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Inhibition of liver metastasis by all-*trans* retinoic acid incorporated into O/W emulsions in mice

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Abstract

All-*trans* retinoic acid (ATRA) was incorporated into lipid emulsions in an attempt to alter its distribution characteristics and improve its inhibition of liver cancer metastasis. Lipid emulsions composed of egg phosphatidylcholine, cholesterol, and soybean oil were the optimized carriers for ATRA delivery, as shown by the submicron particle size and high incorporation efficiency. The particle size and zeta potential of ATRA incorporated into emulsions were about 133 nm and −11 mV, respectively. *In vitro* drug release study demonstrated that the release of ATRA from emulsions was sustained in the absence and present of bovine serum albumin, suggesting that ATRA was stable when incorporated in emulsions. After intravenous administration in mice, [^{3}H]cholesteryl hexadecyl ether incorporated into emulsion, which is the inherent distribution of emulsions, accumulated gradually mainly in the liver. The blood concentration and hepatic accumulation of [^{3}H]ATRA incorporated into emulsion was significantly higher than that of serum dissolving [^{3}H]ATRA, which represent the original distribution characteristic of free ATRA. In a murine liver metastasis model by colon adenocarcinoma, the liver metastasis number and liver weight were significantly reduced and the survival time of mice was prolonged following intravenous injection of ATRA incorporated into emulsions.

Keywords: All-*trans* retinoic acid; Lipid emulsions; Liver metastasis; Biodistribution; Controlled release

1. Introduction

Retinoids, natural or synthetic derivatives of Vitamin A, are normal regulators of embryonic development, vision, reproduction and other physiological processes by affecting differentiation, proliferation and apoptosis (Sun and Lotan, 2002). Low serum retinol levels may be a risk factor for the incidence of second liver tumors, and retinoids have a potential antineoplastic effect on liver cancer (Soprano et al., 2004; Okuno et al., 2004). All-*trans* retinoic acid (ATRA), an active metabolite of retinol, has been shown to exert anti-cancer activities in a number of cancer cells and tissues (Otsuki et al., 2003; Arce et al., 2005). Recently, it has been extensively used for the treatment of acute promyelocytic leukemia (APL) (Lengfelder et al., 2005). However, a gradual decrease in the ATRA concentration in the blood circulation after prolonged treatment (Muindi et al., 1992) and highly variable bioavailability after oral administration was observed (Ozpolat et al., 2003). Therefore, a parenteral formulation may provide a reliable approach for ATRA administration.

It is known that tumor metastasis is an important prognostic factor affecting the survival time of cancer patients and the liver appears to be target organ of tumor metastasis (McCarter and Fong, 2000). Multiple metastases in the liver occur from many primary tumor sites, and these multiple nodules are unresectable (Ravikumar et al., 1990). To overcome liver metastasis, various chemotherapeutic agents and combined therapy with radiotherapy are used (Webber et al., 1978). However, the effect of anti-cancer agents is not enough and is limited by toxicity. An alternative approach using non-cytotoxic anti-cancer agents, such as retinoids, was investigated (Garcia-Alonso et al., 2005).

In recent years, considerable emplasis has been placed on the development of new formulations of ATRA that are suitable for intravenous administration. However, the poor aqueous solubility of ATRA hampers its administration in solution form. Attempts have been made to develop parenteral formulations of ATRA by loading it in lipophilic carriers to overcome its solubility limitation. ATRA dispersal systems such as liposomes (Estey et al., 2005; Kawakami et al., 2006), solid lipid nanoparti-

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cles (Lim et al., 2004), polymeric micelles (Zuccari et al., 2005; Kawakami et al., 2005), and phospholipid-based microemulsions (Hwang et al., 2004) have been developed for parenteral administration purposes. Among these approaches, liposomes have been a promising parenteral delivery system for ATRA because of their superior ability to maintain plasma concentrations of ATRA over oral administration. In particular, clinical trials have demonstrated that parenteral liposomal ATRA offers potential pharmacological advantages over oral administration for APL therapy (Ozpolat and Lopez-Berestein, 2002). However, the limited solubility of ATRA within the liposomal membrane allowed only large vesicle multilamellar-type liposomes to provide efficient ATRA loading. In this regard, other carriers of submicron size and effective ATRA loading will provide an alternative for ATRA parenteral delivery for liver targeting.

Lipid emulsions are considered to be superior to liposomes as they can be produced on an industrial scale, are stable during storage, are highly biocompatible, and have a high solubilizing capacity as far as lipophilic drugs are concerned because they have an oil phase in particulate form, so they can dissolve large amounts of drugs (Kawakami et al., 2000a; Hashida et al., 2005). Moreover, small lipid emulsions have been widely used as long circulating carriers and for the administration of lipid nutrients, and have become useful parenteral drug delivery systems. It has been reported that particular lipid drug carriers mimic the metabolism of plasma lipoprotein or chylomicron and are preferentially taken up by the liver (Tomii, 2002). This phenomenon is also expected in small lipid emulsions with phosphatidylcholine and cholesterol located at the surface since they are the major lipid constituents of plasma lipoprotein. These triglyceride-rich emulsions attract apolipoproteins, are hydrolyzed by lipoprotein lipase, convert into remnants and are subsequently taken up by the liver (Rensen et al., 1997). Therefore, improved solubility, sustained and prolonged blood concentration, and enhanced liver uptake by lipid emulsion could be advantageous to target ATRA to the liver and treatment of liver metastasis.

