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The delivery and antinociceptive effects of morphine and its ester prodrugs from lipid emulsions

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Abstract

Long-acting analgesia is critical for patients suffering from long-acting pain. The purpose of this study was to develop lipid emulsions as parenteral drug delivery systems for morphine and its ester prodrugs. Morphine prodrugs with various alkyl chain lengths, including morphine propionate (MPR), morphine enanthate (MEN), and morphine decanoate (MDE), were synthesized. The prodrugs were stable against chemical hydrolysis in an aqueous solution, but were quickly hydrolyzed to the parent drug when exposed to esterase and plasma. Lipid emulsions were prepared using phosphatidylethanolamine (PE) as an emulsifier, while squalene was used as an inner oil phase. Drug release was found to be a function of the drug/prodrug lipophilicity, with a lower release rate for more-lipophilic drug/prodrugs. The inclusion of morphine and its prodrugs into lipid emulsions retarded their release. Lipid emulsions, which incorporated cholesterol, generally exhibited a drug/prodrug release comparable to that of emulsions without co-emulsifiers. Pluronic F68 (PF68) further slowed down the release of morphine and its prodrugs from the emulsions. The antinociceptive activity through the parenteral administration of these emulsions was examined using a cold ethanol tail-flick study. Compared with an aqueous solution, a prolonged analgesic duration was detected after application of the drug/prodrug emulsions. Incorporation of co-emulsifiers such as PF68 and cholesterol further increased the duration of action. The combination of prodrug strategy and lipid emulsions may be practically useful for improving analgesic therapy with morphine.

Keywords: Morphine; Prodrugs; Lipid emulsions; Drug delivery; Phosphatidylethanolamine

1. Introduction

Morphine is the most widely used opioid analgesic for acute and chronic pain and is the standard against which new analgesics are measured (Lugo and Kern, 2002). The parenteral route for morphine is often chosen because a steady state is reached more rapidly, which avoids the appearance of peaks of pain (Morales et al., 2004). However, the rapid distribution from the central compartment combined with the short half-life results in postoperative therapeutic plasma concentrations for only 1–3 h. The question arises as to whether a water-soluble delivery system for morphine can be obtained with a prodrug approach (Mignat et al., 1996). The prodrug concept involves the chemical modification of a known pharmacologically active compound into a bioreversible form, with the aim of changing its pharmaceutical and/or pharmacokinetic characteristics and thereby enhancing its delivery efficacy and therapeutic value (Chan and Li Wan Po, 1989; Ettmayer et al., 2004).

The use of subcutaneous or intravenous morphine infusions is limited because sustained parenteral access and expensive pump devices are required. Lipid emulsions have been introduced as parenteral drug carriers offering sustained release and organ targeting (Kawakami et al., 2000). These kinds of delivery systems can reduce toxicity, enhance stability of the active substance, and slow down delivery rates (Moinard-Checot et al., 2006). One of the most interesting potential approaches to prolong the retention time in emulsions after an injection is to increase the lipophilicity of the drug by chemical modification leading to a prodrug (Kawakami et al., 2000). The aim of this study was to develop lipid emulsions for delivering morphine

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and its ester prodrugs, and examine the physicochemical characteristics and in vivo analgesic activities of these emulsions. Phosphatidylcholine (PC) is commonly used as an emulsifier for lipid emulsions. However, surface-active proteins such as lipoproteins may bind to the emulsions with PC and destabilize the droplets or vesicles (Saito et al., 1997; Hung et al., 2006). This effect can be inhibited by the incorporation of phosphatidylethanolamine (PE) (Saito et al., 1997). Furthermore, the increased hydrophilicity of the emulsion surface increases the circulation time of the droplets (Liu and Liu, 1995). In the present study, we utilized hydrophilic PE as an emulsifier to substitute for PC in lipid emulsions.

2. Materials and methods

2.1. Materials

Morphine HCl was supplied by the National Bureau of Controlled Drugs (Taipei, Taiwan). Phosphatidylethanolamine, Pluronic F68 (PF68), cholesterol, squalene, and esterase from porcine liver were purchased from Sigma Chemical (St. Louis, MO, USA). Hydrogenated soybean phosphatidylcholine (SPC, Phospholipon® 80H) was obtained from American Lecithin Company (Oxford, CT, USA). Cellulose membranes (Cellu-Sep® T2, with a molecular weight cutoff of 6000–8000) were purchased from Membrane Filtration Products (Seguin, TX, USA).

2.2. Prodrug synthesis

Morphine HCl (10 g, 35 mmol) was dissolved in 141 ml of dichloromethane and triethylamine (8.62 ml, 120 mmol) under argon gas. The solution was placed in an ice bath and stirred. The acid chloride (0.037 mmol) of the desired prodrug moiety was added in a dropwise manner. After adding the acid chloride, the reaction mixture was vigorously stirred overnight. Whether or not the reaction was completed was determined by thin layer chromatography (TLC) with a cosolvent system of ammonium hydroxide:methanol:dichloromethane at 1:6:93. After completion of the reaction, the dichloromethane solvent was dried in a rotary evaporator. An appropriate amount of ethyl acetate was added to the dried product, and it was transferred to a separatory funnel. The resulting solution was washed with 5% aqueous sodium carbonate and then with water. After evaporation of the organic solvent, the crude product obtained was purified by column chromatography. Purity (>99%) was assured through elemental analysis, nuclear magnetic resonance spectroscopy, and gas chromatography with mass spectroscopy. Three prodrugs, morphine propionate (MPR), morphine enanthate (MEN), and morphine decanoate (MDE), were synthesized in this study. The chemical structures and basic characteristics of the prodrugs are shown in Fig. 1.

