JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1357–1363

Received April 10, 2002
Accepted June 14, 2002
ISSN 0022-3573

Effect of oxyethylene moiety in polyoxyethylene sorbitan esters on the pharmacokinetics of menatetrenone incorporated in O/W lipid emulsions prepared with polyoxyethylene sorbitan esters and soybean oil in rats

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Abstract

Oil-in-water (O/W) lipid emulsions are suitable drug carriers for lipophilic drugs; however, the effects of numbers or chains of oxyethylene units within a surfactant molecule such as polyoxyethylene sorbitan esters (PSs) on the biological fate of these lipid emulsions have not yet been clarified. In this study, a series of PSs and soybean oil (SO) were utilized to prepare menatetrenone-incorporated lipid emulsions (SO/PSs), and the biological fate of menatetrenone administered as SO/PSs was studied at a clinical injection volume (0.1 mL kg^{-1}) in rats. The plasma concentration and organ uptake of menatetrenone administered as SO/20OE-PSs (PSs with 20 oxyethylene units) was similar to that of SO/egg-yolk phosphatides (SO/EYP). The plasma concentration of menatetrenone was extensively lower for SO/6OE-PSs (PSs with 6 oxyethylene units) and SO/20OE-3FA-PSs (PSs with 20 oxyethylene units and 3 fatty acid chains) than that for SO/EYP, and menatetrenone uptake by the liver and spleen was higher for SO/6OE-PSs and SO/20OE-3FA-PSs, respectively, than those for SO/EYP. Furthermore, menatetrenone uptake by the lungs was also increased for SO/6OE-PS and SO/20OE-3FA-PS with double bonds in the fatty acid moieties of the PSs. These findings suggested that shortening the oxyethylene units or decreasing the oxyethylene chain numbers of emulsifiers resulted in a rapid clearance of the lipid emulsions from the circulation by extensive uptake via the liver, spleen or lungs.

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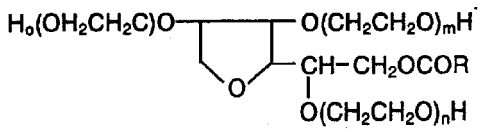
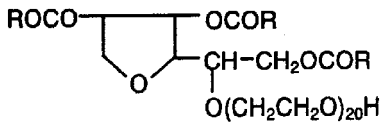
Acknowledgement: We are grateful to Mr Eiji Watanabe, President of Mizuho Industrial Co., Japan, for lending us the microfluidizer system. We also thank Shionogi Pharmaceutical Co., Japan, for generously lending us the Coulter N4 Plus, and Mr Yoshitaka Nishihara of Shionogi Pharmaceutical Co., Japan, for constructive and helpful suggestions.

Introduction

Oil-in-water (O/W) lipid emulsions are now utilized not only as a lipid supply in parenteral nutrition, but also as drug carriers for highly lipophilic agents. The lipid emulsions can be used for solubilizing low water-solubility drugs, stabilizing hydrolytically susceptible compounds, preventing drug adsorption by infusion apparatuses and reducing irritation, pain or toxicity of intravenously administered drugs (Prankerd & Stella 1990; Klang & Benita 1998). Recent studies have also revealed that O/W lipid emulsions could be utilized as drug carriers for sustained release or organ targeting of lipophilic agents (Prankerd & Stella 1990; Klang & Benita 1998). Sakaeda & Hirano (1998a) demonstrated that lipid emulsions consisting of soybean oil (SO) and egg yolk phosphatides (EYP) could be utilized as drug carriers for delivering lipophilic agents with $\log P_{cal} > 8$ to the liver, lungs and spleen, where $\log P_{cal}$ is the calculated logarithm of the partition coefficient between *n*-octanol and water. Recently, we showed that lipid emulsions consisting of SO and EYP were rapidly cleared from the plasma at injection volumes that are normally used clinically for bolus injection, such as 0.1 mL kg^{-1} (i.e. 6 mL for a person weighing 60 kg), via extensive uptake by the liver and spleen (Ueda et al 2001).

