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Notes

Inverse targeting of reticuloendothelial system-rich organs after intravenous administration of adriamycin-loaded neutral proliposomes containing poloxamer 407 to rats

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Abstract

The inverse-targeting of reticuloendothelial system (RES)-rich organs was compared after intravenous (i.v.) injection of free adriamycin, ADM (treatment I) and ADM-loaded neutral proliposomes containing poloxamer 407 (treatment II), 16 mg per kg as free ADM, to rats using the HPLC assay. The amount of ADM remaining per g tissue at 30 min after i.v. administration, and the tissue-to-plasma ratio of ADM in the spleen, a RES-rich organ, were significantly lower, but the values in non-RES organs such as the Kidney, stomach small intestine, large intestine, lung, heart, muscle and mesentery, were significantly higher in treatment II than in treatment I. After 1 min i.v. infusion, the terminal half-life (65.2 vs 406 min), mean residence time (27.5 vs 318 min) and the apparent volume of distribution at steady state (2480 vs 22800 ml kg^{-1}) were significantly higher, but the renal clearance (9.81 vs 0.138 ml min^{-1} kg^{-1}), and the amount of ADM excreted in 48 h urine (496 vs 39.5 μg) were significantly lower in treatment II than in treatment I.

Keywords: Adriamycin; Inverse targeting of RES; Proliposomes; Poloxamer; Adriamycinol; Pharmacokinetics

Liposomes have been used as a drug delivery system for adriamycin, ADM (van Hoesel et al., 1984; Mayer and Tai, 1989; Gabizon, 1992), and have a high affinity for reticuloendothelial system (RES)-rich organs, such as the spleen and liver. Therefore, the ADM loaded in liposomes could be taken up in the RES of liver and spleen after injection of the liposome. In order to inverse the targeting of RES-rich organs, poloxamer, a non-ionic surfactant and a block copolymer of polyoxyethylene and polyoxypropylene, has been used (Illum and Davis, 1984; Illum et al., 1987; Lee et al., 1995b). Payne et al. (Payne et al., 1986a,b) introduced proliposomes to overcome disadvantages (Frϕkjaer et al., 1984) of liposomes. Pro-

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liposomes could be stored sterilized in dry state, which can then be dispersed/dissolved by adding water before use to form an isotonic multilamellar liposomal suspension suitable for administration either intravenously or by other routes (Payne et al., 1986b). The pharmacological effect and stability of amphotericin B- (Payne et al., 1986a,b), indomethacin- (Katare et al., 1991a) and non-steroidal anti-inflammatory analogues- (Katare et al., 1991b) loaded proliposomes have been reported.

The purpose of this note is to compare the inverse-targeting of RES-rich organs after intravenous (i.v.) administration of free ADM and ADM-loaded neutral proliposomes containing poloxamer 407 to rats.

ADM-loaded proliposomes containing poloxamer 407 were prepared by slightly modifying the reported method (Lee et al., 1995a). Egg phosphatidylcholine (Sigma Chemical Company, St. Louis, MO), poloxamer 407 (Junsei Chemical Company, Tokyo, Japan), and ADM (ADM powder was kindly donated from Central Research Lab. of Boryung Pharmaceutical Company, Kun Po-Si, South Korea) at a molar ratio of 1: 0.1: 0.172 were dissolved in a mixture of organic solvent (chloroform/methanol, 50: 50%, v/v) at 40°C. Before use, injectable distilled water was added to the resulting proliposomes and then manually shook twice for 1 min with a 15-min interval for complete hydration, to make 2 mg ml^{-1} as free ADM.

Twenty four, healthy, male Sprague-Dawley rats (245-345 g, Research Lab. of Dong-A Pharmaceutical Company, Yongin, 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 Company, Midland, MI). Both silastic tubings were covered with wire to allow free movement of the rat. The exposed areas were sutured. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea), and allowed to recover from anesthesia for 4–5 h before the study.

Free ADM (i.v. solution as a HCl salt, 10 mg per 5 ml, kindly supplied by Central Research Lab. of Boryung Pharmaceutical Company, treatment I, $n = 7$) or ADM-loaded proliposomes containing poloxamer 407 (hydrated with injectable distilled water before use to make 2 mg ml^{-1} as free ADM, treatment II, $n = 7$), equivalent to 16 mg/kg as free ADM, were administered via the jugular vein by i.v. infusion in 1 min to rats. Total injection volume was approximately 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. 0.25 ml of heparinized 0.9% NaCl injectable solution (20 U ml^{-1}) was used to flush the cannula immediately 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 the freezer prior to the HPLC analysis of ADM and adriamycinol (kindly donated by Adria Labs., Dublin, OH), a metabolite of ADM (Lee et al., 1995a). 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 the HPLC analysis of ADM and adriamycinol (Lee et al., 1995a).

