Distribution of Prostaglandin E_1 in Lipid Emulsion in Relation to Release Rate from Lipid Particles

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The distribution style of prostaglandin E_1 (PGE_1) in injectable lipid emulsion (Lipo-PGE_1) was analyzed using a three-phase model: an aqueous phase, an oil phase and an oil/water interface. A combination of the oil phase and the oil/water interface was obtained as the particle phase. Initial diffusion rates of PGE_1 from lipid particles to the aqueous phase were determined by the dialysis method, and equilibrium concentrations were calculated by the diffusion rates using an equation derived from Fick's theory. Lipo-PGE_1 enclosed in cellulose tubing was immersed in buffer solutions (pH 5.5, 7.4), then incubated at various constant temperatures. Each concentration of PGE_1 in the aqueous phase was measured at appropriate intervals. By using these values, partition coefficients between the particle phase and the aqueous phase were calculated. It was found that a larger portion of PGE_1 was distributed in the particle phase compared with the aqueous phase in Lipo-PGE_1 at each temperature. Furthermore, actual partition coefficients between oil and aqueous phases were measured. From the results of two partition coefficients, the distribution ratio of PGE_1 in Lipo-PGE_1 was determined as: oil : oil/water interface : water = 0.2 : 93.1 : 6.7, that is, 93% PGE_1 was found to be distributed in lipid particles. On the other hand, by diluting PGE_1 one hundred times with transfusion, the distribution ratio of PGE_1 in lipid particles decreased as the pH of the transfusion increased. The ratios after 2 h were 74% and 47% for pH 5.5 and 7.4, respectively.

In conclusion, a large portion of PGE_1 was found to be distributed in the particle phase when Lipo-PGE_1 was diluted with transfusion. Since most PGE_1 exists in an oil/water interface, it is considered that it has a strong affinity for phospholipids. Thus, this affinity may contribute to the remarkable increase in activity in clinical treatment.

Keywords prostaglandin E_1; lipid emulsion; distribution; emulsion; lipid particle; partition

Lipid emulsion formulations of prostaglandin E_1 (PGE_1) show a remarkable increase in activities in the treatment of various vascular disorders,[1-3] as well as a reduction in side effects such as local irritation near the site of injection following parenteral administration.[4-8]

But the behavior of PGE_1 in preparation has not been extensively investigated. Since PGE_1 in injectable lipid emulsion (Lipo-PGE_1) is generally diluted with several kinds of transfusions for treatments, it is important to clarify the distribution of PGE_1 in these transfusions, because it may be released from the lipid particles in the aqueous phase in the transfusion bottle. Minakuchi *et al.* reported that most of the PGE_1 was released in an aqueous medium immediately after diluting it ten times with a saline or buffer (pH 5.6).[9] Based on these results, they reported that Lipo-PGE_1 might not exert its effect as a lipid emulsion in clinical treatment. Many case studies indicate that Lipo-PGE_1 diluted two hundred times with an aqueous solution exerts high activity in clinical treatment. For example, the activity of Lipo-PGE_1 (3 μg) was greater than that of PGE_1-CD (cyclodextrin inclusion compound of PGE_1, 40 μg).[10] The purpose of this study is to identify those factors which account for the high efficacy of Lipo-PGE_1 from the pharmaceutical viewpoint, taking the distribution of PGE_1 in lipid emulsion into consideration.

Theoretical

Derivation of the Diffusion Rate Equations in Relation to Equilibrium Concentrations Fick's theory was applied to explain the transport of a drug in lipid emulsion. The amount, M, of drug flowing through a unit cross section, S, of a membrane in unit time, t, is known as the flux, J, in Fick's first law.