The purpose of this study was to investigate lipid emulsions as a delivery system for ATRA. We hypothesize that ATRA uptake by the liver can be enhanced by incorporating it into optimized emulsions. In this study, we have shown that ATRA was strongly incorporated into emulsions without significant release. The biodistribution characteristics of ATRA delivered by emulsions after intravenous administration showed increased liver accumulation when compared to the injection of free ATRA. Finally, we evaluated the efficiency of ATRA-incorporated emulsions against hepatic metastasis by measuring the number of metastatic colonies on the liver surface, tissue weight of the liver, and survival of mice.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (EggPC) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-polyethylene glycol 2000 (DSPE-PEG_{2000}) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Cholesterol (Chol) and Clear-Sol I were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Soluene-350 was purchased from Packard Co., Inc. (Groningen, The Netherlands). ATRA and soybean oil were obtained from Wako Pure Chemicals Industry Ltd. (Osaka, Japan). HCO-60 was obtained from Nikko Chemical Co. Ltd. (Japan). [^{3}H]Cholesteryl hexadecyl ether (CHE) and [^{3}H]ATRA were purchased from NEN Life Science Products Inc. (Boston, MA, USA). RPMI1640 medium was purchased from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Biowhittaker (Walkersville, MD, USA). Bovine serum albumin, fraction V (BSA) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemicals were of the highest purity available.

2.2. Preparation of ATRA-incorporated emulsions

Emulsions were prepared by the method described previously with slight modification (Takino et al., 1994; Ishida et al., 2004; Managit et al., 2005). Briefly, a mixture of EggPC/Chol (60:40, molar ratio) or EggPC/DSPE-PEG_{2000}/Chol (50:10:40, molar ratio) and ATRA (1:20, molar ratio of total lipid) was first dissolved in chloroform. After vacuum drying and desiccation, soybean oil (7:3, weight ratio of total lipid) was added to dissolve the dry film and sterile phosphate-buffered saline pH 7.4 (PBS) was added for hydration. The emulsions were then sonicated (200 W) at 4 °C under a current of nitrogen gas using an ultrasonic sonicator (US300, Nissei Inc., Tokyo, Japan) for 30 min. The preparations were passed through a 0.45 μm filter to remove precipitated ATRA and protected from light under nitrogen gas at 4 °C and had been used within 1 week. For radiolabeling preparations, [^{3}H]ATRA or [^{3}H]CHE (50 μCi) was added to the lipid mixture before formation of the thin film layer. Other preparation steps were the same as described above.

2.3. Characterization of the preparations

The lipid content of the emulsions was determined by a cholesterol E-test Wako kit (Wako Pure Chemical Industry Ltd., Osaka, Japan). The amount of ATRA in the formulations was determined by UV absorption at 360 nm (UV–vis Spectrophotometer, Shimazu Co. Ltd., Kyoto, Japan) after dissolving the formulations in a mixture of dimethyl sulfoxide (DMSO) and water (DMSO: water = 9:1 by volume). The particle size and zeta potential of the emulsions were measured by Zetasizer Nano Series (Malvern Instruments Ltd., Worcestershire, UK).

2.4. Animals

Male ddY mice (5 weeks old, 20–25 g) and CDF1 mice (male, 4 weeks old, 18–22 g) were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Animals were maintained under conventional housing conditions. All animal experiments were carried out in accordance with the guidelines for Animal Experiments of Kyoto University.

2.5. In vitro release study

The dialysis membranes (Spectra/Por® membrane MWCO 3500, Spectrum Laboratories Inc., Roncho Dominguez, CA, USA) were mounted in diffusion chambers. Three ml of PBS containing 0.5% Tween-20 was added to the receiver side and the diffusion chamber was maintained at 37 °C. ATRA dissolved in 1N NaOH (0.25 mg/ml) or ATRA incorporated into emulsions were diluted with PBS to the concentration of ATRA 33.33 μg/ml and 3 ml of diluted ATRA was added to the donor side. The samples were withdrawn from the receiver side at specific times and analyzed for ATRA concentration by UV spectrophotometer at 360 nm. To investigate the effect of BSA on ATRA release, PBS containing 4% BSA was used instead of PBS at each step of the method described above. These studies were performed in triplicate for each sample.

2.6. Biodistribution study

A biodistribution study was performed by the method described previously (Kawakami et al., 2000b; Yeeprae et al., 2005). [^{3}H]ATRA dissolved in serum or radioactivity labeling emulsions were injected into the tail vein of ddY mice at an ATRA dose of 0.6 mg/kg. At each collection time point, blood was collected from the vena cava under anesthesia, and the mice were then killed. The liver, spleen, kidney and lung were excised, washed with saline, blotted dry and weighed. Ten microliters of blood and a small piece of each tissue were precisely weighed and digested in 0.7 ml Soluene-350 by incubating overnight at 45 °C. Following digestion, 0.2 ml isopropanol, 0.2 ml 30% hydrogenperoxide, 0.1 ml 5N HCl, and 5.0 ml Clear-Sol I were added. The samples were stored overnight and the radioactivity was measured using a liquid scintillation counter (LSA-500, Beckman, Tokyo, Japan).

