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Preparation and evaluation of flurbiprofen-loaded microemulsion for parenteral delivery

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Abstract

The purpose of this study was to improve the solubility of flurbiprofen, a poorly water-soluble drug, in an oil-in-water (o/w) microemulsion that is suitable for parenteral administration. Microemulsions with varying ratios of oil to surfactant were prepared with ethyl oleate, Tween 20 and isotonic solution. The effect of formulation variables on the particle size of microemulsion and solubility of flurbiprofen in microemulsion system was investigated. The pharmacokinetic parameters of flurbiprofen after intravenous administration of flurbiprofen-loaded microemulsion were compared with those of a solution of the drug. The mean droplet diameter of microemulsion containing less than 1% (w/w) of flurbiprofen was below 100 nm. The maximum solubility of flurbiprofen in the microemulsion system was found to be 10 mg/ml. However, the mean droplet diameters of flurbiprofen-loaded o/w microemulsions tend to be increased at room temperature. The pharmacokinetic parameters of flurbiprofen after intravenous administration of flurbiprofen-loaded microemulsion to rats were not significantly different from those of flurbiprofen in phosphate-buffered saline solution. It can be concluded that microemulsions of flurbiprofen prepared with ethyl oleate and Tween 20 can be used as a parenteral drug carrier for this and other poorly water-soluble drugs, provided that physical stability can be properly addressed.

Keywords: Flurbiprofen; Ethyl oleate; Tween 20; Microemulsion; Poorly water-soluble; Pharmacokinetics

1. Introduction

Recently, much attention has been paid to the application of microemulsions as drug delivery systems, since microemulsions are thermodynamically stable and are formed spontaneously by simple mixing of the various components (Prince, 1977).

Water-in-oil (w/o) microemulsions have been described in the literature as drug carriers of water-soluble molecules for oral (Ritschel, 1991; Sarciaux et al., 1995) or intramuscular delivery (Gasco et al., 1990). In the case of w/o microe-

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mulsions, phase inversion is an interesting property and the resulting oil-in water (o/w) microemulsions can be used for parenteral drug delivery (Constantinides et al., 1994; Constantinides and Yiv, 1995; Kim et al., 1997). It has been found that w/o microemulsions can easily be inverted into o/w microemulsions and/or multiple water-in-oil-in-water (w/o/w) emulsions upon dilution with excess aqueous phase (Constantinides and Yiv, 1995). Recently, it was reported that the o/w microemulsions formed spontaneously by simple mixing were prepared as a drug delivery system (Malcolmson et al., 1998; Von Corswant et al., 1998). Some of the advantages of o/w microemulsions as a drug delivery system include improvement of drug solubilization, potential for parenteral use and production on a large industrial scale without high-energy homogenization.

Poorly water-soluble drugs may not be solubilized to the extent necessary (10^3- to 10^5-fold) for the relatively high concentrations (1–100 mg/ml) which are frequently required in parenteral use. Drug-loaded emulsions may be prepared extemporaneously by adding a concentrated solution of drug in a suitable solvent to the drug-free emulsion (El-Sayed and Repta, 1983; Oh et al., 1991). A potential problem in developing these emulsions is precipitation of drug in the aqueous phase prior to its incorporation into the oily phase.

Flurbiprofen, a phenylpropionic acid derivative which has analgesic, anti-inflammatory actions, was employed as a model compound. Flurbiprofen is widely used in the treatment of rheumatoid arthritis and other rheumatic disorders. The intrinsic solubility of flurbiprofen is about 5.0×10^{-5} M (Anderson and Conradi, 1985). Thus, commercial oral dosage forms of flurbiprofen are tablets and sustained release capsules. A parenteral dosage form of flurbiprofen is not available in the market. Chemically modified flurbiprofen derivative, flurbiprofen axetil, was formulated in the form of emulsion for parenteral use rather than flurbiprofen itself (Lipfen®, 50 mg/5 ml as flurbiprofen axetil; Green Cross, Japan). A single dose of flurbiprofen for oral administration is from 50 to 75 mg (Insel, 1996) and the bioavailability of flurbiprofen is about 92% (Davies, 1995). Taken together, a single dose of flurbiprofen for parenteral preparation could be approximately 50 mg.

