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Pharmacokinetics and tissue distribution of adriamycin and adriamycinol after intravenous administration of adriamycin-loaded neutral proliposomes to rats

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Abstract

The pharmacokinetics and tissue distribution of adriamycin (ADM) and its metabolite, adriamycinol were investigated after intravenous (i.v.) injection of free ADM (treatment I), ADM-loaded neutral proliposomes (treatment II) and empty neutral proliposomes mixed manually with free ADM (treatment III), 16 mg per kg as free ADM, to rats, using HPLC assay. After 1 min i.v. infusion, the plasma concentrations of ADM (Cp), area under the plasma concentration-time curve (AUC, 159 vs 351 μg min ml^{-1}), terminal half-life ($t_{1/2}$, 65.2 vs 633 min), mean residence time (MRT, 27.5 vs 541 min) and apparent volume of distribution at steady state (V_{ss}, 2480 vs 22800 ml kg^{-1}) were significantly higher, however, the total body clearance (CL, 101 vs 45.6 ml min^{-1} kg^{-1}), renal clearance (CL_R, 9.81 vs 2.27 ml min^{-1} kg^{-1}), nonrenal clearance (CL_{NR}, 90.8 vs 42.8 ml min^{-1} kg^{-1}) and the amount of ADM excreted in urine (X_u, 496 vs 297 μg) were significantly lower in treatment II than the values from treatment I. This could be due to the fact that some of the ADM-loaded liposomes (formed by hydration of ADM-loaded proliposomes) were entrapped in tissues and the rest were in plasma (higher MRT and V_{ss} in treatment II), and ADM was slowly released from ADM-loaded liposomes (higher $t_{1/2}$ in treatment II). In the present HPLC assay, the concentrations of ADM represent the sum of free ADM and ADM loaded in liposomes (higher C_p and AUC, slower CL in treatment II). After 1 min i.v. infusion, some pharmacokinetic parameters, such as $t_{1/2}$, MRT and X_u were significantly different between treatments I and III, but not as distinct between treatments I and II. 30 min after i.v. infusion, the amount of ADM remaining in lymph node and the lymph node to plasma ratio were significantly higher in treatment II than in treatment I. This suggested that the i.v. administration of ADM-loaded proliposomes might have a better lymph node targeting ability than free ADM. The mean amount of ADM loaded in ADM-loaded proliposomes was 4.09 mg per g proliposome.

Keywords: Adriamycin-loaded neutral proliposome; Adriamycin; Adriamycinol; Pharmacokinetics; Tissue distribution

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1. Introduction

It has been reported that the plasma concentrations of ADM were higher (Van Hoesel et al., 1984; Mayer and Tai, 1989; Gabizon et al., 1989; Gabizon, 1992), the cardiac tissue uptake of ADM was less (Van Hoesel et al., 1984; Mayer and Tai, 1989), the cardiotoxicity of ADM was lower (Van Hoesel et al., 1984), and the therapeutic effect of ADM was stronger (Van Hoesel et al., 1984; Mayer and Tai, 1989; Gabizon, 1992) or the tumor uptake of ADM was greater (Gabizon, 1992) after administration of ADM-encapsulated liposomes having different charges and different lipid compositions to mice or rats than those of free ADM. However, liposomes have the following disadvantages when stored in dispersed aqueous system (Frøkjaer et al., 1984): phospholipid hydrolysis, phospholipid oxidation, decomposition of encapsulated drug, and sedimentation, aggregation and fusion of liposomes. Furthermore, problems encountered in sterilization and large-scale production of liposomes remain to be solved (Frøkjaer et al., 1984). Payne et al. (1986a,b) introduced proliposomes in order to overcome previously mentioned disadvantages of liposomes. Proliposomes are composed of water-soluble porous powder as a carrier to load phospholipids and drugs dissolved in organic solvent. Proliposomes can be stored as sterilized in the dry state and disperse/dissolve to form an isotonic multilamellar liposomal suspension suitable for administration either intravenously or by other routes by adding water before use (Payne et al., 1986b). The pharmacological effect or stability of amphotericin B (Payne et al., 1986a,b), indomethacin (Katare et al., 1991a) and nonsteroidal anti-inflammatory analgesics (Katare et al., 1991b) was reported to be maintained for a long period of time when the drugs were loaded in proliposomes. Recently, it has been reported from our laboratory that almost 'constant' plasma concentrations of propranolol were maintained for a longer period of time when the drug-loaded proliposomes were administered intranasally to rats than those of free propranolol (Ahn et al., 1995b). The plasma concentrations of methotrexate (MTX) were higher, and tissue-to-plasma ratios of MTX in kidney and stomach were significantly lower when MTX-loaded proliposomes were injected intravenously after hydration of the proliposomes to rats than free MTX at a dose of 8 mg kg^{-1} as free MTX (Park et al., 1994).

