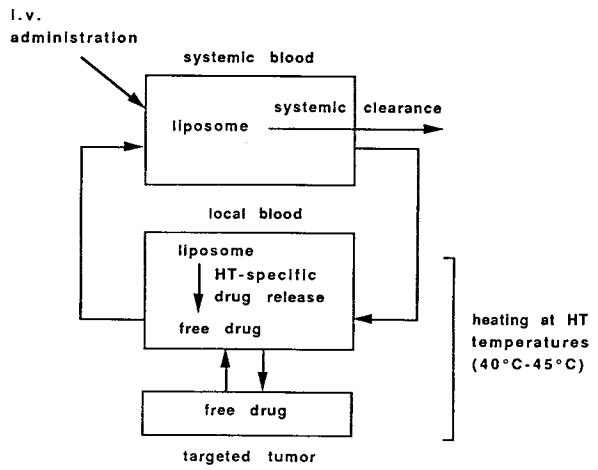

Fig. 1. Anatomical tissue perfusion model for the pharmacokinetics of liposome-encapsulated drug and free drug after administration of a thermosensitive liposome.

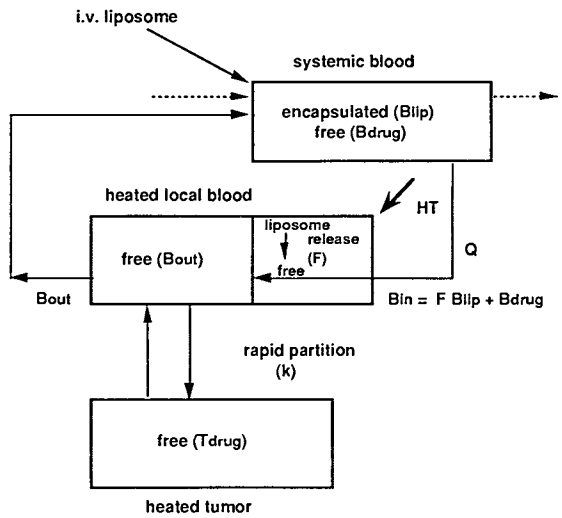

Fig. 2. Distribution of free drug in the tumor after administration of a solution with hyperthermia.

The distribution of drug in the tumor after administration of a thermosensitive liposome can also be expressed by a differential equation similar to Eq. (1). In this case, B_{in} is equal to the concentration of drug released from the liposomes in the blood at or adjacent to the heated tumor in a single pass plus the concentration of free drug which has already been released at the tumor or at the systemic circulation (the concentration very low), and the concentration of the drug released from the liposomes in response to heat is assumed to be the product of the concentration of liposome-encapsulated drug in the arterial blood which is equal to the concentration in the systemic blood (B_{lip}) and the fraction of the drug released from the liposomes in a single pass through the heated tumor (F) (Fig. 3).

Thus,

$$B_{in} = F\,B_{lip} + B_{drug} \quad \text{(except } HT \text{ time, } F = 0) \quad (3)$$

The integration of the differential equation gives

$$\mathrm{AUC}(T_{drug}, \mathrm{lip}) = K\,[F\,\mathrm{AUC}(B_{lip}, \mathrm{lip})_{t_s - t_f} + \mathrm{AUC}(B_{drug}, \mathrm{lip})] \quad (4)$$

where $\mathrm{AUC}(T_{drug}, \mathrm{lip})$, $\mathrm{AUC}(B_{drug}, \mathrm{lip})$, and $\mathrm{AUC}(B_{lip}, \mathrm{lip})_{t_s - t_f}$ refer to the AUC of the concentration of drug in the tumor after liposome administration, the AUC of the concentration of free drug in the systemic blood after liposome administration, and the AUC of the concentration of liposome-encapsulated drug in the systemic blood between the HT starting time (t_s) and the HT finishing time (t_f) after liposome administration, respectively.

