JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 1527–1535

Received July 13, 2004
Accepted September 3, 2004
DOI 10.1211/0022357044959
ISSN 0022-3573

Solid lipid nanoparticles (SLNs) to improve oral bioavailability of poorly soluble drugs

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Abstract

The purpose of this work was to improve the oral bioavailability of poorly soluble drugs by incorporation into solid lipid nanoparticles (SLNs). All-*trans* retinoic acid (ATRA) was used as a poorly soluble model drug. Different formulations of SLNs loaded with ATRA were successfully prepared by a high-pressure homogenization method and using Compritol 888 ATO as lipid matrix. The particle size and distribution, drug loading capacity, drug entrapment efficiency (EE %), zeta potential, and long-term physical stability of the SLNs were investigated in detail. Drug release from two sorts of ATRA-SLN was studied and compared with the diffusion from ATRA solution in 0.1 M HCl, distilled water and phosphate buffer (pH 7.40), using a dialysis bag method. A pharmacokinetic study was conducted in male rats after oral administration of 8 mg kg^{-1} ATRA in different formulations and it was found that the relative bioavailability of ATRA in SLNs was significantly increased compared with that of an ATRA solution. The amount of surfactant also had a marked effect on the oral absorption of ATRA with SLN formulations. Although an emulsion formulation also increased ATRA absorption, it was too unstable for use in clinical situations. The absorption mechanism of the SLN formulations was discussed. These results indicate that ATRA absorption is enhanced significantly by employing SLN formulations. SLNs offer a new approach to improve the oral bioavailability of poorly soluble drugs.

Introduction

Despite having good therapeutic effects, many drugs fail in clinical situations due to their poor water solubility. Attempts to overcome the solubility problem and improve oral absorption have been investigated in many recent studies, and many technologies have been employed to improve the oral absorption of poorly soluble drugs, including complexation (Kouzou et al 2000), solid dispersion techniques (Kohri et al 1999; Leuner & Dressman 2000; Hemant et al 2004), microemulsions (Kawakami et al 2002) and self-emulsifying drug delivery systems (SEDDS) (Ping et al 2003), co-administered with various P-glycoprotein inhibitors (Jong et al 2003). The use of solid lipid nanoparticles (SLNs) offers a new perspective in the formulation of poorly soluble drugs.

SLNs have been reported as an alternative drug delivery system to traditional polymeric nanoparticles (Mühlen et al 1998; Mehnert & Mader 2001). Nanoparticles are in the submicron size range (50–1000 nm) and are composed of physiologically tolerated lipid components; at room temperature the particles are in the solid state (Schwarz et al 1993; Müller & Lucks 1996). SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes (Schwarz & Mehnert 1999). They can be produced on a large industrial scale by high-pressure homogenization (Dingler & Gohla 2002), are toxicologically highly acceptable like emulsions and liposomes, produce sustained release due to their solid matrix, similar to polymeric nanoparticles, and can effectively target specific tissues after parenteral administration (Cavalli et al 1999; Yang et al 1999). In this study, SLNs were employed to investigate the enhancement of the absorption and bioavailability of poorly soluble drugs.

All-*trans* retinoic acid (ATRA), the chemical structure of which is shown in Figure 1, has been shown to exhibit anti-cancer activity in the treatment of human malignant gliomas (Kalmekerian et al 1994; Krupitza et al 1995; Defer et al 1997; Giannini et al

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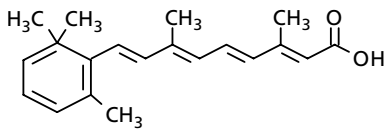

Figure 1 All-*trans* retinoic acid.

1997) and, in particular, acute promyelocytic leukaemia (APL) (Huang et al 1988). ATRA influences the morphological differentiation, proliferation and gene expression of neuroblastoma (Irving et al 1998) and astrocytoma cells (Dirks et al 1997). Because it is practically insoluble in water, its clinical use is greatly restricted by the low plasma concentration reached after oral dosing and so there is a need to improve its poor aqueous solubility to increase its oral bioavailability. An oral formulation with a high degree of oral absorption would, therefore, be highly desirable.

