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Effect of acyl chains of phosphatidylcholines on the pharmacokinetics of menatetrenone incorporated in O/W lipid emulsions prepared with phosphatidylcholines and soybean oil in rats

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Abstract

Oil-in-water (O/W) lipid emulsions were prepared with phosphatidylcholines (PCs) of various acyl chains and soybean oil (SO) using a microfluidizer system, and the pharmacokinetics of menatetrenone incorporated in these oil particles were examined at the clinical injection volume (0.1 mL kg^{-1}) in rats. The plasma half-life of menatetrenone incorporated in the oil particles prepared with SO and dipalmitoylphosphatidylcholine (DPPC) (SO/DPPC) was longer than that prepared with SO and egg-yolk phosphatides (EYP) (SO/EYP) by 3 fold, while those of menatetrenone as oil particles prepared with SO and either dilauroyl phosphatidylcholine (DLPC), dimyristoyl phosphatidylcholine (DMPC), distearoyl phosphatidylcholine (DSPC), dioleoyl phosphatidylcholine (DOPC) or dilinoleoyl phosphatidylcholine (DLoPC) (SO/DLPC, SO/DMPC, SO/DSPC, SO/DOPC and SO/DLoPC, respectively) were similar to that of menatetrenone as SO/EYP. The menatetrenone uptake by the liver was not significantly different from that as SO/EYP in all SO/PCs examined, but the menatetrenone uptake by the spleen as SO/DPPC and SO/DSPC was higher than that as SO/EYP. The menatetrenone uptake by the lungs as SO/DPPC was also higher than that as SO/EYP. These findings suggest that SO/DPPC is a good candidate drug carrier for the prolonged plasma circulation of lipophilic drugs.

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Introduction

Oil-in-water (O/W) lipid emulsions have been widely utilized in parenteral nutrition for several decades, and are also known as drug carriers for lipophilic drugs (Benita 1998). Lipid emulsions consisting of soybean oil (SO) and egg-yolk phosphatides (EYP), commonly used in parenteral nutrition, are known to be useful as drug carriers for lipophilic drugs. It was reported that menatetrenone, a highly lipophilic drug incorporated in SO/EYP, showed prolonged plasma half-life when administered intravenously at an experimental injection volume (3 mL kg^{-1}), and was not released from the oil particles (Sakaeda & Hirano 1998). We reported that the plasma half-life of menatetrenone incorporated into SO/EYP was extensively shorter after intravenous administration at a clinical injection volume (0.1 mL kg^{-1}) than that after administration at the experimental injection volume (3 mL kg^{-1}), even though menatetrenone was not released from the oil particles (Ueda et al 2001). On the other hand, we also reported that the oil particles prepared with SO and hydrogenated castor oils (HCOs), nonionic surfactants with greater than 20 oxyethylene units, showed a prolonged plasma half-life of menatetrenone, irrespective of the particle size, although those prepared with SO and polyoxyethylene sorbitan esters (PSs) quickly disappeared from plasma (Ueda et al 2002, 2003a, b). This prolongation of the plasma circulation time of menatetrenone with HCOs was also observed when the oil particles were prepared with medium-chain triglycerides instead of SO, a mixture of long-chain triglycerides (Ueda et al 2004). However, these nonionic surfactants possessing oxyethylene units may cause some biological effects

when administered to man. In fact, Fluosol-DA, a perfluorocarbon artificial blood substitute emulsion stabilized with poloxamer-188, a nonionic block copolymer surfactant, activated the complement system and damaged the pulmonary endothelium, and poloxamer-188 alone exhibited an antithrombotic and neutrophil-inhibitory effect (Moghimi & Hunter 2000).

EYP is a mixture of phospholipids, including phosphatidylcholines (PCs) with various lengths of acyl chains, and the biological fate of the oil particles depends on the phospholipid used for the preparation of the oil particles. It was reported that oil particles prepared with dipalmitoylphosphatidylcholine (DPPC) showed prolonged plasma circulation of radiolabelled triolein and cholesteryl oleate incorporated into the oil particles (Lenzo et al 1988; Redgrave et al 1992). It was also reported that the lipolysis did not occur with the oil particles prepared with DPPC or distearolyphosphatidylcholine (DSPC) (Clark & Derksen 1987; Redgrave et al 1992). However, no studies have compared the kinetics of the drugs incorporated into the oil particles prepared with various PCs. In other words, the kinetics of the drugs incorporated into the oil particles prepared with PCs of various acyl chains have not been clarified. In this study, the lipid emulsions were prepared with soybean oil (SO) and PCs with various acyl chains. Menatetrenone was chosen as the drug to be incorporated, because it has been reported to be incorporated in oil particles even after entering the circulation (Sakaeda & Hirano 1998). Furthermore, the kinetics of menatetrenone incorporated in these oil particles was studied, and the effect of the acyl chains of the phospholipids on the kinetics of menatetrenone was also discussed.