In general, the pharmacokinetics of lipid emulsions are greatly affected by uptake via the liver, spleen and lungs, especially via the reticuloendothelial system (RES)





PS	R	m+n+o
20OE-PSs		
PS 20	$C_{11}H_{23}$	20
PS 40	$C_{15}H_{31}$	20
PS 60	$C_{17}H_{35}$	20
PS 80	$C_{17}H_{33}$	20
6OE-PSs		
PS 21	$C_{11}H_{23}$	6
PS 61	$C_{17}H_{35}$	6
PS 81	$C_{17}H_{33}$	6

PS	R
20OE-3FA-PSs	
PS 65	$C_{17}H_{35}$
PS 85	$C_{17}H_{33}$

Figure 1 Chemical structures of polyoxyethylene sorbitan esters (PSs).

(Juliano 1988). One of the most important factors that affect the biological fate of the oil particles is the surface property. Both liposomes and lipid emulsions prepared with polyethyleneglycol (PEG) derivatives of lipids have been reported to show a prolonged circulation time (Klibanov et al 1990; Papahadjopoulos et al 1991). Coating the microsphere particles with block co-polymer modulates the particle clearance by preventing RES uptake (Illum et al 1987), and emulsifying with polyoxyethylene-(60)-hydrogenated castor oil also prolongs the plasma half-life of emulsions (Sakaeda & Hirano 1998 b). All of these oil particles were prepared or coated with surfactants that possess the polyoxyethylene (POE) moiety; POE polymer conformations are considered to form a dense statistical cloud over the particle surface even at relatively low polymer concentrations, thereby creating a steric barrier preventing (or decreasing) the opsonization process and the interaction with phagocytic cells (Torchilin et al 1994). Furthermore, it was reported that the length of POE chains are required for prolongation of the plasma half-life of the oil particles (Liu & Liu 1995). Therefore, shortening the POE chains of the emulsifiers might result in extensive uptake by the liver or spleen, although information with respect to this point has not been obtained yet. In this study, lipid emulsions were prepared using a series of polyoxyethylene sorbitan esters (PSs) as emulsifiers, which included those with different numbers of oxyethylene units and various lengths or numbers of fatty acid (FA) chains coupled within a molecule (Figure 1). As a model lipophilic drug, menatetrenone was incorporated in the lipid emulsions because its $\log P_{cal}$ is 9.5 and it has been reported to be kept inside the lipid emulsions even after entering the circulation (Sakaeda & Hirano 1998 a). Furthermore, the biological fate of menatetrenone incorporated in the lipid emulsions was examined at a clinical injection volume (0.1 mL kg^{-1}) in rats, and how numbers of oxyethylene units within a molecule of PS affected the kinetics of menatetrenone incorporated in various lipid emulsions was discussed.

Materials and Methods

Materials

Polyoxyethylene-(20)-sorbitan monolaurate (Polysorbate 20 (Tween 20), PS20), polyoxyethylene-(20)-sorbitan monopalmitate (Polysorbate 40 (Tween 40), PS40), polyoxyethylene-(20)-sorbitan monostearate (Polysorbate 60 (Tween 60), PS60), polyoxyethylene-(20)-sorbitan monooleate (Polysorbate 80 (Tween 80), PS80), polyoxyethylene-(20)-sorbitan trioleate (Polysorbate 85 (Tween 85), PS85) and soybean oil (SO) were purchased from Kanto Chemicals Co. Inc. (Tokyo, Japan). Polyoxyethylene-(6)-sorbitan monolaurate (Polysorbate 21 (Tween 21), PS21), polyoxyethylene-(6)-sorbitan monostearate (Polysorbate 61 (Tween 61), PS61), polyoxyethylene-(6)-sorbitan monooleate (Polysorbate 81 (Tween 81), PS81), polyoxyethylene-(20)-sorbitan tristearate (Polysorbate 65 (Tween 65), PS65) and menatetrenone (vitamin K_2) were purchased from Sigma Chemicals Co. (MO). Hydrophilic–lipophilic balance (HLB) and calculated molecular weight of the PSs utilized in this study is shown in Table 1. Purified egg-yolk phosphatides (EYP, NC-50S), which contained $>95\%$ phosphatidylcholine, were obtained from Nippon

Table 1 HLB values and the molecular weights of the PSs.

