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Inverse targeting of drugs to reticuloendothelial system-rich organs by lipid microemulsion emulsified with poloxamer 338

Mi-Jeong Lee, Min-Hwa Lee, Chang-Koo Shim *

College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea

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Abstract

Lipid microemulsions (LM) consisting of soybean oil and lecithin have been studied as a parenteral drug delivery system for site-specific delivery of non-water-soluble drugs. A major obstacle to targeting to non-RES organs or maintaining high concentrations of LM in vasculature is their rapid and extensive uptake by the RES in the liver and spleen. By replacing lecithin with hydrophilic poloxamer 338, it has been possible to avoid the normal deposition of LM in the liver and spleen (inverse targeting). Poloxamer 338-modified LM (PLM) containing ibuprofen octyl ester was intravenously administered to rats. Ibuprofen concentrations in the plasma and various organs were measured to elucidate the effect of inverse targeting to RES and targeting to other tissues in terms of the incorporated drug rather than the drug carrier. It was suggested that PLM can be exploited to direct lipophilic drugs in LM away from RES in the liver and spleen to other targeting tissues such as inflammatory tissues.

Keywords: Lipid microemulsion; Poloxamer 338; Reticuloendothelial system; Inverse targeting; Ibuprofen; Pharmacokinetics; Tissue distribution

1. Introduction

Lipid microemulsions (LM) consisting of soybean oil and lecithin are widely used in clinical medicine for parenteral nutrition (Intralipid, etc.). They are very stable and can be stored for 2 years at room temperature. They are also autoclavable (Mizushima, 1985). They have no particular side-effects even at dose levels of 500 ml. Thus, they have been investigated as a parenteral drug delivery system especially for non-water-soluble drugs (Rosenkrantz et al., 1974; Tarr et al., 1987; Paborji et al., 1988; Prankerd et al., 1988). They are expected to protect incorporated drugs in the body from enzymatic degradation and relieve probable side-effects through sustained release of drugs (El-Sayed and Repta, 1983; Otomo et al., 1985; Benita et al., 1986; Forster et al., 1987).

Regarding their distribution in the body, they are rapidly captured after intravenous injection by the reticuloendothelial system (RES)-rich organs such as the liver and spleen, and by inflammatory cells like other colloidal carriers (Hallberg, 1965; Koga et al., 1975). This can be advantageous if one is attempting to treat diseases involving the RES (Alving et al., 1978) or using

* Corresponding author.

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therapies which require drug delivery to macrophages (Poste, 1983).

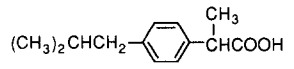
However, in many therapeutic applications, rapid and extensive uptake of the colloidal carriers by the RES is a significant disadvantage. Accumulation of the carriers in the RES with repeated dosings can lead to RES blockade and impairment of an important host defense system (Allen et al., 1984). Accumulation of drugs in the liver and spleen could be hazardous in the case of cytotoxic agents and drugs which have adverse effects on such organs. In addition, rapid removal of the drug carriers from circulation significantly impairs the ability to target drugs to non-RES tissues and prevents, to a large degree, use of the colloidal systems as a depot system for slow release of drugs within the vasculature. Thus, it is sometimes necessary to change the pattern of distribution of the carrier systems in the body to yield high concentration of a drug in the plasma and tissues other than RES-rich organs.

There have been some attempts to avoid RES uptake of colloidal drug carriers (inverse targeting) (Lazo and Hacker, 1985). One way of inverse targeting would be to suppress the function of RES by preinjection of a large amount of blank colloidal carriers (Illum et al., 1986a) or macromolecules like dextran sulfate (Patel et al., 1983). Unfortunately, this approach would not be normally applicable in clinical practice because repeated saturation of the RES can lead to RES blockade and impairment of an important host defense system (Allen et al., 1984). Alternative strategies are modifying the size (Illum et al., 1982; Davis and Illum, 1987; Allen et al., 1989), surface charge (Roerdink et al., 1983; Schwendener et al., 1984; Allen et al., 1989; Nishida et al., 1990), composition and rigidity (Hwang et al., 1980; Utsumi et al., 1983; Allen et al., 1989; Namba et al., 1989, 1990), and hydrophilicity (Van Oss, 1978; Illum and Davis, 1983, 1984; Illum et al., 1986b) of carriers. In LM, however, particle size and rigidity of particle surface cannot be changed easily. Likewise, the surface charge of LM, which is rendered usually by adding ionic surfactants to the LM system, cannot be changed very much since the surfactants may reduce the stability of LM.

Among the remaining approaches, coating of hydrophobic particles with hydrophilic substances reduces RES uptake, thereby allowing the carriers to reach other sites. Illum and Davis (1984) showed that polystyrene particles (50–60 nm) coated with poloxamer 338, a hydrophilic non-ionic surfactant, are diverted away from the RES of the liver and spleen after their i.v. injection into rabbits. Davis and Hansrani (1985) reported that phospholipid microspheres emulsified with poloxamer 338 showed the slowest RES uptake in mouse peritoneal macrophage in vitro. Poloxamine 908, another hydrophilic non-ionic surfactant, was also reported to decrease the uptake of the coated emulsions by the liver and spleen (Illum, 1987). Poloxamine 908 coated polystyrene particles (80 nm) were reported to avoid the normal liver uptake and to provide a strategy for resultant targeting of microspheres to inflammatory sites in rabbits (Illum et al., 1989). Thus, modification of the surface hydrophilicity of the carrier particles seems to be an effective way of inverse targeting of the particles.