The purpose of this paper is to report the pharmacokinetics and tissue distribution of ADM and its metabolite, adriamycinol after intravenous (i.v.) administration of free ADM, ADM-loaded neutral proliposomes and empty neutral proliposomes mixed manually with free ADM to rats.

2. Materials and methods

2.1. Materials

Egg lecithin and desipramine HCl were purchased from Sigma Chemical Co. (St. Louis, MO) and sorbitol was a product of Junsei Chemical Co. (Tokyo, Japan). ADM (both free ADM powder and its HCl salt for i.v. injection, 10 mg per 5 ml) were kindly supplied by Central Research Lab. of Boryung Pharmaceutical Co. (Kun Po-Si, South Korea). Adriamycinol was kindly donated by Adria Labs (Dublin, OH). Daunorubicin, the internal standard in HPLC assay of ADM, was a gift from Dong-A Research Lab. of Dong-A Pharmaceutical Co. (Yongin, South Korea). Other chemicals were of reagent grade or HPLC grade, and used without further purification.

2.2. Preparation of ADM-loaded neutral proliposomes and empty neutral proliposomes

The preparation of ADM-loaded proliposomes is similar to that of MTX-loaded proliposomes (Park et al., 1994). 1 g of egg lecithin and 50 mg of free ADM powder were dissolved in a mixture of organic solvents (chloroform-methanol, 20:80, v/v) at 40° C. Since egg lecithin was employed in the present preparation of proliposomes, neutral liposomes would form when the proliposomes were hydrated before use. Empty proliposomes were similarly prepared using 1 g of egg lecithin without ADM.

2.3. Measurement of the amount of ADM loaded in ADM-loaded neutral proliposomes

To determine the amount of ADM loaded in ADM-loaded proliposomes, the ADM-loaded proliposomes were first hydrated with distilled water to make 4.42 mg per ml proliposome suspension, and then 450 μl of acetonitrile was added to 50 μl of the suspension. After vortex-mixing and centrifugation, 50 μl of the supernatant was injected directly onto the HPLC column.

2.4. Hydration of ADM-loaded neutral proliposomes and empty neutral proliposomes

ADM-loaded proliposomes and empty proliposomes used in the present studies were hydrated immediately before use. Injectable distilled water was added to ADM-loaded proliposomes, followed by manual shaking twice for 1 min with 15-min intervals for complete hydration to give 2 mg ml^{-1} as free ADM. Empty proliposomes were also similarly hydrated.

2.5. Particle size determination of the liposomes formed after hydration of ADM-loaded neutral proliposomes and empty neutral proliposomes

ADM-loaded proliposomes and empty proliposomes were hydrated following the above procedures. The particle size of the resultant liposomes (neutral multilamellar vesicles, MLV) was determined using a particle size analyzer (LPA-3100, Phtal Ostuka Electric Co., Japan).

2.6. Pretreatment of rats

36 healthy male Sprague-Dawley rats (210–330 g, Laboratory Animal Center, Seoul National University, Seoul, South Korea) were employed in this study. The carotid artery and the jugular vein were cannulated with polyethylene tubing (PE-60, Clay Adams, Parsippany, NJ) under light ether anesthesia. Both cannulae were exteriorized to the dorsal side of the neck and terminated with long silastic tubing (Dow Corning Co., Midland, MI). Both silastic tubings were covered with wire to allow free movement of the rat. The exposed areas were surgically sutured. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Co., Seoul, South Korea), and allowed to recover from anesthesia for 4–5 h before the study.