Materials and Methods

Materials

Purified egg-yolk phosphatides (EYP, NC-50S, > 95% phosphatidylcholine, according to the technical data provided by the manufacturer), L-α-dilauroyl phosphatidylcholine (DLPC, MC-2020), L-α-dimyristoyl phosphatidylcholine (DMPC, MC-4040), L-α-dipalmitoyl phosphatidylcholine (DPPC, MC-6060), L-α-distearoyl phosphatidylcholine (DSPC, MC-8080), L-α-dioleoyl phosphatidylcholine (DOPC, MC-8181) and L-α-dilinoleoyl phosphatidylcholine (DLoPC, MC-8282) were supplied by Nippon Oil and Fats (Tokyo, Japan). The purity of DLPC, DMPC, DPPC, DSPC, DOPC and DLoPC was > 99%, according to the technical data provided by the manufacturer. The main gel–liquid crystalline transition temperature and the molecular weight of EYP and PCs, also obtained as technical data provided by the manufacturer, are listed in Table 1. Soybean oil (SO) was obtained from Kanto Chemicals (Tokyo, Japan). The fatty acid composition of SO, according to the technical data provided by the manufacturer, was as follows: palmitoyl acid 11.3%, stearyl acid 3.4%, oleic acid 23.1%, linoic acid 55.8% and linoleic acid 6.4%. Menatetrenone (vitamin K_2) was purchased from Sigma Chemical Co. (MO). All other chemicals were of the highest purity available.

Table 1 Main gel–liquid crystalline transition temperature and molecular weight of the phosphatidylcholines

Phosphatidylcholines	Main gel–liquid crystalline transition temperature (°C)	Molecular weight
EYP	$-15 \sim -17$	773[a]
DLPC	0	622
DMPC	23	678
DPPC	42	734
DSPC	55	790
DOPC	−22	786
DLoPC	—	782

[a]Average molecular weight.

Preparation of lipid emulsions

Lipid emulsions (SO/EYP and SO/PCs (SO/DLPC, SO/DMPC, SO/DPPC, SO/DSPC, SO/DOPC, SO/DLoPC)) were prepared using a high-pressure homogenization system as described previously (Ueda et al 2002) with slight modifications. Briefly, menatetrenone (1.0% (w/w)) was dissolved in SO (20% w/w), mixed with phosphatidylcholines (2.4% w/w) and purified water using a homomixer (model LR-1; Mizuho Industrial Co., Osaka, Japan), and the mixture (100 g) was emulsified using a microfluidizer system (M110-EH; Mizuho Industrial Co., Osaka, Japan; the distributor in Japan) at a pressure of 20 000 psi for 10 min. The procedure was conducted at 35°C, 55°C and 65°C for SO/DMPC, SO/DPPC and SO/DSPC, respectively, and at 15°C for the remainder of the oil particles. The size and distribution of the lipid emulsions in diameter was determined by quasielastic light scattering using Coulter Model N4 Plus (Beckman Coulter, CA). Analysis of the lipid emulsions was performed in triplicate, and the findings from the median size were adopted; all the three independent data of the particle size obtained for each preparation were within ±20% of the median. The lipid emulsions were stored at 4°C in the dark until the animal studies were performed, and no size change in the lipid emulsions was detected at the time of the animal studies. The menatetrenone content in the lipid emulsions was examined by an HPLC method similar to those utilized to detect menatetrenone in the plasma, and the phospholipid and triglyceride content were determined using a diagnostic kit (Phospholipid C-test Wako and Triglyceride E-test Wako; Wako Pure Chemical Industries Ltd, Osaka, Japan).