The purpose of this study was to formulate the parenteral delivery system for poorly water-soluble flurbiprofen without chemical modification using o/w microemulsions. Microemulsions with varying weight ratios of oil to surfactant were prepared with ethyl oleate as an oil, Tween 20 as a surfactant and isotonic solution as an aqueous phase. The effect of formulation variables on the particle size of microemulsions and the solubility of flurbiprofen was investigated. The pharmacokinetic parameters of flurbiprofen after intravenous administration of microemulsion or a solution formulation were also evaluated.

2. Materials and methods

2.1. Materials

Flurbiprofen was supplied by Samil Pharmaceutical (Seoul, South Korea). Ethyl oleate was purchased from Aldrich Chemical (Milwaukee, WI, USA). Polyoxyethylene(20) sorbitan monolaurate (Tween 20) was supplied by ICI Americas (Wilmington, DE, USA). 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, South Korea).

2.2. Preparation and physicochemical evaluation of flurbiprofen-loaded microemulsions

2.2.1. Preparation

Ethyl oleate was blended with Tween 20 in fixed weight ratios (1:2, 1:3, and 1:4). Flurbiprofen (0–20% w/w) was dispersed into the mixture of ethyl oleate and Tween 20 with a constant stirring until the mixture became clear. An aliquot (0.1 g) of flurbiprofen-loaded mixture was then added to 0.9 g of aqueous phase (saline, 2.5% glycerol solution, 5% sorbitol solution and 5% dextrose solution). The mixture was gently shaken and kept at ambient temperature (25°C) to obtain a clear or translucent microemulsion.

2.2.2. Entrapment of flurbiprofen in microemulsions

At 3 days after the preparation of flurbiprofen-loaded microemulsions, the precipitated drug was removed from microemulsions by filtration through a 0.45 μm membrane filter. The amount of flurbiprofen in the resulting clear filtrate was determined using a UV spectrophotometer at 280 nm after appropriate dilution with methanol.

2.2.3. Stability of microemulsions

The physical stability of microemulsions with 1% flurbiprofen kept at ambient temperature was evaluated by measuring particle size changes at designated time intervals.

2.2.4. 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) equipped with 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 aqueous phase, which is the continuous phase of emulsions. The continuous phase of microemulsions did not exhibit light scattering and the particle size of diluted microemulsion (up to 20-fold dilution) was not significantly changed.

2.3. Pharmacokinetic study

Under light ether anesthesia, the femoral arteries and veins of rats were cannulated with PE-50 polyethylene tubing. After complete recovery from anesthesia, flurbiprofen-loaded microemulsion (ethyl oleate:Tween 20 = 1:4, 7.5 mg/ml) and saturated solution of flurbiprofen in pH 7.4 phosphate-buffered saline (PBS) (1.25 mg/ml) were administered intravenously to the femoral vein through the catheter at a dose of 0.33 and 2 ml/kg, equivalent to the dose of 2.5 mg/kg as flurbiprofen, respectively. 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 an aliquot (50 μl) was transferred to an eppendorf tube and stored in a freezer at -20°C prior to the assay for flurbiprofen. The concentrations of flurbiprofen in the plasma were determined by the previously reported high performance liquid chromatography method (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 residence 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 Student t-test between the two means for unpaired data. All results are expressed as mean $\pm$ SD values.

3. Results and discussion

3.1. Particle size analysis and flurbiprofen entrapment

Oil-in-water microemulsions without/with flurbiprofen were prepared with varying compositions of ethyl oleate, Tween 20, flurbiprofen and aqueous phase. The results for the particle size analysis of various microemulsions are shown in Table 1. The numbers in parentheses were the ratios of average diameter of weight distribution to average diameter of number distribution. They represent the homogeneity of droplet distribution. As they are close to 1, the droplet distribution is more homogeneous. In the case of a microemulsion without flurbiprofen, the droplet diameter decreased and the droplet distribution was more homogeneous with increasing the ratio of Tween 20 to ethyl oleate. Regardless of the aqueous phase composition, the average droplet diameter decreased to a certain level and then increased with the incorporation of flurbiprofen. The average droplet diameter reached a minimum value when the content of flurbiprofen was 1.5, 1 and

0.5% in the ratio of 1:2, 1:3 and 1:4 of ethyl oleate to Tween 20, respectively. Currently, it is not clear by which mechanism droplet size decreased. However, the following two possibilities can be considered. First, a certain portion of undissolved drug could act as an emulsifying agent by the deposition of drug particles at the interface of microemulsion (Martin, 1993). Second, by the deposition of drug at the interface of microemulsion, the reduced mobility of surfactant is thought to decrease the particle size of drug-loaded microemulsion.