Surfactant	HLB	Calculated molecular weight
20OE-PSs		
PS20	16.9	1225
PS40	15.6	1282
PS60	14.9	1310
PS80	15.0	1308
6OE-PSs		
PS21	13.0	610
PS61	9.6	695
PS81	10.0	693
20OE-3FA-PSs		
PS65	10.5	1855
PS85	11.0	1853

PS, polyoxyethylene sorbitan ester; HLB, hydrophilic–lipophilic balance; OE, oxyethylene.

Oil & Fats Co. (Tokyo, Japan). All other chemicals were of the highest purity available.

Preparation of lipid emulsions

Lipid emulsions (SO/EYP, SO/PS20, SO/PS40, SO/PS60, SO/PS80, SO/PS21, SO/PS61, SO/PS81, SO/PS65 and SO/PS85) were prepared using a high-pressure homogenization system as described previously (Ueda et al 2001). Briefly, menatetrenone (1.0% w/w) was dissolved in SO (20% w/w) and mixed with EYP or PSs (2.4% w/w) and purified water using a homomixer (model LR-1, Mizuho Industrial Co., Osaka, Japan), and the mixture (100 mL) 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 at 13°C. The size and distribution of the lipid emulsions were determined from the histogram method (SDP analysis) by quasielastic light scattering using a 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. The lipid emulsions were stored at 4°C under no lights until the animal studies were performed, and no size change in the lipid emulsions was detected at the time of the animal studies. The content of menatetrenone in the lipid emulsions was examined by an HPLC method similar to that used to detect menatetrenone in the plasma, and the content of triglycerides was determined using a diagnostic kit (Triglyceride E-test Wako, Wako Pure Chemical Industries Ltd, Osaka, Japan).

Animals

Male slc:Wistar rats, 8–9 weeks old, body weight 200–250 g, with free access to food and water, were used for the experiments. They were anaesthetized by subcutaneous injection of urethane/saline solution (30% w/w, 4.5 mLkg^{-1}), and kept on temperature-regulation 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 Animal Experimental Committee of Kobe Pharmaceutical University.

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

The plasma concentration and organ distribution of menatetrenone after intravenous administration as lipid emulsions were examined as previously described (Ueda et al 2001). SO/EYP or SO/PSs containing menatetrenone were injected into the right femoral vein at an injection volume of 0.1 mL kg^{-1}, and blood samples were collected from the jugular vein at 5, 15, 30, 45 and 60 min using heparinized syringes. Liver, spleen, lungs and kidneys were also collected at 60 min immediately after sacrificing by blood sampling from the abdominal aorta. Blood samples were centrifuged to obtain plasma samples, and liver, spleen, lungs and kidneys were homogenized with water (1:1, 1:2, 1:2 and 1:1, respectively).

Ten-fold and five-fold volumes of ethanol were added to the plasma and organ homogenized samples, respectively, and the supernatant was analysed for menatetrenone by HPLC (Ueda et al 2001). The column and eluent used were Wakosil 5C18 (Wako Pure Chemical Industries Ltd., Osaka, Japan) and acetonitrile–water 95:5, respectively. The flow rate was 3.0 mL min^{-1} and menatetrenone was detected at 270 nm. No peak was detected at the retention time corresponding to menatetrenone in the blank plasma and tissue homogenates.

Pharmacokinetic analysis

The plasma concentration–time curve of menatetrenone administered as SO/PSs was fitted to a mono-exponential equation by non-linear least square regression using Kaleida Graph (Synergy Software, PA), and the plasma half-life ($t_{1/2}$) was calculated from the mono-exponential equation. Volume of distribution (Vd) of menatetrenone was calculated according to equation 1.

$$Vd = (\text{Injected dose of menatetrenone})/C_0 \quad (1)$$

where C_0 is the plasma concentration of menatetrenone at time zero calculated from the mono-exponential equation.