Animals

Male slc:Wistar rats (8–9 weeks old, 200–250 g) with free access to food and water were used for the experiments. They were anaesthetized by a subcutaneous injection of

urethane/saline solution (30% w/w, 4.5 mL kg^{-1}), and kept on temperature-regulated mats for 2 h before starting the experiments to avoid blood pressure fluctuation (Sakaeda & Hirano 1995). All procedures were carried out according to the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science with the permission of the Animal Experimental Committee of Kobe Pharmaceutical University.

Plasma concentration and organ distribution of menatetrenone after administration as lipid emulsions

Lipid emulsions containing menatetrenone were injected into the right femoral vein at an injection volume of 0.1 mL kg^{-1}, and serial blood samples (0.1 mL each) were collected from the jugular vein using heparinized syringes. Liver, spleen and lungs were also collected at 60 min immediately after sacrificing by blood sampling from the aorta. Amount of menatetrenone in plasma and organ homogenates was analysed by HPLC as described previously (Ueda et al 2001). The lower limit for the quantification of menatetrenone in plasma was 0.1 μg mL^{-1}.

A mono-exponential equation was fitted to the plasma concentration data of menatetrenone administered as lipid emulsions by non-linear least-square regression, and the volume of distribution and the plasma half-life were calculated from the mono-exponential equation. The volume of distribution (V) was calculated using the equation 1:

$$V = \text{Dose}/C_0 \quad (1)$$

where C_0 is the menatetrenone concentration at time 0.

Statistical analysis

Statistical analyses were performed on the following parameters: t½, V and organ uptake by the liver, spleen and lungs. After the analyses by the Bartlett's test for equality of variance, a one-way analysis of variance followed by the Dunnet's multiple comparison was employed. Statistical difference was assumed to be $P < 0.05$. Also, the body weight of rats with SO/PCs administration was not significantly different from that with SO/EYP administration (one-way analysis of variance followed by the Dunnet's multiple comparison).

Results

Size distribution and measured phospholipid, triglyceride and menatetrenone content of SO/EYP and SO/PCs

The mean particle sizes of the lipid emulsions prepared in this study were within 131–191 nm in diameter (Table 2). The measured contents of phospholipids, triglycerides and menatetrenone in these emulsions were within ±20% of the amount indicated for all of the emulsions prepared.

Plasma concentration–time profile of menatetrenone after intravenous injection as SO/EYP and SO/PCs

Figure 1 shows the plasma concentration–time profile of menatetrenone after intravenous injection as SO/PCs at a clinical injection volume (0.1 mL kg^{-1}) to rats. Menatetrenone quickly disappeared from plasma after intravenous administration as SO/EYP, as it did after administration as SO/PCs (except SO/DPPC). Figure 2 shows the plasma half-life and volume of distribution of menatetrenone calculated from the plasma concentration–time curve. The menatetrenone half-life for SO/DPPC was significantly longer than that for SO/EYP. The volume of distribution of menatetrenone for SO/DMPC was significantly larger than that for SO/EYP, but those for other SO/PCs were similar to that for SO/EYP, which was similar to the actual plasma volume (36.0 ± 4.3 mL kg^{-1}; $n = 8$) obtained by ^{125}I-albumin from bovine serum (Sakaeda & Hirano 1995).

Organ uptake of menatetrenone after intravenous injection as SO/EYP and SO/PCs

Table 3 shows the organ uptake of menatetrenone at 60 min after intravenous administration as lipid emulsions. Menatetrenone uptake by the liver for SO/EYP was 23% of the dose, and was similar to that for any of the SO/PCs. On the other hand, menatetrenone uptake by the spleen for SO/DPPC and SO/DSPC was, respectively, 6.6-fold and 3.4-fold larger than that for SO/EYP. Menatetrenone uptake by the lungs for SO/PCs was 0.2–0.5% of the dose for all the lipid emulsions examined, except for SO/DPPC, which was 2-fold larger than that for SO/EYP. The

Table 2 Particle size and phospholipid, triglyceride and menatetrenone content of the lipid emulsions

Emulsion	Particle diameter (nm; ± s.d.)	Phospholipids (g/100 mL)	Triglycerides (g/100 mL)	Menatetrenone (g/100 mL)
SO/EYP	152 ± 37	2.5	16.8	0.98
SO/DLPC	131 ± 24	2.5	17.1	0.99
SO/DMPC	151 ± 23	2.4	17.4	0.99
SO/DPPC	153 ± 4	2.4	16.5	0.97
SO/DSPC	191 ± 30	2.4	16.6	0.96
SO/DOPC	160 ± 69	2.4	16.5	0.96
SO/DLoPC	167 ± 49	2.4	16.4	1.00

