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Solid Lipid Nanoparticle Formulation of All Trans Retinoic Acid

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ABSTRACT–All-trans retinoic acid (ATRA), vitamin A acid, has been shown to exert anticancer activity in a number of types of cancers, particularly in acute promyelocytic leukaemia (APL). Due to its highly variable bioavailability and induction of its own metabolism after oral treatment, development of parenteral dosage forms are required. However, its poor aqueous solubility and chemical unstability give major drawbacks in parenteral administration. This study was undertaken to investigate a possibility to develop a parenteral formulation of ATRA by employing solid lipid nanoparticle (SLN) as a carrier. By optimizing the production parameters and the composition of SLNs, SLNs with desired mean particle size (< 100 nm) as a parenteral dosage form could be produced from trimyristin (as solid lipid), Egg phosphatidylcholine and Tween 80 (as SLN stabilizer). The mean particle size of SLN formulation of ATRA was not changed during storage, suggesting its physical stability. Thermal analysis confirmed that the inner lipid core of SLNs exist at solid state. The mean particle size of ATRA-loaded SLNs was not significantly changed by the lyophilization process. ATRA could be efficiently loaded in SLNs, while maintaining its anticancer activity against HL-60, a well-known APL cell line. Furthermore, by lyophilization, ATRA loaded in SLN could be retained chemically stable during storage. Taken together, our present study demonstrates that physically and chemically stable ATRA formulation adequate for parenteral administration could be obtained by employing SLN technology.

Keywords–All-trans retinoic acid, Cancer, Solid lipid nanoparticle, Trimyristin, Stability

All-trans retinoic acid (ATRA, vitamin A acid) has been shown to exert anticancer activities in a number of types of cancer cells and tissues, particularly in acute promyelocytic leukemia (APL).[1] Treatment with ATRA, upon activation of retinoic acid receptors in cancer cells, control the growth, differentiation and apoptosis of cancer cells. ATRA has been given to cancer patients by oral administration. The ATRA concentration in blood circulation after long-term oral treatment, however, has been shown to gradually decrease, probably due to the induced cytochrome P-450-dependent metabolism of ATRA.[2] Moreover, the bioavailability of ATRA is highly variable among patients.[3] Therefore, parenteral administration of ATRA may have advantages over oral administration of ATRA.

The poor aqueous solubility and chemical unstability of ATRA give major drawbacks for its parenteral administration.[4] Attempts have been made to improve the aqueous solubility of ATRA by loading in carriers such as cyclodextrins[4] and liposomes.[5] Currently, liposomal parenteral formulation of ATRA is undergoing clinical trials. In liposomal formulation, to achieve high incorporation efficiency, ATRA was incorporated into the lipid bilayers rather than being solubilzed in the inner aqueous core due to its lipophilicity. These multilamellar types of liposomes are relatively large (mean particle size > 3 μm)[6], physically unstable and thus prone to aggregation, remaining as major drawbacks of these types of formulation. Other types of carriers which can be stable during storage and also be in submicron size range to avoid capillary blockade after intravenous injection will be desirable as a parenteral delivery system of ATRA.

ATRA is very sensitive to oxidation and photolysis[4], being rapidly degraded during storage. ATRA in aqueous solution is inevitably more prone to degradation in comparison with ATRA as solid forms. In this regard, dosage forms that can be stored as dried powder but that can be easily redispersed as nanoparticles will be ideal as a parenteral formulation of ATRA.

Recently, solid lipid nanoparticles (SLN) have attracted increasing attention as a new colloidal drug carrier system, which can overcome the limitations of other colloidal carriers.[7] Advantages of SLNs include biodegradability, high incorporation efficiency, submicron particle size, the possibility of controlled drug release and the possibility of large scale production.[8] Furthermore, entrapment of hydrophobic

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drugs in solid lipids, instead of liquid oils as in emulsions, can improve the stability of drugs.[9] The stability of drugs and drug-incorporated SLNs during storage can be further improved by lyophilization or spray drying.[10]

In this study, we investigated the possibility to develop a new parenteral formulation of ATRA by employing SLN as a carrier. Here we report that physically and chemically stable ATRA formulation could be obtained by optimizing the composition of SLNs and production parameters.