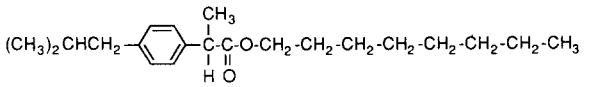
In this study, we tried to modify the surface hydrophilicity of the conventional LM consisting of soybean oil and lecithin (emulsifier) by replacing lecithin with poloxamer 338. Poloxamer 338 was selected due to its high hydrophilicity (HLB value of 25 at 25°C; Wu and Miller, 1990) and low toxicity (BASF Technical Data, 1990). Although lecithin has been used as an excellent water-in-oil emulsifier (dispersed phase is water, and continuous phase is oil), it appears to be less hydrophilic than poloxamer 338, since it is not soluble in water but soluble in many nonpolar solvents. Therefore, the surface of LM emulsified with poloxamer 338 is expected to be more hydrophilic than LM emulsified with lecithin.

Poloxamer 338-lipid microemulsion (PLM) was prepared by emulsifying soybean oil and glycerin in the presence of poloxamer 338. Then, ibuprofen octyl ester (IPO; Scheme 1), a model of lipid-soluble drugs, was incorporated into the PLM. Non-lipid-soluble drugs like ibuprofen cannot be incorporated into the LM system. Thus, a lipid soluble and oily ester of ibuprofen, IPO, was synthesized from ibuprofen and utilized in the incorporation.



$C_{13}H_{18}O_2$
M.W. 206.27

(A) ibuprofen



$C_{21}H_{34}O_2$
M.W. 318.50

(B) ibuprofenoctylester

Scheme 1. Chemical structure of ibuprofen (A) and its lipophilic prodrug, ibuprofen octyl ester (IPO) (B).

In clinical use, the distribution of the incorporated drug(s) rather than the drug carriers is much more important. Thus, we attempted in this study to elucidate in vivo whether the surface modification technique is also effective in inverse targeting of the incorporated drug(s) as well as the carriers. The PLM was administered intravenously to rats, and the body distribution of ibuprofen, especially to the RES tissues and experimental inflammatory sites, was examined in order to evaluate the effect of surface modification with poloxamer 338 on the inverse targeting of drugs. The conventional lipid microemulsions (CLM) were prepared in the same manner as PLM except replacing poloxamer 338 in PLM with lecithin, and served as control.

2. Materials and methods

2.1. Materials

Ibuprofen octyl ester (IPO), a colorless, transparent and oily ester of ibuprofen, was kindly synthesized and supplied by the Shin-Poong Pharm. Co. (Seoul, Korea). Ibuprofen standard was purchased from the Hong-Sung Pharm. Co. (Seoul, Korea). Carrageenan lambda (type IV), soybean oil and lecithin were the products of Sigma Chemical Co. (St. Louis, MO). Poloxamer 338 (Pluronic F-108) was kindly donated by BASF Korea Ltd. Male Wistar rats weighing 210–260 g were obtained from the Experimental Animal Center of Seoul National University.

2.2. Preparation and characterization of IPO-loaded LM

CLM was prepared as follows. A crude emulsion of soybean oil was prepared by mixing soybean oil, lecithin, glycerin and water for injection at 70°C for 20 min using a high shear homogenizer (12 000 rpm, Model AM-8, Nihonseiki Kaisha Ltd, Japan), followed by sonication with an ultrasonic probe system (Branson Cleaning Equipment Co., U.K.) for 20 min in an ice bath (Min et al., 1986). Glycerin was used as an isotonic agent. Then the pH of the emulsion was adjusted to 6.8 using 1 N NaOH and autoclaving performed at 121°C for 15 min (Min et al., 1986). To this blank emulsion, IPO was added dropwise and probe sonication was performed for 20 min in an ice bath. The resultant lipid microsphere served as the lipid microspheres for the control study (CLM). The composition of the final CLM was as follows: 10% (w/v) of soybean oil, 2% (w/v) of lecithin, 2.25% (w/v) glycerin and 1.544% (w/v) of IPO, when expressed as a percentage of the total volume of the emulsion. The concentration of lecithin was critical to obtain a stable emulsion. PLM was prepared in the same manner except that lecithin was replaced with equivalent amounts of poloxamer 338. CLM and PLM were stored in the refrigerator (4°C).

All of IPO seems to be incorporated into the LM, since IPO itself was confirmed not to exist as a dispersed system without forming LM with the blank emulsion. Probably, IPO will dissolve in soybean oil phase of the blank emulsion. Perfect incorporation of IPO in CLM and PLM was supported by the absence of detectable IPO (< 0.1 μg/ml) in the supernatant of both LMs after centrifugation at $3000 \times g$ for 15 min. IPO concentration in the supernatant of the LMs was below the detection limit even after incubation at 37°C for 4 h.

Size distribution of the microspheres were analyzed by the dynamic light scattering method using a particle analyzer (LPA-3000, Otsuka Electronics, Japan) and photon correlator (LPA-3100, Otsuka Electronics, Japan) for the fresh and refrigerator-stored lipid microspheres. Transmission electron microscope (TEM) (Jedel-200 CX, Jeol, Japan) photographs were also taken to observe the shape of LM.

2.3. In vitro release of ibuprofen from IPO-loaded LM

In vitro release of ibuprofen from free IPO, IPO-loaded CLM and IPO-loaded PLM to phosphate-buffered saline (PBS) or to fresh rat plasma was determined as follows. To 50-ml beakers containing 10 ml of PBS or fresh rat plasma, 1 ml of IPO, PLM or CLM was added. The beakers were sealed with parafilm to prevent evaporation of the release medium during the test, and were placed in the water bath to keep them at 37°C. The water bath was put on the magnetic stirring apparatus to facilitate mixing of the samples with the release medium. At 10, 20, 30, 60, 120, 240, 360, 720 and 1440 min after the addition of the release medium to each beaker, 50-μl aliquots were sampled from the 10 ml release medium and stored in the freezer prior to the analysis of ibuprofen by HPLC described below.