In this study, ATRA-loaded SLNs were successfully prepared by a high-pressure homogenization technique and the physicochemical characteristics of the SLNs were investigated. Drug release from the SLNs was studied and compared with the diffusion from an ATRA solution using a dialysis bag method. The oral bioavailability of ATRA in SLNs was compared with that in a solution to investigate the absorption enhancement of SLNs for poorly soluble drugs. In addition, the in-vivo behaviour of an ATRA emulsion was assessed. The effect of surfactant on the oral absorption of ATRA was also studied with SLN formulations. The behaviour and absorption mechanism of the SLN formulations were discussed.

Materials and Methods

Chemicals and reagents

ATRA was supplied by LiangFu Pharmaceutical Co. (Shandong, China). Pluronic F68 was obtained from Shanghai XieTai Chemical Co. (Shanghai, China); soy lecithin was provided by Shanghai TaiWei Pharmaceutical Co. (Shanghai, China). Tween 80 and ethanol were purchased from Shenyang Chemical Reagent Factory (Shenyang, China). Compritol 888 ATO was from Colorcon Co. (Shanghai, China). All other chemicals were of analytical grade.

Preparation of formulations

SLNs were prepared by a high-pressure homogenization (HPH) method. The desired amounts (Table 1) of Compritol 888 ATO and ATRA were mixed with approximately 2 mL ethanol and heated to 80°C; emulsifiers (soy lecithin/Pluronic F 68 or soy lecithin/Tween 80) were dispersed in distilled water with magnetic stirring and heated to the same temperature. After the ethanol had completely evaporated, the water phase was added drop-wise to the oil phase at 80°C followed by magnetic stirring for a few minutes, then the coarse premix was subjected to ultrasonic treatment for 10 min using a high-intensity probe ultrasonicator (Uitra-cell 750 W; Sonics Materials Inc., USA) at 80°C. Then the coarse emulsion was passed through a high-pressure homogenizer (NS10012K; Niro Soavi, Italy) at 800 bar for 3 homogenization cycles. The dispersions were immediately filtered through a 0.45-μm membrane and the final volume was adjusted to 100 mL with distilled water, followed by storage at 4°C. All operations were performed in a dark room.

ATRA solution was prepared by dissolving ATRA in a 20-mL mixture of ethanol, propylene glycol 400 (PEG 400) and Tween 80. The mixture was subjected to ultrasound for 5 min and the volume was adjusted to 40 mL with distilled water.

ATRA emulsion was prepared by dispersing ATRA in a moderate amount of ethanol in an ultrasonic bath, and then mixed with soybean oil after evaporating the ethanol. The coarse emulsion was then treated as described above in the SLN operation. ATRA solution and emulsion were prepared immediately before oral administration.

All the formulations used are shown in Table 1.

Transmission electron microscopy (TEM)

The morphology of SLNs was examined using an electronic transmission microscope (Hitachi H-600; Japan).

Table 1 Composition of formulations for the oral administration study (weight %)

Ingredient	SLN A	SLN B	SLN C	SLN D	SLN E	SLN F	ATRA solution	ATRA emulsion
ATRA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Compritol 888 ATO	4.0	4.0	4.0	4.0	4.0	4.0		
Tween 80	3.0	2.0	1.0				5.0	
Pluronic F68				3.0	2.0	1.0		2.0
Soy lecithin	2.5	2.5	2.5	2.5	2.5	2.5		2.5
Soybean oil								10
Ethanol	Trace	Trace	Trace	Trace	Trace	Trace	4.9	Trace
PEG 400							40	
Water	90.4	91.4	92.4	90.4	91.4	92.4	50	85.4

After diluting 50 fold with the original dispersion medium of the preparation, the samples were negatively stained with 2% (w/v) osmium tetroxide for observation.

Particle size and zeta potential

The mean diameter of SLNs in the dispersion was determined by photon correlation spectroscopy (PCS) using a NICOMP particle sizing system (CW380; Santa Barbara, CA) at a fixed angle of 90° at a temperature of 23°C. The particle size analysis data were evaluated using the volume distribution. Zeta potential measurement was carried out using the same instrument at an electrical field strength of $10\,V\,cm^{-1}$ and at the same temperature. Before measurement, SLN dispersions were diluted 20 fold and 50 fold with the original dispersion preparation medium for size determination and zeta potential measurement, respectively. All the measurements were performed in triplicate.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was performed using a DSC-60 differential scanning calorimeter (Shimadzu, Japan). For DSC measurement, a scan rate of $10°C\,min^{-1}$ was employed in the temperature range of 30–260°C under a nitrogen purge. The samples were placed directly in aluminium pans for analysis. Solvent mixtures were obtained by dissolving ATRA in ethanol and the solvents were removed by rotoevaporation. Lyophilized ATRA SLNs were obtained with 10% mannitol as a cryoprotectant using a freeze-drier (FDU-1100 Freeze Drier; EYELA, Japan).

