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Phospholipid-based microemulsion formulation of all-*trans*-retinoic acid for parenteral administration

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Abstract

All-*trans*-retinoic acid (ATRA) shows anti-cancer activities, especially in patients with acute promyelocytic leukemia. Due to the highly variable bioavailability of ATRA and induction of its own metabolism after oral treatment, development of alternative parenteral dosage form is required. The principal aim of this study was to develop a parenteral formulation of ATRA by overcoming its solubility limitation by utilizing phospholipid-based microemulsion system as a carrier. Microemulsion was prepared with pharmaceutically acceptable ingredients such as soybean oil and phospholipids. The mean particle diameter and polydispersity of ATRA microemulsion could be decreased to be applicable for parenteral administration by modulation of composition of microemulsion. The loading concentration of ATRA in microemulsion increased by increasing the oil contents and also by inclusion of distearoylphosphatidyl-ethanolamine-*N*-poly(ethyleneglycol) 2000 (DSPE-PEG). Furthermore, loading of ATRA in microemulsion improved the chemical stability of ATRA. The pharmacokinetic profile of ATRA after intravenous injection of microemulsion formulation to rats was similar to that of sodium ATRA. The growth inhibitory effects of ATRA on human cancer HL-60 and MCF-7 cell lines were also similar between free ATRA and microemulsion formulation of ATRA, suggesting that its anti-cancer activity was not impaired by loading in microemulsion. Our study herein demonstrates that phospholipid-based microemulsion may provide an alternative parenteral formulation of ATRA.

Keywords: Retinoic acid; Parenteral delivery; Phospholipid; Microemulsion

1. Introduction

All-*trans*-retinoic acid (ATRA), an active metabolite of retinol (Vitamin A), has been shown to exert anti-cancer activities in a number of types of cancer cells and tissues. The anti-cancer activity of ATRA is mediated by ATRA-dependent activation of retinoic acid receptors and retinoid X receptors on the nuclear membrane of cancer cells, leading to the growth inhibition, differentiation and apoptosis of cancer cells (Hansen et al., 2000).

Recently, ATRA has been given to patients mainly by oral administration in the treatment of acute promyelotic leukemia patients (Conley et al., 1997). However, gradual decrease in the ATRA concentration was observed in the blood circulation after

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prolonged oral treatment due to the induction of its own metabolism (usually within 1–6 weeks) (Muindi et al., 1992). Moreover, the bioavailability of ATRA was highly variable among patients probably due to its poor aqueous solubility (Adamson et al., 1993). The oral ATRA treatment may also be difficult for patients who cannot swallow capsules. Therefore, parenteral administration of ATRA may have merits over oral administration in providing reliable potency and duration of ATRA's activity in cancer patients.

The poor aqueous solubility of ATRA also gives major drawbacks in its parenteral administration. To date, no parenteral formulation of ATRA is commercially available. However, attempts have been made to provide parenteral formulation of ATRA by loading in carriers such as cyclodextrins and liposomes as a way to overcome its solubility limitation (Drach et al., 1993; Lin et al., 2000a). Moreover, solid lipid nanoparticles have been applied for parenteral formulation of ATRA (Lim and Kim, 2002). Currently, liposomal parenteral formulation of ATRA is at the stage of clinical trials (Douer et al., 2001). However, in their studies, multilamellar types of liposomes were prepared to provide highly efficient loading of lipophilic drugs such as ATRA. Multilamellar types of liposomes are relatively large, physically unstable and thus, prone to aggregation. In this regard, other types of carriers similar to liposomes but also that can be physically more stable with submicron size to avoid capillary blockade after intravenous injection will be advantageous as a parenteral delivery system of ATRA.

Of various formulations, microemulsion is known to be very stable system compared with liposomes. Furthermore, its oily core, instead of aqueous core of liposomes, allows highly efficient incorporation of lipophilic drugs and it may also have great potential as a parenteral vehicle for sparingly substances because of its high solubilization capacity. Recently, phospholipids-based microemulsion has attracted a great deal of interest as a pharmaceutically acceptable microemulsion (Moreno et al., 2001).