2.7. Intravenous infusion studies

Free ADM (i.v. solution as a HCl salt, 10 mg per 5 ml, treatment I, $n = 7$), ADM-loaded proliposomes (hydrated with injectable distilled water before use to give 2 mg ml^{-1} as free ADM, treatment II, $n = 7$) or empty proliposomes mixed manually with free ADM (hydrated with injectable distilled water before use and mixed manually with free ADM to provide 2 mg ml^{-1} as free ADM, treatment III, $n = 7$), equivalent to 16 mg per kg as free ADM were administered via the jugular vein by i.v. infusion in 1 min to rats. Total injection volume was approx. 2 ml. Blood samples (0.12–0.22 ml) were collected via the carotid artery at 0 (to serve as a control), 1 (at the end of infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 600 and 720 min for treatment I, and at 0, 1, 5, 15, 30, 60, 120, 180, 240, 360, 480, 720, 1080, 1440, 2160 and 2880 min for treatments II and III. 0.25 ml of heparinized 0.9% NaCl injectable solution (20 U ml^{-1}) was used to flush the cannula just after each blood sampling to prevent blood from clotting. Blood samples were centrifuged immediately to reduce the 'blood storage effect' of plasma concentrations of ADM (Lee and Chiou, 1989), and 50–100 μl of each plasma was stored in a freezer prior to the HPLC analysis of ADM and adriamycinol. At the end of 8, 24 and 48 h after i.v. injection, the metabolic cage was rinsed with 10 ml of distilled water and the rinsings were combined with urine. After measuring the exact volume of the combined urine, two 0.1-ml aliquots of the combined urine were frozen prior to HPLC analysis of ADM and adriamycinol. At the end of 48 h after i.v. injection, the whole gastrointestinal (GI) tract (including its contents and feces) was removed, transferred to a beaker containing 40 ml of 0.9% NaCl injectable solution and cut into small pieces using scissors. After stirring with a glass rod for

10 min, two 0.1 ml aliquots of the supernatant were collected from the beaker and stored in the freezer prior to the HPLC analysis of ADM and adriamycinol.

2.8. Tissue distribution studies after intravenous infusion

Free ADM (treatment IV, $n = 5$), ADM-loaded proliposomes (treatment V, $n = 5$) and empty proliposomes mixed manually with free ADM (treatment VI, $n = 5$), equivalent to 16 mg per kg as free ADM, were similarly infused in 1 min via the jugular vein of rats. After 30 min from the start of infusion, as much blood as possible was collected through the carotid artery and each rat was exsanguinated. After centrifugation of the blood, plasma was added to 4 volumes of 0.9% NaCl injectable solution and vortex-centrifuged. Approx. 1 g of heart, lung, spleen, brain, liver, kidney, stomach, small intestine, large intestine, mesentery, fat or thigh muscle was quickly removed, rinsed with cold 0.9% NaCl injectable solution, minced and homogenized with 4 volumes of 0.9% NaCl injectable solution in a tissue homogenizer (Ultra-Turrax T 25, Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany), and then centrifuged immediately. Two 0.1-ml aliquots of plasma or supernatant of tissue homogenate were stored in the freezer prior to the HPLC analysis of ADM and adriamycinol. The ipsilateral iliac lymph nodes were also removed, rinsed, minced and stood overnight at room temperature with 4 volumes of 0.9% NaCl injectable solution. After centrifugation, the supernatant was frozen prior to the HPLC analysis of ADM and adriamycinol.

2.9. HPLC analysis

The concentrations of ADM and adriamycinol in plasma, urine and tissues (or organs) were determined by modification of the reported HPLC method (Bots et al., 1983). The mobile phase consisted of acetonitrile-distilled water-0.1 M H_3PO_4 (31:61:8, v/v) containing 20 μg of desipramine HCl per ml (pH 2.3) and the flow rate was 0.33 ml min^{-1}. Fluorescence detection was employed with an excitation wavelength of 470 nm and an emission wavelength of 565 nm. Quantitation of ADM or adriamycinol was based on peak height ratios using the structural analogue, daunorubicin, as the internal standard. To 50–100 μl of biological sample, 50 μl of internal standard (daunorubicin, 3 μg ml^{-1}, dissolved in methanol), 250–300 μl of methanol and 1 ml of ethyl acetate were added. The mixture was vortexed for 2 min and centrifuged at 10000 rpm for 3 min. 900 μl of the organic layer was transferred and evaporated to dryness under nitrogen gas. 100 μl of Britton-Robinson buffer-methanol (1:2, v/v) was added to reconstitute the residue. After vortex-centrifugation, 80–100 μl of the supernatant was injected directly onto the column. The detection limits for ADM and adriamycinol in plasma were 0.032 and 0.038 μg ml^{-1}, respectively, and the corresponding values in urine were 0.028 and 0.033 μg ml^{-1}.