On the other hand, the increase of microemulsion size with increasing the flurbiprofen content further may be related to the formation of drug aggregate on the surface of oil droplets due to the excess amount of drug undissolved.

The particle size distributions of flurbiprofen-loaded microemulsions (ethyl oleate:Tween 20 = 1:4) are shown in Fig. 1. In the case of microemulsion without flurbiprofen (Fig. 1A), particle size distribution was homogenous and the mean droplet diameter was 91.3 ± 10 nm. In Fig. 1B, smaller micellar particles (mean diameter, around 10 nm) and small microemulsion droplets (mean diameter, 81 nm) were observed in the size distribution of microemulsions with 0.5% (w/w) flurbiprofen. It is already indicated that a certain portion of surfactant on the surface of droplets was replaced by the drug molecules or particles. In the microemulsion system with 1% (w/w) flurbiprofen, micellar particles were not observed as shown in Fig. 1C. In the case of the microemulsion system prepared with less than 1% (w/w) flurbiprofen, the concentration of flurbiprofen was not changed after 3 days as shown in Fig. 2. That is, all of the loading drugs were completely dissolved in three different microemulsion systems. Fig. 1D shows that the microemulsion system prepared with 1.5% (w/w) flurbiprofen has two different size distributions. Larger particles of above 500 nm in particle size distribution curves were observed immediately after preparation (Fig. 1D). At 3 days after preparation, the solubility of flurbiprofen was decreased in the microemulsion

Table 1
Effect of the aqueous phase compositions and drug contents (% w/w) on the mean droplet diameter (nm) of microemulsion

Drug (%)	Aqueous phase			
	0.9% NaCl solution	2.5% Glycerol solution	5% Sorbitol solution	5% Dextrose solution
Ethyl oleate:Tween 20 = 1:2 (weight ratio)				
0	235.6 (3.03)*	272.0 (2.34)	267.4 (2.56)	283.1 (2.26)
0.5	199.8 (1.9)	208.4 (1.55)	182.3 (1.71)	209.2(1.83)
1.0	185.5 (1.03)	176.9 (1.02)	166.8 (1.59)	167.7(1.02)
1.5	248.2 (2.36)	167.8 (1.82)	159.3 (1.76)	173.7(1.61)
2.0	395.7 (3.62)	194.2 (5.62)	272.9 (1.83)	215.0(4.20)
Ethyl oleate:Tween 20 = 1:3 (weight ratio)				
0	110.5 (1.52)	114.7 (1.49)	120.1 (1.73)	115.4 (1.50)
0.5	79.3 (1.38)	86.1 (1.16)	94.4 (1.36)	86.8 (1.84)
1.0	76.5 (1.32)	69.9 (1.60)	79.5 (1.71)	74.9 (1.60)
1.5	253 (2.14)	126.6 (1.85)	174.9 (1.23)	165.5 (1.90)
2.0	195.8 (1.19)	178.5 (1.04)	178.0 (1.68)	158.9 (1.67)
Ethyl oleate:Tween 20 = 1:4 (weight ratio)				
0	81.0 (1.28)	97.4 (1.03)	91.3 (1.06)	85.2 (1.21)
0.5	50.0 (1.58)	81.3 (1.23)	62.0 (1.29)	53.0 (1.23)
1.0	93.3 (1.03)	106.5 (1.25)	89.7 (1.04)	97.9 (1.02)
1.5	305 (1.20)	206.3 (2.06)	244.0 (1.93)	282.1 (1.25)
2.0	253.3 (4.67)	236.2 (15.23)	259.3 (3.82)	232.9 (11.6)

* The numbers in parentheses represent the ratios of the average of number distribution to the average of weight distribution.