$$J=\frac{dM}{S\cdot dt} \tag{1}$$

The flux, in turn, is proportional to the concentration gradient, dC/dx:

$$J=-D\frac{dC}{dx} \tag{2}$$

in which D is the diffusion coefficient of a drug, since the solubility of a drug in the particle phase is different from that in the aqueous phase, and the concentration of the drug is not the same. Accordingly, C_{wmax} is defined as the equilibrium concentration of a drug in the aqueous phase at an infinite time. Then, the concentration gradient dC/dx can be represented as $(C_{wmax}-C_w)/h$. Hence, Eq. 2 can be rewritten as:

$$\frac{dM}{dt}=\frac{D_m\cdot S\cdot K}{h}(C_{wmax}-C_w) \tag{3}$$

where dM/dt is the amount of gradient of drug in the aqueous phase, D_m is the diffusion coefficient of the drug in the interfacial membrane, S is the surface area of the interfacial membrane, K is the partition coefficient between the interfacial membrane and the aqueous phase, h is the thickness of the interfacial membrane, and C_w is the concentration in the aqueous phase at time t. Permeability P is defined as:

$$P=\frac{D_m\cdot S\cdot K}{h} \tag{4}$$

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and, a rearrangement of Eq. 3 shows that

$$\frac{V_w \cdot dC_w}{dt} = P \cdot (C_{wmax} - C_w) \quad (5)$$

in which V_w is the volume in the aqueous phase. Then, Eq. 5 can be integrated to give:

$$\ln \cdot \left(1 - \frac{C_w}{C_{wmax}}\right) = -\frac{P}{V_w} \cdot t \quad (6)$$

$$\frac{C_w}{C_{wmax}} = 1 - \exp\left(-\frac{P}{V_w} \cdot t\right) \quad (7)$$

Since the equilibrium concentration as well as the solubility of the oil phase is different from the equilibrium concentration of the aqueous phase, C_{wmax} can be written as:

$$C_{wmax} = \frac{M_t - C_d \cdot V_o}{V_w + V_o} \quad (8)$$

where the C_d is the difference between the equilibrium concentrations in the oil phase and the aqueous phase, M_t is the amount of the drug, and V_o is the volume of the oil phase. By substituting Eq. 8 into Eq. 7, C_w can be written as follows:

$$C_w = \frac{M_t - C_d \cdot V_o}{V_w + V_o} \cdot \left(1 - \exp\left(-\frac{P}{V_w} \cdot t\right)\right) \quad (9)$$

Experimental

Materials The PGE_1 used was synthesized at Taisho Pharmaceutical Co., Ltd. A Palux Injection® was used to administer Lipo-PGE_1. Prostaglandin A_1 (PGA_1) of analytical standard was obtained commercially from Funakoshi Co., Ltd. Soybean oil was obtained commercially from Ajinomoto, Co., Inc. All other chemicals were of reagent grade. Cellulose tubing (pore size: 24 Å, thickness: 0.03 mm, diameter: 20 mm) used for dialysis was purchased from Viskase Sales Corp. Buffer solutions (pH 3, 4.5, 5.5) were prepared with 0.2 M citric acid and 0.1 M Na_2HPO_4. Other buffer solutions (pH 6.5, 7.4) were prepared using 0.2 M KH_2PO_4 and 0.2 M $NaHCO_3$. The ionic strength of each buffer solution was adjusted to 0.15 with NaCl.

Assay for PGE_1 PGE_1 was measured by high-performance liquid chromatography (HPLC) with a post-column reaction.[11] An aqueous solution of PGE_1 (equivalent to 2.5 μg of PGE_1) was run through a pre-column (Sep pack C18) which had been washed, and eluted with 7 ml of methanol. All of the elution was evaporated at a temperature of not more than 40 °C under vacuum conditions. One ml of the internal standard solution was added to the evaporated sample. This sample was injected to HPLC. The HPLC parameters were as follows: column, TSK gel ODS-LS410; column temperature, about 50 °C; mobile phase, CH_3CN: aqueous 1/150 M phosphate buffer (pH 6.3) = 28 : 72; and flow rate, 1 ml/min. Conditions for the post-column reaction were as follows: column, teflon tube; flow rate, 0.5 ml/min; reaction solution, 1 N KOH.