Free ADM (treatment III, $n = 5$) or ADM-loaded proliposomes containing poloxamer 407 (treatment IV, $n = 5$), equivalent to 16 mg/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. Approximately 1 g of heart, lung, spleen, brain, liver, kidney, stomach, small intestine, large intestine, mesentery, fat, thigh muscle or ipsilateral lymph nodes 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 cen-

trifuged immediately. Other procedures were similar to the method reported previously (Lee et al., 1995a).

The pharmacokinetic parameters, such as the total area under the plasma concentration-time curve from time zero to time infinity (AUC; Chiou, 1978), 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 the time-averaged renal (CL_R) and nonrenal (CL_{NR}) clearances were estimated (Kim et al., 1993) by the standard method (Gibaldi and Perrier, 1982). The mean values of each clearance, V_{SS} and half-life were calculated by the harmonic mean method (Chiou, 1979). Levels of statistical significance were assessed using the t-test between the two means for unpaired data. Significant differences were judged as a P value of less than 0.05. All results were expressed as mean $\pm$ standard deviation.

The mean amount of ADM loaded in ADM-loaded proliposomes containing poloxamer 407 was 19.6 $\pm$ 1.42 mg per g proliposome ($n = 4$) using the method reported previously (Lee et al., 1995a). It has been reported that a liposomal suspension was immediately formed when ADM-loaded (Lee et al., 1995a) or MTX-loaded (Park et al., 1994) proliposomes were hydrated with water. In the present study, a neutral liposomal suspension was also formed immediately when ADM-loaded proliposomes containing poloxamer 407 were hydrated with water. The liposomal suspension formed was found to be multilamellar vesicles under the transmission electron microscopy.

Fig. 1 shows the mean arterial plasma concentration-time profiles of ADM in treatments I and II, and some relevant pharmacokinetic parameters are listed in Table 1. After i.v. infusion of free ADM (treatment I), 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 II, however, the plasma levels of ADM were significantly lower at 5 min post-infusion and were significantly higher from 3 h post-infusion than in treatment I. The plasma levels of ADM decreased slowly at the terminal phase with a mean terminal half-life of 406 min in treatment II. The lower plasma concentrations of ADM at 1 ($P < 0.3236$) and 5 ($P < 0.01$) min post-infusion in treatment II could be due to the entrapment of the ADM-loaded liposomes containing poloxamer 407 (formed from the proliposomes by hydration) into tissues (Table 2). The slow decay of plasma ADM at the terminal phase in treatment II could be due to the slow release of ADM from the ADM-loaded liposomes entrapped in tissues 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 declined rapidly which became undetectable from 3 h onwards after i.v. infusion in treatment I. Therefore, the plasma concentrations of ADM in treatment II suggest that most ADM is in the liposomes at early times after injection, which would be released as free ADM at later times (Fig. 1).

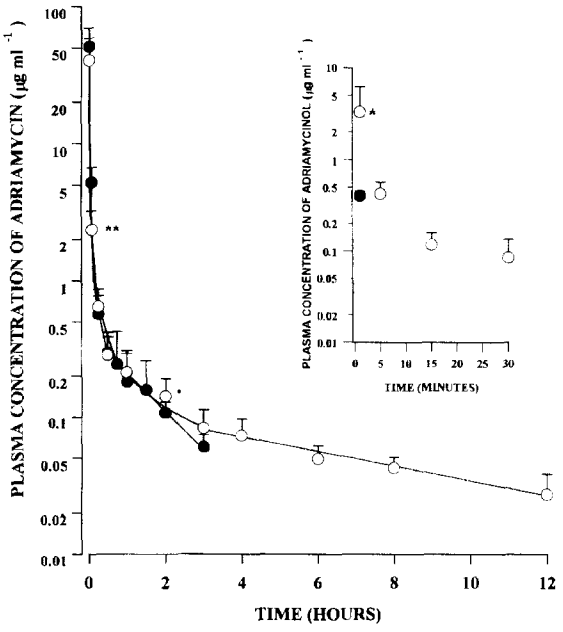

Fig. 1. Mean arterial plasma concentration-time profiles of adriamycin (ADM) after 1 min intravenous infusion of free ADM (treatment I, $n = 7$, ●), ADM-loaded proliposomes containing poloxamer 407 (treatment II, $n = 7$, ○), 16 mg kg^{-1} as free ADM, to rats. Inset shows the plasma profiles of adriamycinol in treatments I and II. Bars represent standard deviation. *: $P < 0.05$, **: $P < 0.01$ and ***: $P < 0.001$.