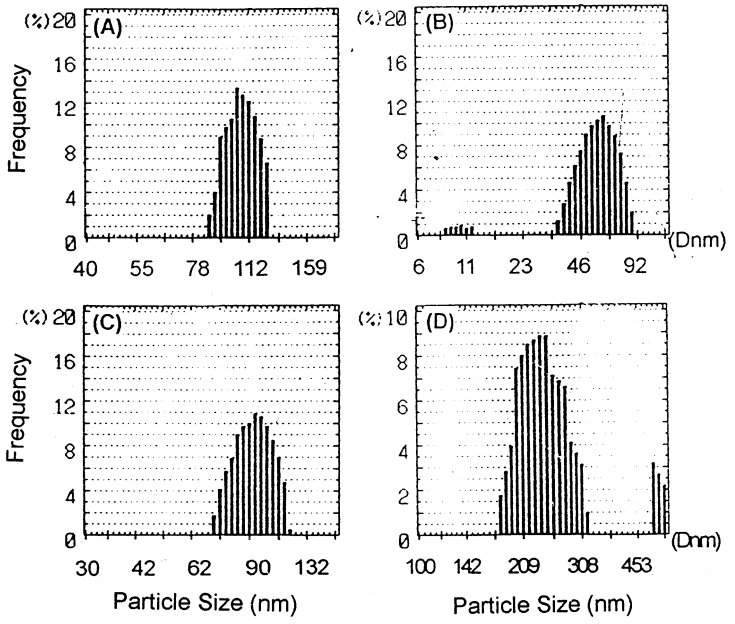

Fig. 1. The particle size distribution of microemulsions (ethyl oleate:Tween 20 = 1:4) containing various amounts of flurbiprofen: (A) 0%, (B) 0.5%, (C) 1% and (D) 1.5% (aqueous phase, 5% sorbitol solution).

systems containing more than 1.5% (w/w) flurbiprofen and the spinal crystals were observed. It is indicated that the excess amount of drug that existed in the interface of the oil–surfactant mixture at preparation was released to the aqueous phase and grew the crystal and/or precipitate of flurbiprofen in the course of time. After the filtration of precipitates through the membrane filter (0.45 μm), the excess amount of Tween 20 did not affect the solubility of flurbiprofen in the flurbiprofen-loaded microemulsions, as shown in Fig. 2.

Taken together, the flurbiprofen-loaded microemulsion system using ethyl oleate and Tween 20 mixture could be prepared with 1% (w/w) flurbiprofen. The microemulsion system using ethyl oleate and Tween 20 mixture could solubilize flurbiprofen up to 10 mg/ml, which is an about 800- and 8-fold increase compared with intrinsic solubility and solubility in PBS, respectively. When 50 mg flurbiprofen is administered via i.v. bolus, the injection volume of flurbiprofen dissolved in PBS is about 40 ml, which is not practical as i.v. bolus. On the other hand, the injection volume of flurbiprofen-loaded microemulsion is 5 ml, which is suitable for i.v. bolus.

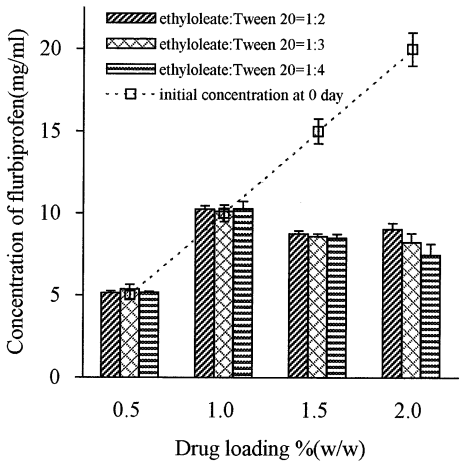

Fig. 2. Concentrations of flurbiprofen in the microemulsion systems with various ratios of Tween 20 to ethyl oleate at 3 days after preparation. Drug loading (%) means flurbiprofen contents (% w/w) in the mixture of ethyl oleate, Tween 20 and flurbiprofen.

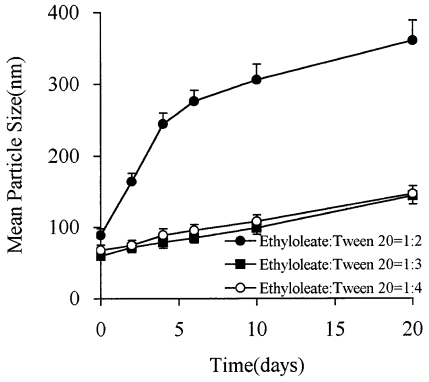

Fig. 3. Changes in mean particle size of microemulsion systems containing 1% (w/w) flurbiprofen as a function of time at room temperature.