2.4. Plasma profile study

The rats were supine during the experiment. Under light ether anesthesia, the femoral arteries and veins of the rats were cannulated with PE-50 polyethylene tubing. After complete recovery (1 h) from anesthesia, CLM, PLM, or aqueous solution of ibuprofen was administered intravenously to the femoral vein through the catheter at a dose of 1 ml/kg (10 mg/kg as ibuprofen). They were prepared on the day of experiment. Blood samples (120 μl) were drawn at 0, 2, 5, 15, 30, 60, 120 and 240 min after the dose from the femoral artery, and were cooled immediately in an ice-bath to prevent further hydrolysis of IPO to ibuprofen by plasma esterase. Plasma samples were separated immediately at 4°C by centrifuging the blood samples at $6000 \times g$ for 1 min and 50-μl aliquots were transferred to conical polypropylene tubes (15 ml) which contained 0.05 μg flurbiprofen, an internal standard, and stored in a deep freezer (−70°C) until HPLC assay for ibuprofen. In a previous study, the cooling to 4°C and maintaining at −70°C was confirmed to prevent hydrolysis of IPO in plasma to ibuprofen. Binding of ibuprofen to blood cells was ignored in this study since more than 99% of blood ibuprofen binds to plasma protein (Shargel and Yu, 1992).

2.5. Tissue distribution study

Rats were injected with 0.2 ml of 1%(w/v) solution of lambda carrageenan subcutaneously into the right hind paw to induce inflammation (Mizushima et al., 1982). Either PLM or CLM was injected into the femoral vein through the catheter 30 min after the carrageenan injection at a dose of 1 ml/kg (10 mg/kg dose as ibuprofen). The rats were killed at 5, 15, and 120 min by carotid artery bleeding. Blood was collected through the carotid artery as much as possible. Then several organs and tissues (liver, lung, spleen, kidney, heart, muscle, paw and adipose tissue) were excised. Approx. 1 g of each organ or tissue was cut and quickly rinsed with ice-cold saline, weighed and homogenized with 3 volumes of saline in a tissue homogenizer (Ika-Ultra-Turrax T25, Janke & Kunkel Ika-Labortechnik, Germany). After centrifugation at $3000 \times g$ for 20 min, aliquots of plasma and the supernatants of tissue homogenates were stored for up to 4 weeks in a deep freezer (−70°C) until HPLC assay for ibuprofen.

2.6. HPLC assay of ibuprofen in plasma and tissues

Ibuprofen in plasma was assayed according to a modified HPLC method (Litowitz et al., 1984). 30 μl of 1 N HCl were added to the conical tubes which contained plasma samples (50 μl) and flurbiprofen (0.05 μg, internal standard). Then they were extracted with 1.5 ml of extraction solvent (iso-octane/isopropyl alcohol = 85 : 15) by vortexing for 5 min. After centrifugation at $3000 \times g$ for

15 min, 1.0-ml aliquots of the supernatant were transferred to other tubes. They were evaporated to dryness under a gentle stream of nitrogen. The residues were dissolved with 100 μl of methanol and aliquots of 50 μl were injected into the HPLC (LC-9A, Shimadzu, Japan).

Ibuprofen in the tissue supernatants was measured by a modified HPLC method for ibuprofen in urine (Chai et al., 1988). To 1.0 ml of the supernatant of the tissue homogenates, 0.5 ml of 1 N HCl and 0.15 μg of flurbiprofen were added and extracted with 5 ml of the extraction solvent mentioned above by vortexing for 5 min. After centrifugation at $3000 \times g$ for 20 min, 4.0-ml aliquots of the supernatants were mixed with 1 ml of 1 N NaOH and vortexed for 5 min. After centrifugation at $3000 \times g$ for 10 min, the organic layer was aspirated and the aqueous layer was mixed with 2 ml of 1 N HCl and they were extracted with 4 ml of the extraction solvent by vortexing for 5 min. After centrifugation at 3000 $\times g$ for 10 min, 3.0-ml aliquots of the organic layer were evaporated to dryness under a gentle stream of nitrogen. Then the residue was dissolved with 200 μl of methanol, and aliquots of 100 μl were injected into the HPLC.

The HPLC system consisted of a precision isocratic pump (LC-9A, Shimadzu, Japan), a C18 reversed-phase column (Shimpak CLC-ODS, 150 $\times$ 4.6 mm i.d., Shimadzu, Japan), and a fluorescence detector (Model RF-535, Shimadzu). The mobile phase was a mixture of acetonitrile, water, orthophosphoric acid (650:350:0.5 by vol.). The flow rate of the mobile phase was 1.5 ml/min and the wavelength of the detector was 250 nm (excitation) and 285 nm (emission).

Calibration curves of ibuprofen were prepared with plasma and respective tissues spiked with known amounts of the drug utilizing its HPLC peak height ratio to the internal standard. Concentrations of ibuprofen in plasma and tissue samples were read from the respective calibration curve. The retention time of ibuprofen was approx. 3.5 min (Fig. 1) and detection limits were 0.1 and 0.5 μg/ml for plasma and tissue homogenates, respectively. The recoveries of ibuprofen from the plasma and tissue samples were more than 90 and 70%, respectively. Inter- and