If the liposomes are not taken up by the RES and V_T is sufficiently small so that almost all the drug encapsulated in the liposomes is released in the blood after repeated circulation, then $\mathrm{AUC}(B_{drug}, \mathrm{lip})$ is approximately equal to $\mathrm{AUC}(B_{drug}, \mathrm{sol})$. Then

$$\mathrm{AUC}(T_{drug}, \mathrm{lip}) = K\,[F\,\mathrm{AUC}(B_{lip}, \mathrm{lip})_{t_s - t_f} + \mathrm{AUC}(B_{drug}, \mathrm{sol})] \quad (5)$$

If we define $\mathrm{DTI} = \mathrm{AUC}(T_{drug}, \mathrm{lip})/\mathrm{AUC}(T_{drug}, \mathrm{sol})$, then from Eqs. (2) and (5),

$$\mathrm{DTI} = 1 + F\,\mathrm{AUC}(B_{lip}, \mathrm{lip})_{t_s - t_f}/\mathrm{AUC}(B_{drug}, \mathrm{sol}) \quad (6)$$

or using the total-body clearance of drug (Cl_T) in place of $\mathrm{AUC}(B_{free}, \mathrm{sol})$, $\mathrm{DTI} = 1 + F\,Cl_T\,\mathrm{AUC}(B_{lip}, \mathrm{lip})_{t_s - t_f}/\mathrm{dose}$.

Upper Limit of DTI as a Function of Blood Liposome Concentration

The aim of targeting delivery systems is to release the drug at the target site (8). Therefore, the concentration of the liposome-encapsulated drug or conjugate in the blood at the targeted site is a primary factor limiting the targeting specificity. The DTI value assuming complete drug release (F = 1) gives an upper limit of DTI (DTI_{max}), which is determined just by the concentration of the liposome during HT and the systemic clearance of the drug:

$$\mathrm{DTI}_{max} = 1 + \mathrm{AUC}(B_{lip}, \mathrm{lip})_{t_s - t_f}/\mathrm{AUC}(B_{drug}, \mathrm{sol}) = 1 + Cl_T\,\mathrm{AUC}(B_{lip}, \mathrm{lip})_{t_s - t_f}/\mathrm{dose} \quad (7)$$

Thus, the DTI_{max} value can be an indicator of how the tar-

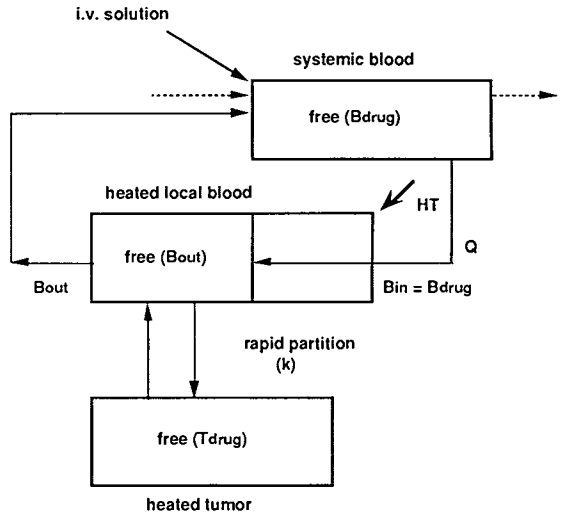

Fig. 3. Distribution of free drug in the tumor after administration of a thermosensitive liposome with hyperthermia.

Table I. The Liposomal Characteristics of LUV-1, LUV-2, LUV-3, and SUV-1 (4)

Liposome	Composition	Type	T_m (°C)[a]	CDDP content (μg/ml)	Heat sensitivity (%)[b]
LUV-1	DPPC[c]/DSPC[d] (9:1)	LUV[e]	41	250	81
LUV-2	DPPC/SMT[f] (9:1)	LUV	41	350	76
LUV-3	DSPC/SMT (10:1)	LUV	58	300	2
SUV-1	DPPC/DSPC (9:1)	SUV[g]	41	150	30

[a] Phase transition temperature.
[b] Determined by the means of the amount released (%) after incubation of the saline-diluted liposomes (1:10) at temperatures from 40 to 47°C for 15 min.
[c] Dipalmitoylphosphatidylcholine (DPPC).
[d] Distearoylphosphatidylcholine.
[e] Prepared by reverse-phase evaporation method. Particle size is approximately 0.2 μm.
[f] Sodium stearoylmethyltaurate.
[g] Prepared by sonicating multilamellar vesicles. Particle size is less than 0.1 μm.

geting specificity of the present targeting delivery system is limited by the systemic clearance of the liposome or by the systemic clearance of the drug.