3.2. Stability of the microemulsion

The stability of the 1% (w/w) flurbiprofen-loaded microemulsion was evaluated by monitoring mean droplet diameter changes. As shown in Fig. 3, the droplet diameter of 1% (w/w) flurbiprofen-loaded microemulsion tends to be increased with respect to time. Nevertheless, at 20 days after preparation, the mean droplet diameters of microemulsions with 1:3 and 1:4 ratio of ethyl oleate to Tween 20 were 144 and 147 nm, respectively. These microemulsion droplets are much smaller than the diameter of red blood cells (8–10 μm) and, therefore, this system is suitable for injectable applications.

3.3. Pharmacokinetic evaluation

Fig. 4 shows the plasma concentration–time profiles of flurbiprofen after intravenous administration of flurbiprofen-loaded microemulsion and flurbiprofen dissolved in PBS to rats at a dose of 2.5 mg/kg as flurbiprofen, respectively. The noncompartmental pharmacokinetic parameters in Table 2 were calculated based on the observed plasma levels of the drug. Overall plasma concentrations of flurbiprofen upon administration of flurbiprofen-loaded microemulsion were slightly higher than those after the administration of flurbiprofen in PBS, and the calculated pharmacokinetic parameters were not significantly different between the two drug formulations.

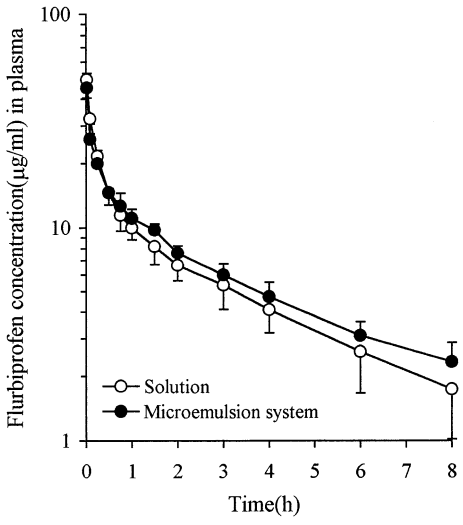

Fig. 4. The plasma concentration–time profiles of flurbiprofen after intravenous administration of flurbiprofen dissolved in PBS (pH 7.4, 1.25 mg/ml) and flurbiprofen-loaded microemulsion (ethyl oleate:Tween 20 = 1:4, 7.5 mg/ml) to rats ($n = 5$) with varying injection volume to adjust the dose of 2.5 mg/kg as flurbiprofen.

4. Conclusions

Oil-in-water microemulsions prepared with ethyl oleate and Tween 20 could solubilize flurbiprofen up to 10 mg/ml, which is an 8-fold increase compared with the flurbiprofen solubility in PBS solution. Increased solubility of flurbiprofen in this microemulsion implies the reduction of injection volume. The pharmacokinetics of

Table 2
Noncompartmental pharmacokinetic parameters of flurbiprofen after intravenous administration of 2.5 mg/kg of flurbiprofen in PBS (pH 7.4) solution and microemulsion (ethyl oleate:Tween 20 = 1:4) to rats ($n = 5$)[a]

Parameters	Formulation	
	Solution	Microemulsion
$T_{1/2}$ (h)	2.78 ± 0.52	3.51 ± 0.62
AUC (μg/h/ml)	54.08 ± 9.78	63.02 ± 4.15
MRT (h)	3.40 ± 0.78	4.41 ± 0.74
V_{ss} (ml/kg)	156.78 ± 15.82	174.93 ± 23.02
CL (ml/hkg)	47.94 ± 10.65	39.25 ± 3.06

[a] $P < 0.05$, when compared with solution.

flurbiprofen after intravenous administration of flurbiprofen-loaded microemulsion to rats was not significantly different from that of flurbiprofen in PBS. Therefore, it is suggested that this microemulsion system could be used as a parenteral drug carrier for lipophilic drugs without chemical modification, especially flurbiprofen, provided that the long-term stability of microemulsions is addressed.

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