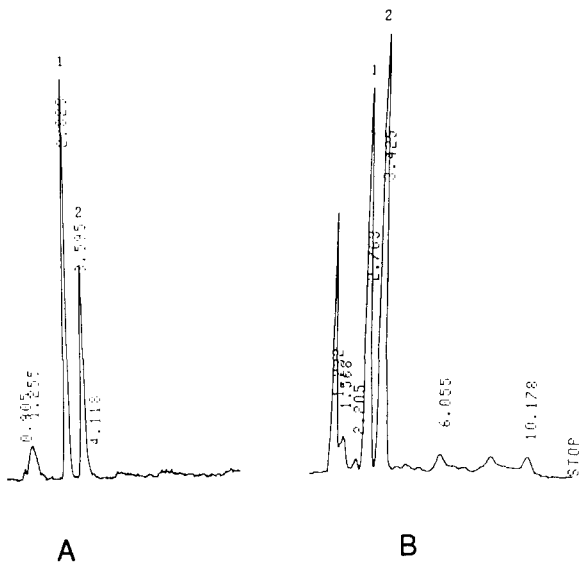


Fig. 1. HPLC chromatograms of ibuprofen in rat plasma (A) and rat liver homogenate samples (B). 1, flurbiprofen (internal standard); 2, ibuprofen.

intra-day variations were less than 6% for plasma samples and 10% for tissue samples.

Ibuprofen in the plasma and tissue samples may be overestimated in this assay if IPO which may coexist in the samples is hydrolyzed to ibuprofen during the extraction process mentioned above. In order to examine this possibility, 50 μl of fresh blank plasma and 200 μl of 1 N HCl were added simultaneously to 50 μl of IPO. Then the mixtures were extracted and assayed according to the above methods for plasma and tissue samples. No detectable peak of ibuprofen was found from the resultant HPLC chromatograms. This indicates that ibuprofen is not formed from IPO during the assay procedure. Therefore, the possibility of overestimation of ibuprofen from IPO could be eliminated. The assay method of this study was concluded to represent free ibuprofen only, and not to reflect total ibuprofen (free ibuprofen and IPO) in the plasma and tissue samples.

2.7. Pharmacokinetic analysis

Total plasma clearance (CL_t) and distribution volume at steady state (Vd_{ss}) of ibuprofen after

i.v. administration were calculated based on Eq. 1 and 2:

$$CL_t = D/AUC \quad (1)$$

$$Vd_{ss} = D \cdot AUMC/AUC^2 \quad (2)$$

where D, AUC, and AUMC, respectively, denote dose, area under the plasma ibuprofen concentration-time curve from time 0 to infinity, and area under the moment of the plasma ibuprofen concentration-time curve from time 0 to infinity. The AUC and AUMC were calculated by the trapezoidal method from time 0 to 4 h and extrapolated from 4 h to infinity using the elimination rate constant (β). β is the slope of the terminal phase obtained after fitting the plasma concentration data to a conventional two-compartment model using the program MULTI (Yamaoka et al., 1981).

2.8. Statistical analysis

The statistical significance of the differences between CLM and PLM in terms of concentration ratio (tissue/plasma), and pharmacokinetic parameters of ibuprofen after i.v. administration was determined using the one-way analysis of variance (ANOVA) for unpaired data with Duncun's multiple range comparison procedure. A p value of < 0.05 was chosen as the level of statistical significance. All results are expressed as mean $\pm$ standard error.