F as a Function of DTI and DTI_{max}

Combining Eqs. (6) and (7) gives an equation for *F*:

$$F = (DTI - 1)/(DTI_{max} - 1) \quad (8)$$

This equation enables us to estimate *F* when we know the values of DTI_{max} and DTI.

MATERIALS AND METHODS

Experimental Data Used in Calculation of *F* and DTI_{max}

The *F* and DTI_{max} for four different types of thermosensitive liposomes containing CDDP (LUV-1, LUV-2, LUV-3, and SUV-1; Table I) (4) were estimated using the present method and the following reported data on blood CDDP levels and tumor CDDP levels (DTI values) in mice (4). The liposomes were administered to mice bearing Meth-A-fibrosarcoma (dose, 40 μg/mouse), and HT was conducted using a heater (heater temperature of 47°C) at 15 min after liposome administration and was continued for 30 min (T_s = 15 min; t_f = 45 min). The blood Pt levels and tumor Pt levels after administration were examined by sacrificing the mice at appropriate time intervals. The DTI values (Table II) were calculated using the AUC of the tumor Pt levels from 0 to 4 hr, assuming

$$DTI = AUC(T_{drug}, lip)/AUC(T_{drug}, sol) = AUC(T_{drug}, lip)_{0\text{–}4\ hr}/AUC(T_{drug}, sol)_{0\text{–}4\ hr}$$

Calculation of *F* and DTI_{max}

First, the DTI_{max} values were calculated using Eq. (7) and the AUC of the blood total Pt levels (approximately equal to the encapsulated Pt levels) from 15 to 45 min. Then the *F* values were calculated using Eq. (8) and the DTI values and DTI_{max} which were obtained above.

RESULTS AND DISCUSSION

Upper Limit of Targeting Index

The values of DTI_{max} after administration of SUV-1, LUV-1, LUV-2, and LUV-3 with HT treatment are listed in Table III. The values after administration of LUV-1 and SUV-1 are about 6. The DTI_{max} value after administration of LUV-2 is less than half, and that after administration of LUV-3 is intermediate.

As described under Theoretical, DTI_{max} can be an indicator of how the targeting specificity of the delivery system is limited by the systemic clearance of the liposomes. Assuming that the liposomes are not eliminated from the systemic circulation, the resulting DTI_{max} (about 10) indicates the upper limit of DTI for the present drug delivery system:

$$DTI_{max} = 1 + Cl_T\,(t_f - t_s)/\text{blood volume} = 10$$
(Cl_T = 20 ml/hr; $t_f - t_s$ = 0.5 hr; blood volume = 1 ml) (4).

The values of DTI_{max} for LUV-1 and SUV-1 are about 60% of this limit, whereas the value of DTI_{max} for LUV-2 is only about 20%. The small DTI_{max} obtained with LUV-2 is due to the large systemic clearance of the liposomes, and thus, a small DTI in spite of high heat sensitivity for LUV-2 is attributed to the large systemic clearance of the liposomes.

Table II. The Values of DTI After Administration of SUV-1, LUV-1, LUV-2, or LUV-3 With or Without HT Treatment (4)

Liposome	DTI
SUV-1	0.97
SUV-1 + HT	2.66
LUV-1	0.99
LUV-1 + HT	4.63
LUV-2	0.84
LUV-2 + HT	2.55
LUV-3	0.15
LUV-3 + HT	0.24

Table III. The Values of DTI_{max} After Administration of SUV-1, LUV-1, LUV-2, or LUV-3 With HT Treatment