Experimental

Materials

ATRA, tricaprin (TC), trimyristin (TM) and MTT (3-(4,5-dimethylthazol- 2-yl)-2,5-diphenyl tetrazolium bromide) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Egg phosphatidylcholine (EggPC) and dimyristoyl-phosphatidylcholine (DMPC) were provided by Avanti Polar lipids (Alabasster, AL, USA). Poloxamer 188 (Pluronic F68) were obtained from BASF (Aktiengesellschatt, Germany). Tween 80 was purchased from ICI Americas (Wilmington, DE, USA). RPMI 1640, Antibiotic-antimycotic (100X), Dulbecco's phosphate buffered saline (PBS) and fetal bovine serum were Gibco products from Life Technologies (Paisely, UK). HL-60 cell line was obtained from Korean Cell Line Bank (KCLB). Soy bean oil was kindly provided by Green Cross Co (Yongin, KOREA). All other chemicals were of reagent grade and used without further purification.

Preparation of solid lipid nanoparticles

SLNs were prepared by melt homogenization method[11] with slight modification. Briefly, 200 mg of solid lipid (TC or TL), 54 mg of EggPC, 6 mg of ATRA and 46 mg of Tween 80 or 46 mg of Poloxamer 188 were mixed and dissolved by 4 ml of tertiary butyl alcohol. After rapid freezing in liquid nitrogen tank, mixtures were dried in Ultra 35EL freezer dryer (Virtis, USA). Finely-dispersed cakes were obtained after overnight drying and then cakes were put in water bath at 70°C. After a few minutes of incubation, lipid melts containing ATRA, PC and surfactants were obtained. Preheated (70°C) water for injection was slowly added to the melts (2 g of total weight) and sonicated in bath type sonicator for 1 h at 60°C until crude and milky emulsions were obtained. These crude emulsions were homogenized for 7 cycles at 60~70°C and 100 MPa using a high pressure homogenizer (Emulsiflex® EF-B3, Avestin Inc., Canada) wired with heating tape (Thermolyne®). SLNs were produced by subsequent cooling of homogenized emulsions at varying temperature. SLNs were then thawed at room temperature and stored at 4°C until use.

When required, SLNs were freeze-dried to obtain dried product. After 1:1 dilution with 5% of sucrose as a cryoprotectant, SLNs were rapidly frozen in liquid nitrogen tank and then lyophilized in Ultra 35EL feezer dryer. Freeze-dried SLNs were stored at 4°C. Just prior to use, SLNs were redispersed by adding water.

Measurement of particle size and zeta potential

The mean particle size and distribution of ATRA-loaded SLNs were determined by dynamic light scattering method using Submicron Particle Sizer (Nicomp 370, Particle Sizing System Inc. CA, USA) at a fixed angle of 90°C and at room temperature. SLN dispersions were diluted with filtered water to give an intensity of 300 Hz according to the manufacturer's protocol.

The electrophoretic mobility and zeta potential were determined using electrophoretic light scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). Zeta potential measurements were performed in filtered distilled water at room temperature (n = 3).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was performed using VP-DSC Micro Calorimeter (Microcal TM Incorporated). A scan rate of 1°C/min was employed in the 15~65°C temperature range. For DSC measurement, 80 μl of SLNs stored for 2 weeks at 4°C, were diluted with 1ml of distilled water. The base line was adjusted by using distilled water as a reference.

Determination of concentration of ATRA loaded in SLNs

After homogenization, the hot ATRA-containing SLNs were immediately filtered through a 0.45 μm membrane filter to remove precipitated ATRA. The amount of ATRA in the resultant filtrate containing SLN fractions was determined by high performance liquid chromatographic (HPLC) method.[4,12] The HPLC system consisted of mobile phase delivery pump (LC-10AS, Shimadzu, Japan), UV detector (SPD-10A, Shimadzu, Japan) and Chromatopac integrator (CR6-A). The C18 reverse phase column (Phenomenex Luna 5u C18 (25 cm × 4.6 mm 5u), Germany) was used. The eluent was 50.6 : 24.4 : 25.6 (v/v) mixture of acetonitrile : methanol : 2.5% aqueous ammonium acetate, and detected at 340 nm. The injection volume was 20 μl and the flow rate was 1.0 ml/min. Under these conditions, the linear calibration curve of ATRA was obtained in the range of 0.1~5 μg/ml (r^2>0.999).