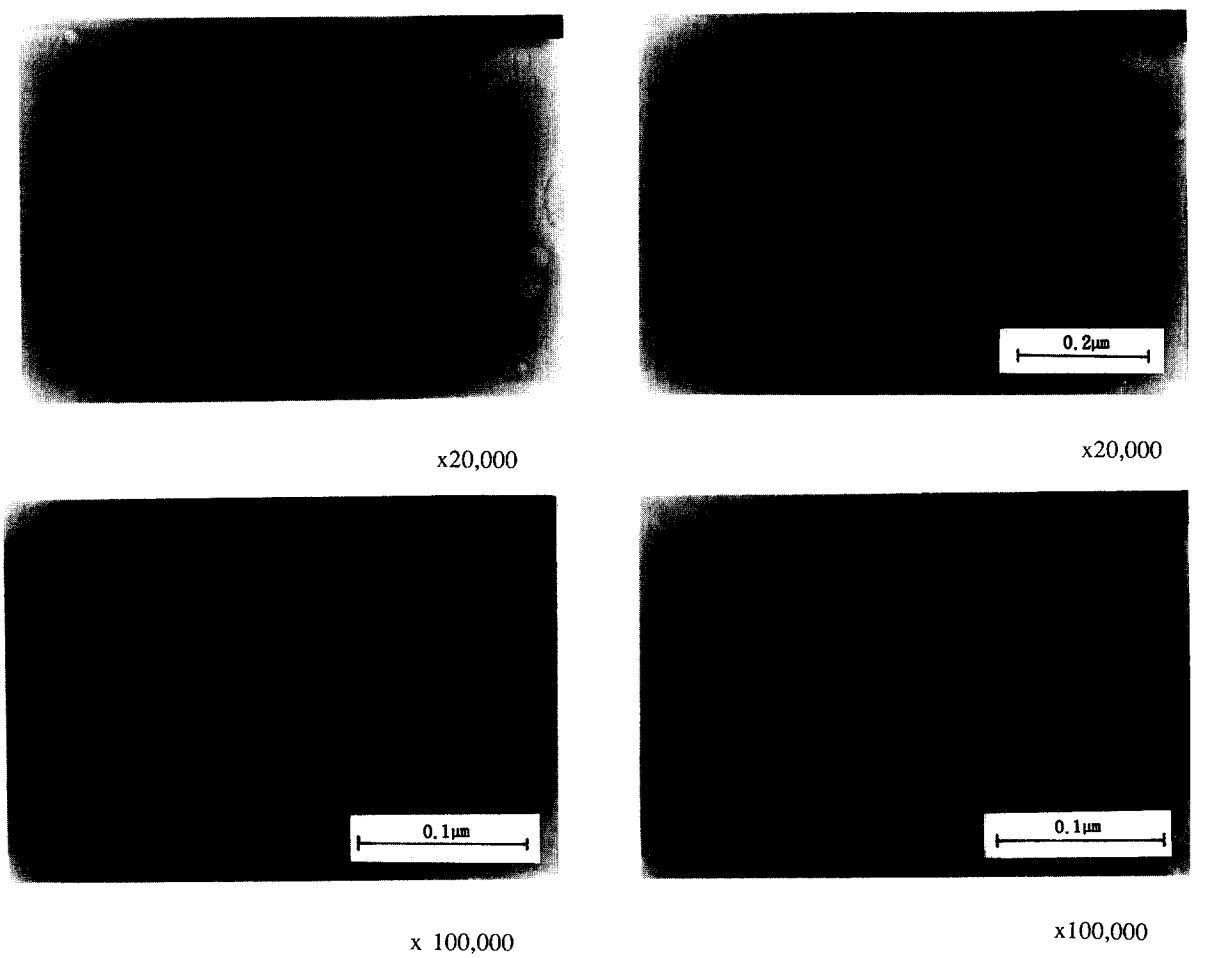


Fig. 2. Transmission electron microscope (TEM) photographs of CLM (left) and PLM (right).

3. Results and discussion

3.1. Size distribution of CLM and PLM

Microspheres of very similar shape and size were observed from TEM photographs of both emulsions (Fig. 2). Size distribution analysis by the dynamic light scattering method revealed that the two microemulsions prepared in this study were almost the same in size distribution of microspheres regardless of drug loading. Mean diameters of CLM and PLM were 126.03 ± 3.17 and 126.87 ± 3.35 nm, respectively, when expressed as mean $\pm$ SD of the three determinations. Smaller particles of around 30 nm in mean diameter were also observed in the size distribution curves of both CLM and PLM, but they were almost negligible in gamma ratio ($< 1\%$) to the larger particles. Therefore, the contribution of small particles to the overall mean diameter or to the body distribution of the microspheres seemed to be almost negligible. The largest diameter of the microspheres was around 0.3 μm. This is much smaller than the diameter (4–6 μm) that increases the incidence of emboli blood pressure changes (Atik et al., 1965). The size distribution and TEM photographs of both LM remained unchanged for 5 months when stored in the refrigerator, indicating substantial stability of the LM system.

Body distribution of colloidal drug carriers is influenced by the size of the colloidal particles in addition to their surface characteristics (Davis and Illum, 1987). Since the size distribution of the microspheres in both microemulsions was almost equal in this study, the size of the LM would not be a cause of different body distribution of the microspheres and incorporated drug, if any, between PLM and CLM.

3.2. In vitro release of ibuprofen

Fig. 3 shows the in vitro release of ibuprofen from IPO, IPO-loaded CLM and IPO-loaded PLM to PBS and rat plasma kept at 37°C as a function of time. The release of ibuprofen to PBS from all the preparations was below the detection limit (0.1 μg/ml) of the HPLC method during the test for 24 h. The release to fresh plasma, however, was marked. It indicates that IPO is hydrolyzed to ibuprofen by some component(s) in the plasma, possibly esterase. Among the three preparations, free IPO showed the slowest release of ibuprofen probably due to the most hydrophobic surface characteristics of IPO, which might be an unfavorable condition for hydrophilic plasma esterase to interact with. Much faster release of ibuprofen was observed from the emulsified microspheres, CLM and PLM. Microspheres emulsified with poloxamer 338, PLM, showed the fastest release of ibuprofen.

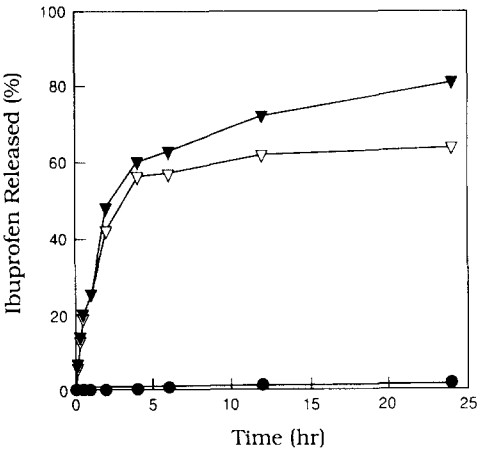


Fig. 3. In vitro release of ibuprofen from free IPO (●), IPO-loaded CLM (△) and IPO-loaded PLM (▲) to fresh rat plasma at 37°C as a function of time. Each point represents the mean of three determinations. The release of ibuprofen to PBS was below the detection limit (< 0.1 μg/ml) of the assay method adopted, and is thus not shown.

3.3. Effect of poloxamer 338 modification on the plasma profile of ibuprofen

Plasma levels of ibuprofen after i.v. administration of the aqueous ibuprofen solution, IPO-loaded CLM and IPO-loaded PLM to rats at a dose of 1 ml/kg (10 mg/kg as ibuprofen) were plotted as a function of time and are expressed as mean $\pm$ standard errors of three experiments in Fig. 4. The plasma level of ibuprofen showed its maximum even at the first sample taken at 2 min after the administration in all the preparations and decayed biexponentially.