The profiles of MPR were mp: 101–103 °C. IR υ^{KBr} (cm^{-1}) = 3511 (OH), 1753 (C=O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 172.6, 149.2, 134.7, 132.9, 132.6, 132.4, 127.9, 121.6, 120.3, 92.6, 66.2, 59.4, 46.8, 43.3, 42.9, 40.6, 35.4, 27.8, 21.3, 9.5. EI-MS *m/z* (rel. int. %): 341 (M^+, 66), 285 (100), 215 (33), 162 (74), 57 (97).

The profiles of MEN were mp: 79–81 °C. IR υ^{KBr} (cm^{-1}) = 3512 (OH), 1752 (C=O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 171.9, 149.3, 134.8, 132.4 (x3), 127.7, 121.7, 120.3, 92.6, 66.2, 59.5, 46.9, 43.3, 42.9, 40.5, 35.3, 34.5, 31.8, 29.1, 25.3, 22.9, 21.3, 14.4. EI-MS *m/z* (rel. int. %): 397 (M^+, 22), 285 (100), 267 (18), 215 (16), 162 (38).

The profiles of MDE were IR υ^{KBr} (cm^{-1}) = 3520 (OH), 1747 (C=O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 172.0, 149.3, 135.1, 132.6, 132.4, 132.0, 127.2, 122.5, 120.4, 92.4, 66.1, 59.7, 47.0, 43.1, 42.7, 40.1, 34.9, 34.5, 32.2, 29.8, 29.7, 29.6, 29.4, 25.3, 23.1, 21.5, 14.5. EI-MS *m/z* (rel. int. %): 439 (M^+, 14), 285 (100), 267 (17), 215 (13), 162 (35), 43 (39).

2.3. In vitro hydrolysis

The in vitro sensitivity to enzymatic hydrolysis of the prodrugs was carried out using esterase and human plasma. Each prodrug was dissolved in pH 7.4 citrate–phosphate buffer to give a concentration of 0.4 mM. Esterase at 1.92 IU/ml in pH 7.4 buffer with respective volumes of 10 μl and 15 μl was added to the prodrug solution which had a volume of 500 μl. Human plasma with volumes of 10 μl and 15 μl was also used as the hydrolytic medium in this study. The resulting solution was incubated at 37 °C. At predetermined intervals, the reaction mixture was withdrawn and rapidly frozen to stop the enzymatic reaction. After thawing and filtration, the concentration of prodrugs in the medium was measured by high-performance liquid chromatography (HPLC).

2.4. HPLC analysis

Morphine and its prodrugs were quantified using an HPLC system consisting of a Hitachi (Tokyo, Japan) L-7100 pump, a Hitachi L-7200 sample processor, and a Hitachi L-7400 UV detector. A 25-cm long, 4-mm inner diameter stainless C18 column (LiChrospher® in LiChrocart® column, Merck, Darmstadt, Germany) was used. The mobile phase was a mixture of acetonitrile and a 20 mM phosphate aqueous solution with 1 mM sodium dodecylsulfate at ratios of 32:68, 35:65, 55:45 and 70:30 for morphine, MPR, MEN and MDE, respectively. The flow rate was set to 1 ml/min, and the wavelength of the UV detector was 212 nm.

2.5. Preparation of lipid emulsions

Squalene (5% of the final product, w/v) and PE or SPC were dissolved in an appropriate volume of chloroform:methanol (2:1). PF68 and cholesterol (3%) as the co-emulsifiers were incorporated into this organic solvent if necessary. The organic solvent was evaporated in a rotary evaporator at 50 °C, and solvent traces were removed by maintaining the lipid film under a vacuum for 6 h. The films were hydrated with double-distilled water (to 85% in the formulation without co-emulsifiers and 82% in the formulation with co-emulsifiers) using a high-shear