Drug content and entrapment efficiency

The SLN dispersions were dissolved in methanol to preferentially precipitate the lipid. After centrifugation ($4000\,rev\,min^{-1}$ for 15 min), the drug content in the supernatant was measured by HPLC. The HPLC system consisted of a mobile phase delivery pump (L-7100; Hitachi, Japan) and a UV-Vis detector (L-7420; Hitachi, Japan). A C18 reverse-phase column (Diamonsil TM 5 μ C18, 200×4.6 mm; Beijing, China) was used. The eluant was methanol–0.05 M aqueous $NH_4H_2PO_4$ (90:10, v/v) and detection was carried out at 348 nm. The injection volume was 10 μL and the flow rate was $1.0\,mL\,min^{-1}$.

The SLN dispersions were subjected to ultracentrifugation (Hitachi CS120GXL micro ultracentrifuge; Japan) at $50\,000\,rev\,min^{-1}$ for 3 h at 4 °C in vacuum. The supernatant containing the free drug was withdrawn for HPLC analysis as described above. The precipitate in the ultracentrifuge tube was desiccated to give an exact weight.

The equations for the drug content and loading efficiency are as follows:

$$\text{Drug content (\% w/w)} = [(\text{Amount of ATRA in the SLNs})/(\text{Weight of SLNs})] \times 100 \quad (1)$$

$$\text{Drug entrapment efficiency (\%)} = [(\text{Amount of ATRA in the SLNs})/(\text{Amount of ATRA used in formulation})] \times 100 \quad (2)$$

Stability studies

Four formulations(SLN A, SLN D, ATRA solution and ATRA emulsion) were injected into 10-mL ampoules and sealed for storage at 4°C in the dark for one year, The average size, zeta potential and entrapment efficiency were determined.

Drug release properties of SLN

The drug release from both SLN formulations and the diffusion test of an ATRA solution were performed in 0.1 M HCl, distilled water, pH 6.95 and phosphate buffer, pH 7.4, using the dialysis bag method. The dialysis bag retains nanoparticles and allows the transfer of the drug into the dissolution media with a cut-off of 12 000–14 000. The bags were soaked in double-distilled water for 12 h before use then 2 mL SLN dispersion was poured into the bag with the two ends fixed by clamps. The bags were placed in a conical flask and 50 mL dissolution medium was added. The conical flasks were placed into a thermostatic shaker (HZQ-C; Haerbin Dongming Medical Instrument Factory, Haerbin, China) in an air bath, and then shaken horizontally at 37°C at a rate of 140 times per min. At fixed time intervals, the medium in the conical flask was completely removed by filtration for analysis and fresh dissolution medium was then added to maintain sink conditions. The filtrate was analysed by the HPLC method described above. All the operations were performed in the dark and experiments were carried out in triplicate.

Oral administration

Male Wistar rats (provided by China Medical University Animals Center), 250 ± 20 g, were used for the oral administration study. All animal experiments complied with the requirements of the National Act on the use of experimental animals (People's Republic of China). All the rats were fasted for 12 h before the experiments but had free access to water. After light anaesthetization with ether, each formulation was administered to 6 rats by gavage at an ATRA dose of $8\,mg\,kg^{-1}$. Blood (0.5 mL) was collected via the orbital vein at 0, 0.33, 0.66, 1, 2, 3, 4, 5, 6 and 8 h after administration. Blood samples were placed into heparinized tubes and separated immediately by centrifugation. After centrifugation, the plasma obtained was stored at −20°C until analysis.

Quantification of plasma concentration

ATRA plasma concentrations were determined by HPLC analysis as described above. A 0.2-mL plasma sample was placed into a centrifuge tube and then 0.8 mL methanol was added. After vortexing for 5 min, the mixture was centrifuged at $4000\,rev\,min^{-1}$ for 10 min. The clear supernatant

(20 μL) was injected for HPLC analysis. Calibration curves were prepared by linear regression analysis of the plot of the peak area against the concentration of ATRA covering the range of 25–5000 ng mL^{-1}. The concentration of plasma samples was determined from the area of the chromatographic peak using the calibration graph. The limit of detection was 25 ng mL^{-1}.

