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Phospholipid-based microemulsions of flurbiprofen by the spontaneous emulsification process

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Abstract

The purpose of this study was to investigate the possibility for parenteral delivery of flurbiprofen without chemical modification using a phospholipid-based microemulsion system. Microemulsions composed of ethyl oleate, lecithin and distearoylphosphatidyl-ethanolamine-*N*-poly(ethyleneglycol) 2000 (DSPE-PEG) were prepared using ethanol as a cosolvent. The effect of formulation variables on the particle size of the microemulsion was investigated. Flurbiprofen concentrations in plasma and various organs after the intravenous administration of flurbiprofen-loaded microemulsion were measured and compared with those after the intravenous administration of flurbiprofen axetil-entrapped emulsion (Lipfen®, 50 mg/5 ml as flurbiprofen axetil) and flurbiprofen solution. Phospholipid-based microemulsions could solubilize more than 10 mg ml^{-1} of flurbiprofen at the ratio of vehicle to drug at least 10:1, if the oil contents (10 or 20%) of common parenteral emulsions were used. The half-life, AUC and MRT of flurbiprofen loaded in microemulsion (ethyl oleate:lecithin:DSPE-PEG:flurbiprofen = 8:3:1:1.2) increased significantly. The biodistribution of flurbiprofen loaded in this microemulsion was quite different from others. Reticuloendothelial uptake of flurbiprofen loaded in microemulsion decreased compared with that in solution or Lipfen®. It is concluded that the current microemulsion system might be applicable to formulate the parenteral dosage form of poorly water-soluble flurbiprofen without chemical modification.

Keywords: Phospholipid; Distearoylphosphatidylethanolamine-*N*-poly(ethyleneglycol) 2000; Spontaneous emulsification; Flurbiprofen; Parenteral dosage form.

1. Introduction

Recently much attention has been paid to utilization of the phospholipids in formulating pharmaceutically acceptable microemulsions (Mag-

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dassi and Siman-Tov, 1990; Lundberg, 1994; Wheeler et al., 1994). Their non-toxicity makes them an ideal choice, especially for injectable microemulsions. The characteristic properties of lecithin in solution must be considered in preparing phospholipid-based microemulsions for parenteral use. Lecithin exhibits a very strong hydrophobicity due to two long hydrocarbon chains as well as a strong hydrophilicity due to the zwitterionic polar head groups which have dipole moments. There is a close balance between hydrophilic and lipophilic properties (Shinoda and Kaneko, 1989). Lecithin is too lipophilic to form spontaneously the lipid layer of zero interfacial tensions necessary for the formation of microemulsions. Thus, a cosurfactant such as a short chain alcohol is needed to achieve the ultra-low interfacial tensions necessary for microemulsions. Some recent reports described the phase study of phospholipid-based microemulsion (Shinoda et al., 1991; Attwood et al., 1992; Aboofazei and Lawrence, 1993). The majority of microemulsions was produced in the presence of a cosurfactant (Kim et al., 1997; Gao et al., 1998). The most commonly used cosurfactants are low molecular weight alcohols such as butanol and propanol; unfortunately, such alcohols are generally considered to be unacceptable pharmaceutically. Recently, Saint Ruth et al. (1995) reported the possibility of using ethanol as a cosurfactant for the formation of oil-in-water microemulsions composed of lecithin, isopropyl myristrate and water. It was reported that the phospholipid-based microemulsions could be prepared with more than 60% (w/w) ethanol in water as an aqueous phase, consequently, the total percentage of ethanol in the microemulsion was about 10–12%. However, it is hard to use microemulsions with higher concentration of ethanol in aqueous phase owing to patient compliance, especially for parenteral use. To overcome this problem, the excess amount of ethanol should be removed from the dispersion medium to prepare the parenteral emulsion.