Preparations of Lipo-PGE_1 The formulas of Lipo-PGE_1 are shown in Table I. The preparation of Lipo-PGE_1 was as follows: 7 mg of PGE_1, 18 mg of egg yolk lecithin and 2.4 g of oleic acid were added to 100 g of soybean oil. This mixture was heated and stirred until it was completely clear. Eight hundred ml of water for injection and 22.1 g of glycerol were added to the oil mixture. This total mixture was preemulsified using a Physcotron homogenizer (model NS-60, Niti-on Medical & Physical Instruments MFG. Co., Ltd.) at 8000 rpm for 10 min. The pH of the coarse emulsion was then adjusted to a pH of 4.5—6.0 with sodium hydroxide aqueous solution. Final emulsification was completed by passing the coarse emulsion through a high pressure homogenizer (model 15 M, APV Gaulin, Inc.). The volume of emulsion was adjusted to 1000 ml with water for injection.[12] In all the processes, nitrogen gas was bubbled into the emulsion. The mean particle size of the Lipo-PGE_1 thus obtained was established as being 0.2 μm using a light scattering spectrometer (Nicomp model 370 Submicron particle sizer, Pacific Scientific Instrument Division). The pH of Lipo-PGE_1 was determined to be 5.4 by a pH meter (model HM-30, Toa Denpa Kogyo Co. Ltd.).

Diffusion Rate Diffusion rates of PGE_1 from the particle phase to the aqueous phase were obtained by the dialysis method. Four ml of Lipo-PGE_1 enclosed in cellulose tubing was immersed in 36 ml of pH 5.5 or 7.4 buffer solution, and incubated at constant temperatures (5, 20, 37 °C). Each concentration of PGE_1 in the aqueous phase was measured at appropriate intervals by HPLC. Similarly, 2 ml of Lipo-PGE_1 enclosed in cellulose tubing was immersed in 198 ml of pH 5.5 or 7.4 buffer solution and incubated at 20 °C.

Oil/Water Partition Coefficient Five-tenths mg of PGE_1 was dissolved in 50 ml of various pH of buffer solutions. Ten ml of each solution was added to 10 ml of soybean oil, and the oil/water mixture was shaken for 3 or 24 h. The mixture was then centrifuged at 3000 rpm for 15 min to separate each layer, and the aqueous layer was assayed for PGE_1 by HPLC.

Table I. Formulas for Lipo-PGE_1

PGE_1	7 μg
Soybean oil	100 mg
Egg yolk lecithin	18 mg
Oleic acid	2.4 mg
Glycerol	22.1 mg
Sodium hydroxide for pH adjustment to 4.5—6.0	
Water for injection	a.q.[a]

a) Adjusted to 1 ml. a.q. = adequate quantity.

Results and Discussion

Diffusion Rates of PGE_1 from the Particle Phase The release profiles of PGE_1 from lipid particles are shown in Figs. 1 to 3, when Lipo-PGE_1 was diluted ten times with the buffer solution. A small amount of PGA_1 was yielded during experimentation as a decomposition product (Table II). But since the amount was so small, this effect was considered to be negligible. The plots show the concentrations of PGE_1 in the aqueous phase at appropriate intervals, and the full lines are the theoretical curves obtained from Eq. 9. The values of the characteristic parameter in Eq. 9 are shown in Table III, and the values of C_d and P were obtained by regressing Eq. 9 to the experimental measurements. Since good correlation was observed between experimental and theoretical data, the values of C_d and P were considered reasonable. The particle/water partition coefficient could be calculated

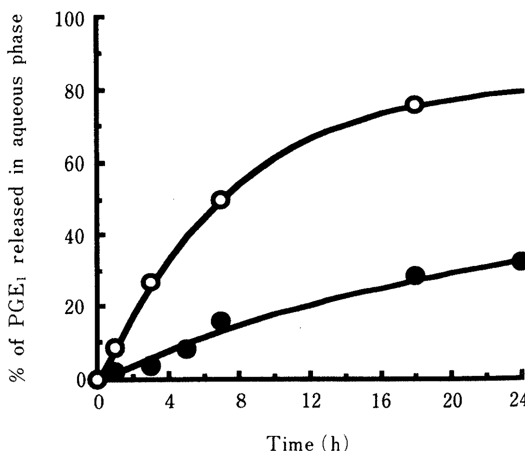

Fig. 1. Release Profiles of PGE_1 from Particles in Lipo-PGE_1 Diluted Ten Times with Buffer Solutions at 5 °C

●, pH 5.5; ○, pH 7.4. The full lines are theoretical curves of Eq. 9.