The HPLC system consisted of a Model 7125 injector (Rheodyne, Cotati, CA), a Model 400 solvent delivery system pump (Applied Biosystems, San Jose, CA.), a reversed-phase column (220 × 2.1 mm i.d., 5 μm, Applied Biosystems), a fluorescence detector (FS-980, Applied Biosystem, Foster, CA) and a Model 1200 recorder (Linear, Reno, NV).

2.10. Pharmacokinetic analysis

The total area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated according to the trapezoidal rule-extrapolation method (Kim et al., 1993); this method employed the logarithmic trapezoidal rule for the calculation of area during the declining plasma-level phase (Chiou, 1978) and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

A standard method (Gibaldi and Perrier, 1982) was used to calculate the following pharmacokinetic parameters; the time-averaged total body clearance (CL), area under the first moment of the plasma concentration-time curve (AUMC),

mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}), and time-averaged renal (CL_R) and nonrenal (CL_{NR}) clearances (Kim et al., 1993).

The mean values of each clearance, V_{ss} and half-life were calculated by the harmonic mean method (Chiou, 1979).

2.11. Statistical analysis

Levels of statistical significance were assessed using the analysis of variance (ANOVA) test between the two means for unpaired data. Significant differences were judged as a p value of less than 0.05. All results are expressed as mean ± standard deviation.

3. Results and discussion

3.1. Measurement of the amount of ADM loaded in ADM-loaded neutral proliposomes and determination of particle size of the liposomes formed after hydration of ADM-loaded neutral proliposomes and empty neutral proliposomes

The amount of ADM loaded in ADM-loaded proliposomes was 4.09 ± 0.572 mg per g of proliposome ($n = 4$). It has been reported that a liposomal suspension was immediately formed when propranolol-loaded (Ahn et al., 1995a) and MTX-loaded (Park et al., 1994) proliposomes were hydrated with water. In the present study, a liposomal suspension was also formed immediately when ADM-loaded proliposomes or empty proliposomes were hydrated with water. The liposomal suspension formed from ADM-loaded proliposomes or empty proliposomes was found to be neutral multilamellar vesicles under transmission electron microscopy. The mean particle sizes of the liposomes formed after hydration of ADM-loaded proliposomes and empty proliposomes were 785 and 268 nm, respectively.

3.2. Pharmacokinetics of ADM after i.v. administration

Fig. 1 shows the mean arterial plasma concentration-time profiles of ADM in treatments I–III, and the relevant pharmacokinetic parameters are listed in Table 1. After i.v. infusion of ADM, the plasma concentrations of ADM declined polyexponentially in all rats studied, and the levels decayed rapidly with a mean terminal half-life of 65.2 min in treatment I. The $t_{1/2}$, 65.2 min, was considerably shorter than the reported 38 h in rats (Balis, 1986), and this could be due to our HPLC assay sensitivity. However, the plasma levels of ADM declined slowly in treatments II and III: they were detected up to approx. 24 and 8 h in treatments II and III, respectively. The plasma concentrations of ADM were also detected up to 24 h after i.v. administration of ADM-loaded liposomes in humans (Rahman et al., 1990; Gabizon et al., 1991). The slow decay of plasma ADM in treatment II could be due to the slow release of ADM from ADM-loaded liposomes (formed from proliposome hydration) entrapped in tissues (will be discussed later in a tissue distribution study; Table 2) or present in plasma. In the present HPLC assay, the plasma concentrations of ADM represent both free ADM and ADM loaded in proliposomes, and plasma levels of free ADM rapidly declined and was undetected from 3 h onwards after i.v. infusion (treatment I). Therefore, the plasma concentrations of ADM in treatment II suggest that at early times after injection, most of ADM is in liposomes, whereas a considerable amount of free ADM would be present at later times (Fig. 1). It should be noted that the in vitro release of ADM from free ADM, ADM-loaded neutral proliposomes and empty neutral proliposomes mixed manually with free ADM did not seem to be any different (data not shown) after incubation in rat plasma using a dialysis bag (Park et al., 1994).