To investigate the possibility for parenteral delivery of drug in microemulsion systems, flurbiprofen was employed as a model compound. Flurbiprofen, a phenylpropionic acid derivative which has analgesic, anti-inflammatory actions, is widely used in the treatment of rheumatoid arthritis and other rheumatic disorders. The solubility of flurbiprofen is very low in acidic media and water, but relatively high in alkaline media. Thus, commercial dosage forms of flurbiprofen are oral tablets and sustained release capsules. Parenteral dosage form of flurbiprofen is not available in the market. Chemically modified flurbiprofen, flurbiprofen axetil, was formulated in the form of emulsion and commercialized (Lipfen®, 50 mg/5 ml as flurbiprofen axetil, Green Cross, Japan).

In our previous study, microemulsions prepared with ethyl oleate and Tween 20 could solubilize flurbiprofen up to 10 mg ml^{-1}, but they had a limit to use because of uncertainty of long-term stability of microemulsion and toxicity of Tween 20 (Park and Kim, 1999).

The purpose of this work was to prepare parenteral dosage form of poorly water-soluble flurbiprofen without chemical modification using phospholipid-based microemulsion system to improve the stability and safety. In this study, microemulsions composed of ethyl oleate, lecithin and distearoylphosphatidylethanolamine-*N*-poly(ethyleneglycol) 2000 were prepared using ethanol by the spontaneous emulsification process. Then, ethanol was removed after the preparation so as to contain less than 0.2% of ethanol in microemulsions. The effect of formulation variables on the particle size of these phospholipid-based microemulsions was investigated. Flurbiprofen concentrations in plasma and various organs after intravenous administration of flurbiprofen-loaded microemulsion were measured and compared with those after intravenous administration of flurbiprofen axetil-entrapped emulsion (Lipfen®, 50 mg/5 ml as flurbiprofen axetil) and flurbiprofen solution.

2. Materials and methods

2.1. Materials

Flurbiprofen was supplied by Samil Pharmaceutical (Seoul, Korea). Ethyl oleate (EO) was

purchased from Aldrich Chemical (Milwaukee, WI). Egg lecithin (PC content 60%) and calcein were purchased from Doosan–Serdary Research Laboratories (Kyungki-Do, Korea) and Sigma Chemical (St. Louis, MO), respectively. Distearoyl-phosphatidylethamolamine-*N*-poly(ethyleneglycol) 2000 (DSPE-PEG) was purchased from Avanti Polar Lipids (Alabaster, AL). All other chemicals were of reagent grade and used without further purification. Male Sprague–Dawley rats weighing 280 ± 30 g were obtained from the Experimental Animal Center of Seoul National University (Seoul, Korea)).

2.2. Preparation of phospholipid-based microemulsion

Phospholipid-based microemulsions without or with drug were prepared by modifying the reported method (Yu et al., 1993). Briefly, both of EO and egg lecithin were dissolved in ethanol (oily ethanolic solution). Flurbiprofen was dissolved in oily ethanolic solution. DSPE-PEG was dissolved in water (aqueous phase). The oily ethanolic solution with/without flurbiprofen was then slowly added into the DSPE-PEG solution under moderate magnetic stirring. The aqueous phase immediately turned milky with opalescence as the result of microemulsion produced. The ethanol was then removed under the reduced pressure (360 mmHg) at 50°C. The microemulsion was concentrated to the desired final volume by the removal of water under the reduced pressure.

After the preparation of flurbiprofen-loaded microemulsions, the precipitated drug was removed from the microemulsion systems by centrifugation for 5 min at $3000 \times g$. The amount of flurbiprofen in the resulting supernatant was determined using a UV spectrophotometer at 280 nm after appropriate dilution with methanol.

2.3. Particle size determination

The particle size distribution and the average droplet size of microemulsions with/without flurbiprofen were measured at 25 ± 1°C by photon correlation spectroscopy. A light scattering spectrophotometer (LPA-3100, Otsuka Electronics, Osaka, Japan) and a data processing unit (LPA-3000, Otsuka Electronics) were used for characterizing the particle size in the 3–5000 nm range using the dynamic light scattering method. For measuring the particle size by photon correlation spectroscopy, microemulsions were diluted with water to the concentration range, 8000–12 000 counts per second (cps). Then, particle size measurement was validated after the dilution of samples to the above concentration range. The continuous phase of the emulsion did not exhibit light scattering, and the particle size distribution of diluted samples was not significantly changed.