Size and distribution of lipid emulsions after incubation in plasma

To examine whether the size of SO/PSs had changed soon after entering the circulation, SO/PSs were incubated with a 20-fold volume of fresh rat plasma for 5 min at 37°C, and

the particle size and distribution were examined by quasi-elastic light scattering using Coulter Model N4 Plus (Beckman Coulter, CA).

Statistical analysis

Statistical analyses were performed by Kruskal–Wallis test followed by Dunnet's multiple comparison.

Results

Size distribution and measured concentration of components of lipid emulsions

The size distribution and the measured concentration of components of the lipid emulsions prepared with EYP and PSs are shown in Table 2. The mean particle size of SO/PSs was 165–206 nm, which was 1.1–1.4-fold greater than that of SO/EYP. The content of menatetrenone and triglycerides was 0.96–1.00% and 16.7–17.8% of the total weight, respectively, in all of the lipid emulsions prepared.

Plasma concentration–time profile of menatetrenone after intravenous injection as lipid emulsions

Figure 2 shows the plasma concentration–time profile of menatetrenone after intravenous injection as SO/EYP or SO/PSs. The plasma concentration–time profiles of menatetrenone after administration as SO/20OE-PSs (PSs with 20 oxyethylene units) were similar to that as SO/EYP. However, the plasma concentration of menatetrenone after administration as SO/6OE-PSs (PSs with 6 oxyethylene units) was extensively lower than that as SO/EYP; the plasma concentration of menatetrenone at 5 min after the administration as SO/PS21 and SO/PS61 was about one-fourth that of SO/EYP, and that after the administration as SO/PS81 was about one-tenth that as SO/EYP. The plasma concentration of menatetrenone after administration as SO/20OE-3FA-PSs (PSs with 20 oxyethylene units and 3 FA chains) was also lower than that as SO/EYP.

Plasma half-life and volume of distribution of menatetrenone after intravenous injection as lipid emulsions

The plasma half-life of menatetrenone after intravenous administration as SO/EYP or SO/PSs is shown in Table 3. The plasma half-life of menatetrenone for SO/PSs was 6.6–15.5 min and was not significantly different from that for SO/EYP.

The volume of distribution of menatetrenone after administration as SO/EYP or SO/PSs is also shown in Table 3. The volume of distribution of menatetrenone for SO/20OE-PSs was similar to that for SO/EYP, which was also similar to the plasma volume of rats examined by ^{125}I-bovine serum albumin (Sakaeda & Hirano 1995). However, the volume of distribution of menatetrenone for SO/6OE-PSs and SO/20OE-3FA-PSs was larger than that for SO/EYP, although the difference in the values were not significantly different except for SO/PS81, where the volume of distribution of menatetrenone was 29 times that with SO/EYP.

Organ uptake of menatetrenone after intravenous injection as lipid emulsions

The organ uptake of menatetrenone by the liver, spleen, lungs and kidneys 60 min after the intravenous injection as SO/EYP or SO/PSs is shown in Table 4. The organ uptake of menatetrenone by the liver, spleen, lungs and kidneys

Table 2 Size distribution and measured concentration of components of SO/EYP and SO/PSs.

Emulsion	Size distribution (nm; ± s.d.)	Menatetrenone (g/100 mL)	Triglycerides (g/100 mL)
SO/EYP	148 ± 32	0.98	16.8
SO/20OE-PSs			
SO/PS20	178 ± 19	0.99	17.8
SO/PS40	176 ± 26	0.96	17.0
SO/PS60	170 ± 42	0.96	17.3
SO/PS80	165 ± 21	0.97	17.6
SO/6OE-PSs			
SO/PS21	194 ± 27	0.97	17.5
SO/PS61	206 ± 34	1.00	16.7
SO/PS81	199 ± 32	0.98	16.9
SO/20OE-3FA-PSs			
SO/PS65	192 ± 49	0.98	16.7
SO/PS85	177 ± 20	0.96	17.0

SO, soybean oil; EYP, egg-yolk phosphatides; PS polyoxyethylene sorbitan ester; OE, oxyethylene; FA, fatty acid.