2.7. Experimental liver metastasis and treatment with ATRA

CT26, mouse colon adenocarcinoma cells were routinely growth with RPMI1640 medium supplemented with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (All from Invitrogen Co., Carlsbad, CA, USA) in 5% CO_2, humidified air at 37 °C. The cells were harvested from 2-day-old subconfluent cultures by trypsin and the cell concentration was adjusted to 10^6 cells/ml by Hank's balance salt solution (HBSS). CT26 cells (0.1 ml) were then implanted by intrasplenic injection to CDF1 mice under pentobarbital anesthesia. ATRA dissolved in 5% HCO-60 solution, ATRA incorporated into emulsions, and empty emulsions were repeatedly injected through the tail vein at an ATRA dose of 0.6 mg/kg/day or total lipid dose of 120 mg/kg/day, from days 3 to 7 after tumor inoculation (6–8 mice per group). The survival of mice was recorded up to 28 days after tumor inoculation. In a different set of experiments, mice were sacrificed on day 14 and the number of tumor nodules on the liver surface and the liver weight were measured.

2.8. Statistical analysis

Statistical comparisons were performed by Student's *t*-test for two groups, and one-way ANOVA for multiple groups. Statistical analysis of survival curves was done with the log-rank test. $P < 0.05$ was considered significant.

3. Results

3.1. Physicochemical properties of ATRA incorporated into emulsions

The particle size intensity of the prepared ATRA emulsions is shown in Fig. 1. The particle size of ATRA incorporated into emulsions was 133.2 ± 3.41 nm ($n = 3$), similar to preparations without ATRA. The zeta potential of emulsions was -11 ± 4.5 mV ($n = 3$), shifted from the neutral or slightly negative sign of empty emulsions (data not shown). The particle size and zeta potential of the emulsions remained constant over 1 month at 4 °C but the particle size was slightly increased at room temperature at 30 days as shown in Fig. 2. The percent recovery of ATRA in emulsions when compared with the initial concentration of ATRA was $88.9 \pm 4.3\%$ ($n = 3$) with the final concentration of ATRA up to 0.123 mg/ml of emulsions.

3.2. Release of ATRA from ATRA incorporated into emulsions

In order to investigate ATRA binding with the emulsions, the release of ATRA was performed using a dialysis membrane since it retained the emulsions and allowed the transfer of only free ATRA into the receiver side. Fig. 3 shows the *in vitro* diffusion of ATRA through the dialysis membrane. ATRA in the free form rapidly diffused through the membrane and reach equilibrium within 72 h whereas the diffusion of ATRA incorporated into the emulsions was very slow, less than 10% at 72 h. In the presence of 4% BSA in the donor and receiver sides, the diffusion of ATRA incorporated into the emulsions was almost the same as without

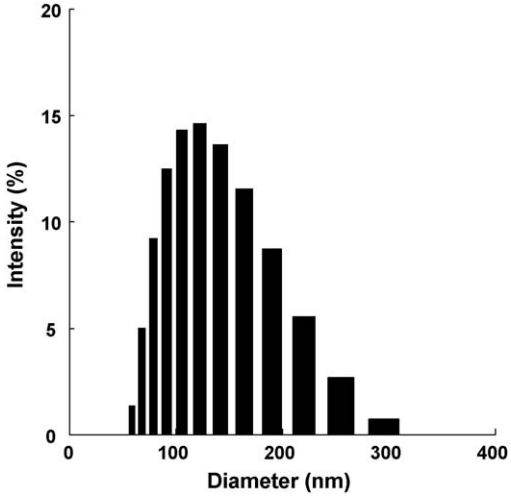

Fig. 1. Size distribution of ATRA-incorporated emulsions.

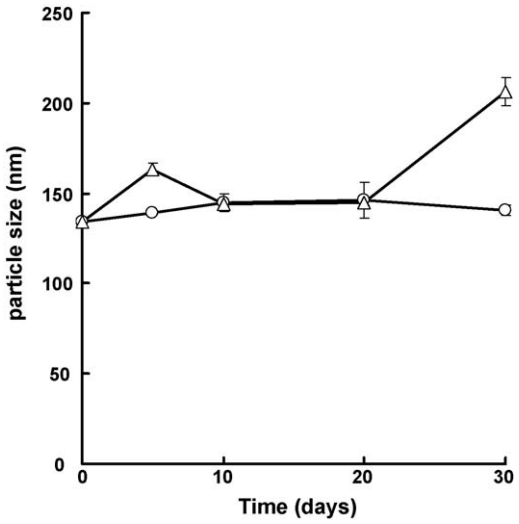

Fig. 2. The mean particle sizes of ATRA in emulsions within 30 days. Emulsions were protected from light under nitrogen gas and stored at 4 °C (◯) or room temperature (△) until measurement.

BSA. The results imply that ATRA was stably incorporated into the emulsions even in the presence of BSA.

3.3. Biodistribution of [^{3}H]CHE and [^{3}H]ATRA labeling emulsions

The distribution characteristics of empty emulsions, free ATRA, and ATRA incorporated into emulsions after intravenous administration into mice was investigated. The empty emulsions labeled with [^{3}H]CHE were eliminated from the circulating blood and accumulated in the liver for up to 60 min, and were retained in the liver for over 240 min (Fig. 4).