Table 1
Pharmacokinetic parameters of ibuprofen after i.v. administration of ibuprofen solution and IPO-incorporated lipid microspheres [a]

Parameters	Ibuprofen solution	CLM	PLM
Vd_{ss} (ml kg^{-1})	736.1 ± 94.9	2167.6 ± 970.4	364.3 ± 101.6
CL_t (ml h^{-1} kg^{-1})	127.1 ± 39.7	348.6 ± 29.8 [b]	246.7 ± 17.9 [b]
C_0 (μg ml^{-1})	63.1 ± 2.5	31.2 ± 5.3	138.3 ± 18.3 [b,c]
AUC_0^{4h} (μg h ml^{-1})	46.9 ± 7.1	16.9 ± 3.7 [b]	35.8 ± 1.2 [c]

[a] CLM, PLM or aqueous solution was administered intravenously at a dose of 1 ml/kg (10 mg/kg as ibuprofen), and all the parameters are shown as mean ± SE of three experiments.
[b] Significantly different from the solution ($p < 0.05$).
[c] Significantly different from CLM ($p < 0.05$).

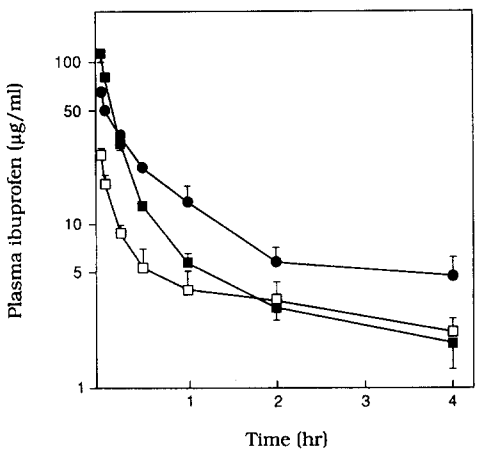


Fig. 4. Mean arterial plasma concentration-time profiles of ibuprofen after i.v. administration of ibuprofen solution (●), IPO-loaded CLM (□) and IPO-loaded PLM (■) to rats at a dose of 1 ml/kg (10 mg/kg as ibuprofen). Bars represent standard error of three determinations.

The appearance of ibuprofen in the plasma was much faster than expected from the in vitro release study (Fig. 3). Hydrolysis of IPO in the plasma samples to ibuprofen during the assay procedure could be excluded as the cause of the rapid appearance, since IPO was confirmed not to undergo acidic or alkaline hydrolysis. Contamination of the CLM or PLM from free IPO or leaky imperfect micelle or emulsions seemed negligible, if any, since free IPO itself could not exist as a dispersed system and the gamma ratio of smaller particles (around 30 nm in diameter) to the overall size distribution contribution was negligible (< 1%).

Therefore, the rapid appearance of ibuprofen in plasma should be explained by the much faster hydrolysis of IPO in LMs to ibuprofen in vivo than in vitro. The faster in vivo hydrolysis might be attributed, at least in part, to the rapid capture of the microspheres by the RES-rich organs after i.v. administration (Hallberg 1965; Koga et al., 1975) and subsequent hydrolysis there. If the hydrolysis there is immediate, ibuprofen will appear in the plasma more rapidly than expected from the in vitro hydrolysis in the plasma (Fig. 3). More studies are necessary to verify the above speculation and to elucidate the real mechanism of the rapid in vivo release of ibuprofen.

Overall plasma levels of ibuprofen following i.v. administration of the LMs were much lower than those after i.v. administration of ibuprofen solution implying removal of microspheres from the blood circulation probably by the RES-rich organs (Fig. 4). The levels following PLM administration, however, were significantly ($p < 0.005$) higher than those following CLM administration during the early phase of 30 min after administration, implying the possibility that poloxamer 338 modification of LM reduces RES uptake of the microemulsions (Hallberg, 1965; Koga et al., 1975).

Fig. 5. Mean tissue concentration-time profiles of ibuprofen in various organ tissues after i.v. administration of IPO-loaded CLM (□) and IPO-loaded PLM (■) to rats at a dose of 1 ml/kg (10 mg/kg as ibuprofen). Bars represent standard error of three determinations. * $p < 0.05$ between CLM and PLM.