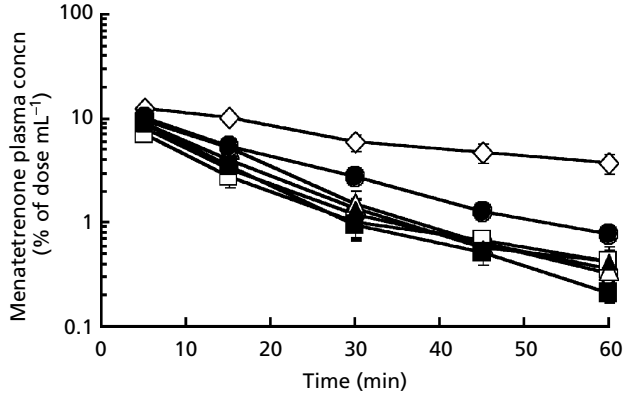

Figure 1 Plasma concentration–time profiles of menatetrenone after intravenous injection as SO/EYP and SO/PCs. Lipid emulsions (SO/EYP (○, n = 6), SO/DLPC (△, n = 3), SO/DMPC (□, n = 4), SO/DPPC (◇, n = 3), SO/DSPC (●, n = 3), SO/DOPC (▲, n = 4) and SO/DLoPC (■, n = 4)) were administered intravenously at an injection volume of 0.1 mL kg^{-1}. Each point represents the mean ± s.d.

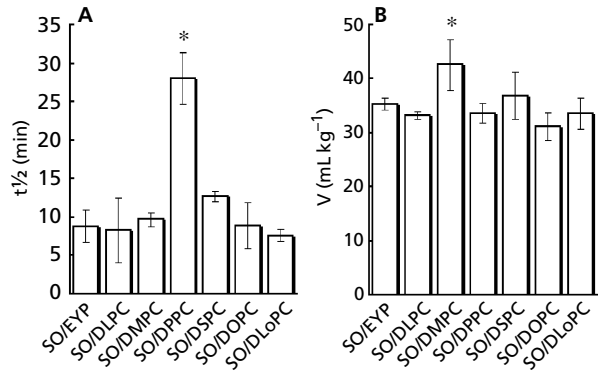

Figure 2 Plasma half-life (t½, A) and volume of distribution (V, B) of menatetrenone after intravenous injection of SO/EYP and SO/PCs. See Figure 1 for the number of rats for each bar. Each point represents the mean ± s.d. *$P < 0.05$, compared with SO/EYP.

Table 3 Organ uptake of menatetrenone after intravenous injection of SO/PCs

Emulsion	Organ uptake (% of dose)		
	Liver	Spleen	Lungs
SO/EYP (n = 6)	23.4 ± 8.2	0.8 ± 0.3	0.4 ± 0.0
SO/DLPC (n = 3)	35.0 ± 3.1	0.5 ± 0.1	0.2 ± 0.1
SO/DMPC (n = 5)	35.5 ± 12.2	0.8 ± 0.0	0.3 ± 0.1
SO/DPPC (n = 3)	35.9 ± 2.4	5.3 ± 0.3*	0.8 ± 0.0*
SO/DSPC (n = 3)	30.9 ± 2.6	2.7 ± 0.4*	0.3 ± 0.1
SO/DOPC (n = 4)	28.8 ± 4.9	1.3 ± 0.2	0.5 ± 0.1
SO/DLoPC (n = 4)	22.6 ± 3.5	0.8 ± 0.2	N.D.

Values are the means ± s.d. Tissues were excised at 60 min after intravenous injection of lipid emulsions at 0.1 mL kg^{-1}. *$P < 0.05$, compared with SO/EYP. N.D., not detected.

menatetrenone uptake by the lungs for SO/DLoPC could not be detected.

Discussion

We found that the plasma half-life of menatetrenone as SO/DPPC was longer than that as SO/EYP, while those as the oil particles prepared with SO and other PCs were similar to that as SO/EYP. Previously, Redgrave et al (1992) demonstrated that the plasma circulation time of triolein and cholesteryl oleate in oil particles prepared with DPPC was longer than that in oil particles prepared with 1-palmitoyl-2-oleoylphosphatidylcholine (POPC). These findings together suggest that not only the plasma half-life of the oil particles of SO/DPPC but also that of the drug incorporated into SO/DPPC was prolonged, compared with those of SO/EYP, and that SO/DPPC is a good candidate as a drug carrier for prolonged plasma circulation.