Here, we prepared a parenteral formulation of ATRA by loading in phospholipid-based microemulsion and evaluated its physicochemical properties and stability. This study includes pharmacokinetics in a rat model and anti-cancer activity in human cancer cell lines.

2. Materials and methods

2.1. Materials

ATRA and MTT (3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide) were obtained from Sigma (St. Louis, MO, USA). Distearoylphosphatidyl ethanolamine-*N*-poly(ethyleneglycol) 2000 (DSPE-PEG), egg phosphatidylcholine (EPC), dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and distearoylphosphatidylcholine (DSPC) were provided by Avanti Polar lipids (Albaster, AL, USA). Tween 80 was purchased from ICI Americas (Wilmington, DE, USA). RPMI 1640, Antibiotic–antimycotic (100×), Dulbecco's phosphate buffered saline (PBS) and fetal bovine serum (FBS) were Gibco products from Life Technologies (Paisely, UK). Pharmaceutical grade Soybean oil was kindly provided by Green Cross Co. (Yongin, South Korea). Acitretin was gifted by Roche Company (Seoul, South Korea) as an internal standard for the HPLC determination of ATRA (Agadir et al., 1995). All other chemicals were of reagent grade and used without further purification.

2.2. Cell lines

HL-60 cells (human acute promyelocytic leukemia cells) were obtained from Korean Cell Line Bank (Seoul, South Korea). MCF-7 cells (human breast carcinoma cells) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were routinely cultured in RPMI medium supplemented with 10% heat-inactivated FBS and antibiotics in a humid atmosphere containing 5% CO_2.

2.3. Preparation of phospholipid-based microemulsion

Phospholipid-based microemulsion was prepared by following methods. Briefly, PC, DSPE-PEG, and ATRA were dissolved in soybean oil. Preheated (50 °C) water for injection was slowly added to the mixture (2 g of total weight) and sonicated in bath type sonicator at 50 °C for 3 h until crude and milky emulsions were obtained. These crude emulsions were homogenized for eight cycles at 150 MPa using

a high pressure homogenizer (Emulsiflex® EF-B3, Avestin Inc., Canada) to reduce the droplet size to the submicron range (Vandamme, 2002). After homogenization, the hot dispersions were immediately filtered through a 0.45 μm membrane filter to remove precipitated ATRA. ATRA is known to be rapidly degraded upon exposure to light, oxidants, and heat. Therefore, the formulations were stored in the dark at 4 °C prior to injection.

2.4. Measurement of particle diameter and zeta potential

The mean particle diameter and polydispersity index (PI) of ATRA microemulsion were determined by dynamic light scattering method using electrophoretic light scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan) at a fixed angle of 90 °C and at room temperature. Prior to the measurement, ATRA microemulsion was diluted with filtered water to an adequate scattering intensity. The system was used in the auto-measuring mode. The laser diffraction particle diameter analysis data were evaluated using volume distribution to detect even a few large particles. The PI is a measure of the distribution of nanoparticle population (Koppel, 1972).

The electrophoretic mobility and zeta potential were determined using electrophoretic light scattering spectrophotometer. Zeta potential measurements were performed after dilution with filtered distilled water at room temperature.

2.5. Determination of ATRA

The concentration of ATRA was determined by high performance liquid chromatography (HPLC) method (Lin et al., 2000a). The HPLC system consisted of mobile phase delivery pump (LC-10AS, Shimazu, Japan), UV detector (SPD-10A, Shimazu), and Chromatopac integrator (CR6-A, Shimazu). The C_{18} reverse phase column (Luna 5μ C18, 250 mm × 4.6 mm, Phenomenex®, Germany) was used. The mobile phase consisted of acetonitrile–methanol–2.5% aqueous ammonium acetate (50.6:24.4:25 v/v) and the flow rate was 1.0 ml/min. The injection volume was 20 μl. In this system, ATRA was eluted at around 18 min. Under this condition, the linear calibration curve of ATRA was obtained in the concentration range of 0.1–5 μg/ml ($r^2 > 0.999$).