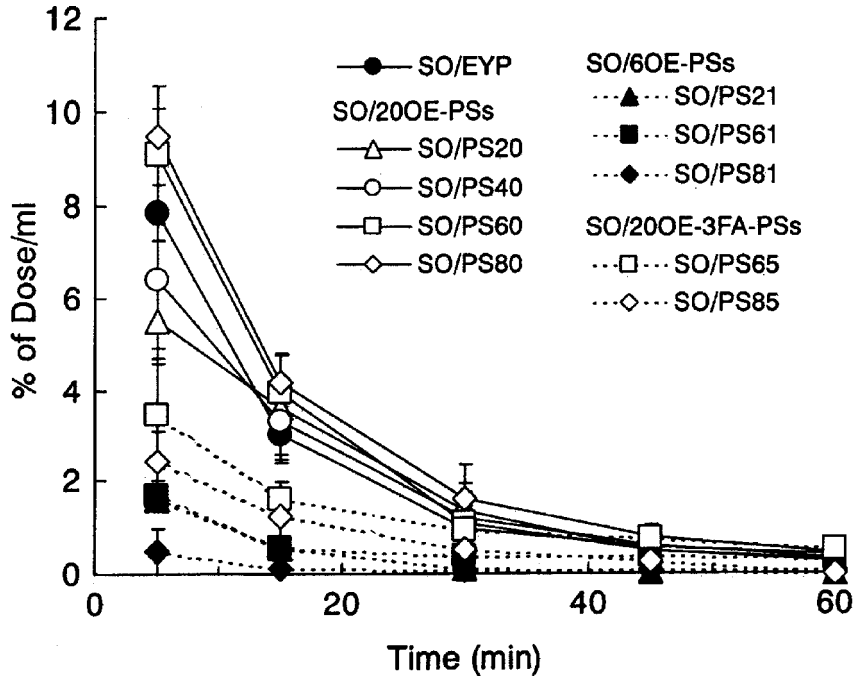


Figure 2 Plasma concentration–time profiles of menatetrenone in rats after intravenous injection as soybean oil/egg-yolk phosphatide (SO/EYP) and soybean oil/polyoxyethylene sorbitan esters (SO/PSs) at an injection volume of 0.1 mL kg^{-1}. The values are the means±s.d. of data from 3–5 rats.

Table 3 Plasma half-life ($t_{1/2}$) and volume of distribution (Vd) of menatetrenone in rats after intravenous injection as SO/EYP or SO/PSs at an injection volume of 0.1 mL kg^{-1}.

Emulsion	$t_{1/2}$ (min)	Vd (mL kg^{-1})
SO/EYP	7.8±1.0	35.3±1.5
SO/20OE-PSs		
SO/PS20	11.3±1.5	62.8±12.5
SO/PS40	10.8±1.3	53.8±13.5
SO/PS60	8.6±1.4	35.2±7.8
SO/PS80	9.5±1.5	35.3±4.1
SO/6OE-PSs		
SO/PS21	6.6±0.8	173.3±11.8
SO/PS61	11.1±3.4	210.6±59.2
SO/PS81	8.8±4.8	1024.8±717.4*
SO/20OE-3FA-PSs		
SO/PS65	15.5±7.2	117.6±60.8
SO/PS85	10.8±1.4	148.4±49.6

SO, soybean oil; EYP, egg-yolk phosphatides; PS polyoxyethylene sorbitan ester; OE, oxyethylene; FA, fatty acid. Values are the mean±s.d. of data from 3–5 rats. *$P < 0.05$, compared with SO/EYP.

after administration as SO/20OE-PSs was not significantly different from that after administration as SO/EYP. On the other hand, menatetrenone uptake by the liver for SO/6OE-PSs was significantly higher than that for SO/EYP by 3–4 fold. Menatetrenone uptake by the spleen was significantly higher for SO/PS21 than that for SO/EYP by 5 fold. Uptake of menatetrenone by the spleen for SO/20OE-3FA-PSs was significantly higher than that for SO/EYP by 6–8 fold. Furthermore, among SO/6OE-PSs and SO/20OE-3FA-PSs, significantly higher uptake of menatetrenone by the lungs was observed for the lipid emulsions with a double bond within the FA moiety of PSs, such as SO/PS81 and SO/PS85, than that for SO/EYP by more than 30 fold. Kidney uptake of menatetrenone could not be detected for any of the emulsions examined except for SO/PS85.