Liposome	DTI_{max}
SUV-1 + HT	5.83
LUV-1 + HT	6.08
LUV-2 + HT	2.32
LUV-3 + HT	3.57

Rate of Heat-Specific Drug Release

Table IV shows the F values after administration of SUV-1, LUV-1, LUV-2, and LUV-3 with HT treatment. The F values after administration of LUV-1 and LUV-2 with HT treatment were 0.71 and 1.17, respectively. In contrast, the F values after administration of these liposomes without HT treatment were 0.08 and −0.12, respectively. The F value after administration of SUV-1 with HT treatment was 0.34. In spite of some error, the values obtained with the thermosensitive liposomes seem to reflect their *in vitro* heat sensitivity (Table I). The results suggest that unlike the small DTI obtained with LUV-2, the small DTI with SUV-1 can be attributed to the small release rate. The F value after administration of LUV-3 with or without HT was negative with a large absolute value (approximately −0.3). Theoretically F must not be negative. The value obtained with LUV-3 may indicate an error beyond that of normal data variation. It was assumed that liposomes are not to be taken up by the RES, but actually about 20% of the dose after administration of LUV-1 and LUV-2 and about 50% of the dose after administration of LUV-3 were taken up by the RES (4). If we assume that the fraction of the dose taken up by the RES is α, then the fraction of the dose released in the systemic circulation is $1 - \alpha$, and Eq. (6) can be rewritten as

$$\mathrm{DTI} - (1 - \alpha) = F\,\mathrm{AUC}(B_{\mathrm{lip}}, \mathrm{lip})_{t_s - t_f} / \mathrm{AUC}(B_{\mathrm{free}}, \mathrm{sol})$$

F can be recalculated using this equation. The F values for LUV-1 + HT and LUV-1 alone (α = 0.2) are 0.75 and 0.04, respectively, and the F value for LUV-2 + HT and LUV-2 alone (α = 0.2) are 1.32 and 0.03, respectively. These values are not much different from those above. The F values for LUV-3 + HT and LUV-3 alone (α = 0.5) are −0.1 and −0.13, respectively, and these are more realistic than those above in light of the *in vitro* release characteristics.

Table IV. The Values of F After Administration of SUV-1, LUV-1, LUV-2, or LUV-3 With or Without HT Treatment

Liposome	F
SUV-1	0.0
SUV-1 + HT	0.34
LUV-1	0.08
LUV-1 + HT	0.71
LUV-2	−0.12
LUV-2 + HT	1.17
LUV-3	−0.33
LUV-3 + HT	−0.29

Correlation Between F and *in Vitro* Heat Sensitivity

In the theory, F is assumed to be constant during HT. However, the fraction released in the first pass is likely to be larger than the fraction released from the same liposomes in each additional pass. Our earlier study showed thermosensitive LUV liposomes release encapsulated carboxyfluorescein within a few seconds (3). Therefore, highly heat-sensitive liposomes are supposed to release almost all the drug in the first pass. This, however, does not contradict the present theory because, with highly heat-sensitive liposomes, B_{lip} in Eq. (3) represents the concentration of only the liposomes which are going to experience the first pass through the tumor.

Temperature distribution in the tumor is another factor to be considered in estimating theoretically the extent of heat-specific drug release. If the temperature distribution in the tumor is not uniform, the fraction of drug released will be different at different sites in the tumor. Our study on tumor heating with a PTC heater (heater temperature of 47°C) revealed that the temperature shows a linear gradient in the direction of the depth of the tumor (4). The temperature at the surface of the tumor was almost the same as the heater temperature, while the temperature at the bottom was about 40°C. Therefore, the F value represents the average fraction of drug release. Thus, we can compare the values of F with the mean of the *in vitro* drug release rates at the temperatures from 40 to 47°C as shown under Materials and Methods, and these are in good agreement. This result indicates not only that the present method is reliable but also that the phase transition of the liposomes in response to the heat occurs quickly enough for complete drug release when the liposomes pass through the heated tumor (3).