2.4. Determination of liposomes in microemulsion systems

The proportion of liposomes in the microemulsion systems was determined by slight modification of the method of Lundberg (1994) using calcein as a water soluble marker. Egg lecithin (7.5 mg) and varying amounts of EO were dissolved in 1.5 ml of ethanol (oily ethanolic solution). A total of 30 mg of calcein and 2.5 mg of DSPE-PEG were dissolved in 3 ml of water (aqueous phase). The calcein-loaded microemulsion was prepared with the addition of oily ethanolic solution into the aqueous phase. As a reference, ethanolic solution without EO instead of oily ethanolic solution was added to the aqueous phase and the ethanol was removed under the reduced pressure. Under such conditions (without EO), liposomes were formed and calcein was entrapped inside the liposomes. Both the liposome and microemulsions were then loaded separately on a Sepharose CL-4B column (1×25 cm) to separate free calcein from that entrapped inside the liposomes. Aliquots of both liposomes and microemulsions were analyzed in the presence of 0.2% Triton-X 100 for the fluorescence intensity in a spectrofluorometer with excitation and emission wavelengths of 490 and 520 nm, respectively. The concentration of phospholipid in the preparations was determined by the Stewart assay (Stewart, 1959). The fluorescence intensity of calcein from each microemulsion was compared with the fluorescence intensity of calcein from the pure liposome preparation (phospholipid only). The

ratio of fluorescence intensity given by microemulsions (as measured by the phospholipid concentration) to that of the pure liposome (composed of equal amounts of phospholipid and microemulsion) was presented as the percentage of liposomal fraction in the microemulsion system.

2.5. Pharmacokinetic study

Under light ether anaesthesia, the femoral arteries and veins of rats were cannulated with PE-50 polyethylene tubing. After complete recovery from anaesthesia, flurbiprofen solution, microemulsions and commercial product (Lipfen®, Green Cross, Japan) (equivalent to 2.5 mg kg^{-1} as free flurbiprofen) were administered to the rats via the femoral vein. Blood samples (0.15 ml) were collected via the femoral artery at designated time intervals after the dose. The blood samples were centrifuged immediately at $3000 \times g$ for 1 min and 0.05 ml aliquots were transferred to Eppendorf tubes (1.5 ml) and stored in a freezer at -20°C prior to the analysis of flurbiprofen. The concentrations of flurbiprofen in the plasma were determined by HPLC (Park et al., 1997).

The non-compartmental pharmacokinetic parameters such as area under the drug concentration–time curve (AUC), biological half-life ($T_{1/2}$), mean resident time (MRT), total clearance (CL) and apparent volume of distribution at steady state (V_{ss}) were calculated based on the reported method (Gibaldi and Perrier, 1982). Levels of statistical significance ($p < 0.05$) were assessed using the one-way ANOVA followed by Duncan's multiple range test. All results are expressed as mean ± standard deviation (S.D.).

2.6. Tissue distribution

The carotid artery and the jugular vein were cannulated with polyethylene tubing (PE-60, Clay Adams, Parsippany, NJ) under light ether anaesthetization. Both cannulas were exteriorized to the dorsal side of the neck, where each cannula terminated with a long silastic tubing (Dow Corning, Midland, MI). The silastic tubing was covered with wire to allow free movement of the rat. The exposed areas were closed using a surgical suture. Each rat was housed in a rat cage. Flurbiprofen solution, microemulsions and commercial product, equivalent to 2.5 mg kg^{-1} as free flurbiprofen, were injected through the jugular vein to rats. At 2 and 8 h after the dose, blood was collected as much as possible through the carotid artery and each rat was exsanguinated. The blood was centrifuged, and the plasma was stored in the freezer prior to the measurement of flurbiprofen. Approximately 1 g of the liver, kidney, heart, lung and spleen was quickly removed, rinsed, minced, homogenized with four volumes of normal saline in a tissue homogenizer (Ultra-Turrax T 25, Janke and Kunkel, IKA-Labortechnik, Germany), and centrifuged immediately. Two 0.05 ml aliquots of plasma or supernatant of tissue homogenates were stored in the freezer prior to the analysis of flurbiprofen. The concentrations of flurbiprofen in the organs were determined by the HPLC method.