2.6. Stability of ATRA

The chemical stability of ATRA in microemulsion was examined by monitoring the concentration of intact ATRA during incubation at room temperature. For comparison, the stability of ATRA dissolved in methanol or 1% Tween 80 solution (in PBS, pH 7.4) was also tested. All the ATRA preparations were adjusted to the same concentration of ATRA (30 μg/ml). A 60 W light source was placed at an 80 cm distance from the samples. Aliquots were taken from each sample at designated time intervals and then analyzed by HPLC.

2.7. Pharmacokinetic study

To administer as a free form of ATRA, sodium ATRA was prepared by dissolving 14 mg of ATRA in 10 ml of 0.9% NaCl–0.3% NaOH (w/v) solution as described in other studies (Lin et al., 2000b).

Male Sprague–Dawley rats weighing 260 ± 30 g were obtained from Experimental Animal Breeding Center of Seoul National University (Seoul, South Korea). The Seoul National University guideline of experimental animal care was observed. The rats were fed with commercial rodent chow (Samyang Co., Seoul, South Korea) and tap water ad libitum. The rats were fasted overnight, and the femoral artery and vein were cannulated with polyethylene tubings (PE-50, Becton Dickinson, NJ, USA) under light anesthesia with diethylether. After complete recovery from anesthesia, a single dose of sodium ATRA or microemulsion formulation of ATRA equivalent to 4 mg/kg as ATRA was intravenously administrated via the femoral vein of rats. Blood samples (0.2 ml) were collected via the femoral artery at designated time intervals after the dose. Blood samples were centrifuged immediately to obtain plasma, and stored at −20 °C prior to the analysis of ATRA.

The proteins in the plasma samples were precipitated by addition of acetonitrile to the plasma samples. After brief centrifugation, the resulting upper organic layer containing ATRA and internal standard was subjected to the HPLC analysis for the quantitative determination of ATRA concentration. The limit

of detection of this assay was 40 ng/ml. The standard curve of ATRA was obtained over the calibration range between 100 and 8000 ng/ml ($r^2 > 0.99$).

2.8. Pharmacokinetic analysis

The non-compartmental pharmacokinetic parameters including biological half-life ($t_{1/2}$), area under the drug concentration–time curve (AUC), mean resident time (MRT), apparent volume of distribution at steady state (V_{ss}), and total clearance (CL) were calculated by the trapezoidal rule-exploration with the software program WINNONLIN version 1.1 (Scientific Consulting Inc., Lexington, KY, USA).

All data were expressed as mean ± standard deviation (S.D.). Levels of statistical significance ($P < 0.05$) were assessed using the Student's *t*-test between the two means for unpaired data.

2.9. Anti-cancer activity of ATRA

In many cases, the anti-cancer activity of ATRA is mediated by the inhibition of proliferation of cancer cells (Hansen et al., 2000). The growth inhibitory effects of ATRA were investigated in HL-60 and MCF-7 cell lines.

The cells were seeded in 96-well plates at 2×10^4 and 2×10^3 cells/well densities for HL-60 and MCF-7 cells. After 4 h (HL-60) or overnight (MCF-7) incubation, varying concentration of free ATRA or ATRA microemulsion formulation was added to the wells after dilution with PBS. Free ATRA was prepared by dissolving in DMSO at a concentration of 3 mg/ml and then by dilution with culture medium to obtain varying concentration of ATRA. The cells were also separately treated with ATRA-free microemulsion or DMSO dilution as a control. After 4 days of incubation under 5% CO_2 at 37 °C, cell viability was determined by MTT assay following the manufacturer's protocol. The formation of MTT formazan crystals was determined by microplate spectrophotometer (MCC340, Multiskan, Belgium). Each experiment was performed in triplicate.