To separate the fraction of ATRA dissolved in outer water phase rather than incorporated in SLNs, ATRA-loaded SLN dispersions were ultrafiltered or dialyzed against distilled water and then amount of ATRA was redetermined by HPLC.

Stability of ATRA-loaded SLNs

The physical stability of ATRA-loaded SLNs were assessed by monitoring changes in the particle size of SLNs during storage for 60 days at 4°C.

The chemical stability of ATRA loaded in SLNs was determined by monitoring the concentration of intact ATRA in SLN dispersions or freeze-dried SLNs during storage for 4 weeks at 4°C. For comparison, the stability of ATRA loaded in microemulsions prepared with 27:23:100 (mg/g) of EggPC :Tween 80:soybean oil was determined under the same condition. The concentration of intact ATRA was determined by HPLC method as described above.

Anticancer activity of ATRA

ATRA has been shown to exert anticancer activity in acute promyelytic leukaemia (APL) cells.[1] In order to examine whether the anticancer activity of ATRA was fully retained after incorporation in SLNs, HL-60 cells, a well-known APL cell line, were used. HL-60 cells were cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B. Cells were seeded at 20000 cells/ well density in 96-well plates in 0.1 ml of RPMI medium supplemented with 10% of fetal bovine serum. After 4 h, ATRA loaded in SLNs or dissolved in dimethylsulfoxide (2 mg/ml) was added to the wells after appropriate dilution with Dulbecco's PBS. After 4 days of incubation under 5% CO_2 at 37°C, 20 μl of MTT solution (5 mg/ml in PBS) was added to each well. Three hour later, 120 μl of isopropyl alcohol was added and then vigorously mixed to dissolve the insoluble MTT formazan crystals. The optical density at 570 nm was determined by microplate spectrophotometer (MCC340, Multiskan, Belgium). Each experiment was performed in triplicate and repeated at least three times.

The cell growth and viability was calculated according to the following equation;

$$\text{Cell growth and viability } (\%) = (OD_{570}\ (\text{sample}) / OD_{570}\ (\text{control})) \times 100$$

Where the OD 570 (sample) represents the absorbance from the wells treated with ATRA and the OD 570 (control) represents the absorbance from the wells treated with medium only.

Table I–*Effect of Composition of Solid Lipid Nanoparticles loaded with ATRA on the Resultant Particle Size. After High Pressure Homogenization, Solid Lipid Nanoparticles were cooled Overnight at -20°C to Solidify the Lipid Core and then thawed at Room Temperature*

Composition of SLN (mg/g)	Mean particle size (nm)
EggPC:Tween80:TC = 27:23:100	187 ± 36.4
EggPC:Poloxamer 188:TM = 27:23:100	439 ± 141
EggPC:Tween80:TM=27:23:100	73.5 ± 20.7
DMPC:Tween80:TM=27:23:100	177 ± 17.6

Results and Discussion

Effect of SLN composition on the particle size

Among various types of solid lipids, TC or TM was selected as a solid lipid core in this study. These triglycerides have been employed for SLN prodcution in other studies.[13] SLNs prepared with combination of stabilizers tend to have smaller particle size and higher storage stability by preventing particle agglomeration more efficiently.[14,15] Therefore, Poloxamer 188 or Tween 80, which is pharmaceutically acceptable for parenteral administration, was combined with PC as a SLN stabilizer and then the particle sizes of resultant SLNs were compared.

The mean particle sizes of all the SLNs, except poloxamer 188-containing SLNs, were smaller than that of commercially available parenteral lipid emulsions (200~400 nm)[16] (Table I). TM-containing SLNs were smaller than TC-containing SLNs. This result is inconsistent with other study demonstrating that the mean particle size of SLN dispersions produced by melt homogenization method tends to increase with increasing melting point of core lipid [14] since the melting point of TM (m.p. 54°C) is higher than that of TC (m.p. 31-32°C). The discrepancy may be due to the molecular characteristics of ATRA loaded in SLNs.

Among TM-containing SLNs, combination of EggPC and Tween 80 resulted in the smallest particle size (< 100 nm). Therefore, this combination was used for the preparation of SLNs in the subsequent study.