Serum dissolved in [^{3}H]ATRA was rapidly eliminated from the blood circulation and recovered in the liver within the first

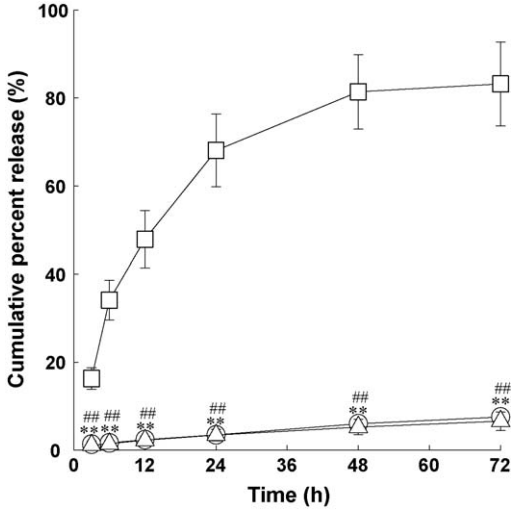

Fig. 3. The diffusion of ATRA through a dialysis membrane within 72 h (□) and *in vitro* release of ATRA from ATRA incorporated into emulsions in the presence (△) or absent (◯) of 4% BSA. Each value represents the mean ± S.D. of three experiments. Statistically significant differences from the diffusion of free ATRA in the presence or absent of BSA ($^{**}P < 0.001$ or $^{\#\#}P < 0.001$, respectively).

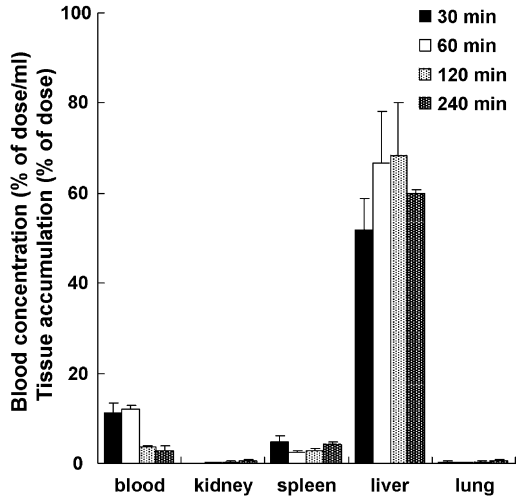

Fig. 4. Distribution profiles of [^{3}H]CHE incorporated into emulsions after intravenous injection in mice. Results are expressed as the means + S.D. of three mice.

30 min, and was rapidly eliminated from the liver (Fig. 5A). When ATRA was incorporated into emulsions, ATRA was retained in the blood circulation longer than free ATRA and increasingly accumulated in the liver and spleen for up to 60 min

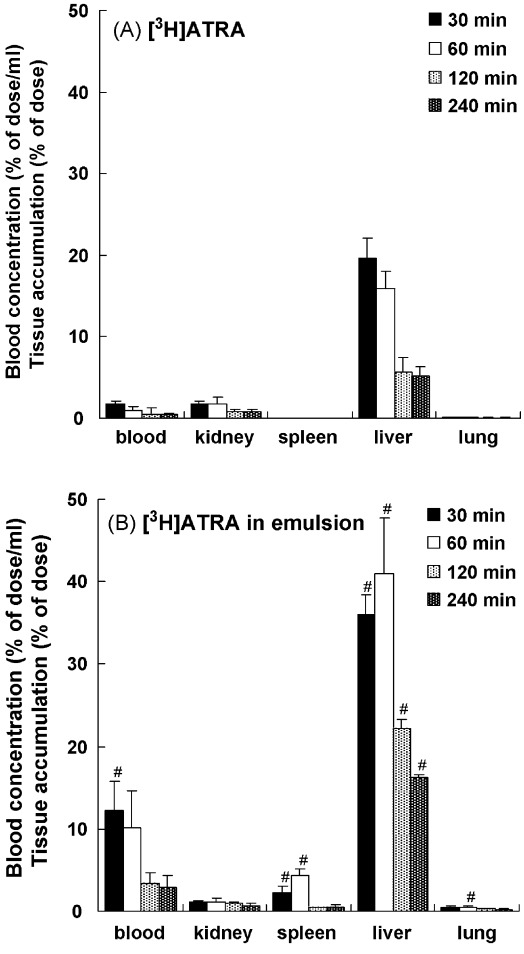

Fig. 5. Distribution profiles of serum dissolved in [^{3}H]ATRA (A) and [^{3}H]ATRA-incorporated emulsions (B) after intravenous injection in mice. Results are expressed as the means + S.D. of three mice. Statistically significant differences from the distribution of [^{3}H]ATRA ($^{\#}P < 0.01$).