The cell viability was calculated according to the following equation

$$\text{Cell viability (\%)} = \left(\frac{\text{OD}_{570}\text{ (sample)}}{\text{OD}_{570}\text{ (control)}}\right) \times 100$$

where the OD_{570} (sample) represents the absorbance from the wells treated with samples and the OD_{570} (control) means the absorbance from the wells treated with medium alone.

3. Results and discussion

Microemulsion system composed of various kinds of oils and surfactants has been employed in developing parenteral formulations of drugs with limited aqueous solubility (Park and Kim, 1999; Lee et al., 2002). In this study, to obtain pharmaceutically-acceptable parenteral formulation, phospholipids and soybean oil were selected as surfactants and oil phase constituting microemulsion.

3.1. Effect of formulation variables on the particle diameter of microemulsion

The mean particle diameter and the PI of microemulsion are important parameters predicting the physical stability (Charman et al., 1992) and the in vivo fate of colloidal drug carriers such as microemulsion (Tarr and Yalkowsky, 1989). Therefore, we investigated the changes in those parameters by varying surfactant components and oil amount to get a stable microemulsion formulation of ATRA applicable for parenteral administration.

The mean particle diameter and PI of ATRA microemulsion were affected by the type of PC (Fig. 1). The mean particle diameter and PI tended to increase with the increase in the chain length of fatty acid in phospholipids. The mean particle diameter and PI of EPC- and DLPC-containing microemulsion were within the value range reported for parenteral fat emulsions (200–400 nm of mean particle diameter, 0.100–0.250 of PI) (Muller and Heinemann, 1992). In subsequent studies, microemulsion was prepared with EPC, considering the hemolytic toxicity of DLPC.

The particle diameter of microemulsion was also influenced by the presence of DSPE-PEG included in microemulsion as a surfactant (Fig. 2a). Previous studies have shown that DSPE-PEG can reduce or increase the particle diameter of colloidal carriers depending on the content of DSPE-PEG (Park et al., 1999; Hong et al., 2001). The inclusion of 0.6% (w/w) of DSPE-PEG in ATRA-microemulsion slightly reduced the mean microemulsion particle

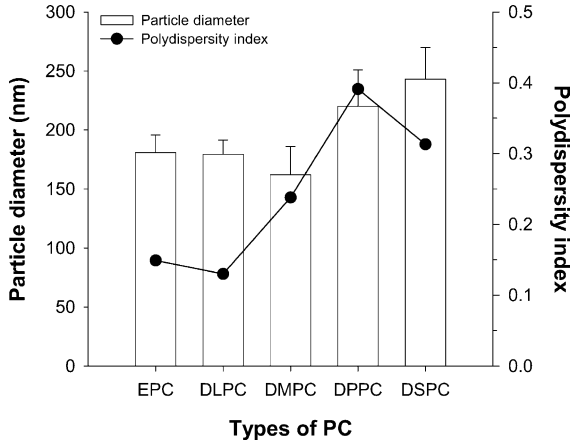

Fig. 1. Effect of the type of PC on the resultant mean particle diameter and polydispersity index of ATRA microemulsion. ATRA microemulsion were prepared with 3:120:24:6 (mg/g) of ATRA:soybean oil:PC:DSPE-PEG as described in Section 2. Each point represents the mean ± S.D. ($n = 3$).

diameter. When 12% oil was added to microemulsion, the particle diameter reduced from 236.8 ± 26.9 nm to 201.15 ± 21.1 nm. In case of microemulsion with 15% oil, the particle diameter changed from 282.6 ± 27.6 nm to 233.48 ± 27.7 nm. The PI of microemulsion was similarly low regardless of whether DSPE-PEG was included or not (0.166 versus 0.170 (12% oil) and 0.219 versus 0.213 (15% oil)).