Levels of statistical significance ($p < 0.05$) were assessed using the one-way ANOVA followed by Duncan's multiple range test. All results are expressed as mean ± S.D.

3. Results and discussion

3.1. Effect of formulation variables on physical characteristics of microemulsion

It is known that the particle size distribution is one of the most important characteristics of emulsion for the evaluation of stability (Charman et al., 1992) and the in vivo fate of emulsion (Tarr and Yalkowsky, 1989). The effect of each component of the microemulsion systems on the resultant droplet size has been investigated.

Fig. 1 shows the mean particle size of microemulsions versus DSPE-PEG content in lecithin and DSPE-PEG mixtures (S_{mix}) as a surfactant for the preparation of microemulsions with various ratios of EO to the S_{mix}. The droplet size tended to be decreased with increasing ratio of S_{mix} to EO. The droplet size also increased dramatically with increasing DSPE-PEG content in S_{mix}. It indicates that the droplet size of these

microemulsion systems was greatly affected by the ratio of S_{mix} to EO and the DSPE-PEG content in S_{mix}. The mean droplet size of the microemulsion prepared with less than 33% DSPE-PEG content in S_{mix} was less than 200 nm, which is the optimal droplet size for an injectable emulsion. The smallest droplet size microemulsion was obtained with 20 ~ 25% DSPE-PEG content in S_{mix}.

Fig. 2 shows the plots of mean particle size of the microemulsion versus the EO content (%, w/w) in the mixture of EO and S_{mix} for the preparation of microemulsions with various ratios of lecithin to DSPE-PEG in S_{mix}. The smallest droplet size microemulsion was obtained from 33, 20 and 20% EO content in the mixture of EO and S_{mix} for 4:1, 3:1 and 2:1 ratio of lecithin to DSPE-PEG, respectively. The mean droplet size of the resultant microemulsions prepared with 33% EO content in the mixture of EO and S_{mix} was not changed for at least 10 weeks at 4°C.

The fraction of phospholipid in the form of liposomes, the by-product of microemulsions, was produced using ethanol as a cosolvent and by the spontaneous emulsification process. Thus, the liposomal fraction was quantified with calcein as a liposome marker. When the weight ratio of lecithin to DSPE-PEG was fixed at 3:1, a large liposomal fraction was found in the microemulsion system as shown in Fig. 3. When the EO content was 20% in the EO and S_{mix} mixture, about 30% liposomal fraction was found in the microemulsion systems as shown in Fig. 3. With increasing EO content, the liposome fraction decreased. Less than 15% of the liposomal fraction was obtained when EO content was higher than 50% in the EO and S_{mix} mixture. To reduce the

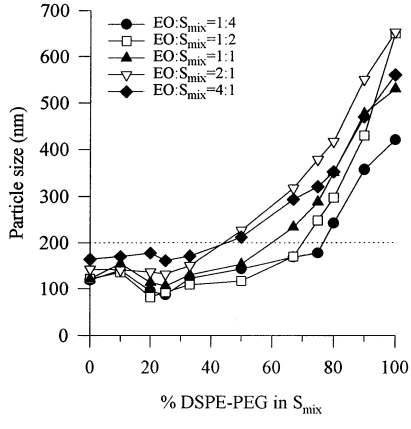

Fig. 1. Mean particle size of microemulsion versus DSPE-PEG content in S_{mix} for the preparation of microemulsions with various ratios of EO to the S_{mix}. EO means ethyl oleate and S_{mix} means the mixture of lecithin and DSPE-PEG.