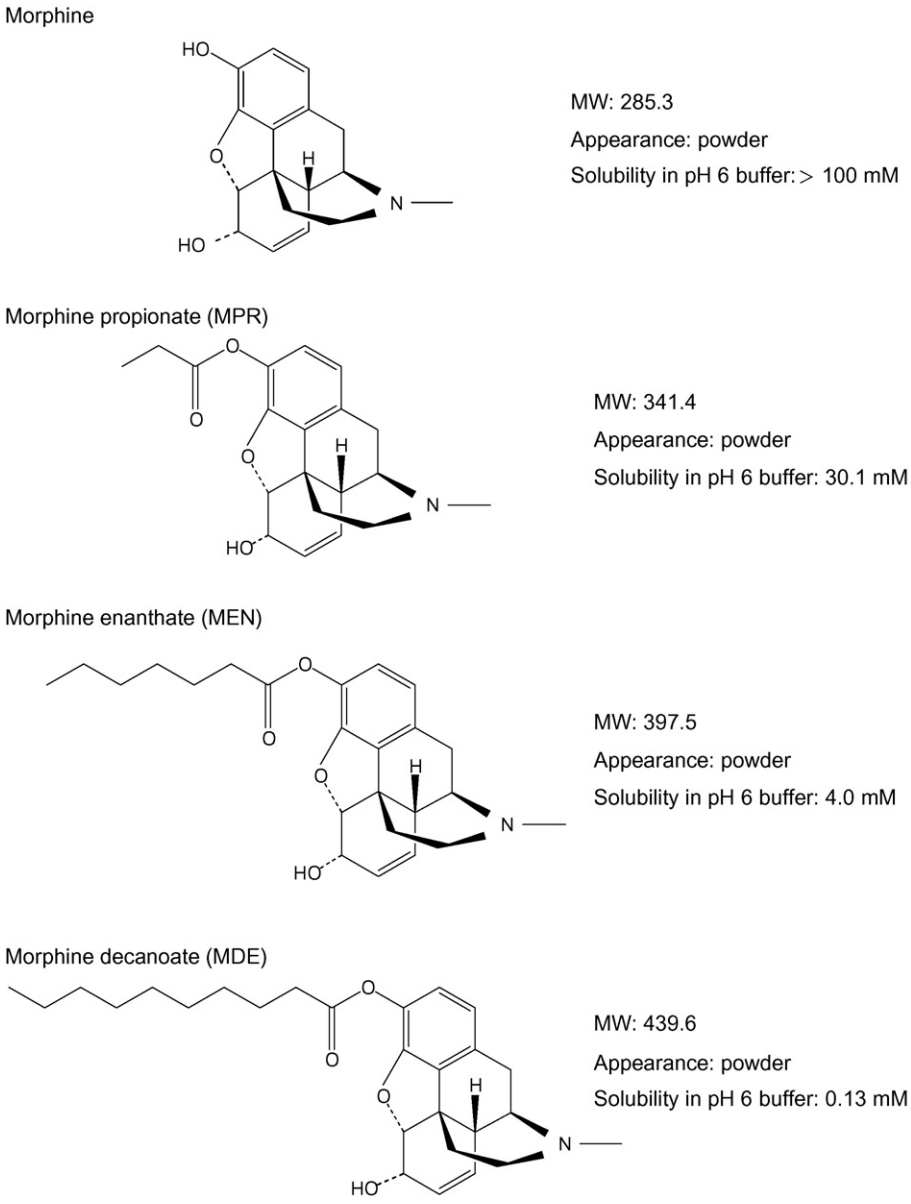

Fig. 1. Chemical structures and physicochemical characteristics of morphine, morphine propionate (MPR), morphine enanthate (MEN), and morphine decanoate (MDE).

homogenizer (Pro Scientific Pro 250, Monroe, CT, USA) for 10 min at 60 °C. Then the resulting solution was sonicated using a probe-type sonicator (Sonics and Materials VCX 600, CT, USA) for 30 min at 60 °C. Morphine and its prodrugs (at 3.5 mM in the final product) were respectively dissolved in the aqueous phase and oil phase. The total volume of the final emulsions was 10 ml.

2.6. Physicochemical characteristics of the lipid emulsions

The mean vesicle size (z-average) and zeta potential of the lipid emulsions were measured by photon correlation spectroscopy (Malvern Nano ZS® 90, Worcestershire, UK) using a helium–neon laser with a wavelength of 633 nm. Photon correlations of spectroscopic measurements were carried out at a scattering angle of 90°. A 1:100 dilution of the emulsions was made using double-distilled water before the measurement.

The emulsions with drug/prodrugs were centrifuged at 48,000 × *g* and 4 °C for 30 min in a Beckman Optima max® ultracentrifuge (Beckman Coulter, Fullerton, CA, USA) in order to separate the incorporated drug from the non-incorporated drug. The non-incorporated drug concentration of the supernatants was analyzed by HPLC to determine the encapsulation percentage.

2.7. In vitro release

The release of morphine and its prodrugs was measured using a Franz diffusion assembly. A cellulose membrane was mounted between the donor and receptor compartments. The donor medium consisted of 0.5 ml of vehicle containing nal-

buphine or its prodrugs. The receptor medium (5.5 ml) for morphine and MPR was pH 7.4 citrate–phosphate buffer. The receptor medium consisted of ethanol/pH 7.4 buffer (3:7) for MEN and MDE to maintain the sink condition. The available diffusion area between cells was 1.13 cm^2. The stirring rate and temperature were respectively kept at 600 rpm and 37 °C. At appropriate intervals, 300-μl aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh buffer. The amounts of the drug/prodrugs were determined by HPLC.

2.8. In vivo cold ethanol tail-flick test

Male Sprague–Dawley rats weighing 200 ± 25 g were used in this study. All tests were performed in accordance with the recommendations and policies of the International Association for the Study of Pain for the handling and use of experimental animals. The apparatus used was a temperature circulation system (Neslab RTE-140D, Newington, NH, USA) with a bath solution of 95% ethanol. A temperature of −30 °C was chosen for testing. Vehicles containing 3.5 mM drug/prodrugs were injected intravenously into the tail vein at a dose of 5 μmol/kg. Normal saline was used as a control.

Animals were firmly held over the opening of the bath with their tails submerged approximately half way into the bath. The nociceptive threshold was taken as the latency until the rat flicked its tail from the bath. The time from immersion to tail flicking was measured to the nearest tenth of a second with a laboratory timer. To minimize damage to the animal's tail, a predetermined cutoff time of 12 s was used, and was considered to be the maximum latency. Under this condition, neither frostbite nor skin color change was found on the tail throughout the experiment. The number of animals in each group was four.

2.9. Statistical analysis

Statistical analysis of differences between different treatments was performed using unpaired Student's *t*-test. A 0.05 level of probability was taken as the level of significance. An analysis of variance (ANOVA) test was also used.

3. Results

3.1. In vitro hydrolysis

By analogy with heroin or codeine, whose opioid-like effects are considered to be mediated by released morphine, one can assume that the pharmacological activity of morphine prodrugs may also be related to the release of morphine by enzymatic degradation (Mignat et al., 1996). Fig. 2 shows that the bioconversion of MPR proceeded in a dose-dependent manner of esterase to the hydrolytic level. The disappearance of the ester prodrugs was accompanied by a corresponding increase in morphine. The conversion of MPR in plasma was so rapid that no prodrug was detectable immediately after adding MPR to the plasma. MEN and MDE were completely hydrolyzed during a 20-min treatment in either esterase or plasma. Incubation of