Increased content of oil phase in microemulsion may be effective in enhancing the loading capacity of highly lipophilic drugs such as ATRA. However, it often results in an unstable microemulsion formulation with too big particle diameter (Park and Kim, 1999). In our study, the mean particle diameter of microemulsion was not affected by the increase in the oil content in the range from 4.5 to 15% (Fig. 2b). The mean particle diameter was slightly increased only when oil was included at above 15% but it was still below 300 nm. Neither the PI values were greatly affected by the oil content (0.296, 0.216, 0.195, and 0.213 for microemulsion containing 4.5, 9, 12, and 15% oil).

Taken together, microemulsion formulation of ATRA adequate for parenteral administration could be obtained with soybean oil (up to 15%) and phospholipid mixture composed of EPC and DSPE-PEG. The mean particle diameter and PI of this microemulsion formulation were only slightly increased during storage at ambient temperature for 60 days. The

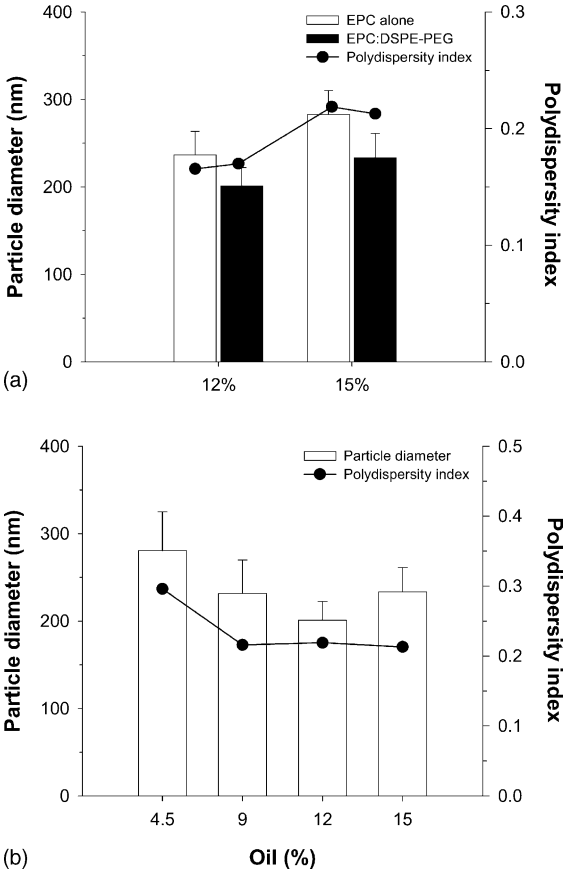

Fig. 2. Effect of the content of DSPE-PEG (a) and oil (b) on the resultant mean particle diameter and polydispersity index of ATRA microemulsion. (a) ATRA microemulsion were prepared with 12 or 15% of soybean oil together with 3 mg/g of ATRA and 30 mg/g of surfactants composed of EPC alone or 4:1 mixture of EPC:DSPE-PEG. (b) ATRA microemulsion were prepared with 4.5, 9, 12, or 15% of soybean oil together with 3 mg/g of ATRA and 30 mg/g of surfactant (4:1 mixture of EPC:DSPE-PEG). Each point represents the mean ± S.D. ($n = 3$).

zeta potentials of these microemulsion formulation were −22.75 ± 4.06 mV. These values are slightly lower than the reported value of parenteral emulsions (>−30 mV) to get full stabilization by electrostatic stabilization (Muller and Heinemann, 1992).