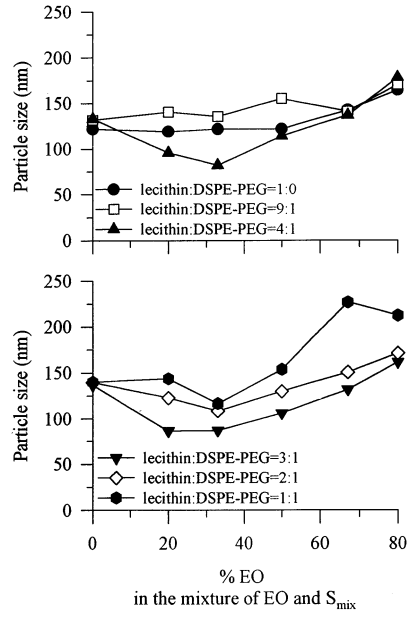

Fig. 2. Mean particle size of microemulsion versus EO contents in the mixture of EO and S_{mix} for the preparation of microemulsions with various ratios of lecithin to DSPE-PEG.

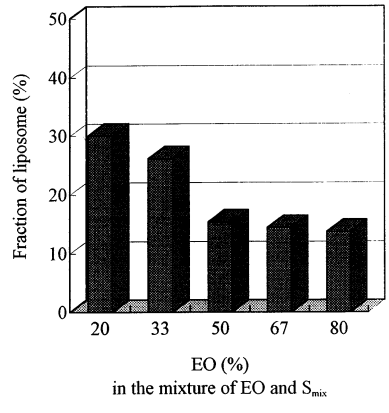

Fig. 3. Fraction of phospholipid in the form of liposomes as a by-product of microemulsions versus EO contents (%, w/w) (leithin:DSPE-PEG = 3:1).

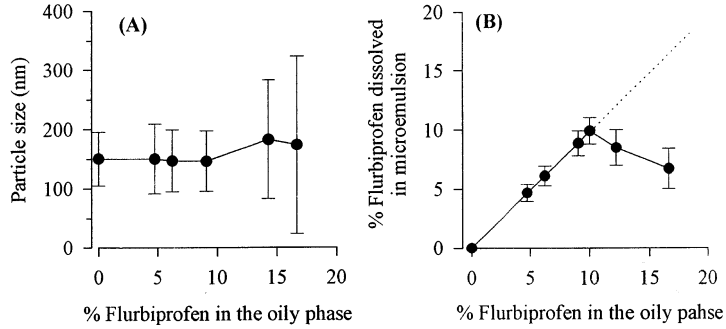

Fig. 4. Particle size distributions of microemulsions (A) and percentage of flurbiprofen dissolved in microemulsions (B) as a function of flurbiprofen contents (%, w/w) in the oily phase.

liposomal fraction in the system, EO content should be higher than 50% in the mixture of EO and $S_{mix.}$

3.2. Solubilization of flurbiprofen in the microemulsion systems

The flurbiprofen-loaded microemulsions were evaluated in particle size and solubility of flurbiprofen. Fig. 4(A) shows the particle size distribution of microemulsions against the loading amount of flurbiprofen in microemulsions prepared with the 8:3:1 weight ratio of EO:lecithin:DSPE-PEG. The mean droplet sizes of microemulsions prepared with less than 10% (w/w) of flurbiprofen were around 150 nm and the droplet size distributions were relatively uniform. On the other hand, the mean droplet size of microemulsion prepared with more than 15% (w/w) of flurbiprofen was slightly increased and the particle size distribution broaden. It is indicated that the excess amount of flurbiprofen unincorporated in EO core and/or lipids layers formed aggregates with phospholipids (Park and Kim, 1999). It was also confirmed by measuring the amount (%) of flurbiprofen dissolved in the microemulsion as shown in Fig. 4(B). When the microemulsions were prepared with less than 10% of flurbiprofen in the oily phase, all of the loading drugs were completely dissolved in the microemulsion system. On the other hand, when the microemulsions were prepared with more than 10% of flurbiprofen in the oily phase, flurbiprofen was not completely dissolved. Flurbiprofen precipitates were observed after the removal of ethanol. Therefore, this microemulsion system could solubilize flurbiprofen up to about 10% in the oily phase. The concentration of flurbiprofen axetil in the marketed dosage form, Lipfen®, is 10 mg ml^{-1} and a total volume of 5 ml is usually injected intravenously as a single dose. Thus, the desired concentration of flurbiprofen (10 mg ml^{-1}) was achieved in this microemulsion system with the 1:9 ratio of oily phase to aqueous phase.