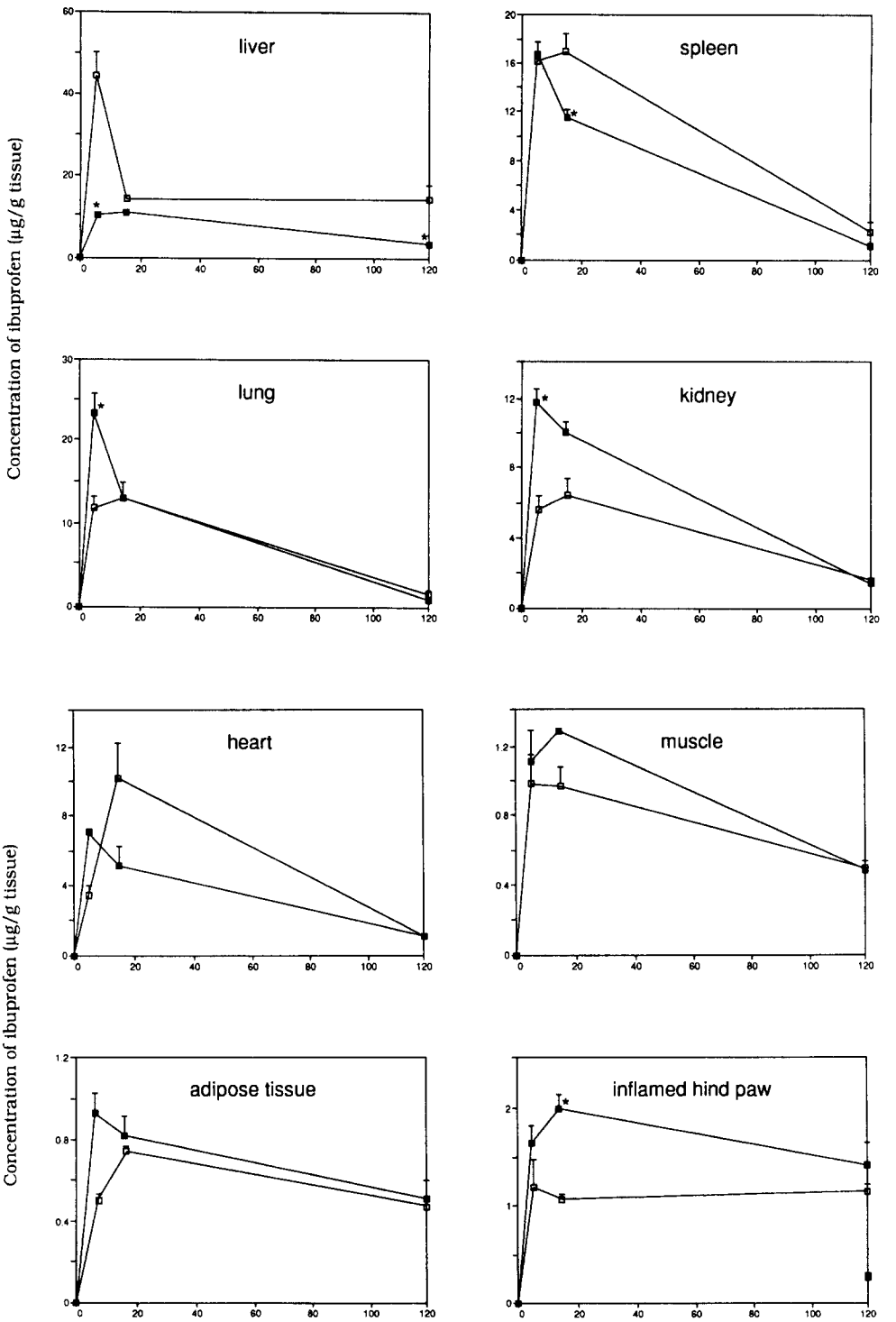

liver
spleen
lung
kidney
heart
muscle
adipose tissue
inflamed hind paw
Concentration of ibuprofen (μg/g tissue)
Concentration of ibuprofen (μg/g tissue)
Time (min)

Table 1 summarizes the effect of poloxamer modification of the surface of the lipid microspheres on the pharmacokinetic parameters of ibuprofen. The pharmacokinetic parameters following i.v. administration of ibuprofen solution are also shown for comparison. The plasma level of ibuprofen at time zero (C_0) after PLM administration, which was calculated from extrapolation of the fitting of the plasma concentration-time data to the conventional two-compartment model, was 4-times greater than that after CLM administration ($p < 0.05$). The AUC of CLM from time zero to 4 h ($AUC_0^{4\,h}$) was significantly ($p < 0.05$) smaller than that of ibuprofen solution, but AUC^4_{h0} of PLM was significantly ($p < 0.05$) larger than that of CLM. This implies that CLM is more readily cleared from the blood circulation than ibuprofen solution probably by RES uptake, and that the uptake can be reduced by surface modification of the microspheres with hydrophilic substance such as poloxamer 338.

In spite of the significant difference in C_0 and $AUC_0^{4\,h}$ between CLM and PLM, there were no significant differences between them in the overall pharmacokinetic parameters such as Vd_{ss} and CL_t. This might be due to the similar plasma profiles of ibuprofen of the preparations at the post-distributive phase which dominates the overall pharmacokinetics of the drug. It implies that microspheres are taken up by the RES mainly during the early phase of the systemic circulation and that poloxamer 338 modification can reduce the uptake of the microspheres during this phase.

3.4. Effect of poloxamer modification on the tissue distribution of ibuprofen

Tissue levels of ibuprofen after i.v. administration of each LM at a dose of 1 ml/kg (10 mg/kg as ibuprofen) were plotted as a function of time and are shown in Fig. 5. In Fig. 4, plasma levels of ibuprofen after PLM administration were much higher than those after CLM administration during the early phase. This could be a result of reduced distribution of ibuprofen to certain tissues. Actually, ibuprofen concentration in the liver at 5 min after PLM administration was only one-quarter of that after CLM administration. This indicates that surface modification with hydrophilic poloxamer 338 significantly decreases the hepatic uptake of ibuprofen probably through reducing hepatic uptake of LM. The higher plasma levels of ibuprofen following PLM administration could be confirmed again by the decreased spleen concentration of the drug at 15 min. In contrast to liver and spleen, ibuprofen levels after PLM administration became higher than those after CLM administration in other