Parameter Analysis

The response of F, $\mathrm{AUC}(B_{\mathrm{lip}}, \mathrm{lip})_{t_s - t_f}$ and Cl_T to DTI can be seen in Fig. 4. A larger DTI is achieved with a larger area A or a smaller area B and, accordingly, with a larger

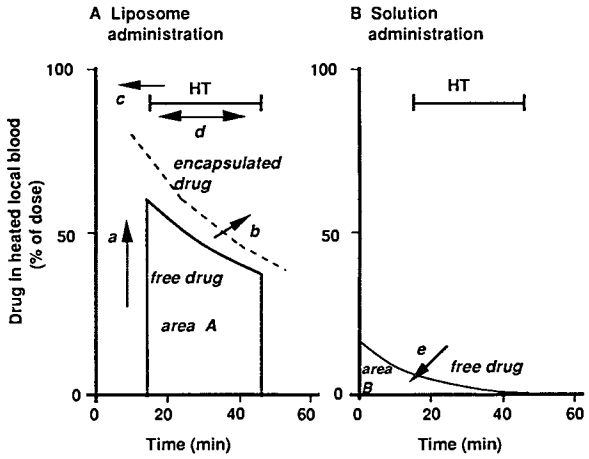

Fig. 4. Hypothetical drug concentration in the blood at or adjacent to the targeted tumor (A, liposome administration; B, solution administration) and the response of the heat-specific drug release (F), the AUC of the blood liposome level during hyperthermia $[\mathrm{AUC}(B_{\mathrm{lip}}, \mathrm{lip})_{t_s - t_f}]$ and the systemic clearance of a drug (CL_T) to the drug targeting index (DTI).

drug release rate (arrow a), a smaller systemic clearance of the liposomes (arrow b), an earlier (arrow c) or longer (arrow d) HT period, or a larger systemic clearance of the drug (arrow e). Therefore, it is important not only to prepare a highly heat-sensitive liposome with a small systemic clearance, but also to choose a drug with a large systemic clearance. In this respect, CDDP is a suitable drug.

CONCLUSION

The present method using pharmacokinetic data obtained after administration of a thermosensitive liposome enabled us to evaluate F and DTI_{max}. The value of DTI_{max} after administration of LUV-1 and SUV-1 was about 6. The values of F (0.71 for LUV-1 and 0.34 for SUV-1) are in good agreement with the *in vitro* drug release characteristic. This result confirms that the encapsulation of CDDP in a thermosensitive LUV liposome which is highly heat sensitive and has a small systemic clearance is preferable for the present drug delivery system.

NOMENCLATURE

CDDP	Cisplatin
HT	Hyperthermia
F	Fraction of drug released at the heated tumor when liposomes pass concurrently with blood through heated tumor
DTI	Drug targeting index
DTI_{max}	Upper limit of drug targeting index assuming complete drug release (maximum drug targeting index)
RES	Reticuloendothelial system
AUC	Area under a curve (μg hr/ml)
V_T	Volume of the tumor (ml)
T_{drug}	Concentration of drug in the tumor (μg hr/ml)
Q	Blood flow rate (ml/hr)
B_{drug}	Concentration of drug in the systemic blood (μg/ml)
B_{in}	Concentration of drug in the arterial blood which is equal to B_{drug} (μg/ml)
B_{out}	Concentration of drug in the venous outflow (effluent) blood from the tumor compartment (μg/ml)
K	Ratio of tissue concentration to the venous outflow blood concentration
AUC(T_{drug}, sol)	AUC of the concentration of drug in the tumor after solution administration (μg hr/ml)
AUC(B_{drug}, sol)	AUC of the concentration of drug in the systemic blood after solution administration (μg hr/ml)
AUC (T_{drug}, lip)	AUC of the concentration of drug in the tumor after liposome administration (μg hr/ml)
t_s	HT starting time after liposome administration (hr)
t_f	HT finishing time after liposome administration (hr)
AUC(B_{lip}, lip)$_{t_s - t_f}$	AUC of the concentration of liposome-encapsulated drug in the systemic blood between t_s and t_f (μg hr/ml)
Cl_T	Total-body clearance of drug (ml/hr)

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