Effect of cooling temperature on the particle size of SLNs

For preparing SLNs, cooling is a critical step to solidify the lipid core. The cooling rate can affect the solidifying process and thereby change the particle size of SLNs. In this study, the particle sizes of SLN dispersions after cooling overnight at RT or 4°C were much smaller than those at -20 or in liquid nitrogen (-196°C) (Table II). However, after lyophilization of these SLN dispersions, the particle size of redispersed SLNs appeared to decrease with lower cooling temperature. In other words, the

Table II–*Effect of Cooling Temperature on the Mean Particle Size of ATRA-incorporated Solid Lipid Nanoparticles. SLNs were Prepared with 3:27:23:100 (mg/g) of ATRA:EggPC: Tween 80:TM*

Cooling temperature (°C)	Before freeze-drying	After freeze-drying
	Mean particle size (nm)	Mean particle size (nm)
RT	44.5 ± 17.8	122.5 ± 27.7
4	43.5 ± 6.9	118.9 ± 37.7
-20	73.5 ± 20.7	104.6 ± 16.0
Liquid nitrogen	83.4 ± 19.7	93.0 ± 25.6

change in the particle size by lyophilization was the smallest in SLNs cooled at -196 (from 83.4 to 93.0 nm) but the biggest in SLNs cooled at RT (from 44.5 to 122.5 nm). These data indicate that the physical state of SLNs cooled in liquid nitrogen was affected to the least extent by lyophilization process.

It has been shown that the SLNs prepared with TM (Dynasan 114) recrystallize at much lower temperature (8 °C) than its melting point (54°C).[17] We cannot exclude the possibility that the smaller particle size of SLNs cooled at RT or 4 °C may be due the fact that they exist in different physical state (supercooled melts) from those cooled at -20 or -196°C (solids). The significant increase in the particle size of SLNs cooled at RT or 4°C, after being processed to freeze-drying, may be due to the change of physical state of lipid core from supercooled melts to solid.

Characteristics of optimized SLN formulation of ATRA

As shown in Table I and II, by optimization of formulation parameters, ATRA-loaded SLNs could be produced with small particle size in the injectable range even after lyophilization. When SLNs were prepared with 3:27:23:100 (mg/g) mixture of ATRA:EggPC: Tween 80:TM, the mean particle size was 83.4 ± 19.7 nm before lyophilization and 93.0 ± 25.6 nm after lyophilization. The polydispersity index slightly increased after lyophilization (0.168 versus 0.202), but still indicated a narrow size distribution.

When SLNs were prepared with 3 mg/g of ATRA, ATRA could be loaded at 2.59 ± 0.348 mg/ml of concentration in SLNs. This value is approximately 49000-fold higher than the reported solubility of ATRA in PBS buffer (0.21 μM).[18] No ATRA was detectable in the filtrate after ultrafiltration or dialysis, suggesting more than > 99% of ATRA in SLN dispersions was incorporated in the solid lipid nanoparticles rather than being dissolved in outer aqueous phase. After freeze-drying, no significant decrease in ATRA content incorporated in SLNs was observed, suggesting that ATRA retained

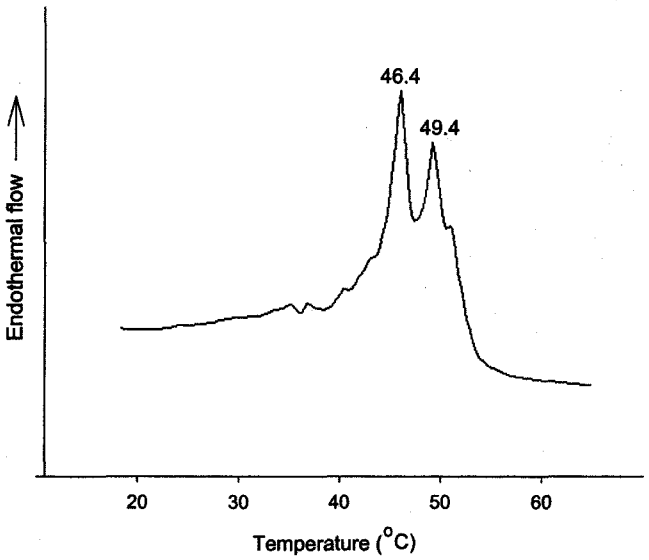

Figure 1–DSC curves of ATRA-incorporated SLNs. SLNs were prepared as described in material and methods. Prepared SLNs were cooled in liquid nitrogen and then stored at 4°C before DSC measurement.

in the inner solid lipid core during lyophilization process.