3.3. Stability of flurbiprofen-loaded microemulsions

Fig. 5(A) shows the plots of mean droplet size of flurbiprofen-loaded microemulsion as a function of DSPE-PEG contents in S_{mix} when the weight ratio of EO to S_{mix} was fixed at 2:1. The mean droplet size of flurbiprofen-loaded microemulsion prepared with lecithin alone was about 500 nm. On the other hand, the mean droplet size decreased markedly with increasing the DSPE-PEG content in S_{mix}. It was less than 200 nm when the content of DSPE-PEG was 20–50% in S_{mix}. It was observed that the sedimentation occurred in microemulsion system prepared with less than 20% DSPE-PEG in S_{mix} and creaming took place in microemulsion system prepared with more than 50% DSPE-PEG in S_{mix}. These might be due to the instability of the system and the increase in droplet size. Fig. 5(B) shows the particle distribution of the microemulsion system (EO:lecithin:DSPE-PEG: flurbiprofen = 8:3:1:1.2) after 6 months storage at 4°C. The mean droplet

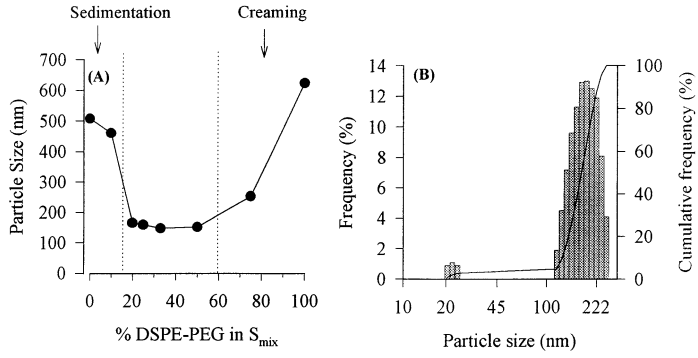

Fig. 5. Stability of flurbiprofen-loaded microemulsion at 4°C. (A) Changes in mean particle size of flurbiprofen-loaded microemulsion as a function of DSPE-PEG concentration in the surfactant mixture (EO: S_{mix} = 2:1) at 3 weeks. (B) Particle size distribution of microemulsion (EO:lecithin:DSPE-PEG: flurbiprofen = 8:3:1:1.2) at 6 months.

size of this microemulsion was about 180 nm. Smaller micellar particles of around 20 nm in mean diameter were observed in the size distribution curves, but they were almost negligible (< 1%) compared to the average particles. The largest diameter of the microemulsion droplet was around 250 nm, which is much smaller than the diameter (4000–6000 nm) that increases the incidence of emboli. Taken together, this flurbiprofen-loaded microemulsion was very stable, hence this system would be applicable as a flurbiprofen carrier.

3.4. Pharmacokinetics and tissue distribution of flurbiprofen

Fig. 6 shows the plasma concentration–time profiles of flurbiprofen after intravenous administration of aqueous flurbiprofen solution (PBS, pH 7.4, 1.25 mg ml^{-1}), flurbiprofen-loaded microemulsion (EO:lecithin:DSPE-PEG:flurbiprofen = 8:3:1:1.2) and commercial Lipfen® (flurbiprofen axetil, 50 mg/5 ml) to rats at a dose of 2.5 mg kg^{-1} as flurbiprofen. In the case of Lipfen®, flurbiprofen axetil, the prodrug of flurbiprofen, could not be detected in the plasma sample. This might be due to the fast hydrolysis of flurbiprofen axetil to flurbiprofen in rat plasma (Sciesaka et al., 1988). The early plasma levels of flurbiprofen following intravenous administration were about the same in all preparations. The plasma levels following Lipfen® administration were higher than those following flurbiprofen solution. However, there were no significant differences between them. When compared with Lipfen®, overall plasma levels of flurbiprofen following flurbiprofen-loaded microemulsion administration were not significantly different. But, plasma levels following microemulsion administration were significantly ($p < 0.05$) higher than those following flurbiprofen solution except for the early phase (15 min) after administration.