The oil particles that resemble chylomicrons are known to be eliminated mainly via two routes after entering the circulation: one is the hydrolysis by the lipoprotein lipase followed by uptake via apolipoprotein E-specific receptors on the liver parenchymal cells; the other is the uptake by the reticuloendothelial system (Nishikawa et al 1998). Since the volume of the lipid emulsions administered into rats was as small as 0.1 mL kg^{-1} in this study, which equates to 6 mL for a person weighing 60 kg, it might be that the uptake via apolipoprotein E-mediated receptors would not have been saturated by the oil particles by this injection volume (Iriyama 1996). Therefore, it is probable that menatetrenone not only as SO/EYP but also as SO/DLPC, SO/DMPC, SO/DOPC and SO/DLoPC was eliminated by both of the routes. For the oil particles prepared with DSPC, it was reported that they were not hydrolysed by the lipoprotein lipase and that triolein and cholesteryl oleate in these DSPC emulsions were taken up greatly by the liver, probably via the reticuloendothelial system (Clark & Derksen 1987; Redgrave et al 1992). The main gel–liquid crystalline transition temperature of DSPC is higher than body temperature and DSPC is considered as solid after entering the circulation. Therefore, menatetrenone as SO/DSPC was likely to be eliminated mainly via the reticuloendothelial system.

On the other hand, the mechanisms for the prolongation of plasma circulation of menatetrenone as SO/DPPC could not be explained in this study. The uptake of menatetrenone as SO/DPPC by the liver, spleen or lungs was similar to, or larger than, those as SO/EYP despite the prolonged plasma circulation of menatetrenone as SO/DPPC compared with that as SO/EYP in this study (Table 2), although the prolonged plasma circulation time of oil particles such as those coated with poloxamine 908 is often achieved by the evasion of the reticuloendothelial system (i.e. the liver and spleen) (Stolnik et al 1995). It was reported previously that oil particles prepared with DPPC were not hydrolysed by lipoprotein lipase, and that liver uptake of cholesteryl oleate as oil particles prepared with DPPC was lower than that as oil particles prepared with POPC or EYP (Redgrave et al 1992). These previous findings did not fully support the findings obtained in our study, possibly due to the difference in the

contents of the oil particles (cholesteryl oleate and cholesterol included in Redgrave et al (1992) but not included in our study) in spite of the similar particle size. Furthermore, the amount of menatetrenone that was not in plasma, liver, spleen or lungs at 60 min after administration as SO/DPPC was less than half that as SO/EYP in this study when the mass balance was taken into consideration ($31.9 \pm 3.9\%$ of dose for SO/DPPC vs $73.0 \pm 8.9\%$ of dose for SO/EYP, when the plasma volume was calculated from the blood weight (assumed to be 7% of total body weight) and the hematocrit value (0.478)). This difference in the amount of menatetrenone that was outside the plasma, liver, spleen and lungs implies that the uptake of menatetrenone as SO/DPPC by some of the organs and tissues other than the liver, spleen or lungs would have been lower than that as SO/EYP. The main gel–liquid crystalline transition temperature of DPPC, higher than but close to body temperature, might have affected the menatetrenone kinetics, but we have not examined this in detail. It might also be that the particle size affected the kinetics of menatetrenone as SO/DSPC, which was the largest of the oil particles examined, since the particle size was reported to affect the kinetics of the lipid emulsions (Lundberg et al 1996; Takino et al 1994).

Conclusions

We found that the plasma half-life of menatetrenone as SO/DPPC was longer than that as SO/EYP, while the half-lifes of menatetrenone as SO/DLPC, SO/DMPC, SO/DSPC, SO/DOPC and SO/DLoPC were similar to that as SO/EYP. The menatetrenone uptake by the liver was not significantly different from that as SO/EYP in all SO/PCs examined, but the menatetrenone uptake by the spleen as SO/DPPC and SO/DSPC was higher than that as SO/EYP. The menatetrenone uptake by the lungs as SO/DPPC was also higher than that as SO/EYP. These findings suggest that SO/DPPC is a good candidate drug carrier for the prolonged plasma circulation of lipophilic drugs.

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