Data analysis

The pharmacokinetic parameters were calculated based on a non-compartmental model. The area under the concentration–time curve from time zero to time t (AUC_{0-t}) was calculated using the trapezoidal method. Peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma concentration–time profiles. The area from time zero to infinity was calculated by: $AUC_{0-\infty} = AUC_{0-t} + C_t/K_e$, where C_t is the ATRA concentration observed at last time, and K_e is the apparent elimination rate constant obtained from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The relative bioavailability F_r at infinity at the same dose was calculated as: $F_r = AUC_{sln,0-\infty}/AUC_{sol,0-\infty}$. The mean residence time (MRT) was estimated from $MRT = AUMC_{0-\infty}/AUC_{0-\infty}$.

The data obtained from the release rate and pharmacokinetic parameters were analysed statistically by one-way analysis of variance and Student's *t*-test using SPSS version 11.0 software. Statistically significant differences were assumed when $P < 0.05$. All values are expressed as their mean ± s.e.

Results

Characterization of SLNs

The size of ATRA-SLN prepared with different formulations ranged from 80 to 300 nm (Table 2). The SLN formulations containing Tween 80 showed a relatively small size distribution compared with those containing similar amounts of Pluronic F68.

Table 2 The mean diameter, zeta potential and entrapment efficiency of different SLN formulations (0 day)

Formulation	Mean diameter (nm)	Zeta potential (mV)	Entrapment efficiency (%)
SLN A	89.3 ± 11.2	−32.8 ± 1.9	96.0 ± 0.3
SLN B	134.3 ± 5.9	−27.1 ± 0.6	95.1 ± 0.2
SLN C	174.2 ± 6.2	−21.8 ± 0.5	97.4 ± 0.4
SLN D	158.3 ± 8.8	−24.3 ± 1.1	96.8 ± 0.3
SLN E	258.5 ± 3.9	−21.6 ± 0.5	96.7 ± 0.3
SLN F	328.8 ± 8.4	−17.5 ± 0.5	97.1 ± 0.5
ATRA emulsion	269.8 ± 10.7	−3.86 ± 1.4	ND

Data are shown as mean ± s.d., n = 4. ND, mean not determined.

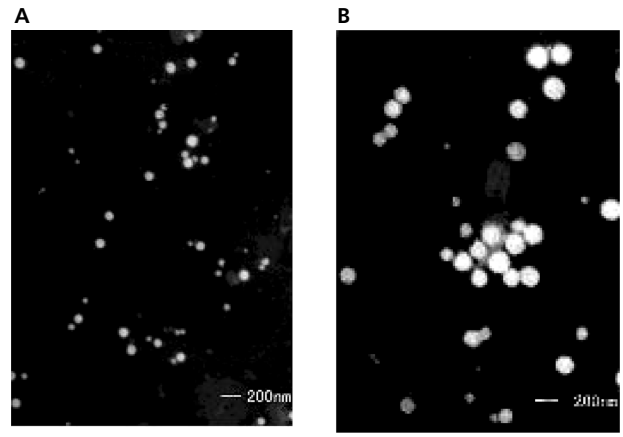

Figure 2 TEM of ATRA-SLN obtained in an aqueous system. A, SLN A. B, SLN D.

The results show that ATRA has a high entrapment efficiency in SLNs. When the amounts of Tween 80 or Pluronic F68 increased, the entrapment efficiency decreased due to the solubilization of both emulsifiers. The entrapment efficiency of formulations containing Tween 80 was a little lower than that of formulations containing Pluronic F68. These results may have contributed to the higher solubilization of Tween 80 compared with Pluronic F68 in the aqueous system for ATRA.

The SLNs had a zeta potential ranging from −17 mV to −40 mV. As the amount of Pluronic F68 and Tween 80 increased, the zeta potential decreased significantly.

TEM shows that the particles had round and uniform shapes. The mean diameters of SLN A and SLN D were in the range of approximately 150–200 nm and 50–100 nm, respectively (Figure 2).