To obtain full electrostatic stabilization of SLNs, zeta potential should be higher than -30 mv.[19] The zeta potentials of SLN formulation of ATRA:EggPC: Tween 80:TM (3:27:23:100) were -14.1 ± 11.2 (n = 3). These values are not enough to be fully stabilized by electrostatic stabilization. Rather, these correspond to the region of limited flocculation.[20]

Thermal analysis was performed to verify the solidification of the lipid core in SLNs after being cooled overnight. When SLNs were cooled in liquid hitrogen, they presented two melting peaks at 46.4 and 49.4 °C (Figure 1). To contrast, in SLNs cooled at 4°C, no melting peak was observed within the scanned temperature range (data not shown).

TM has been known to exist as three forms in solid states (α (33.0°C), β(54.5°C) and β'(57.0°C)).[21] Generally, the melting peak of lipid core in SLNs is observed at lower temperature than that of bulk lipid due to the nanocrystaline size of lipids in SLNs.[22] The observed two peaks in our study may correspond to β and β' forms in SLNs. Since β, β' forms are more stable than α forms[17], the TM in SLNs cooled in liquid nitrogen is thought to exist at stable solid state. In contrast, the TM-containing SLNs cooled at 4 °C may still exist as supercooled melts, in which the crystallization process is being retarded.

The stability of ATRA-loaded SLNs

As a parameter to assess the physical stability of SLNs, the changes in particle size of SLN dispersions were monitored during storage at 4°C after cooling at -20°C and in liquid

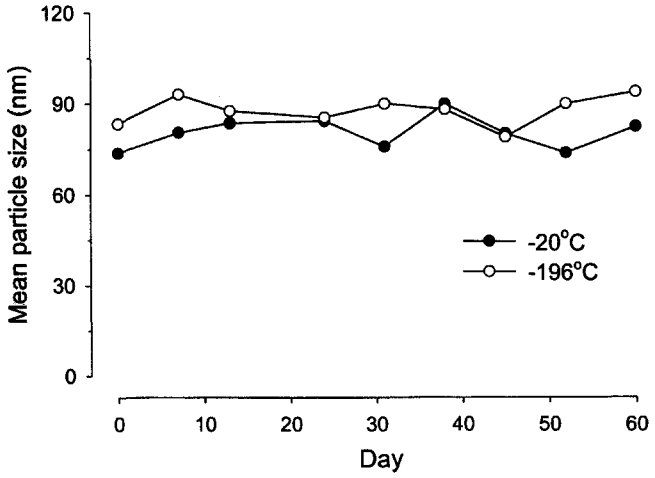

Figure 2–The changes in mean particle size of ATRA-incorporated SLNs during storage at 4°C. Before storage, SLNs were cooled overnight at -20°C or in liquid nitrogen.

nitrogen, respectively. Regardless of cooling temperature, the mean particle size of ATRA-incorporated SLNs was not changed during storage (Fig ure 2). These data demonstrate that the prepared SLNs were physically stable without particle aggregation, even though the zeta potential was not high enough to get full electrostatic stabilization. Probably SLNs could be further sterically stabilized by Tween 80.

We also examined whether ATRA could be maintained chemically stable during storage at 4 °C by being immobilized in lipid core of SLNs and also by protection given by emulsifier films. Unfortunately, the stability of ATRA in SLN dispersions during 4 weeks of storage was not significantly higher than that in microemulsion dispersions in which ATRA is solubilized in liquid oil core instead of solid lipid core (Table III). In other words, the protective effect of SLN dispersions was not so much high as expected. However, when ATRA-loaded SLN dispersions were further subjected to lyophili - zation and then stored as a dried form, the intact concentration of ATRA measured after reconstitution of SLNs remained unaltered during storage (Table III). These data demonstrate that the SLN formulation of ATRA could provide improved stability of ATRA mainly by lyophilization.