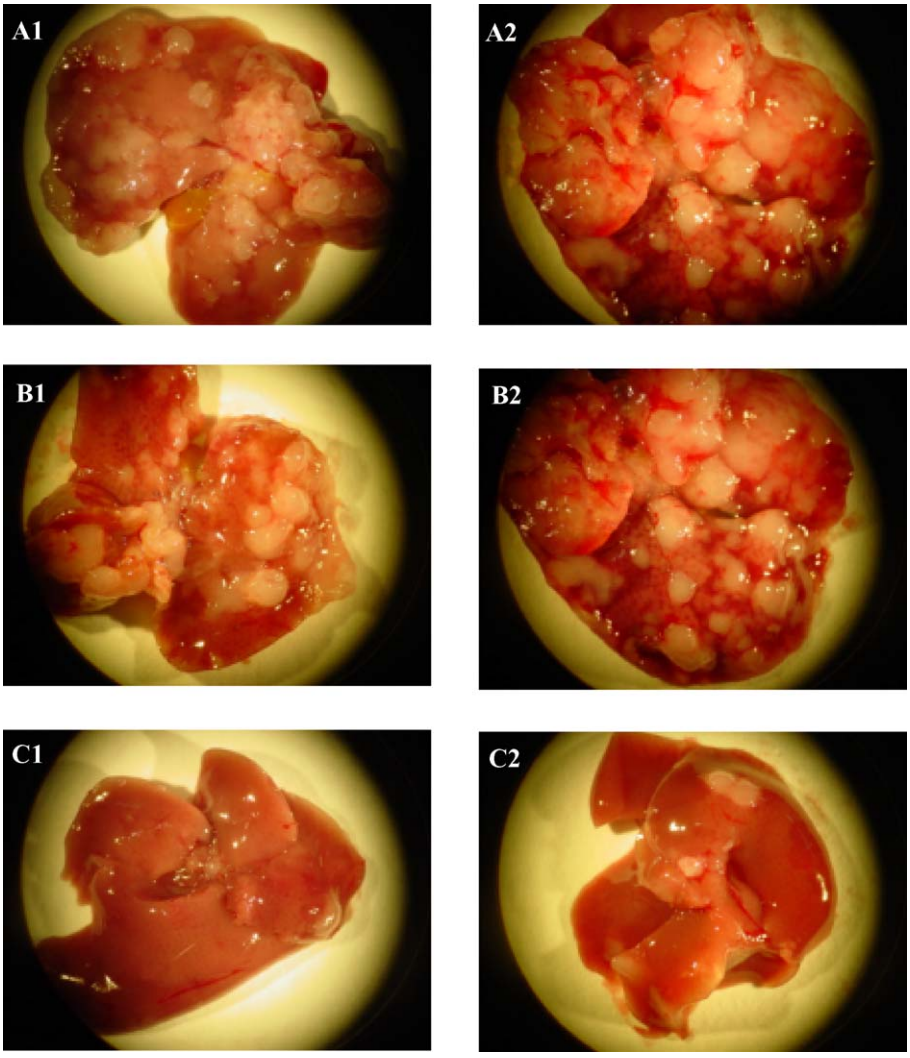

Fig. 6. Metastatic colonies of CT26 tumor cells on the liver surface of mice injected with saline (A), ATRA in 5% HCO-60 micelles (B) or ATRA in emulsion (C) on days 3–7 after tumor inoculation. Mice were sacrificed at day 14 after tumor inoculation. Two different mice in the same treatment group are shown in the figure.

before being gradually eliminated (Fig. 5B). Modification of the emulsion surface by DSPE-PEG_{2000} did not further increase the liver accumulation of ATRA although it was retained in the blood circulation longer than free ATRA (data not shown). For both free ATRA and ATRA incorporated into emulsions, no significant accumulation in the kidney and lung was observed.

3.4. Anti-liver cancer metastasis activity of ATRA emulsions

The antitumor efficiency of several formulations of ATRA in the liver metastasis model was studied. When CT26 tumor cells were injected into the spleen of CDF1 mice, metastatic colonies were visibly observed on the surface of the liver, which corresponds to the progressive stage of liver metastasis lesions. On day 14, the number of metastatic colonies on the liver surface was significantly higher in the control mice injected with saline when compared with mice treated with ATRA in 5% HCO-60 micelles or emulsions (Figs. 6 and 7A). Moreover, the ATRA incorporated into the emulsion-treated group showed the most prominent effect over the HCO-60 micelle group as far as the inhibition of liver metastases was concerned. The liver weights correlated with the number of metastatic colonies in the liver (Fig. 7A and B). As shown in Fig. 8, all mice treated with saline or empty emulsions had died by day 18 after tumor inoculation (mean survival time = 16.0 ± 1.41 and 15.9 ± 1.95 days, respectively) while treatment with ATRA in 5% HCO-60 micelles slightly increased the survival time (mean survival time = 19.6 ± 2.30 days). In contrast, the survival time of mice treated with ATRA incorporated into emulsions was much longer than any other treatments with the mean survival time 21.2 ± 5.12 days.

4. Discussion

Most conventional drugs diffuse freely throughout the body and show relatively even tissue distribution due to their low molecular weight. The methodology for manipulating drug distribution in the body and the use of lipid dispersion carrier systems as carriers of lipophilic drugs has attracted particular interest. However, these dispersal systems pose a number of

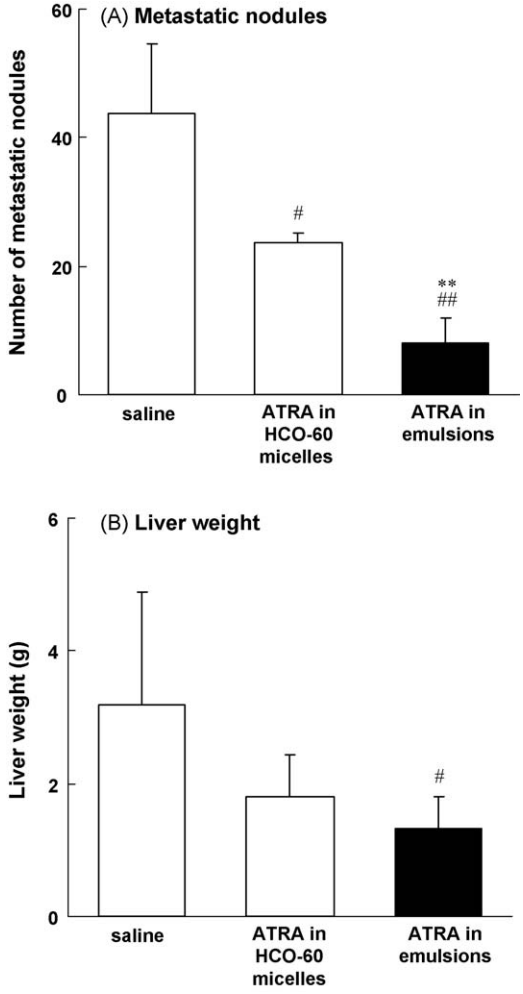

Fig. 7. Number of metastatic nodules of CT26 tumor cells on the liver surface (A) and liver weight (B) of mice intrasplenically injected with cells on day 14. Results are expressed as the mean + S.D. of three mice. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, significantly different from mice injected with saline, and $^{**}P < 0.01$ significantly different from mice injected with ATRA in HCO-60 micelles.