3.2. Effect of formulation variables on the loading capacity of microemulsion

Table 1 shows that the ATRA-loading capacity of microemulsion was affected by the oil content and also

Table 1
The loading capacity of ATRA in microemulsion with varying content of oils ($n = 3$)

Oil (%)	ATRA in microemulsion (mg/ml)	
	Without DSPE-PEG	With DSPE-PEG
4.5	ND[a]	0.854 ± 0.011
12	0.924 ± 0.026	1.232 ± 0.364
15	0.770 ± 0.156	1.586 ± 0.220

Microemulsion was prepared with 3 mg/g of ATRA, 24 mg/g of EPC and varying amount of soybean oil with or without DSPE-PEG (6 mg/g).
[a] ND: not determined.

by the inclusion of DSPE-PEG. The ATRA-loading capacity increased with increasing oil contents. Our data suggest that ATRA was incorporated in the inner oil core of microemulsion. The ATRA-loading capacity of microemulsion was also increased by the inclusion of DSPE-PEG. Currently, it is not clear how DSPE-PEG increased the ATRA-loading in microemulsion but presumably it may be due to the fact that the combination of EPC and DSPE-PEG was more active as a surfactant than EPC alone. Since the inclusion of DSPE-PEG also reduced the particle diameter of microemulsion, it was advantageous in obtaining parenteral microemulsion formulation of ATRA.

The concentration of ATRA in 15% oil-containing microemulsion (1.586 ± 0.220 mg/ml) is approximately 25,000-fold higher than the reported solubility of ATRA in PBS buffer (0.21 μM) (Szuts and Harosi, 1991). Although the ATRA-loading efficacy in microemulsion was calculated less than 32% in any formulations, the separation of free ATRA from microemulsion could be easily done by membrane filtration, since free ATRA was immediately precipitated during preparation due to its extremely low solubility in the outer aqueous medium.

3.3. Stability of ATRA

Since ATRA is readily degraded upon exposure to light, oxidants and heats (Brisaert et al., 2000), the stability of ATRA is one of the important facts in formulation. Indeed our study showed that the concentration of intact ATRA dissolved in methanol rapidly decreased during incubation at room temperature under light exposure (Fig. 3). Within 1 h, less

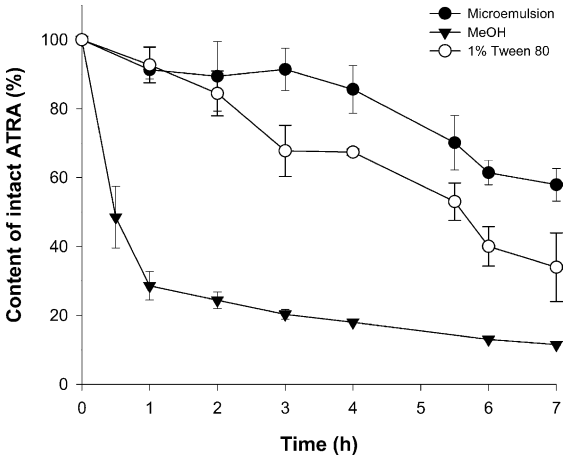

Fig. 3. Comparison of stability of ATRA in various preparations. Microemulsion formulation of ATRA were prepared with 3:150:24:6 (mg/g) of ATRA:soybean oil:EPC:DSPE-PEG. The stability of ATRA was compared among ATRA-microemulsion, methanol and 1% Tween 80 preparation. The chemical stability of ATRA was determined by HPLC. Each point represents the mean ± S.D. ($n = 3$).

than 30% of ATRA remained intact in methanol solution. In contrast, the rate of degradation of ATRA incorporated in microemulsion was greatly retarded compared with that in methanol. Only 9% of ATRA were degraded after 1 h of incubation and approximately 59% of ATRA found intact up to 7 h of incubation. Microemulsion formulation was also more effective in protecting ATRA compared with ATRA in 1% Tween 80, although no significant difference was found (Fig. 3). When the samples were protected from light, no decrease in the concentration of ATRA was observed in any samples, suggesting the rapid degradation of ATRA in our experimental settings was mainly caused by photolysis. Therefore, it indicates that the loading of ATRA in microemulsion may be an effective mean to protect ATRA against light. However, the slow but still significant degradation of ATRA in microemulsion formulation suggests that further protection is required to get chemically stable formulation of ATRA. Addition of other compounds such as anti-oxidants as a component of microemulsion formulation may be effective for this purpose.