DSC study

Figure 3 shows DSC thermograms of ATRA, Compritol 888 ATO, lyophilized ATRA-SLN and the physical mixture and solvent evaporated mixture of ATRA and Compritol 888 ATO. The thermograms of the lyophilized ATRA-SLN did not show the melting peak for ATRA at around 182°C. The disappearance of the endothermic peak of the lyophilized ATRA-SLN demonstrates that ATRA dispersed homogeneously in an amorphous state and no ATRA crystallized out of the dispersion. An endothermic peak of mannitol used as cryoprotectant was observed at 166°C. DSC analysis of camptothecin SLN prepared by high-pressure homogenization also showed that camptothecin was in an amorphous state (Yang & Zhu 2002).

Stability data

After one year of storage at 4°C, no dramatic increase in the size of SLN A and SLN D occurred (Table 3). The entrapment efficiencies of both SLN formulations had

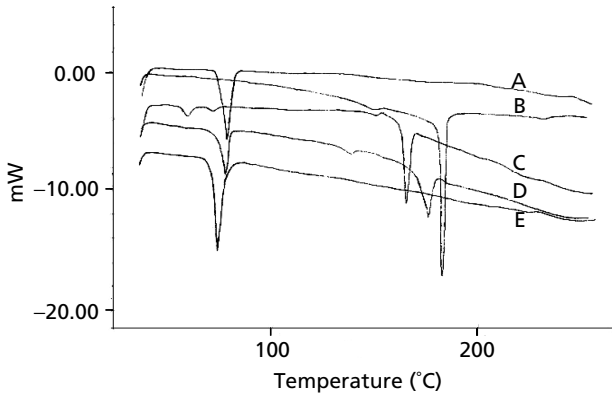

Figure 3 Overlaid DSC thermograms of Compritol 888 ATO (A), ATRA (B), lyophilized ATRA-SLN (C), physical mixture of ATRA and Compritol 888 ATO (D) and solvent evaporated mixture of ATRA and Compritol 888 ATO (E).

Table 3 Effects of time of storage (at 4°C) on particle size and entrapment efficiency of two SLN formulations

SLN	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
SLN A			
0 day	89.3 ± 11.2	−32.8 ± 1.9	96.0 ± 0.32
6 months	101.3 ± 4.1	−31.8 ± 1.7	95.1 ± 0.30
One year	105.0 ± 10.1	−26.8 ± 2.5	94.7 ± 0.45
SLN D			
0 day	158.3 ± 8.8	−24.3 ± 1.1	96.8 ± 0.25
6 months	195.1 ± 7.3	−21.6 ± 0.4	96.2 ± 0.36
One year	226.8 ± 5.7	−21.7 ± 0.3	95.1 ± 0.15

Data are means ± s.d, n = 4.

fallen by about 2%. Other SLN formulations showed similar results (data not shown).

The ATRA emulsion, after storage under the same conditions for only few days, was highly unstable with the appearance of orange crystals in the emulsion. The ATRA solution was more labile because microcrystals appeared about 1 h after preparation.

Release of SLNs

ATRA release from the two SLN formulations and diffusion from ATRA solution are shown in Figures 4A–C. The rate at which ATRA was released from SLN A and SLN D was affected by the pH of the dissolution medium; the release rate increased as the pH increased. From the experimental data, we can see that in the same dissolution media, SLN A always released ATRA faster than SLN D. The difference was significant for 96 h with SLN A and SLN D ($P < 0.05$). This may be due to the stronger solubilization of Tween 80 compared with Pluronic F 68. The diffusion rate of ATRA solution was slow in all three