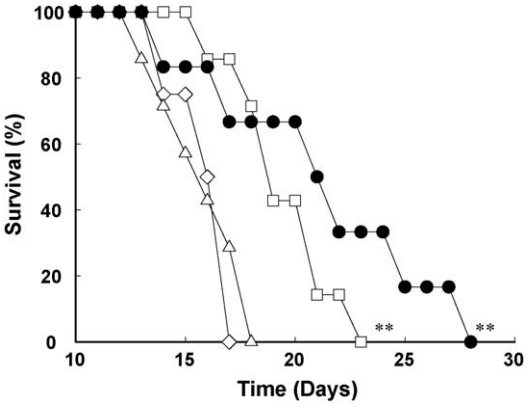

Fig. 8. Survival time of mice inoculated with CT26 tumor cells followed by intravenous injection of saline (△), empty emulsions (◇), ATRA in 5% HCO-60 micelles (□), or ATRA in emulsions (●) on days 3–7 after tumor inoculation. Survival of mice was observed daily for 28 days. $^{**}P < 0.05$ significantly different from mice injected with saline.

problems in their application as drug formulations. The main problems are whether it is possible to include sufficient amounts of the drug and the expected effect of the carrier might not be achieved because there is a possibility that the drug might be released immediately after administration. In this regard, lipid emulsions were investigated as alternative carriers to improve solubility and to enhance the liver uptake of ATRA.

In order to prepare small-sized emulsions, which are considered to prolong retention in blood, 30% EggPC and cholesterol in a molar ratio 60:40 and 70% soybean oil were used. This optimized emulsion system improved the solubility of ATRA and exhibit submicron size which were advantage for parenteral administration. We confirmed that the emulsion had a particle size less than 200 nm with narrow size distribution (Fig. 1). The particle size remained constant over 1 month at 4 °C but not at room temperature suggested the appropriated storage condition of the emulsion at 4 °C. The small size and lipid components of the emulsions also benefited the selective accumulation of emulsions in the liver (Fig. 4). This phenomenon corresponds with previous reports that triglyceride-rich emulsions exhibit distribution characteristics similar to plasma lipoproteins of submicron particle size for apolipoprotein binding, lipoprotein lipase-mediated lipolysis and subsequently uptake by the liver (Arimoto et al., 1998; Tomii, 2002).

Since the water solubility of ATRA is lower than 0.1 μg/ml, the poor aqueous solubility of ATRA is the principal obstruction of a parenteral formulation. Therefore, the advantage of lipid emulsions, which posses an oil phase in particulate form and have a high solubilizing capacity for lipophilic drugs like ATRA, might be an interesting alternative. After removing precipitated ATRA by membrane filtration, the recovery of ATRA in the formulations of almost 90% suggested that ATRA was successfully dissolved in the lipophilic phase of the emulsions. For the *in vitro* release study, a lower diffusion of ATRA from the emulsions across the dialysis membrane was observed (Fig. 3), suggesting that ATRA was strongly bound to the emulsions and had sustained and prolonged release characteristics. The results also implied that most of the ATRA was effectively incorporated into the emulsion core because no rapid diffusion of ATRA across the membrane, which was a characteristic of free ATRA, was detected during ATRA release from emulsion. Moreover, the release of ATRA did not alter in the presence of BSA, which is a model hydrophobic component in serum, suggesting that ATRA was effectively retained in the emulsions even after entering the blood circulation.

In order to examine the effect of emulsion formulation on the distribution of ATRA, the biodistribution of serum dissolved in [^{3}H]ATRA and [^{3}H]ATRA incorporated into emulsions was compared. As shown in Fig. 5A, [^{3}H]ATRA was rapidly eliminated from the blood when dissolved in serum, which represents the original distribution of ATRA (Hattori et al., 2000; Kawakami et al., 2002). In contrast, the blood concentration of [^{3}H]ATRA was significantly delayed (Fig. 5B) and this slower elimination of ATRA contributed to longer and higher liver accumulation when ATRA was incorporated into emulsions. This distribution characteristic of [^{3}H]ATRA incorporated into emulsions corresponded to [^{3}H]CHE-incorporated emulsions

(Fig. 4), which is the inherent distribution of emulsions (Takino et al., 1998; Ishida et al., 2004). These results lead us to believe that when ATRA was incorporated in small-sized emulsions, sustained liver targeting could be achieved.

To investigate whether emulsions can be used to target ATRA to the liver for the treatment of hepatogenic diseases, the antitumor activity of ATRA against liver metastasis was studied. The number of metastatic nodules (Fig. 7A), the liver weight (Fig. 7B), and survival of liver metastasis mice (Fig. 8) demonstrated that ATRA incorporated into emulsion exhibited superior activity against liver metastasis when compared with untreated animals, empty emulsions and ATRA dissolved in HCO-60 micelles. The improved efficacy of ATRA in emulsions is attributed to its strong association and preferential accumulation and retention in the liver. The results indicated the potential of emulsions as carriers for ATRA targeting to liver tissue in order to improve the outcome in liver diseases.