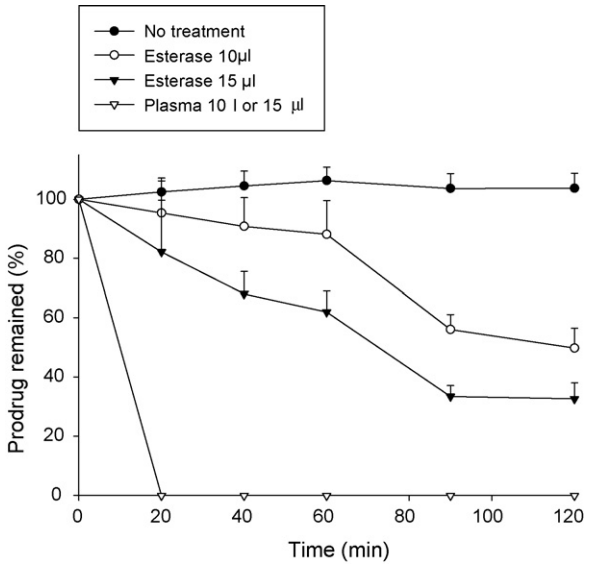

Fig. 2. Hydrolysis of morphine propionate in double-distilled water (no treatment), esterase from porcine liver, and human plasma at 37 °C for 120 min. Morphine enanthate, and morphine decanoate were completely hydrolyzed during a 20-min treatment in either esterase or plasma. All data are presented as the mean ± S.D. of four experiments.

the three prodrugs in pH 7.4 buffer without esterase showed no hydrolysis for up to 120 min. This suggests that morphine prodrugs are stable in an aqueous solution, confirming their applicability in clinical situations.

3.2. Physicochemical characterization of lipid emulsions

Morphine and its prodrugs were incorporated into lipid emulsions with various components. To characterize the physicochemical properties of the various emulsion formulations, the droplet size and zeta potential were determined and are shown in Table 1. The emulsions utilized here were formulations incorporating morphine. The physicochemical characteristics of emulsions with prodrugs were generally similar to those with morphine. The size of PE-containing emulsions was significantly smaller ($p < 0.05$) than that of SPC-containing emulsions. PF68 is a hydrophilic non-ionic block copolymer of polyoxyethylene–polyoxypropylene. The addition of 3% PF68 in PE emulsions further decreased the droplet size ($p < 0.05$). Cholesterol was another co-emulsifier used in this study. The addition of cholesterol to PE emulsions led to an initial increase in the droplet size ($p < 0.05$). On the other hand, changes in size were not significant ($p > 0.05$) when adding co-emulsifiers to the SPC emulsion systems.

As depicted in Table 1, the zeta potentials of PE emulsions were −63 mV to −65 mV. No effect of co-emulsifiers on the zeta potential was observed. Zeta potentials of SPC emulsions ranged from −43 mV to −50 mV. Cholesterol incorporation significantly reduced ($p < 0.05$) the zeta potential of SPC emulsions. As shown in Table 2, approximately 20–30% of the total morphine content was found in the oil phase of PE emulsions. The morphine prodrugs basically showed higher encapsulation efficiencies than morphine. It can be observed that the incorporation

Table 1
The composition and characterization of lipid emulsions loaded with morphine by particle size, polydispersity index, and zeta potential

Phospholipid	Co-emulsifier	Size (nm)	Polydispersity index	Zeta potential (mV)
PE	None	159.7 ± 1.5	0.19 ± 0.02	−65.2 ± 0.3
	Pluronic F68	118.3 ± 0.6	0.21 ± 0.02	−63.5 ± 1.2
	Cholesterol	169.3 ± 3.1	0.22 ± 0.02	−63.5 ± 1.8
SPC	None	181.3 ± 3.5	0.20 ± 0.02	−48.2 ± 0.6
	Pluronic F68	189.3 ± 13.1	0.19 ± 0.03	−50.3 ± 1.9
	Cholesterol	199.0 ± 1.0	0.17 ± 0.04	−43.2 ± 3.6

PE: phosphatidylethanolamine; SPC: soybean phosphatidylcholine. Each value represents the mean ± S.D. ($n = 3$).

Table 2
The encapsulation percentage (%) of morphine and its prodrugs within the oil phase of phosphatidylethanolamine emulsions

Co-emulsifier	Morphine	MPR	MEN	MDE
None	17.2 ± 6.7	25.2 ± 1.9	91.7 ± 0.6	99.5 ± 0.1
Pluronic F68	19.3 ± 4.0	10.4 ± 1.3	67.9 ± 2.8	61.4 ± 2.7
Cholesterol	25.6 ± 5.6	36.9 ± 0.9	97.5 ± 0.3	90.6 ± 2.6

MRP: morphine propionate, MEN: morphine enanthate, and MDE: morphine decanoate. Each value represents the mean ± S.D. ($n = 4$).

of PF68 generally reduced the oil loading of drug/prodrugs, especially for morphine and MPR. The incorporation of cholesterol either maintained or increased drug/prodrug encapsulation into the oil phase.

3.3. In vitro release

When developing emulsions encapsulating drugs in an in vivo status, it is important to optimize the ability to release the drug from the emulsions. The ability of lipid emulsions to deliver morphine and its prodrugs was investigated by determining the release rate across a cellulose membrane. The release of each