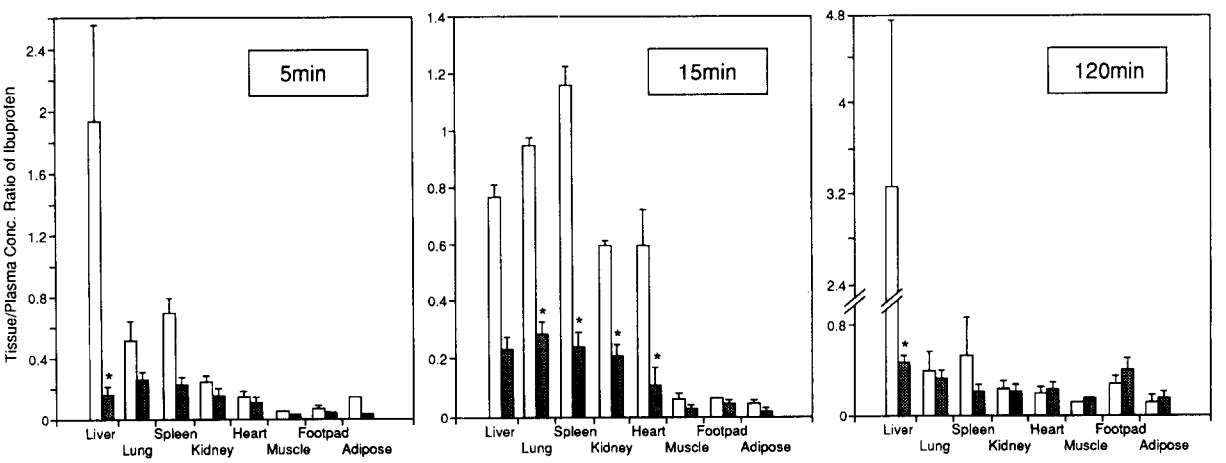


Fig. 6. Concentration ratio of ibuprofen between various tissues and plasma (mean ± SE, $n = 3$) after i.v. administration of IPO-loaded CLM (□) and IPO-loaded PLM (■) to rats at a dose of 1 ml/kg (10 mg/kg as ibuprofen) at three time points after the administration. Bars represent standard error of three determinations. * $p < 0.05$ between CLM and PLM.

tissues such as lung, kidney, and inflamed hind paw. However, it does not necessarily mean that the uptake of the drug by these tissues is increased by the poloxamer modification. It could be just a reflection of the elevated plasma levels of the drug following PLM administration with subsequent partition to the organs.

In order to clarify whether and how the poloxamer 338 modification affects the uptake characteristics of ibuprofen by these tissues, tissue levels of the drug at each time point were normalized by the plasma drug levels of the corresponding time point in each rat; tissue-to-plasma ratios of ibuprofen concentrations at 5, 15 and 120 min were calculated and are shown in Fig. 6. Fig. 6 clearly shows that the ratios after PLM administration are much lower in most tissues examined, especially in liver, spleen and lung, than those after CLM administration. The decrease of the ratio in muscle, inflamed hind paw, and adipose tissue, however, was not as significant at each time point. For example, the liver/plasma ratios at 5, 15, and 120 min after CLM administration were 1.89 ($\pm$0.62), 0.77 ($\pm$0.03), and 3.27 ($\pm$1.52), respectively. After PLM administration, they were reduced to 0.13 ($\pm$0.03) ($p < 0.05$), 0.22 ($\pm$0.04), and 0.47 ($\pm$0.04) ($p < 0.05$), respectively. On the other hand, the ratios in the inflamed hind paw after PLM administration were not significantly reduced by administration of PLM: the ratios of 0.05 ($\pm$0.01), 0.06 ($\pm$0.00), 0.28 ($\pm$0.03) at 5, 15, and 120 min after CLM administration were decreased, respectively, to 0.03 ($\pm$0.01), 0.04 ($\pm$0.01), and 0.40 ($\pm$0.11) by PLM administration. Therefore, it was concluded that uptake into the RES-rich tissues, especially to the liver, could be avoided substantially by modification of LM with poloxamer 338 without significantly affecting the distribution of ibuprofen to the other tissues, such as inflamed hind paw and adipose tissue.

The benefit of the inverse targeting will be explained more clearly by the ratio of drug concentration between the target organ and RES-rich organ. For this purpose, we introduced a term of 'index of inverse targeting (IIT)' which was defined as the concentration ratio of ibuprofen between the exemplified target organ (inflamed hind paw in this study) and liver. The calculated IIT values are listed in Table 2. The larger IIT values indicate greater efficiency of inverse targeting to RES-rich organs or of targeting to a target organ. As is clearly shown in Table 2, the index following PLM administration was much higher than that following CLM administration at each time point. At 120 min after administration, a 7-fold increase in IIT was observed ($p < 0.05$) on poloxamer 338 modification. This indicates that more efficient LM therapy to the inflamed hind paw, for example, can be conducted through poloxamer 338 modification under the same probable hazard or toxicity to the liver.

Table 2
Effect of poloxamer 338 modification on the index of inverse targeting (IIT) of ibuprofen as a function of time [a]

Time after administration (min)	IIT	
	CLM (Control)	PLM
5	3.2 $\pm$ 1.6	20.0 $\pm$ 0.8
15	8.5 $\pm$ 0.5	19.4 $\pm$ 2.1
120	10.8 $\pm$ 3.6	84.0 $\pm$ 13.3 [b]

[a] IIT was calculated from the following equation, and expressed as mean $\pm$ SD of three experiments: IIT = 100 $\times$ [concentration of ibuprofen in the inflamed hind paw]/[concentration of ibuprofen in the liver].
[b] Significantly different from the control ($p < 0.05$).

In short, inverse targeting of ibuprofen avoiding RES uptake could be achieved by modifying the surface characteristics of the conventional LM with poloxamer 338. The inverse targeting effect may be attributed to the surface hydrophilicity of LM induced by the modification (Illum and Davis, 1984). Surface hydrophilicity may reduce or even eliminate the adhesion of opsonic material onto the surface of LM which is believed to be an essential process for phagocytosis and subsequent uptake of LM by the Kupffer cells (Van Oss, 1978; O'Mullane et al., 1990).

These findings are consistent with those of Illum and Davis (1984) where inverse targeting of polystyrene microspheres could be achieved by coating the surface with poloxamer 338. It implies that the body distribution of the drug in the LM system is controlled mainly by the body distribution of the drug carriers. Of course, properties of

drugs incorporated into the LM system, such as lipophilicity and systemic clearance, may also influence the body distribution of the drugs additionally.

In conclusion, our data suggest that the judicious use of non-ionic hydrophilic surfactants can be exploited to direct drug(s) in LM system away from the RES-rich tissues so that they are able to reach other target tissues. It should also be noted that decrease of uptake of LM by RES possibly reduces the potency of the drug in the LM preparation through decreased uptake by macrophages in the inflamed tissues.

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