In order to enhance the circulation time of ATRA emulsion, DSPE-PEG_{2000} was added to the emulsion system. Although the blood circulation of ATRA delivery by this PEGylated emulsion was longer than free ATRA, the liver accumulation did not further increased. The results are in accordance with the report that modification of emulsion with PEG increase the hydrophilicity of emulsion and sterically prevent the affinity for the reticuloendothelial system and liver uptake (Liu and Liu, 1995). Our results imply that the inclusion of PEG into emulsion may retain the drug in the circulation but not advantageous for the target drug delivery to the liver.

Recently, Hwang et al. (2004) reported that the pharmacokinetic profile of ATRA after the intravenous administration of emulsions was similar to that of sodium ATRA. This discrepancy in the distribution characteristics may be explained by the effect of different emulsion compositions on their stability and the release of ATRA in blood. It has been reported that the cholesterol content in emulsion is important factor to stabilize emulsion against lipolysis in blood (Maranhao et al., 1986; Clark et al., 1991); therefore, our emulsions, which contained more cholesterol, may more effectively deliver ATRA to the target tissue (liver) without degradation of emulsion.

Although the results reveal that bare emulsions could target the drug to the liver, efficiency could be further improved by attaching targeting molecules to their surface that cognate moieties at the site of interest and further enhance their targeting ability. Moreover, the effective delivery of ATRA to the liver by emulsions could be advantageous for various retinoid-associated conditions because the majority (up to 80%) of the total body Vitamin A is stored in the liver, serving as the main source of retinoids that are utilized throughout the body (Kmiec, 2001). In addition, ATRA has been shown to be toxic in long-term use since it has very low tissue specificity. Taking this into consideration, emulsion formulation of ATRA, which control the release of the drug into the blood and accumulate in the liver, is a promising approach to increase efficacy in the liver and decrease side-effects.

In conclusion, the delivery of ATRA by emulsions can reduce the elimination of ATRA from the blood circulation and preferentially accumulate in the liver after intravenous injection. The retention of ATRA in the liver can successfully suppress the progression of liver metastasis in mice injected with colon carcinoma cells. These findings indicate that the effective delivery and retention of ATRA in hepatocytes by emulsion is an efficient approach for the treatment of liver metastasis.

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References

Arce, F., Gatjens-Boniche, O., Vargas, E., Valverde, B., Diaz, C., 2005. Apoptotic events induced by naturally occurring retinoids ATRA and 13-cis retinoic acid on human hepatoma cell lines Hep3B and HepG2. Cancer Lett. 229, 271–281.

Arimoto, I., Saito, H., Kawashima, Y., Miyajima, K., Handa, T., 1998. Effects of sphingomyelin and cholesterol on lipoprotein lipase-mediated lipolysis in lipid emulsions. J. Lipid Res. 39, 143–151.

Clark, S.B., Derksen, A., Small, D.M., 1991. Plasma clearance of emulsified triolein in conscious rats: effects of phosphatidylcholine species, cholesterol content and emulsion surface physical state. Exp. Physiol. 76, 39–52.

Estey, E., Koller, C., Tsimberidou, A.M., O'Brien, S., Beran, M., Cortes, J., Tirado-Gomez, M., Lopez-Berestein, G., Kantarjian, H., 2005. Potential curability of newly diagnosed acute promyelocytic leukemia without use of chemotherapy: the example of liposomal all-trans retinoic acid. Blood 105, 1366–1367.

Garcia-Alonso, I., Palomares, T., Alonso-Varona, A., Castro, B., Del Olmo, M., Portugal, V., Mendez, J., 2005. Effects of all-trans retinoic acid on tumor recurrence and metastasis. Rev. Esp. Enferm. Dig. 97, 240–248.

Hashida, M., Kawakami, S., Yamashita, F., 2005. Lipid carrier systems for targeted drug and gene delivery. Chem. Pharm. Bull. 53, 871–880.

Hattori, Y., Kawakami, S., Yamashita, F., Hashida, M., 2000. Controlled biodistribution of galactosylated liposomes and incorporated probucol in hepatocyte-selective drug targeting. J. Control. Release 69, 369–377.

Hwang, S.R., Lim, S.J., Park, J.S., Kim, C.K., 2004. Phospholipid-based microemulsion formulation of all-trans-retinoic acid for parenteral administration. Int. J. Pharm. 276, 175–183.

Ishida, E., Managit, C., Kawakami, S., Nishikawa, M., Yamashita, F., Hashida, M., 2004. Biodistribution characteristics of galactosylated emulsions and incorporated probucol for hepatocyte-selective targeting of lipophilic drugs in mice. Pharm. Res. 21, 932–939.

Kawakami, S., Ohshima, N., Hirayama, R., Hirai, M., Kitahara, T., Sakaeda, T., Mukai, T., Nishida, K., Nakamura, J., Nakashima, M., Sasaki, H., 2002. Biodistribution and pharmacokinetics of *O*-palmitoyl tilisolol, a lipophilic prodrug of tilisolol, after intravenous administration in rats. Biol. Pharm. Bull. 25, 1072–1076.

Kawakami, S., Opanasopit, P., Yokoyama, M., Chansri, N., Yamamoto, T., Okano, T., Yamashita, F., Hashida, M., 2005. Biodistribution characteristics of all-trans retinoic acid incorporated in liposomes and polymeric micelles following intravenous administration. J. Pharm. Sci. 94, 2606–2615.

Kawakami, S., Suzuki, S., Yamashita, F., Hashida, M., 2006. Induction of apoptosis in A549 human lung cancer cells by all-*trans* retinoic acid incorporated in DOTAP/cholesterol liposomes. J. Control. Release. 110, 514–521.