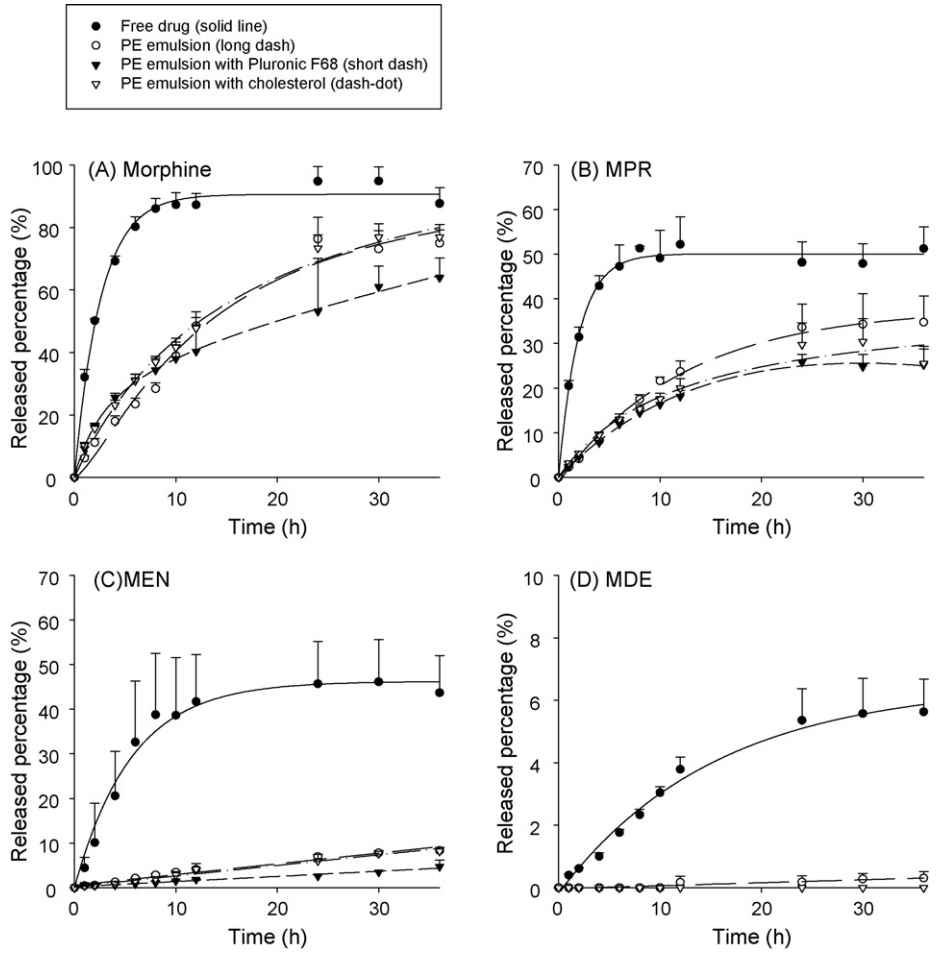

Fig. 3. In vitro release of morphine (A), morphine propionate (B), morphine enanthate (C), and morphine decanoate (D) across a cellulose membrane from double-distilled water (free drug) and PE emulsions with or without co-emulsifiers. All data are presented as the mean ± S.D. of four experiments.

drug and prodrug in double-distilled water was studied as a control. As shown in Fig. 3A, the release of morphine from the aqueous solution showed an initial burst that gradually leveled off after 8 h of administration. Around 95% of the morphine in the donor phase had been released to the receptor phase by the end of experiment (36 h). Practically 50% of the MPR dose in the aqueous solution had crossed the membrane after 36 h (Fig. 3B). The released amounts of MEN and MDE were relatively low, with only less than 45% and 6% of the respective compounds released in 36 h (Fig. 3C and D).

Preparations of morphine and its prodrugs in lipid emulsions had slower drug-releasing profiles than those prepared in the aqueous solution (free form) as shown in Fig. 3. The reductions in release by lipid emulsions were more significant for MEN and MDE than for morphine and MPR. PE emulsions without co-emulsifiers released morphine more slowly in the beginning. A zero-order release (correlation coefficient, $r = 0.99$) was suitable to fit the curves of MEN and MDE in PE emulsions without co-emulsifiers during 36 h of administration, indicating the sustained release of the two prodrugs. The release rates of morphine and its prodrugs were found to change depending on the nature of the co-emulsifiers used in the PE emulsions as shown in Fig. 3. The drug/prodrug release rates from PF68-containing emulsions were slower than those from cholesterol-containing emulsions for morphine, MPR, and MEN. It was noted that there was no MDE released from PE emulsions with co-emulsifiers during the 36-h experiment (Fig. 3D).

3.4. In vivo cold ethanol tail-flick test

The prodrugs should be converted to the parent drug to exhibit pharmacological activity. The antinociceptive activity of morphine and its prodrugs was examined by a cold ethanol tail-flick test. The latency–time profiles of morphine and its prodrugs in an aqueous solution (free form) are shown in Fig. 4. The average baseline latency for drug-blank rats (treatment with normal saline) was 2–3 s. Morphine in aqueous solution showed a quick and significant effect on the latency of the tail-flick response. After a 1.5-h duration, the analgesic ability of morphine had vanished (Fig. 4A). Prolonged analgesia was obtained by MPR aqueous solution treatment for at least 2.5 h (Fig. 4B). There was no significant peak of latency for MEN in the free drug form. The latency–time curves of MDE and morphine in an aqueous solution were similar (Fig. 4A and D). The area under the curve (AUC) of the latency–time profiles for each formulation was