The improved stability of ATRA has been reported in cyclodextrin (Lin et al., 2000a) or liposome formulations (Singh and Das, 1998). As in these for-

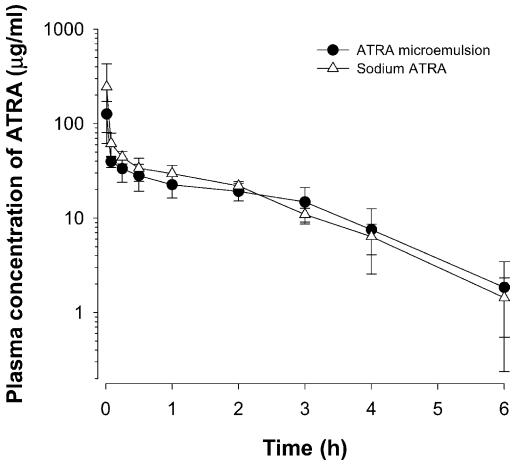

Fig. 4. Plasma concentration–time profiles of ATRA in rats after intravenous administration of ATRA microemulsion formulation or sodium ATRA at a dose equivalent to 4 mg/kg as ATRA. Each point represents the mean ± S.D. ($n = 5$).

mulations, the stability of ATRA in microemulsion formulation is thought to be due to the presence of protective films surrounding ATRA, being provided by phospholipid layers.

3.4. Pharmacokinetics of ATRA after intravenous administration

From the data obtained from optimization studies, ATRA microemulsion was prepared with 3:24:6:150 (mg/g) mixture of ATRA:EPC:DSPE-PEG:soybean oil for subsequent pharmacokinetic studies.

Fig. 4 describes the plasma concentration-time profiles of ATRA after intravenous administration of sodium ATRA or ATRA microemulsion. The initial plasma concentration of ATRA in microemulsion was slightly higher but the overall plasma concentration profile of ATRA was not significantly different from that of sodium ATRA. The non-compartmental pharmacokinetic parameters are summarized in Table 2. No statistical difference was found in all pharmacokinetic parameters between sodium ATRA and ATRA microemulsion after intravenous administration except V_{ss} ($P < 0.05$). The obtained parameters are similar to those values reported in other studies (Lin et al., 2000b). The kinetics of ATRA are known to be non-linear. Previously, Takino et al. (1994) reported the pharmacokinetics of retinoic acid using emulsions in rats. Moreover, several papers showed the non-linear kinetics of ATRA in rats after intravenous administration (Lin et al., 2000b; El Mansouri et al., 1995).

Table 2
Non-compartmental pharmacokinetic parameters of ATRA after intravenous administration of sodium ATRA or ATRA microemulsion formulation, equivalent to 4 mg/kg as ATRA, to rats ($n = 5$)

Parameters	Formulation	
	Sodium ATRA	Microemulsion formulation
$t_{1/2}$ (h)	1.31 ± 0.50	1.20 ± 0.39
AUC (μg·h/ml)	8.27 ± 2.27	9.69 ± 1.39
MRT (h)	1.89 ± 0.13	1.73 ± 0.13
V_{ss} (ml/kg)	850.26 ± 145.91	651.18 ± 106.71*
CL (ml/h)	450.73 ± 178.47	376.84 ± 63.56

* $P < 0.05$, when compared with sodium ATRA.