Effect of plasma on particle size of lipid emulsions

To examine the possibility of coalescence of the lipid emulsions soon after entering the circulation, SO/PS80, SO/6OE-PSs and SO/20OE-3FA-PSs were incubated with fresh rat plasma for 5 min at 37°C, and the particle size and distribution were examined by quasielastic light scattering (Table 5). The mean particle size in the incubation mixture of plasma and SO/PS80 was similar to that of SO/PS80 without plasma. The mean particle size of SO/PS61 increased by 25% after incubating with plasma. On the other hand, most of the lipid emulsions of SO/PS65 more than doubled in particle size after incubation with plasma. Furthermore, the particle size of more than half of the lipid emulsions became larger than 500 nm after incubating SO/PS21, SO/PS81 and SO/PS85 with plasma.

Table 4 Organ uptake of menatetrenone in rats after intravenous injection as SO/EYP or SO/PSs.

Emulsion	Organ uptake (% of dose)			
	Liver	Spleen	Lungs	Kidneys
SO/EYP	21.3±7.3	0.8±0.3	0.3±0.1	ND[a]
SO/20OE-PSs				
SO/PS20	33.9±7.0	1.2±0.2	0.5±0.1	ND[a]
SO/PS40	26.4±4.4	1.1±0.3	0.2±0.1	ND[a]
SO/PS60	25.2±4.6	1.1±0.3	0.3±0.1	ND[a]
SO/PS80	31.0±11.8	1.0±0.2	0.4±0.1	ND[a]
SO/6OE-PSs				
SO/PS21	71.4±12.7*	3.9±2.1*	1.8±0.9	ND[a]
SO/PS61	84.0±4.3*	1.7±0.1	2.4±0.6	ND[a]
SO/PS81	65.4±41.4*	3.2±2.4	10.2±7.5*	ND[a]
SO/20OE-3FA-PSs				
SO/PS65	28.7±13.8	6.5±3.1*	2.2±0.9	ND[a]
SO/PS85	37.2±7.1	4.6±0.3*	11.6±3.7*	0.7±0.3

SO, soybean oil; EYP, egg-yolk phosphatides; PS polyoxyethylene sorbitan ester; OE, oxyethylene; FA, fatty acid. Tissues were excised at 60 min after intravenous injection of lipid emulsions at 0.1 mL kg^{-1}. Values are the mean±s.d. of data from 4–5 rats. [a]Could not be detected. *$P < 0.05$, compared with SO/EYP.

Table 5 Effect of rat plasma on particle size of SO/PSs.

Emulsion	Particle size (nm; ±s.d.)
SO/20OE-PSs	
SO/PS80	169±50 (100%)
SO/6OE-PSs	
SO/PS21	1009±116 (78%), 142±33 (19%), 59±10 (3%)
SO/PS61	243±56 (100%)
SO/PS81	811±113 (51%), 199±36 (37%), 60±12 (12%)
SO/20OE-3FA-PSs	
SO/PS65	517±86 (87%), 96±19 (11%), 34±6 (2%)
SO/PS85	1063±147 (86%), 166±33 (13%), 25±5 (1%)

SO, soybean oil; PS polyoxyethylene sorbitan ester; OE, oxyethylene; FA, fatty acid. The lipid emulsions were incubated with a 20-fold volume of fresh rat plasma for 5 min at 37°C, and the particle size and distribution of the incubation mixture was determined by quasielastic light scattering. Typical values of three determinations are presented. Numbers in parentheses represent the percentage of each intensity peak.