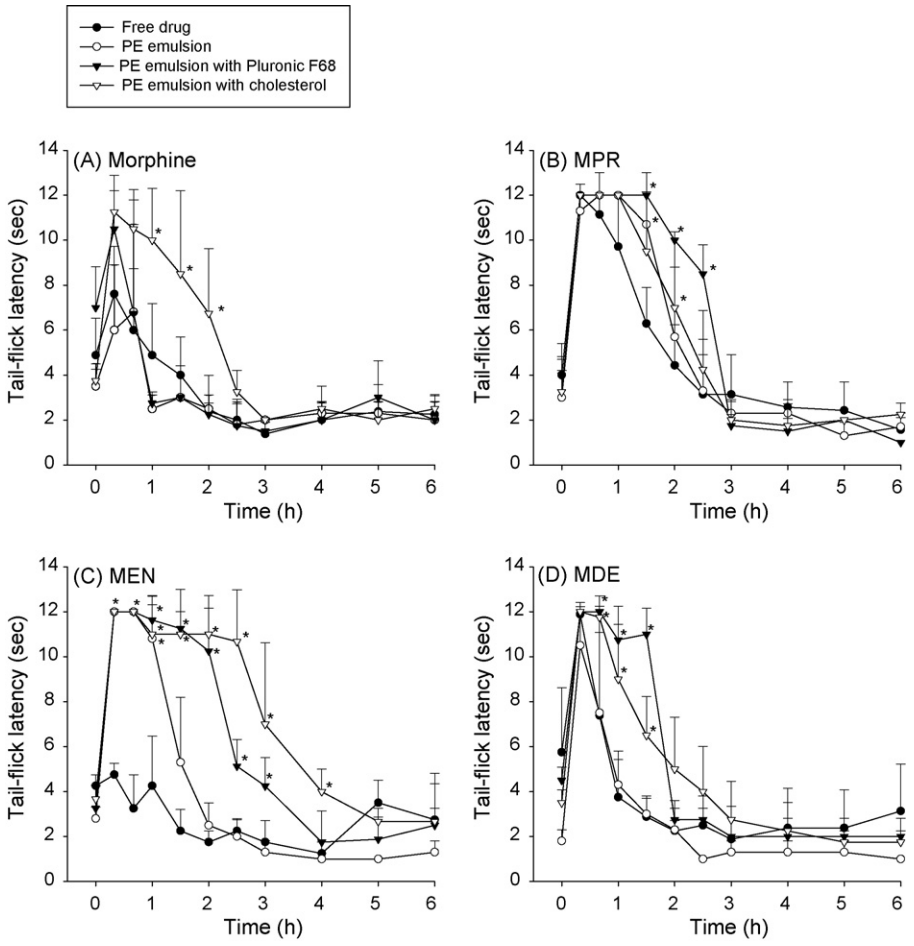

Fig. 4. Antinociceptive activity of morphine (A), morphine propionate (B), morphine enanthate (C), and morphine decanoate (D) from double-distilled water (free drug) and PE emulsions with or without co-emulsifiers after intravenous administration in rats according to the cold ethanol tail-flick test. All data are presented as the mean ± S.D. of four experiments.

Table 3
The area under the curve (AUC) of tail-flick latency–time curves after in vivo administration of morphine and its prodrugs in free or phosphatidylethanolamine emulsion form during 6 h

Co-emulsifier	Morphine	MPR	MEN	MDE
Free drug	18.1 ± 4.6	27.6 ± 3.9	15.7 ± 2.0	20.4 ± 5.9
None	16.5 ± 2.0	29.4 ± 2.7	21.4 ± 1.8	15.1 ± 2.7
Pluronic F68	18.7 ± 5.5	31.0 ± 0.8	34.8 ± 9.5	23.5 ± 7.2
Cholesterol	28.6 ± 3.2	30.3 ± 4.9	42.8 ± 4.3	26.9 ± 4.9

MRP: morphine propionate, MEN: morphine enanthate, and MDE: morphine decanoate. Each value represents the mean ± S.D. ($n = 4$).

calculated, and results are summarized in Table 3. Differences among the AUC of morphine, MEN, and MDE in a free form were not significant ($p > 0.05$) when the data were subjected to statistical analysis.

The analgesic profiles of morphine in PE emulsions without co-emulsifiers did not show significant differences to those in aqueous solution (Fig. 4A). The same phenomenon was observed with MDE. Although the AUC values of MPR in the free form and in PE emulsions without co-emulsifiers were comparable ($p > 0.05$), the analgesic effect of MPR in emulsions lasted longer than that in aqueous solution (Fig. 4B). Both the AUC values and analgesic intensities of MEN in emulsions without co-emulsifiers were significantly higher ($p < 0.05$) than those in the free form (Fig. 4C, Table 3). The incorporation of PF68 or cholesterol in emulsions increased the analgesic intensity or duration of morphine and its prodrugs by different levels (Fig. 4). However, there was no significant difference ($p > 0.05$) among the AUC values of MPR among the three emulsions tested.

4. Discussion

Morphine was esterified by corresponding acid chloride to produce MPR, MEN, and MDE. Morphine possesses two hydroxyl groups (at the 3- and 6-positions) that provide suitable sites for esterification. Due to the importance of the free phenolic hydroxyl group for both opioid receptor binding and analgesic activity (Chen et al., 1991), masking of this position is expected to result in morphine derivatives with prodrug properties.

Esterase activity is localized in erythrocytes, the liver, and brain tissue. The in vitro hydrolysis data showed that MPR was readily converted to the parent drug in the presence of esterase. MEN and MDE with longer alkyl chains resulted in faster enzyme hydrolysis compared to MPR. Although the longer alkyl chains have bulkier moieties than the shorter chains, it was reported that the hydrolysis rates of some esters are enhanced by increasing the lipophilicity (Ahmed et al., 1997; Ostacolo et al., 2004).

The most common lipid injectable emulsion used clinically is a soybean oil formulation (Driscoll, 2006). The principal long-chain fatty acids found in soybean oil include linolenic, linoleic, oleic, palmitic, and stearic acids. The parenteral administration of this type of emulsion is now being discouraged because of the problems related to a "fat overload syndrome" or inflammation to soybean oil (Cury-Boaventura et al., 2006). Hence we used squalene instead of soybean oil as the oil phase in the present study. Squalene is an all-*trans* isoprenoid containing six isoprene units, which is a naturally occurring substance found in plants, animals, and humans. Clinical studies of injectable squalene systems have been performed without an evidence of safety concerns according to the World Health Organization Weekly Epidemiological Record (on 14 July 2006).