Table 1
Mean (± standard deviation) pharmacokinetic parameters of adriamycin (ADM) or adriamycinol after 1 min intravenous infusion of free ADM (treatment I) or ADM-loaded proliposomes containing poloxamer 407 (treatment II), 16 mg kg^{-1} as free ADM, to rats. The numbers in parenthesis represent the percentages of intravenous dose excreted in urine expressed in terms of adriamycin

Parameters	Treatment I ($n = 7$)	Treatment II ($n = 7$)
Body weight (g)	305 ± 42.1	287 ± 21.8
AUC (μg min ml^{-1})	159 ± 33.4	188 ± 83.1
$t_{1/2}$ (min)	65.2 ± 32.0	406 ± 296*
MRT (min)	27.5 ± 12.2	318 ± 216**
V_{SS} (ml kg^{-1})	2480 ± 1510	22800 ± 14200**
CL (ml min^{-1} kg^{-1})	101 ± 22.5	85.1 ± 33.0
CL_R (ml min^{-1} kg^{-1})	9.81 ± 3.05	0.138 ± 0.0469***
CL_{NR} (ml min^{-1} kg^{-1})	90.8 ± 20.6	84.6 ± 32.9
Xu (μg)		
as adriamycin	496 ± 133	39.5 ± 13.7***
(% of dose)	(10.0 ± 1.82)	(0.857 ± 0.283***)
as adriamycinol[a]	32.0 ± 8.62	17.3 ± 7.29**
(% of dose)[a]	(0.651 ± 0.121)	(0.371 ± 0.156**)

[a]Expressed in terms of adriamycin
*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$

The mean arterial plasma concentration-time profiles of adriamycinol in treatments I and II are also shown in Fig. 1. Adriamycinol was detected in plasma up to 1 and 30 min after i.v. administration in treatments I and II, respectively. The plasma concentrations of adriamycinol were significantly higher in treatment II than in treatment I.

The ADM loaded in the liposomes may be neither excreted via the kidney nor metabolized in the liver, but slowly released from the liposomes entrapped in tissues and/or present in plasma. Therefore, the MRT (27.5 vs 318 min), $t_{1/2}$ (65.2 vs 406 min) and V_{SS} (2480 vs 22 800 ml kg^{-1}) were significantly higher in treatment II than in treatment I (Table 1). The MRT and V_{ss} of ADM were also significantly higher when ADM-loaded proliposomes not containing poloxamer 407 (Lee et al., 1995a) were injected to rats. However, the AUC and therefore, CL were not significantly different between treatments I and II (Table 1).

ADM was reported to be transformed to adriamycinol and other metabolites in rats and rabbits (Gewirtz and Yanovich, 1987), and human (Balis, 1986). Since nonlinear disposition of ADM has also been suggested in human (Powis et al., 1981; Boston and Phillips, 1983), the free ADM released slowly from the liposomes entrapped in tissues and/or present in plasma (treatment II) might have been metabolized faster than those in treatment I. This was proved by the significantly smaller amount of ADM excreted in 48 h urine (496 vs 39.5 μg, 10.0 vs 0.857% of i.v. dose of ADM) in treatment II, resulting in a significantly smaller CL_R (9.81 vs 0.138 ml min^{-1} kg^{-1}) than in treatment I (Table 1). Similar results were also obtained from ADM-loaded proliposomes not containing poloxamer 407 (Lee et al., 1995a).

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 III) or ADM-loaded proliposomes containing poloxamer 407 (treatment IV), 16 mg kg^{-1} as free ADM, to rats. The numbers in parenthesis represent mean (± standard deviation) values of tissue to plasma ratio (T/P).