Discussion

PS80, one of the 20OE-PSs, is a non-ionic surfactant that has been used for several decades, and is permitted as an additive or emulsifier in Japan for oral and intraveneous dosages. Recently, PS80 was utilized as a useful co-surfactant for stable sub-micron O/W emulsions or self-emulsifying oil formulations (Gershanik et al 1998; Constantinides et al 2000). PS20, another 20OE-PS, has also been used as an emulsifier for parenteral administration of flurbiprofen (Park & Kim 1999). The usefulness of these PSs for stabilizing the particles and reducing the particle size has been reported (Krishna et al 1998). However, it has not yet been clarified how these surfactants affect the pharmacokinetics of the lipid emulsions. In our pharmacokinetic studies, the biological fate of menatetrenone administered as SO/20OE-PSs was similar to that administered as SO/EYP, and menatetrenone uptake by the liver and spleen was higher for SO/6OE-PSs and SO/20OE-3FA-PSs, respectively, than those for SO/EYP. In other words, we were able to classify the biological fate of the lipid emulsions by the PSs utilized – 20OE-PSs, 6OE-PSs and 20OE-3FA-PSs. Although there was an apparent difference in the pharmacokinetics of lipid emulsions prepared with PSs with HLB values above 14 and those below 13, no obvious rule was observed for organ specificity concerning the lipid emulsions prepared with PSs with HLB values below 13 (Table 4). The length of the FA chains did not affect the kinetics of the lipid emulsions. These findings suggest that the length and number of the oxyethylene chains plays a crucial role in determining the biological fate of the lipid emulsions. Furthermore, menatetrenone uptake by the lungs was also increased for SO/PS81 and SO/PS85, SO/6OE-PS and SO/20OE-3FA-PS with double bonds in the FA moieties of their PSs. The double bond in the FA moieties tend to raise the fluidity of the surfactants, which might also have affected the organ distribution of menatetrenone in these lipid emulsions. The reason that SO/PS81 showed an extremely large volume of distribution is not clear, but it is possible that the high lipophilicity and the fluidity due to the double bond in the FA moiety caused high binding to the surface of various

tissues and organs. The sampling time (60 min after administration) for measuring the uptake by the RES organs for SO/PS81 might have been too long to evaluate the surface binding of menatetrenone or menatetrenone-incorporated lipid emulsions.

It was reported that lipid emulsions with large particle size were rapidly distributed to the RES organs such as the liver, spleen and lungs (Takino et al 1994). Since 6OE-PSs and 20OE-3FA-PSs are less soluble in water than 20OE-PSs, SO/6OE-PSs and SO/20OE-3FA-PSs might have coalesced in plasma and been taken up by the RES organs. The particle sizes of SO/6OE-PSs and SO/20OE-3FA-PSs appeared to become larger after entering the circulation, suggesting that the organ distribution of menatetrenone incorporated in these lipid emulsions depended at least partly upon the particle size after entering the circulation. However, a clear relation between the organ uptake of menatetrenone and the particle size could not be observed; although the size distribution of SO/PS21 and SO/PS85 was similar even after incubating with plasma, menatetrenone incorporated in SO/PS21 was taken up by the liver and spleen while that in SO/PS85 was taken up by the spleen and lungs. It is probable that some of the coalesced particles became greater than 3 μm, which might have contributed to the uptake by the spleen and lungs, but were not able to be detected by the quasielastic light scattering method used in this study. Opsonization or dysopsonization, also known to affect the biological fate of the oil particles (Moghimi & Patel 1998), might have differed within the lipid emulsions studied, although we did not examine these factors.

Conclusion

In this pharmacokinetic study, the biological fate of menatetrenone administered as SO/20OE-PSs was similar to that administered as SO/EYP, and menatetrenone uptake by the liver and spleen was higher with SO/6OE-PSs and SO/20OE-3FA-PSs, respectively, than with SO/EYP. Furthermore, menatetrenone uptake by the lungs was also increased when administered as SO/6OE-PS and SO/20OE-3FA-PS with double bonds in the fatty-acid moieties of their PSs. These findings suggested that shortening the oxyethylene units or decreasing the oxyethylene chain numbers of emulsifiers resulted in a rapid clearance of the lipid emulsions from the circulation by extensive uptake via the liver, spleen or lungs. It is of great interest whether a similar phenomenon can be observed with other families of emulsifiers. The information revealed in this study, on the structures of the PSs that affected the biological fate of the incorporated drugs, should be helpful in synthesizing new emulsifiers for better carriers of lipophilic drugs.

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