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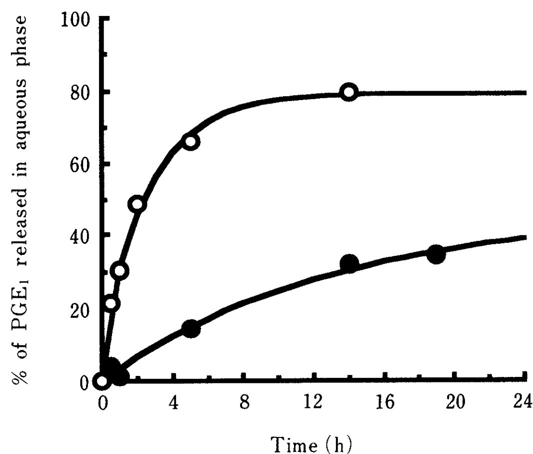

Fig. 2. Release Profiles of PGE_1 from Particles in Lipo-PGE_1 Diluted Ten Times with Buffer Solutions at 20 °C

●, pH 5.5; ○, pH 7.4. The full lines are theoretical curves of Eq. 9.

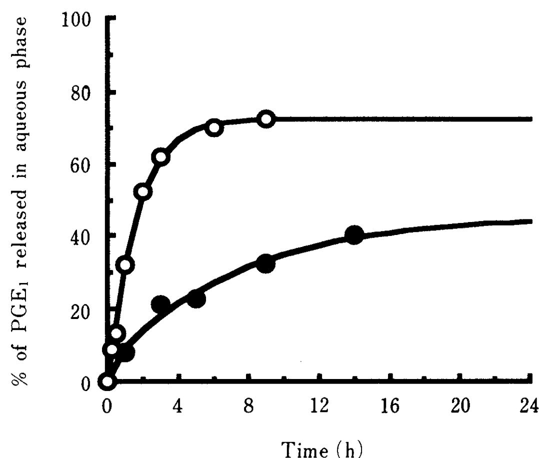

Fig. 3. Release Profiles of PGE_1 from Particles in Lipo-PGE_1 Diluted Ten Times with Buffer Solutions at 37 °C

●, pH 5.5; ○, pH 7.4. The full lines are theoretical curves of Eq. 9.

Table II. Amount of PGA_1 during Experimentation as a Decomposition Product of PGE_1 (%)

pH	Temperature (°C)		
	5	20	37
5.5	0.3	1.4	5.3
7.4	0.4	1.8	5.9

from the values of C_d obtained. Each equilibrium concentration of the particle phase and the aqueous phase under the various temperatures was calculated. The results are shown in Table IV. It was found that most of the PGE_1 was distributed in lipid particles independent of temperature, but that the partition coefficient was increased as the pH of the buffer increased. This may be due to the increase of ionic PGE_1 as the pH of the buffer solution increased, since the pK_a of PGE_1 was reported to be 5.02.[13)]

The effect of temperature on the distribution ratio between the particle phase and the aqueous phase was calculated from the partition coefficient, as shown in Table IV, and the volume ratio (aqueous volume : particle volume = 0.89 : 0.11). The results are shown in Table V.

Table III. Characteristic Parameters of Lipo-PGE_1

Dilution	Total PGE_1 M_t (μg)	Volume (ml)			
		Lipo-PGE_1 (ml)	Buffer (ml)	Aqueous phase V_w (ml)	Oil phase[a)] V_o (ml)
× 10	28	4	36	39.56	0.44
× 100	14	2	198	199.78	0.22

a) V_o = total oil amount/density of oil (0.91).