The slow decay of plasma concentrations of ADM in treatment III could be rationalized by the following explanations. It had been reported that a lipid (egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine) dose of 100 mg per kg was high enough to saturate the reticuloendothelial system (RES) of rats (Hwang, 1987). The dose of lipid administered was approx. 354 mg per kg of rat in treatment III (lipid loss during proliposome preparation was assumed to be negligible).

Therefore, the dose of lipid in empty proliposomes in treatment III might have been high enough to saturate the RES of rats. Then, some of the free ADM might have become entrapped in empty liposomes saturated in RES, which then are slowly released, while the rest of the free ADM in treatment III might have been excreted and/or metabolized, like the free ADM in treatment I. Therefore, the amount of ADM entrapped in RES (treatment III) should be less than those loaded in proliposomes (treatment II). As a result, the plasma concentrations of ADM at the terminal phase in treatment III were higher than those in treatment I but lower than in treatment II (Fig. 1). It should be noted that the partitioning of ADM into the empty liposomes after manual mixing of empty proliposomes and free ADM (treatment III) may at least partly contribute to the entrapment of free ADM into the liposomes; the apparent partition coefficient of ADM between 1-octanol and Tris buffer at pH 7.0 with constant ionic strength ($I = 0.1$) was reported to be 0.52 after shaking for 15 h at room temperature (Vigevani and Williamson, 1980). Plasma concentrations of ADM were not detected after approx. 3, 24 and 8 h in treatments I–III, respectively, and this might be due to our HPLC assay sensitivity.

The mean arterial plasma concentration-time profiles of adriamycinol in treatments I–III are also shown in Fig. 1. Adriamycinol was detected in plasma up to 1, 30 and 15 min after i.v. administrations in treatments I–III, respectively. The plasma concentrations of adriamycinol were the highest in treatment II.

The ADM loaded in liposomes may be neither excreted via the kidney nor metabolized in the liver, but slowly released from the ADM-loaded liposomes entrapped in tissues and/or present in plasma. In the present HPLC assay, the concentrations of ADM in plasma represent both free ADM and ADM loaded in liposomes. In treatment II, some of the ADM-loaded liposomes could be entrapped in tissues (or organs) after i.v. administration and ADM would be released slowly from the liposomes at later time. There-

Table 1
Mean (± standard deviation) pharmacokinetic parameters of adriamycin (ADM) or adriamycinol after 1 min intravenous infusion of free ADM (treatment I), ADM-loaded proliposomes (treatment II) and empty proliposomes mixed manually with free ADM (treatment III), 16 mg kg^{-1} as free ADM to rats

Parameters	Treatment I ($n = 7$)	Treatment II ($n = 7$)	Treatment III ($n = 7$)
Body weight (g)	305 ± 42.1	281 ± 16.0	266 ± 5.35
AUC (μg min ml^{-1})	159 ± 33.4	351 ± 92.1 [e]	194 ± 45.7
$t_{1/2}$ (min)	65.2 ± 32.0	633 ± 371 [d]	335 ± 197 [d]
MRT (min)	27.5 ± 12.2	541 ± 235 [e]	180 ± 158 [c]
V_{SS} (ml kg^{-1})	2480 ± 1510	22800 ± 8040 [e]	8840 ± 11900
CL (ml min^{-1} kg^{-1})	101 ± 22.5	45.6 ± 12.0 [e]	82.3 ± 23.2
CL_R (ml min^{-1} kg^{-1})	9.81 ± 3.05	2.27 ± 1.63 [e]	2.61 ± 4.44 [d]
CL_{NR} (ml min^{-1} kg^{-1})	90.8 ± 20.6	42.8 ± 11.7 [e]	78.7 ± 20.3
X_u (μg) [a]			
As adriamycin	496 ± 133	297 ± 112 [c]	214 ± 157 [d]
	(10.0 ± 1.82)	(6.53 ± 2.18 [d])	(5.02 ± 3.64 [d])
As adriamycinol [b]	32.0 ± 8.62	32.0 ± 16.1	10.3 ± 1.46 [d]
	(0.651 ± 0.121)	(0.709 ± 0.361)	(0.242 ± 0.0347 [d])
% of i.v. dose recovered from whole GI tract			
As adriamycin	6.09 ± 2.31	5.72 ± 3.14	5.90 ± 4.46
As adriamycinol [b]	1.09 ± 1.20	0.815 ± 0.770	0.434 ± 0.191