Tissues	Treatment III ($n = 5$)	Treatment IV ($n = 5$)
Plasma	0.189 ± 0.0641	0.354 ± 0.0771**
Spleen	3.85 ± 1.41	1.83 ± 0.224**
	(22.5 ± 12.2)	(5.25 ± 0.911**)
Kidney	1.97 ± 1.06	5.01 ± 1.27**
	(11.0 ± 6.57)	(14.9 ± 6.36**)
Stomach	0.515 ± 0.0815	2.63 ± 1.22**
	(2.96 ± 1.05)	(7.57 ± 3.65*)
Small Intestine	2.60 ± 2.56	11.6 ± 0.919**
	(12.0 ± 8.30)	(32.7 ± 2.62**)
Large Intestine	0.191 ± 0.0721	2.43 ± 0.721***
	(1.18 ± 0.790)	(6.95 ± 1.93***)
Lung	0.866 ± 0.158	10.4 ± 2.57***
	(4.84 ± 1.24)	(29.5 ± 6.35***)
Lymph nodes	0.190 ± 0.0822	2.52 ± 0.831***
	(1.06 ± 0.601)	(7.11 ± 2.15)***
Liver	1.98 ± 0.997	2.13 ± 0.686
	(10.4 ± 2.79)	(6.36 ± 2.44*)
Heart	1.17 ± 1.42	3.22 ± 0.695*
	(7.41 ± 10.3)	(9.27 ± 2.41)
Fat	0.206 ± 0.126	0.219 ± 0.0706
	(1.43 ± 1.13)	(0.629 ± 0.203)
Muscle	0.423 ± 0.200	1.45 ± 0.520**
	(2.19 ± 0.866)	(4.39 ± 1.60*)
Mesentery	0.565 ± 0.383	1.20 ± 0.145**
	(3.41 ± 2.69)	(3.83 ± 1.14)

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$

The amount of adriamycinol excreted in 48 h urine were negligible; 32.0, and 17.3 μg (0.651 vs 0.371% of i.v. dose of ADM), expressed in terms of ADM, in treatments I and II, respectively.

The mean amount of ADM remaining per g tissue (μg per g tissue) at 30 min after i.v. administration, and tissue-to-plasma ratio (T/P) in treatments III and IV 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 III as reflected by a greater-than-unity T/P values in those organs (22.5, 11.0, 12.0, 10.4 and 7.41). The amount of ADM (3.85 vs 1.83 μg) and T/P ratio (22.5 vs 5.25) in the spleen, a RES-rich organ, was significantly reduced in treatment IV than in treatment III. The amount of ADM (3.85 vs 5.03 μg) in spleen seemed to be higher after i.v. administration of ADM-loaded proliposomes not containing poloxamer 407 (Lee et al., 1995a). However, the amount of ADM and/or T/P ratios of ADM in non-RES organs were significantly higher in treatment IV compared with treatment III, except in the fat. The amount of ADM or T/P ratio in non-RES organs, such as kidney, stomach, small intestine, large intestine, lung, heart, muscle and mesentery seemed to be considerably higher in treatment IV than after i.v. administration of ADM-loaded proliposomes not containing poloxamer 407 (Lee et al., 1995a). Above data indicate that the inverse-targeting of RES-rich organs could be performed by the addition of poloxamer 407 into the proliposomes.

The mean amount of adriamycinol remaining per g tissue (μg per g tissue) in treatments III and IV are listed in Table 3. The amount of adriamycinol remaining was highest in the liver and fat for the treatments III and IV, respectively. In treatment IV, the amount of adriamycinol was significantly lower in the liver and spleen, but increased significantly in the kidney, fat, muscle and mesentery than in treatment III.

Table 3
Mean ($\pm$ standard deviation) amount of adriamycinol remaining in g tissue (μg per g tissue) at 30 min after intravenous infusion of free adriamycin, ADM (treatment III) or ADM-loaded proliposomes containing poloxamer 407 (treatment IV), 16 mg kg^{-1} as free ADM, to rats.

Tissues	Treatment III (n = 5)	Treatment IV (n = 5)
Liver	5.28 $\pm$ 1.87	1.85 $\pm$ 0.742**
Heart	0.214 $\pm$ 0.112	0.304 $\pm$ 0.133
Lung	0.491 $\pm$ 0.347	1.92 $\pm$ 1.67
Spleen	1.05 $\pm$ 0.483	0.0720 $\pm$ 0.0101**
Kidney	1.40 $\pm$ 0.307	4.35 $\pm$ 2.32*
Fat	0.0840 $\pm$ 0.0598	4.79 $\pm$ 0.618***
Muscle	0.0715 $\pm$ 0.0229	0.101 $\pm$ 0.0140*
Mesentery	0.220 $\pm$ 0.179	2.59 $\pm$ 0.441***

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

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References

Balis, F.M., Pharmacokinetic drug interactions of commonly used anticancer drugs. *Clin. Pharmacokinet.*, 11 (1986) 223–235.