Table IV. Equilibrium Concentrations of Two Phases and Partition Coefficients when Lipo-PGE_1 Was Diluted Ten Times

pH of buffer	Temp. (°C)	Particle phase (μg/ml)	Aqueous phase (μg/ml)	Partition particle/water
5.5	5	35.5	0.3	115
	20	35.1	0.3	110
	37	35.6	0.3	130
7.4	5	10.1	0.6	17
	20	20.5	0.5	43
	37	14.9	0.5	28

Table V. Distribution of PGE_1 in Lipo-PGE_1 at Various Temperatures

Temp. (°C)	Particle phase (%)	Aqueous phase (%)
5	93.3	6.7
20	93.2	6.8
37	94.1	5.9

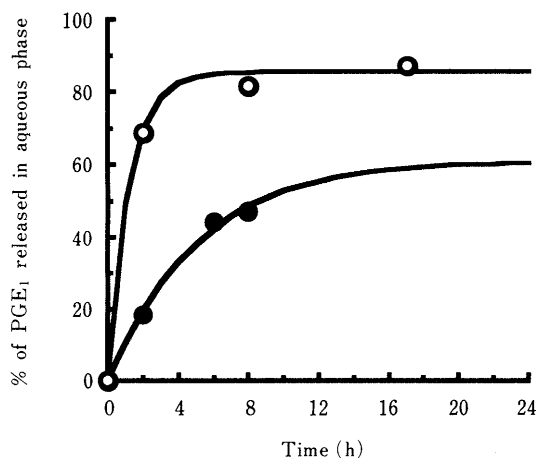

Fig. 4. Release Profiles of PGE_1 from Particles in Lipo-PGE_1 Diluted One Hundred Times with Buffer Solutions at 20 °C

●, pH 5.5; ○, pH 7.4. The full lines are theoretical curves of Eq. 9.

Figure 4 shows the release profiles of PGE_1 from lipid particles at 20 °C, where Lipo-PGE_1 was diluted one hundred times with buffer solutions (pH 5.5, 7.4). As shown in this figure, most of the PGE_1 was distributed in the aqueous phase after equilibrium was reached.

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Table VI. Amount of PGE_1 Remaining in Particles when Lipo-PGE_1 Was Diluted One Hundred Times at 20 °C

pH	PGE_1 (%) Time after dilution (min)			
	Initial	30	60	120
5.5	93.2	87.8	82.9	74.4
7.4	93.2	73.7	60.9	46.9

Table VII. Partition Coefficient of PGE_1 between Soybean Oil and Aqueous Buffers at Various Temperatures

Temp. (°C)	pH				
	3.0	4.5	5.5	6.5	7.4
5	—	—	0.25	—	0.02
20	0.63	0.55	0.22	0.01	0.03
37	—	—	0.24	—	0.02

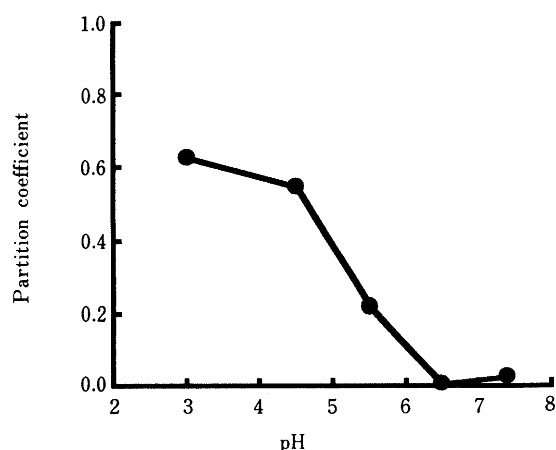

Fig. 5. The pH–Partition Profiles of PGE_1 between Soybean Oil and Water at 20 °C

However, it took from 6—8 h to reach the equilibrium concentration; and as the dilution ratio as well as the pH of the buffer solution increased, the equilibrium concentration increased. The amount of PGE_1 which remained in lipid particles at appropriate intervals is shown in Table VI. The amounts of PGE_1 remaining in lipid particles were 74% and 47% for pH 5.5 and 7.4, respectively, after 2 h, when Lipo-PGE_1 was diluted one hundred times at 20 °C.