Numbers in parentheses represent the percentages of dose excreted in urine expressed in terms of adriamycin.
[a] 0–48 h for treatments I–III.
[b] Expressed in terms of adriamycin.
[c] $p < 0.05$, [d] $p < 0.01$ and [e] $p < 0.001$ when compared with the values in treatment I.

fore, the plasma concentrations of ADM in treatment II were significantly higher than those in treatment I except for just after i.v. injection (Fig. 1), and this resulted in a significantly higher AUC in treatment II (159 vs 351 μg min ml^{-1}) than in treatment I (Table 1). Higher plasma concentrations of ADM were also reported when ADM-loaded liposomes having different charges and different lipid compositions were administered (Van Hoesel et al., 1984; Gabizon, 1992) rather than just free ADM. As expected, CL (101 vs 45.6 ml min^{-1} kg^{-1}) was significantly slower in treatment II (Table 1). The significantly slower CL in treatment II than in treatment I could be due to both significantly slower CL_{NR} (90.8 vs 42.8 ml min^{-1} kg^{-1}) and CL_R (9.81 vs 2.27 ml min^{-1} kg^{-1}) (Table 1). As stated earlier, some of the ADM-loaded liposomes were entrapped in tissues while the rest were in plasma, and since ADM was released slowly from ADM-loaded liposomes, the MRT (27.5 vs 541 min) and V_{ss} (2480 vs 22800 ml kg^{-1}) were significantly higher in treatment II than in treatment I (Table 1). The MRT and V_{ss} of MTX were also significantly greater when MTX-loaded neutral (Bae et al., 1993), negatively charged (Jeong et al., 1994) and positively charged liposomes (Kim et al., 1995), and neutral proliposomes (Park et al., 1994) were injected to rats, and when MTX-rabbit serum albumin conjugates (some of the conjugates were

Table 2
Mean (± standard deviation) amount of adriamycin (ADM) remaining in g tissue (μg per g tissue) at 30 min after intravenous infusion of free ADM (treatment IV), ADM-loaded proliposomes (treatment V) and empty proliposomes mixed manually with free ADM (treatment VI), 16 mg kg^{-1} as free ADM to rats

Tissues	Treatment IV ($n = 5$)	Treatment V ($n = 5$)	Treatment VI ($n = 5$)
Plasma	0.189 ± 0.0641	0.364 ± 0.116 [a]	0.331 ± 0.0478 [b]
Spleen	3.85 ± 1.41	5.03 ± 1.00	4.46 ± 1.00
	(22.5 ± 12.2)	(14.9 ± 5.36)	(13.8 ± 3.91)
Kidney	1.97 ± 1.06	1.02 ± 0.125	0.636 ± 0.0760 [a]
	(11.0 ± 6.57)	(3.16 ± 1.51 [a])	(1.97 ± 0.444 [a])
Stomach	0.515 ± 0.0815	0.840 ± 0.287 [a]	0.479 ± 0.168
	(2.96 ± 1.05)	(2.71 ± 1.67)	(1.55 ± 0.655)
Small intestine	2.60 ± 2.56	2.00 ± 1.46	2.19 ± 1.01
	(12.0 ± 8.30)	(5.32 ± 3.26)	(6.94 ± 3.92)
Large intestine	0.191 ± 0.0721	0.218 ± 0.121	0.148 ± 0.0691
	(1.18 ± 0.790)	(0.718 ± 0.580)	(0.459 ± 0.182)
Lung	0.866 ± 0.158	1.59 ± 0.276 [b]	0.915 ± 0.233
	(4.84 ± 1.24)	(4.93 ± 2.30)	(2.86 ± 1.01 [a])
Lymph nodes	0.190 ± 0.0822	0.951 ± 0.595 [a]	0.607 ± 0.235 [b]
	(1.06 ± 0.601)	(2.73 ± 1.42 [a])	(1.89 ± 0.805)
Liver	1.98 ± 0.997	1.63 ± 0.322	1.22 ± 0.318
	(10.4 ± 2.79)	(4.99 ± 2.20 [b])	(3.84 ± 1.53 [b])
Brain	U.D.	U.D.	U.D.
Heart	1.17 ± 1.42	1.51 ± 0.42	0.695 ± 0.105
	(7.41 ± 10.3)	(4.51 ± 2.08)	(2.12 ± 0.301)
Fat	0.206 ± 0.126	0.166 ± 0.0723	0.0722 ± 0.0158 [a]
	(1.43 ± 1.13)	(0.586 ± 0.474)	(0.223 ± 0.0647 [a])
Muscle	0.423 ± 0.200	0.557 ± 0.116	0.504 ± 0.0738
	(2.19 ± 0.866)	(1.73 ± 0.945)	(1.56 ± 0.406)
Mesentery	0.565 ± 0.383	0.444 ± 0.677	0.107 ± 0.0351 [a]
	(3.41 ± 2.69)	(1.06 ± 1.48)	(0.321 ± 0.0810 [a])