The non-compartmental pharmacokinetic parameters of flurbiprofen after intravenous ad-

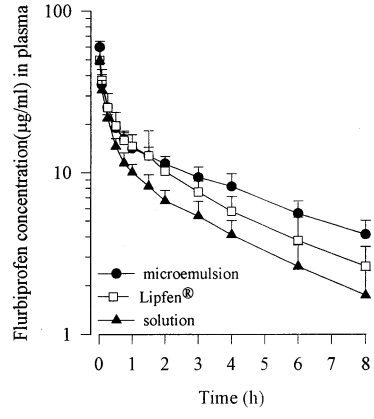

Fig. 6. The plasma concentration–time profiles of flurbiprofen after intravenous administration of flurbiprofen solution, commercial product (Lipfen®, flurbiprofen axetil-loaded emulsion) and flurbiprofen-loaded microemulsion (EO:lecithin:DSPE-PEG:flurbiprofen = 8:3:1:1.2) equivalent to 2.5 mg kg^{-1} as flurbiprofen to rats ($n = 5$).

Table 1
Noncompartmental pharmacokinetic parameters of flurbiprofen after intravenous administration of solution, commercial Lipfen® and microemulsion system[a] equivalent to 2.5 mg kg^{-1} of flurbiprofen to rats ($n = 5$)

Formulation parameters	Solution	Lipfen®	Microemulsion
$T_{1/2}$ (h)	2.78 ± 0.52	3.04 ± 0.63	$4.19 \pm 0.55^{*,**}$
AUC (μg h ml^{-1})	54.08 ± 9.78	79.12 ± 16.43*	$102.39 \pm 17.06^{*,**}$
MRT (h)	3.40 ± 0.78	3.92 ± 0.76	$5.54 \pm 0.77^{*,**}$
V_{ss} (ml kg^{-1})	156.78 ± 15.82	124.96 ± 14.52*	136.39 ± 13.73
CL (ml h^{-1} Kg^{-1})	47.94 ± 10.65	32.64 ± 6.34*	24.91 ± 4.47*

[a] EO:lecithin:DSPE-PEG:flurbiprofen = 8:3:1:1.2. The values are expressed as mean ± S.D. ($n = 5$).
* $p < 0.05$, relative to solution (one-way ANOVA followed by Duncan's multiple range test).
** $p < 0.05$, relative to Lipfen® (one-way ANOVA followed by Duncan's multiple range test).

ministration of the aqueous flurbiprofen solution, flurbiprofen-loaded microemulsion and commercial Lipfen® are listed in Table 1. Flurbiprofen incorporated in emulsions (Lipfen® and microemulsion) resulted in a significantly higher AUC and lower CL compared with flurbirprofen solution ($p < 0.05$). Lipfen® resulted in significantly higher AUC and lower CL but $T_{1/2}$ and MRT were not significantly different compared with the flurbiprofen solution. The AUC of flurbiprofen axetil entrapped lipid emulsion (Lipfen®) increased 1.5-fold compared with that of the flurbiprofen solution (79.12 ± 16.43 vs. 54.08 ± 9.78). The $T_{1/2}$, AUC and MRT of flurbiprofen incorporated in the microemulsion increased 1.5-, 1.9- and 1.6-fold compared with those of flurbiprofen solution (4.19 ± 0.55 vs. 2.78 ± 0.52; 102.39 ± 17.06 vs. 54.08 ± 9.78. 5.54 ± 0.77 vs. 3.40 ± 0.78); 1.4-, 1.3-, and 1.4-fold compared with those of Lipfen® (4.19 ± 0.55 vs. 3.04 ± 0.63; 102.39 ± 17.06 vs. 79.12 ± 16.43; 5.54 ± 0.77 vs. 3.92 ± 0.76). These results indicate that the removal of flurbiprofen-loaded microemulsion from circulation is delayed with PEG-lipid derivatives, since PEG-lipid derivatives are known to reduce the RES uptake and stabilize the vesicles (Allen et al., 1991; Klibanov et al., 1990).