Anticancer activity of ATRA loaded in SLNs

To check whether the anticancer efficacy of ATRA retained after loading in SLNs, the growth inhibitory effect of ATRA on HL-60 cells was investigated. The growth inhibitory effect of ATRA loaded in SLNs was not significantly different from that of free ATRA (Figure 3). Both SLN-loaded and free ATRA inhibited the proliferation of HL-60 cells in a dose-dependent manner in the range of 1~1000 nM. Empty SLNs without

Table III–*Stability of ATRA Loaded in Solid Lipid Nanoparticles during Storage at 4°C. ATRA-loaded SLNs were prepared with 3:27:23:100 (mg/g) of ATRA:EggPC:Tween 80:TM. For Comparison, ATRA- loaded Microemulsions were Prepared with 3:27:23:100 (mg/g) of ATRA:EggPC:Tween 80:Soybean Oil*

Weeks at 4°C	ATRA loaded in SLNs (% of initial amount)		ATRA loaded in microemulsions[c] (% of initial amount)
	without freeze-drying[a]	with freeze-drying[b]	
1 week	94.4 ± 4.9	98.7 ± 2.2	91.2 ± 5.0
2 weeks	79.9 ± 2.4	94.4 ± 4.3	77.3 ± 3.7
4 weeks	57.8 ± 3.1	97.7 ± 2.8	51.4 ± 1.9

[a]ATRA-incorporated SLNs were cooled in liquid nitrogen and thawed at room temperature. After dilution with filtered distilled water to 0.8 mg/ml of ATRA, ATRA- incorporated SLNs were stored as dispersions at 4°C.
[b]ATRA-incorporated SLN dispersions were further subjected to lyophilization and then stored as dried products at 4°C. At the designated time points, the dried SLNs were redispersed in filtered distilled water.
[c]After dilution with filtered distilled water to 0.8 mg/ml of ATRA, ATRA- incorporated microemulsions were stored as dispersions at 4°C.

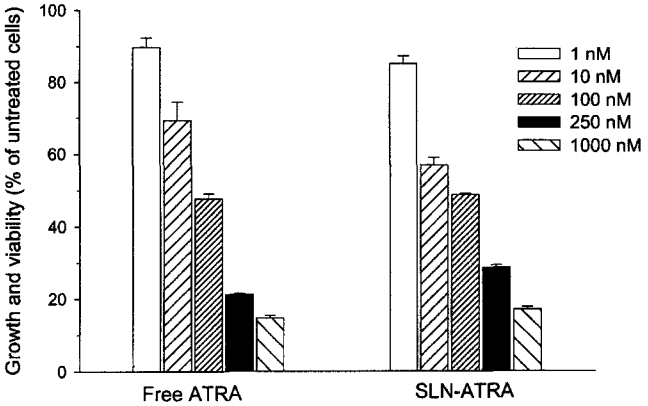

Figure 3–The growth inhibitory effect of ATRA as free or being incorporated in SLNs. SLNs were prepared as described in material and methods section.

ATRA only slightly (< 15%) inhibited the proliferation of HL-60 cells at the dilution corresponding to the highest concen - tration of ATRA used (1000 nM of ATRA). These results indicate that ATRA loaded in SLNs can exert its anticancer activity, probably after being released from SLNs outside or inside of cells, and thus binding to retinoic acid receptors on the nuclear membrane of cells.

Conclusion

In this study, by varying the production parameters and the components constituting SLNs, SLNs with desired mean particle size (< 100 nm) as a parenteral dosge form for ATRA could be produced. Under optimized condition, the mean

particle size of SLN after lyophilization was almost similar to that of initial one. The SLN formulation of ATRA in this study was physically stable when stored at 4 °C.

ATRA could be loaded in these SLNs at high incorporation efficiency, while maintaining its anticancer activity. Furthermore, by lyophilization of ATRA-loaded SLNs, the chemical stability ATRA could be retained during storage. Taken together, our present study demonstrates that physically and chemically stable ATRA formulation adequate for parenteral administration could be obtained by employing SLN technology. SLN formulation may have potential as an alternative parenteral dosage from of ATRA in the treatment of cancer. SLNs in this study may also be applicable as carriers for other poorly water-soluble and/or chemically unstable drugs.

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References

1) M.S. Tallman, J.W. Anderson, C.A. Schiffer, F.R. Appelbaum, J.H. Feusner, A. Ogden, L. Shepherd, C. Willman, C.D. Bloomfield, J.M. Rowe and P.H. Wiernik, All-trans-retinoic acid in acute promyelocytic leukemia, *N. Eng. J. Med.*, **337**, 1021-1028 (1997).

2) J. Muindi, S.R. Frankel, W.H. Jr., Miller, A. Jakubowski, D.A. Scheinberg, C.W. Young, E. Dmitrovsky and R.P.Jr. Warrell, 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 (1992).