PE further reduced the droplet size of emulsions compared to SPC (Table 1). This may have been due to SPC not being used as an emulsifier by itself because it does not produce emulsion over a wide range of oil and water compositions (Brime et al., 2002). The addition of co-emulsifiers such as PF68 and cholesterol even could not reduce the droplet size of SPC emulsions. PE may improve the insufficient emulsification of SPC, thus reducing the droplet size. Smaller emulsions disappear from the blood more slowly than do larger emulsions (Kurihara et al., 1996). Smaller emulsions (~100 nm) show reduced hepatic uptake and prolonged blood circulation times (Kawakami et al., 2000). PF68 reduced the droplet size of PE emulsions to an ideal diameter of near 100 nm (Table 1). This indicates that a mixture of PE and PF68 emulsified the oil phase more effectively than did the single emulsifier. Although cholesterol has been previously used as a stabilizer for PE membranes (Litzinger and Huang, 1992), it caused a slight size increase in PE emulsions in our case.

The *n*-octanol/water partition coefficients ($\log P$) of PF68 and cholesterol were −1.7 and 7.5, respectively. This suggests that the more-hydrophilic PF68 contributed to a smaller-sized droplet. One parameter for the surfactant film separating the water and oil domains is the spontaneous mean curvature, H_0. H_0 expresses the natural tendency of a monolayer to bend away from a flat geometry (von Corswant et al., 1998). H_0 is positive for co-emulsifiers with a large polar head group and a small nonpolar group and decreases with the number and size of alkyl chains of the nonpolar group. Cholesterol may be too highly lipophilic to form stable PE emulsions. The addition of a hydrophilic co-emulsifier was required to increase the hydrophilicity in the films, favoring interfacial film curvature.

PC is a neutral or zwitterionic phospholipid over a pH range from strongly acidic to strongly alkaline. The SPC used in this study contained 80% PC. The other components (20%) of SPC include phosphatidylserine, phosphatidic acid, phosphatidyl glycerol, and phosphatidylinositol, all of which are negatively charged. On the other hand, the charge of PE is negative at a pH of near 7.0 (Vance and Vance, 2002). This may have contributed to the highly negative charges of the PE emulsions. The profiles of the zeta potential may confirm the smaller size of the PE emulsions compared to the SPC ones, since the greater ionization at the interface of PE emulsions tends to increase the electrostatic repulsion among droplets, which avoids aggregation (Driscoll, 2006).

Encapsulation of 20–30% by the oil phase was considered not to be low because morphine is a molecule with a high aqueous solubility. The oil phase alone did not contribute to sufficient entrapment of morphine, while the aqueous phase may play a role in drug loading (Wang et al., 2006). The oil/water emulsion is complex, as the drug may be present in four different environments: the oil phase, oil/water interface, aqueous micelles and water (Pongcharoenkiat et al., 2002). Although the possibility of

the morphine loading within oil phase was low, morphine resided in the oil/water phase may be expected. PE, which possesses a hydrophilic moiety, may provide some affinity to morphine molecules. Moreover, under the conditions of emulsions using phospholipids as the emulsifiers, liposomal vesicles are also found in the aqueous phase for entrapping the drugs (Liu and Liu, 1995). The amounts of MEN and MDE in the oil phase were significantly larger ($p < 0.05$) than those of morphine and MRP (Table 2). This can be attributed to the higher lipophilicities and lower aqueous solubilities of MEN and MDE as compared to morphine and MPR. Although the exact trend could not be determined, the loading of morphine and its prodrugs in the oil phase is expected to increase from morphine to MDE. Another reason is that the long alkyl chains of MEN and MDE may render them surface active. As a result, they tend to adsorb onto the oil/water interface with phospholipids, thus increasing the possibility for encapsulation. A similar result was found in the prodrugs of paraben and nalbuphine entrapped in lipid emulsions stabilized by phospholipids (Pongcharoenkiat et al., 2002; Wang et al., 2006). Less morphine or its prodrugs resided in the oil phase when PF68 was present in the emulsion. The positive H_0 for PF68-containing emulsions may have contributed to this effect. A high H_0 value favors the formation of an elastic or flexible surfactant film, whereas a decrease of H_0 leads to the formation of a more rigid or stiffer film (Wheeler et al., 1994). The flexible film may result in greater leakage of molecules from the inner phase to the outer phase. This mechanism also explains the higher drug encapsulation with cholesterol incorporation than with PF68 incorporation.

The release of morphine and its prodrugs from the aqueous solution (free form) was found to be a function of the lipophilicity, with lower release rates for more-lipophilic drug/prodrugs (Fig. 3). MEN and MDE at doses of 3.5 mM did not completely dissolve in double-distilled water. Since the compound should be released in a dissolved form, their low solubilities may have retarded the release of MEN and MDE from the aqueous solution. In addition to the influence of lipophilicity, the steric structure may be another factor affecting drug/prodrug release. The moiety of a longer alkyl chain is bulkier than a shorter one. The steric hindrance by this functional group may retard the release rate from an aqueous solution.

An aqueous solution or suspension is thermodynamically unstable. Sedimentation is always present, but it is efficiently cancelled by Brownian motion in the case of emulsions (Moinard-Checot et al., 2006). The drug may be stably retained in droplets of emulsions for a determined duration, followed by its slow release into the external phase. The high lipophilicity is a prerequisite for the stable retention of a drug in the oil core of a lipid emulsion system (Lundberg et al., 2003). The stable inclusion of drugs with high lipophilicity is reflected in the fact that the release experiments revealed very low release rates of MEN and MDE from lipid emulsions in contrast to free MEN and MDE (Fig. 3C and D).