Numbers in parentheses represent mean (± standard deviation) values of tissue to plasma ratio (T/P).
U.D., under detection limit.
[a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$, when compared with the values in treatment IV.

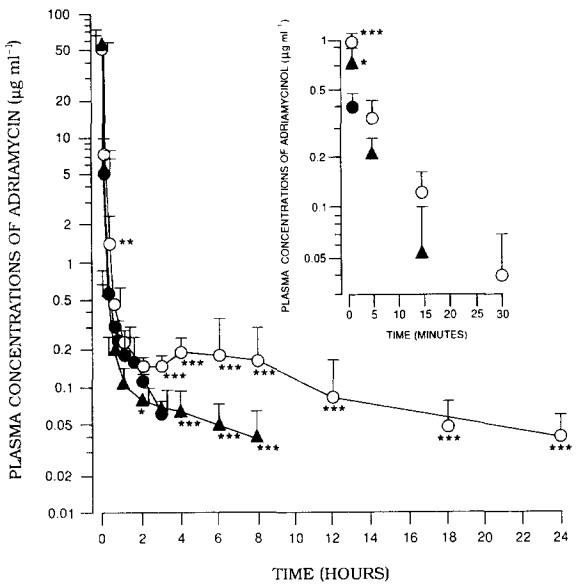

Fig. 1. Mean arterial plasma concentration-time profiles of adriamycin (ADM) after 1 min intravenous infusion of free ADM (treatment I, $n = 7$, ●), ADM-loaded proliposomes (treatment II, $n = 7$, ○) and empty proliposomes mixed manually with free ADM (treatment III, $n = 7$, ▲), 16 mg kg^{-1} as free ADM to rats. Inset shows the plasma profiles of adriamycinol in treatments I–III. Bars represent standard deviation. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, when compared with the values in treatment I.

entrapped in tissues where MTX was slowly released) were injected to rabbits (Yoon et al., 1991) rather than free MTX.

ADM was reported to be transformed to adriamycinol and other metabolites in rats and rabbits (Gewirtz and Yanovich, 1987), and humans (Balis, 1986). Since nonlinear disposition of ADM has also been suggested in humans (Powis et al., 1981; Boston and Phillips, 1983), the free ADM released slowly from ADM-loaded liposomes entrapped in tissues or present in plasma (treatment II) might have been metabolized faster than those in treatment I. This was proved by the smaller amount of ADM excreted in urine (496 vs 297 μg) in treatment II, resulting in a significantly smaller CL_R (9.81 vs 2.27 ml min^{-1} kg^{-1}) than in treatment I (Table 1). Similar results were also obtained from MTX-loaded liposomes (Jeong et al., 1994; Kim et al., 1995) or proliposomes (Park et al., 1994). The amounts of adriamycinol excreted in urine were negligible: 32.0, 32.0 and 10.3 μg (expressed in terms of ADM) in treatments I–III, respectively. The percentages of i.v. dose recovered from the whole GI tract as ADM (6.09 vs 5.72%) and adriamycinol (1.09 vs 0.815%) – expressed in terms of ADM – were not significantly different between treatments I and II (Table 1).