Boston, R.C. and Phillips, D.R., Evidence of possible dose-dependent doxorubicin plasma kinetics in man. *Cancer Treat. Rep.*, 67 (1983) 63–69.

Chiou, W.L., Critical evaluation of potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level-time curve. *J. Pharmacokinet. Biopharm.*, 6 (1978) 539–546.

Chiou, W.L., New calculation method for mean apparent drug volume of distribution and application to rationale dosage regimens. *J. Pharm. Sci.*, 68 (1979) 1067–1069.

Frϕkjaer, S., Hjorth, E.L. and Wϕrts, O., Stability testing of liposomes during storage. *Liposome Technology*, G. Gregoriadis Eds., CRC press Inc., Boca Raton, Florida, Vol. 1, 1984, pp. 235–245.

Gabizon, A.A., Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. *Cancer Res.*, 52 (1992) 891–896.

Gewirtz, D.A. and Yanovich, S., Metabolism of adriamycin in hepatocytes isolated from the rat and the rabbit. *Biochem. Pharmacol.*, 36 (1987) 1793–1798.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, 2nd Ed. Marcel-Dekker, New York, 1982.

Illum, L. and Davis, S.S., The organ uptake of intravenously administered colloidal particles can be altered using a non-ionic surfactant (poloxamer 338). *FEBS Lett.*, 167 (1984) 79–92.

Illum, L., Davis, S.S., Müller, R.H., Mak, E. and West, P., The organ distribution and circulation time of intravenously injected colloidal carriers sterically stabilized with a blockcopolymer-poloxamine 908. *Life Sci.*, 40 (1987) 367–374.

Katare, O.P., Vyas, S.P. and Dixit, V.K., Proliposomes of indomethacin for oral administration. *J. Microencap.*, 8 (1991a) 1–7.

Katare, O.P., Vyas, S.P. and Dixit, V.K., Preparation and performance evaluation of plain proliposomal systems for cytoprotections. *J. Microencap.*, 8 (1991b) 295–300.

Kim, S.H., Choi, Y.M. and Lee, M.G., Pharmacokinetics and pharmacodynamics of furosemide in protein-calorie malnutrition. *J. Pharmacokinet. Biopharm.*, 21 (1993) 1–17.

Lee, H.J., Ahn, B.N., Yoon, E.J., Paik, W.H., Shim C.K. and Lee, M.G., Pharmacokinetics and tissue distribution of adriamycin and adriamycinol after intravenous administration of adriamycin-loaded neutral proliposomes to rats. *Int. J. Pharm.*, 121 (1995a) 1–10.

Lee, H.-J. and Chiou, W.L., Erythrocytes as barriers for drug elimination in the isolated rat liver. I. Doxorubicin. *Pharm. Res.*, 6 (1989) 833–839.

Lee, M.J., Lee, M.H. and Shim, C.-K., Inverse targeting of drugs to reticuloendothelial system-rich organs by lipid microemulsion emulsified with poloxamer 338, *Int. J. Pharm.*, 113 (1995b) 175–187.

Mayer, L.D. and Tai, L.C.L., Influence of vesicle size, lipid composition and drug-to- lipid ratio on the biological activity of liposomal doxorubicin in mice. *Cancer Res.*, 49 (1989) 5922–5930.

Park, J.M., Ahn, B.-N., Yoon, E.J., Lee, M.G., Shim, C.-K. and Kim, C.-K., Pharmacokinetics of methotrexate after intravenous administration of methotrexate-loaded proliposomes to rats. *Biopharm. Drug Disposit.*, 15 (1994) 391–407.

Payne, N.I., Browning, I. and Hynes, C.A., Characterization of proliposome. *J. Pharm. Sci.*, 75 (1986a) 330–333.

Payne, N.I., Timmis, P., Ambrose, C.V., Warel, M.D. and Ridgway, F., Proliposomes: A novel solution to an old problem. *J. Pharm. Sci.*, 75 (1986b) 325–329.

Powis, G., Ames, M.M. and Kovach, J.S., Dose-dependent pharmacokinetics and cancer chemotherapy. *Cancer Chemother. Pharmacol.*, 6 (1981) 1–9.

van Hoesel, Q.G.C.M., Steerenberg, P.A., Crommelin, D.J.A. and van Dijk, A., Reduced cardiotoxicity and nephrotoxicity with preservation of antitumor activity of doxorubicin entrapped in stable liposomes in the LOU/M Wsl rat. *Cancer Res.*, 44 (1984) 3698–3705.