Although the incorporation of PF68 generally showed lower entrapment of morphine and its prodrugs compared to the incorporation of cholesterol, PF68-containing emulsions produced slower drug/prodrug release from the inner phase to the external phase compared to cholesterol-containing emulsions. This suggests that the drug/prodrugs were retained in the PF68-containing systems for a longer time. The combination of phospholipids and PF68 leads to the formation of a closely packed mixed film; this confers improved stability after preparation, which is attributed to the steric stabilization of the non-ionic surfactant (Weingarten et al., 1991; Jumaa and Müller, 2002). On the other hand, monolayer rigidity may be decreased by the presence of cholesterol, resulting in a less-stable interface after preparation. Another observation is the initial burst of morphine from PF68-containing systems (Fig. 3A). This may be due to the existence of some drug molecules in the interface, since the hydrophilic PF68 may associate with morphine. The release profiles suggest that by altering the composition of the lipid emulsions, the release of morphine and its prodrugs can be well controlled. This is important for the development of a system for use as a drug carrier for parenteral use.

Due to the low lipid solubility of morphine, penetration of the blood-brain barrier is delayed and the peak effect does not occur until 20 min after intravenous administration (Lugo and Kern, 2002). This demonstration corresponds to our results that morphine in a free drug form showed a peak latency of the tail-flick response at 20 min. MPR in an aqueous solution exhibited a longer antinociceptive duration compared to morphine. However, the analgesic durations of MEN and MDE were limited. The more-rapid hydrolysis of MEN and MDE compared to MPR means that determined proportions of MEN and MDE convert to the parent drug, which enters brain tissue with more difficulty than the lipophilic prodrugs. The low solubilities of MEN and MDE in the aqueous solution also limit the availability of these two prodrugs. It was verified that the development of formulations to encapsulate the prodrugs is necessary. A previous study proved that an oil/water submicron emulsion is effective in protecting the prodrug from enzymatic attack (Nicolaos et al., 2003).

Although the emulsions without co-emulsifiers were able to slowly release morphine and its prodrugs, they did not increase the analgesic activity as expected from the release profiles except for MEN (Fig. 4). This suggests that emulsions without co-emulsifiers insufficiently protected the drug/prodrugs from enzymes. This defect was overcome by incorporating PF68 or cholesterol in the emulsions, especially for morphine, MEN, and MDE. Although drug/prodrug release from emulsions with cholesterol approximated that without co-emulsifiers (Fig. 3), cholesterol was effective in providing higher antinociceptive activities for morphine, MEN, and MDE. The interaction between lipid emulsions and apolipoproteins plays an important role in the half-life of lipid emulsions in the blood circulation (Kawakami et al., 2000). Cholesterol in the interface of oil droplets decreases the binding capacity to apolipoproteins (Ibdah and Phillips, 1988; Saito et al., 1997), preventing the destruction of the emulsion systems in the circulation.

The good potency of PF68-containing emulsions for prolonging the analgesic duration may have been due to an increased hydrophilicity and the formation of a steric barrier on the emul-

sion surface, which is responsible for the increased circulation time (Kawakami et al., 2000). However, this effect was not found in the case of morphine. A possible reason is that PF68-containing emulsions showed the slowest morphine release among the systems tested (Fig. 3A), and this may have led to a failure to surmount the therapeutic concentration in circulation. Optimization of drug release from the emulsions may be needed in order to develop the emulsion formulations. The incorporation of MPR in the lipid emulsions did not further increase the AUC values of the latency–time profiles ($p > 0.05$), although a longer analgesic duration was obtained by the emulsions. MPR itself exhibited good antinociception in a free form in an aqueous solution. Hence the efficiency of encapsulating MPR in emulsions for an enhanced antinociception is confirmed.

Toxicity is another important issue for developing lipid emulsions. One practical limitation of using lipid emulsions is the hemolysis caused by the interaction between erythrocytes and phospholipids (Ishii and Nagasaka, 2004). In vitro hemolysis of PE emulsions interacting with erythrocytes was carried out as a preliminary test of the formulation safety. The test was a spectroscopic method as described previously (Hung et al., 2006). All PE emulsions tested showed a tolerable hemolysis of <5% to erythrocytes. This result suggests the potential of therapeutic applications of PE emulsions.

5. Conclusions

Although a syringe or patient-controlled analgesic pump (PCA) may be useful to provide sustained analgesia in patients who require it, a single intravenous injection of a long-acting preparation has advantages over those methods: it reduces the use of related medical products and enhances patient convenience and compliance in daily activities. The aim of this study was to combine the prodrug strategy and lipid emulsions for prolonging the analgesic activity of morphine. PE was used as an emulsifier in the emulsions because of the need to increase the half-life in circulation. Squalene is the choice for the oil phase to avoid the "fat overload syndrome" or inflammation caused by soybean oil. PE produced smaller droplet sizes than did SPC. The addition of co-emulsifiers such as PF68 and cholesterol altered the PE emulsion size. However, this effect was not observed with SPC emulsions. Greater drug entrapment in the inner phase of the emulsions was observed with more-lipophilic drug/prodrugs. Co-emulsifier incorporation further reduced the release of morphine and its prodrugs from the lipid emulsions, being especially true with PF68. The incorporation of co-emulsifier was only evaluated at one concentration in this study. It should be further investigated to optimize the drug release based on the choice of co-emulsifiers over a range of concentrations in the future. The inclusion of morphine and its prodrugs in the PE emulsions prolonged the analgesic duration and/or analgesic activity in rats. The controlled-release ability and drug/prodrug protection afforded by the systems may have contributed to the high efficacy of antinociception. The emulsions with co-emulsifiers showed better efficiency of tail-flick latency than did the emulsions without co-emulsifiers. The lipid emulsions developed in this study may be useful for the therapeutic application of morphine. The drug delivery characteristics and analgesic activity could be controlled via optimal selection of drug/prodrug lipophilicity and the composition of the emulsions.

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