It should be noted that some pharmacokinetic parameters of ADM could be affected by i.v. administration of empty proliposomes (treatment III): the mean values of $t_{1/2}$ (65.2 vs 335 min), MRT (27.5 vs 180 min), CL_R (9.81 vs 2.61 ml min^{-1} kg^{-1}) and the amount of ADM excreted in urine (496 vs 214 μg) were significantly differ-

Table 3
Mean (± standard deviation) amount (μg per g tissue) of adriamycinol remaining in g tissue (or organ) at 30 min after intravenous infusion of free adriamycin (treatment IV), ADM-loaded proliposomes (treatment V) and empty proliposomes mixed manually with free ADM (treatment VI), 16 mg kg^{-1} as free ADM to rats

Tissues	Treatment IV ($n = 5$)	Treatment V ($n = 5$)	Treatment VI ($n = 5$)
Liver	5.28 ± 1.87	5.93 ± 5.17	4.25 ± 1.89
Heart	0.214 ± 0.112	0.386 ± 0.439	0.145 ± 0.120
Lung	0.491 ± 0.347	0.972 ± 1.13	0.283 ± 0.136
Spleen	1.05 ± 0.483	1.50 ± 1.12	1.45 ± 0.0424
Kidney	1.40 ± 0.307	1.04 ± 0.633	0.827 ± 0.448 [a]
Stomach	0.0988 ± 0.0203	0.318 ± 0.269	0.122 ± 0.0588
S.I.	0.059 ± 0.003	0.237 ± 0.152 [a]	0.667 ± 0.401 [b]
Fat	0.0840 ± 0.0598	0.0906 ± 0.0698	0.0748 ± 0.0315
Muscle	0.0715 ± 0.0229	0.144 ± 0.0809	0.108 ± 0.003 [b]
Mesentery	0.220 ± 0.179	0.214 ± 0.128	0.102 ± 0.0482

[a] $p < 0.05$ [b] $p < 0.01$ and [c] $p < 0.001$, when compared with the values in treatment IV.

ent between treatments I and III, but the differences, as expected, seemed to be smaller than or similar to those between treatments I and II (Table 1). The significantly higher values of $t_{1/2}$, MRT and V_{ss} in treatment III than in treatment I could be due to the slow release of ADM loaded in empty liposomes entrapped in RES, as explained earlier.

3.3. Tissue distribution of ADM after i.v. administration

The mean amount of ADM remaining per g tissue (μg per g tissue), and tissue-to-plasma ratio (T/P) in treatments IV–VI are listed in Table 2. ADM was highly concentrated in spleen, kidney, small intestine, liver and heart (3.85, 1.97, 2.60, 1.98 and 1.17 μg per g tissue) in treatment IV as reflected in greater-than-unity T/P values in those organs (22.5, 11.0, 12.0, 10.4 and 7.41). The amount of ADM remaining in tissues and thus the T/P of kidney and liver were significantly lower, but those of plasma, stomach, lung, and lymph nodes were significantly higher in treatment V than in treatment IV (Table 2). However, the corresponding value for heart was not significantly different between treatments IV and V. Cardiac tissue uptake of ADM was reported to be lower after administration of ADM-loaded liposomes (Rahman et al., 1980; Van Hoesel et al., 1984; Mayer and Tai, 1989). The area under the tissue concentration of ADM-time curves of heart, lung, liver, kidney, spleen is known to increase or decrease after i.v. injection of ADM-loaded positively or negatively charged liposomes to mice (Rahman et al., 1980). The above differences in tissue distribution of ADM could be due to differences in the charges of liposomes and/or compositions of liposomes used. Many studies have been reported (Park et al., 1994 and references therein) on factors determining the tissue distribution of liposomes used as a drug delivery system, emphasizing the importance of particle diameter, surface charge and doses of liposomes.

The tissue distribution of ADM could also be affected by i.v. administration of empty proliposomes (treatment VI); the amount of ADM remaining in tissues and/or the T/P were significantly different between treatments IV and VI in plasma, kidney, lung, lymph nodes, liver, fat and mesentery.

The mean amount of adriamycinol remaining per g tissue (μg per g tissue) in treatments IV–VI are listed in Table 3. The amount of adriamycinol remaining was highest in liver and was highly concentrated in spleen and kidney. It is of interest to note that the tissue distribution of adriamycinol was not significantly different between treatments IV and V except in the small intestine.

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