Kawakami, S., Wong, J., Sato, A., Hattori, Y., Yamashita, F., Hashida, M., 2000b. Biodistribution characteristics of mannosylated, fucosylated, and galactosylated liposomes in mice. Biochim. Biophys. Acta 1524, 258–265.

Kawakami, S., Yamashita, F., Hashida, M., 2000a. Disposition characteristics of emulsions and incorporated drugs after systemic or local injection. Adv. Drug Deliv. Rev. 45, 77–88.

Kmiec, Z., 2001. Cooperation of liver cells in health and disease. Adv. Anat. Embryol. Cell Biol. 161, 1–151.

Lengfelder, E., Saussele, S., Weisser, A., Buchner, T., Hehlmann, R., 2005. Treatment concepts of acute promyelocytic leukemia. Crit. Rev. Oncol. Hematol. 56, 261–274.

Lim, S.J., Lee, M.K., Kim, C.K., 2004. Altered chemical and biological activities of all-trans retinoic acid incorporated in solid lipid nanoparticle powders. J. Control. Release 100, 53–61.

Liu, F., Liu, D., 1995. Long-circulating emulsions (oil-in-water) as carriers for lipophilic drugs. Pharm. Res. 12, 1060–1064.

Managit, C., Kawakami, S., Yamashita, F., Hashida, M., 2005. Uptake characteristics of galactosylated emulsion by HepG2 hepatoma cells. Int. J. Pharm. 301, 255–261.

Maranhao, R.C., Tercyak, A.M., Redgrave, T.G., 1986. Effects of cholesterol content on the metabolism of protein-free emulsion models of lipoproteins. Biochim. Biophys. Acta 875, 247–255.

McCarter, M.D., Fong, Y., 2000. Metastatic liver tumors. Semin. Surg. Oncol. 19, 177–188.

Muindi, J., Frankel, S.R., Miller Jr., W.H., Jakubowski, A., Scheinberg, D.A., Young, C.W., Dmitrovsky, E., Warrell Jr., R.P., 1992. Continuous treatment with all-trans retinoic acid causes a progressive reduction in plasma drug concentrations: implications for relapse and retinoid "resistance" in patients with acute promyelocytic leukemia. Blood 79, 299–303.

Okuno, M., Kojima, S., Matsushima-Nishiwaki, R., Tsurumi, H., Muto, Y., Friedman, S.L., Moriwaki, H., 2004. Retinoids in cancer chemoprevention. Curr. Cancer Drug Target 4, 285–298.

Otsuki, T., Sakaguchi, H., Hatayama, T., Wu, P., Takata, A., Hyodoh, F., 2003. Effects of all-trans retinoic acid (ATRA) on human myeloma cells. Leuk. Lymphoma 44, 1651–1656.

Ozpolat, B., Lopez-Berestein, G., 2002. Liposomal-all-trans-retinoic acid in treatment of acute promyelocytic leukemia. Leuk. Lymphoma 43, 933–941.

Ozpolat, B., Lopez-Berestein, G., Adamson, P., Fu, C.J., Williams, A.H., 2003. Pharmacokinetics of intravenously administered liposomal all-trans-retinoic acid (ATRA) and orally administered ATRA in healthy volunteers. J. Pharm. Pharm. Sci. 6, 292–301.

Ravikumar, T.S., Olsen, C.O., Steele, G., 1990. Resection of pulmonary and hepatic metastasis in the management of cancer. Crit. Rev. Oncol. Hematol. 10, 111–130.

Rensen, P.C., Herijgers, N., Netscher, M.H., Meskers, S.C., van Eck, M., van Berkel, T.J., 1997. Particle size determines the specificity of apolipoprotein E-containing triglyceride-rich emulsions for the LDL receptor versus hepatic remnant receptor in vivo. J. Lipid Res. 38, 1070–1084.

Soprano, D.R., Qin, P., Soprano, K.J., 2004. Retinoic acid receptors and cancers. Annu. Rev. Nutr. 24, 201–221.

Sun, S.Y., Lotan, R., 2002. Retinoids and their receptors in cancer development and chemoprevention. Crit. Rev. Oncol. Hematol. 41, 41–55.

Takino, T., Konishi, K., Takakura, Y., Hashida, M., 1994. Long circulating emulsion carrier systems for highly lipophilic drugs. Biol. Pharm. Bull. 17, 121–125.

Takino, T., Koreeda, N., Nomura, T., Sakaeda, T., Yamashita, F., Takakura, Y., Hashida, M., 1998. Control of plasma cholesterol-lowering action of probucol with various lipid carrier systems. Biol. Pharm. Bull. 21, 492–497.

Tomii, Y., 2002. Lipid formulation as a drug carrier for drug delivery. Curr. Pharm. Des. 8, 467–474.

Webber, B.M., Soderberg Jr., C.H., Leone, L.A., Rege, V.B., Glicksman, A.S., 1978. A combined treatment approach to management of hepatic metastasis. Cancer 42, 1087–1095.

Yeeprae, W., Kawakami, S., Higuchi, Y., Yamashita, F., Hashida, M., 2005. Biodistribution characteristics of mannosylated and fucosylated O/W emulsions in mice. J. Drug Target. 13, 479–487.

Zuccari, G., Carosio, R., Fini, A., Montaldo, P.G., Orienti, I., 2005. Modified polyvinylalcohol for encapsulation of all-trans-retinoic acid in polymeric micelles. J. Control. Release 103, 369–380.