The tissue levels of flurbiprofen at each time point were divided by the plasma levels of the corresponding time point in each rat. Tissue-to-plasma (T/P) ratios of flurbiprofen concentrations at 2 and 8 h were calculated and are shown in Fig. 7. The T/P ratios after the dose of flurbiprofen-loaded microemulsion were significantly lower in tissues examined than those after the dose of flurbiprofen solution. For example, the liver/plasma ratios at 2 and 8 h after the dose of flurbiprofen solution, Lipfen® and flurbiprofen-incorporated microemulsion were 0.202 ± 0.015, 0.165 ± 0.012 and 0.156 ± 0.003; 0.263 ± 0.047, 0.218 ± 0.013 and 0.127 ± 0.013, respectively. At 2

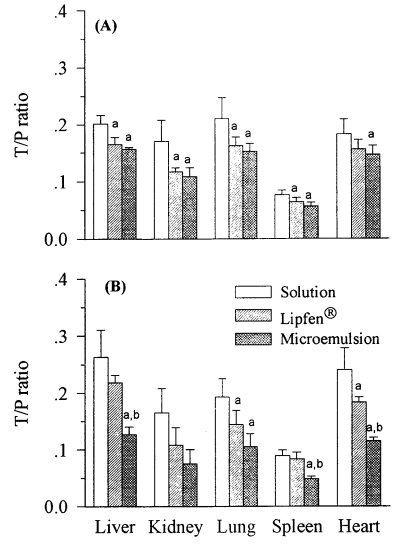

Fig. 7. Tissue/plasma concentration (T/P) ratio of flurbiprofen at 2 h (A) and 8 h (B) after intravenous administration of solution, commercial product (Lipfen®, flurbiprofen axetil-loaded emulsion) and flurbiprofen-loaded microemulsion (EO:lecithin:DSPE-PEG:flurbiprofen = 8:3:1:1.2) equivalent to 2.5 mg kg^{-1} as flurbiprofen to rats ($n = 4$). Each point represents the mean ± S.D. a $p < 0.05$, relative to solution (one-way ANOVA followed by Duncan's multiple range test), b $p < 0.05$, relative to Lipfen® (one-way ANOVA followed by Duncan's multiple range test)

h after the dose, there were no significant differences in T/P ratios between Lipfen® and the microemulsion in the tissues examined. At 8 h after the dose of flurbiprofen-loaded microemulsion, however, the T/P ratios in liver, lung, spleen and heart were significantly lower than those after the dose of flurbiprofen solution. The T/P ratios in liver and spleen, RES-rich tissues, after the dose of flurbiprofen-loaded microemulsion were significantly reduced compared with those after the dose of Lipfen®. This result might be due to the increase in the surface hydrophilicity of the microemulsion by PEG-lipid derivatives. Surface hydrophilicity may reduce or even eliminate the adhesion of opsonic material onto the surface of emulsion which is believed to be an essential process for phagocytosis and subsequent uptake of the emulsion by Kupffer cells (Torchilin et al., 1994; Wheeler et al., 1994).

4. Conclusions

Phospholipid-based microemulsions were prepared using ethanol as a cosolvent by the spontaneous emulsification process. The mean droplet size of microemulsion system (EO:lecithin:DSPE-PEG: flurbiprofen = 8:3:1:1.2) was about 180 nm and no changes were noted for at least 6 months. The plasma concentrations of flurbiprofen following the intravenous administration of the microemulsion were similar to those following commercial Lipfen®. However, the $T_{1/2}$, AUC and MRT of flurbiprofen-loaded microemulsion were significantly increased. This microemulsion could also reduce uptake into RES-rich organs, which is one of the characteristics of this particular drug carrier system, due to the increase in the surface hydrophilicity of the microemulsion by inclusion of DSPE-PEG.

This system has merit to formulate the parenteral dosage form of flurbiprofen without chemical modification, synthesis of flurbiprofen axetil derivative. This formulation approach might also be applicable to the development of the parenteral dosage form of other poorly-water soluble drugs.

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