Strong binding affinity between ATRA and lipids had been demonstrated (Parthasarathy et al., 1994). Thus, substantial amount of ATRA may exist in microemulsion as bound form to phospholipids, when microemulsion formulation of ATRA is intravenously administered, the lipid exchange between lipid-based microemulsion and lipoproteins may rapidly induce the dissociation of ATRA from microemulsion. It might result in the similar pharmacokinetic behavior between sodium ATRA and ATRA microemulsion. However, sodium ATRA in NaOH solution cannot be a pharmaceutically acceptable parenteral formulation due to its irritability. Moreover, the anti-cancer efficacy of ATRA as salt form has never been proven (Brisaert et al., 2000; Parthasarathy et al., 1994). Our previous study has also reported that microemulsion formulation induced significantly less pain on injection than salt form (Lee et al., 2002). Therefore, our results demonstrate that the microemulsion formulation of ATRA could be an alternative effective parenteral dosage form.

3.5. Anti-cancer activity of ATRA

To study whether the anti-cancer efficacy of ATRA was affected by loading in microemulsion, the growth inhibitory effects of ATRA were investigated in HL-60 and MCF-7 cells. The growth inhibitory effects of ATRA were very similar between free- and microemulsion-ATRA (Fig. 5). Both of them inhibited

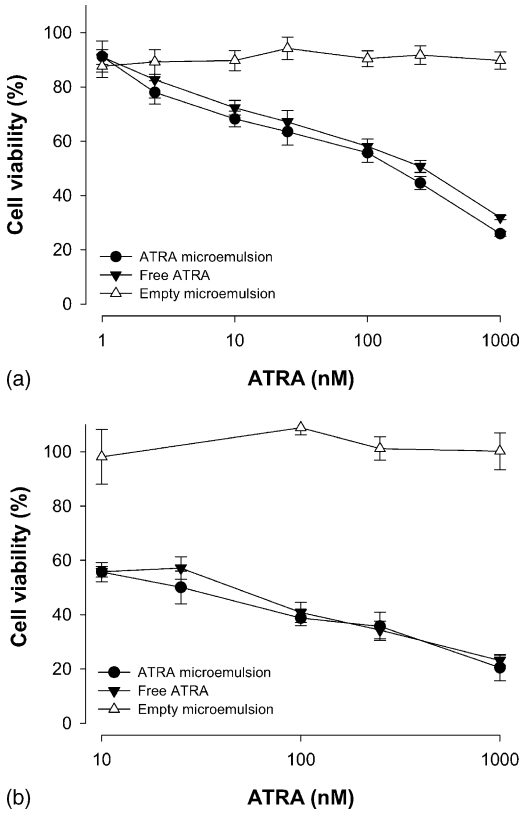

Fig. 5. The growth inhibitory effects of ATRA on human cancer cell lines. ATRA microemulsion formulation of ATRA was prepared from 3:150:24:6 (mg/g) mixture of ATRA:soybean oil:EPC:DSPE-PEG. Varying dilutions of ATRA microemulsion, free ATRA or empty microemulsion were incubated with HL-60 (a) and MCF-7 cells (b) for 4 days until MTT assay was performed. Each point represents the mean ± S.D. ($n = 3$).

the proliferation of HL-60 and MCF-7 cells in a dose-dependent manner in the range of 1–1000 nM concentration of ATRA (Mangiarotti et al., 1998; Toma et al., 1994). DMSO dilution and empty (ATRA-free) microemulsion only slightly (<1% with DMSO dilutions and <15% with empty microemulsion) inhibited the proliferation of HL-60 and MCF-7 cells at the dilution corresponding to the highest concentration of ATRA used (1000 nM of ATRA). All of these results suggest that the ATRA in microemulsion can exert its anti-cancer activity, probably after being released from microemulsion outside or inside of cells, and thus, binding to retinoic acid receptors and retinoid X receptors on the nuclear membrane of cells.

4. Conclusion

In this study, phospholipid-based microemulsion formulation of ATRA applicable for parenteral administration could be obtained by optimizing the composition of microemulsion and production parameters. Microemulsion formulation of ATRA improved the solubility and stability of ATRA while maintaining its pharmacokinetic profile and anti-cancer efficacy. Microemulsion formulation in this study may also have a potential as a dosage form of various retinoid analogues as well as ATRA.

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