3) P.C. Adamson, H.C. Pitot, F.M. Balis, J. Rubin, R.F. Murphy and D.G. Poplack, Variability in the oral bioavailability of all-trans-retinoic acid, *J. Natl. Cancer Inst.*, **85**, 993-996 (1993).

4) H.S. Lin, C.S. Chean, Y.Y. Ng, S.Y. Chan and P.C. Ho, 2-hydroxypropyl- beta-cyclodextrin increases aqueous solubility and photostability of all-trans-retinoic acid, *J. Clin. Pharm. Ther.*, **25**, 265-269 (2000).

5) J. Drach, G. Lopez-Berestein, T. McQueen, M. Andreeff and K. Mehta, Induction of differentiation in myeloid leukemia cell lines and acute promyelocytic leukemia cells by liposomal all-trans-retinoic acid, *Cancer Res.*, **53**, 2100-2104 (1993).

6) R. Parthasarathy, B. Gilbert and K. Mehta, Aerosol delivery of liposomal all-trans-retinoic acid to the lungs, *Cancer Chemother. Pharmacol.* **43**, 277-283 (1994).

7) C. Schwarz and W. Mehnert, Solid lipid nanoparticles (SLN) for controlled drug delivery. II. Drug incorporation and physicochemical characterization, *J. Microencapsul.*, **16**, 205-213 (1999).

8) R. H. Muller, K. Mader and S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery : a review of the state of the art, *Eur. J. Pharm. Biopharm.*, **50**, 161-177 (2000).

9) R.H. Muller and S.A. Runge, Solid lipid nanoparticles (SLN) for drug delivery, *In: S. Benita (Ed.),* Submicron emulsions in Drug Targeting and Delivery, Harwood Academic Publishers, Amsterdam, pp. 219-234 (1998).

10) W. Mehnert and K. Mader, Solid lipid nanoparticles production, characterization and applications, *Adv. Drug Deliv. Rev.*, **47**, 165-196 (2001).

11) H. Heiati, R. Tawashi, R.R. Shivers and N.C. Phillips, Solid lipid nanoparticles as drug carriers I. Incorporation and retention of the lipophilic prodrug 3'-azido-3'-deoxythymidine palmitate, *Int. J. Pharm.*, **146**, 123-131 (1997).

12) R. Wyss, Chromatography of retinoids, *J. Chromatogr.*, **531**, 481-508 (1990).

13) N. Scholer, C. Olbrich, K. Tabatt, R.H. Muller, H. Hahn and O. Liesenfeld, Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages, *Int. J. Pharm.*, **221**, 57-67 (2001).

14) B. Sielmann and K. Westesen, Submicron-sized parenteral carrier systems based on solid lipids, *Pharm. Pharmacol. Lett.*, **1**, 123-126 (1992).

15) C. Olbrich and R.H. Muller, Enzymatic degradation of SLN-effect of surfactant and surfactant mixtures, *Int. J. Pharm.*, **180**, 31-39 (1999).

16) R.H. Muller and S. Heinemann, Fat emulsions for parenteral nutrition. I: Evaluation of microscopic and laser light scattering methods for the determination of the physical stability, *Clin. Nutrition.*, **11**, 223-236 (1993).

17) K. Westesen, H. Bunjes and M.H.J. Koch, Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential, *J. Controll. Release*, **48**, 223-236 (1997).

18) E.Z. Szuts and F.I. Harosi, Solubility of retinoids in water, *Arch. Biochem. Biophys.*, **287**, 297-304 (1991).

19) R.H. Muller and S. Heinemann, Fat emulsions for parenteral nutrition. III : Liposundin MCT regimens for TPN with low and medium electrolyte load, *Int. J. Pharm.*, **101**, 175-189 (1994).

20) C. Schwarz, W. Mehnert, J.S. Lucks and R.H. Muller, Solid lipid nanaoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization, *J. Controll. Release*, **30**, 83-96 (1994).

21) L. Hernqvist, Crystal structures of fats and fatty acids. In:*N. Garti, K. Sato, (Eds.)*, Crystallization and polymorphism of fats and fatty acids, Marcel Dekker, New York, Basel, pp. 97-138 (1998).

22) K. Westesen and H. Bunjes, Do nanoparticles prepared from lipids soild at room temperature alwaays possess a soilid lipid matrix?, *Int. J. Pharm.*, **115**, 129-131 (1995).