In contrast, Minakuchi *et al.* reported that 50% and 10% of PGE_1 remained in the lipid particles immediately after being diluted ten times with a saline (pH 5.6) and phosphate buffer solution, respectively. The inconsistency of the data may be due to the different methods used to separate the aqueous solution from the lipid emulsion. That is, cellulose tubing with a pore size of 24 Å was used in this study, whereas an ultrafilter with a pore size equal to a molecular weight 30000 was used by Minakuchi *et al.* In general, the amount of substance in filtrate is influenced by the pore size of the filter. Since the pore size of the ultrafilter was greater than that of the cellulose tubing, small lipid particles might have been passed through the ultrafilter, which may be the reason why different data were obtained.

Table VIII. Distribution of PGE_1 in Lipo-PGE_1 at Various Temperatures

Temp. (°C)	Oil phase (%) (soybean oil)	Oil/water phase (%) (lecithin)	Water phase (%)
5	0.2	93.1	6.7
20	0.2	93.0	6.8
37	0.2	93.9	5.9

Partition Coefficient of PGE_1 The partition coefficients of PGE_1 between soybean oil and aqueous buffer solutions were obtained in the range of pH 3.0—7.4 at several temperatures (5, 20, 37 °C). The results are shown in Table VII and Fig. 5. Partition coefficients had no influence on the temperature, but did influence the pH value. That is, when the pH of the buffer solution was lower than 6.5, the partition coefficient decreased as the pH value linearly increased. On the other hand, when the pH of the solution was more than 6.5, the partition coefficient became constant and remained at a low level. Since the pK_a of PGE_1 is 5.02, the pH–partition coefficient profile in Fig. 5 can be explained from the relationship between the pH and the ionic PGE_1 ratio.

Distribution of PGE_1 in Lipo-PGE_1 The distribution style of PGE_1 in Lipo-PGE_1 was analyzed using a three-phase model, that is, PGE_1 distribution in an aqueous phase, an oil phase and an oil/water interface. The schematic model for partition was assumed where C_1, C_2 and C_3 were defined as equilibrium concentrations in a water (aqueous) phase, a lecithin (oil/water interface) phase, and an oil phase, respectively. The concentration ratios of the lecithin/water and oil/lecithin are defined as $P_{(L/W)}$ and $P_{(O/L)}$, respectively. If the concentrations of a drug were lower than the solubility in each phase, the values of $P_{(L/W)}$ and $P_{(O/L)}$ became constant. Therefore, C_2, C_3 can be written as follows:

$$C_2 = C_1 \cdot P_{(L/W)} \tag{8}$$

$$C_3 = C_1 \cdot P_{(O/L)} = C_1 \cdot P_{(L/W)} \cdot P_{(O/L)} \tag{9}$$

The particle phase is constituted with soybean oil and lecithin. Therefore, by measuring the concentration ratio between the particle phase and the water phase, and the partition coefficient between the oil phase and the water phase, the concentration ratio in the three phases can be obtained.

From the data in Table IV and VII, the partition coefficients of PGE_1 in the three phases (water, lecithin, oil) were calculated. Furthermore, the distribution ratios of PGE_1 in Lipo-PGE_1 were obtained at various temperatures (Table VIII). Most of the PGE_1 in Lipo-PGE_1 was distributed in lecithin and the ratio was not influenced by temperature. Since a large portion of the PGE_1 was found in the oil/water interface, PGE_1 is believed to have a high affinity with phospholipids. These results are considered to be the apparent distribution of the liposome in the lipid emulsion. Teagarden *et al.* reported that the distribution ratio of PGE_1 in the oil/water interface changed under the influence of ionic form.[14]

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In our study, the distribution of PGE_1 was measured at pH 5.5 (pH of Lipo-PGE_1), but it was considered that the distribution, partition coefficient and release rate were thought to be due to ionic charges and solubilities. Therefore, we are planning to clarify the relationship between pH and distribution of PGE_1.

In conclusion, a study was carried out of the release rate of PGE_1 from lipid particles to an aqueous solution, when Lipo-PGE_1 was diluted with aqueous solution. The equilibrium concentration was obtained using a modified form of Fick's equation. This method allowed the residual ratio of PGE_1 in the lipid particles to be determined, which clarified the distribution of PGE_1 in the transfusion bottle. This may be one of the factors which explains the high activity in clinical treatment.

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