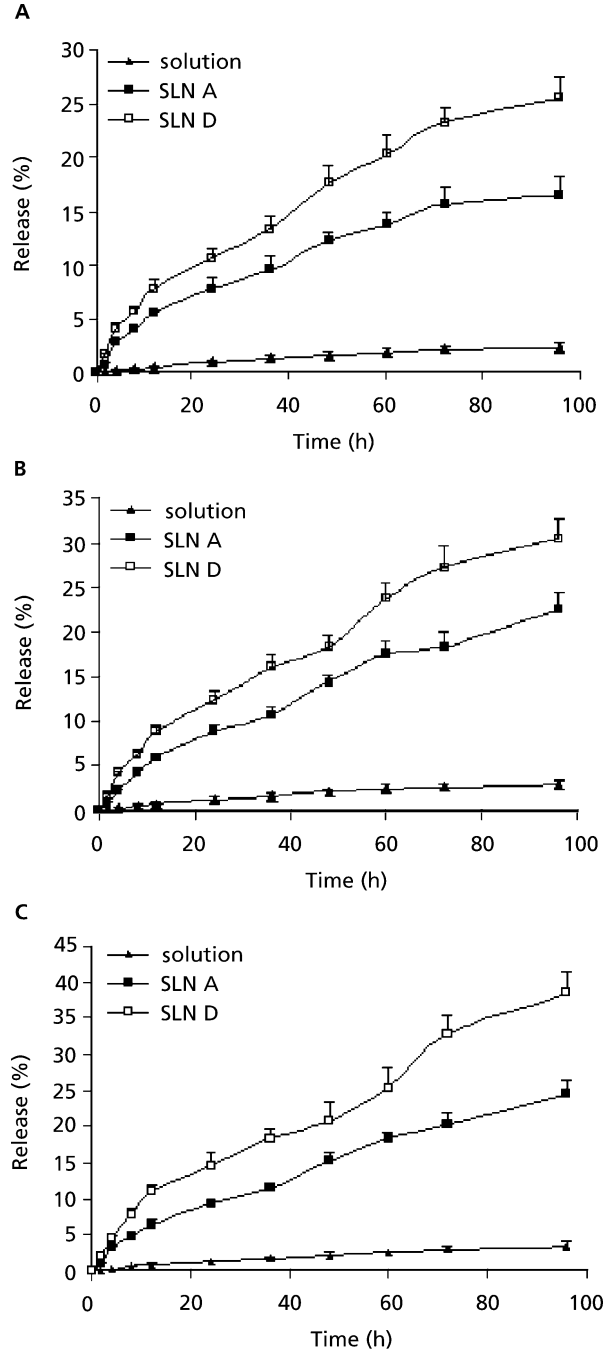

Figure 4 In-vitro release of ATRA from SLN A, SLN D and ATRA solution in three different dissolution media: 0.1 M HCl (A); distilled water (B); and phosphate buffer, pH 7.4 (C). Data are means ± s.d., n = 3.

media. From these results, we concluded that the surfactants (Tween 80 and Pluronic F 68) made an important contribution to the differences between the release from the two SLN formulations and diffusion from ATRA solution. Surfactants diffused into the receiver side altered the barrier properties of the aqueous boundary layer and the permeability of the membrane, resulting in

a high release velocity of ATRA in SLN dispersion. The ATRA solution would not have this effect. In addition, the concentration of ATRA in SLN dispersion was close to saturation (maximal thermodynamic activity), while in ATRA solution, although the overall concentration of ATRA was identical with that in SLN dispersion, with the appearance of microcrystals the real concentration of drug dissolved in solution would be greatly lowered, since thermodynamic activity is the driving force for transport, so the diffusion of ATRA into the receiver side was slow compared with that in the SLN dispersion.

In-vivo pharmacokinetic study

The oral concentration–time curve after a single dose of different ATRA formulations in rats is shown in Figure 5A–C. The oral pharmacokinetic parameters are listed in Table 4.

Figure 5A shows the concentration–time curve of SLN A, SLN D, ATRA solution and ATRA emulsion. At all time points, the ATRA plasma concentrations were significantly higher ($P < 0.05$) for rats treated with ATRA-SLN than for those treated with ATRA solution. The C_{max} values of ATRA in SLN A and SLN D were higher ($7.68\,\mu g\,mL^{-1}$ and $6.46\,\mu g\,mL^{-1}$, respectively) than that obtained with the solution ($1.81\,\mu g\,mL^{-1}$). Twelve hours after oral administration, the ATRA plasma concentrations were still $0.35\,\mu g\,mL^{-1}$ and $0.24\,\mu g\,mL^{-1}$, whereas they were undetectable 6 h after administration of ATRA solution. Incorporation into SLNs resulted in increased absorption of ATRA. The $AUC_{0-\infty}$ values of ATRA after oral administration of SLN A and SLN D were 5.12- and 4.13-fold higher than those obtained with the ATRA solution. From these results, we can conclude that ATRA absorption was enhanced significantly by employing the SLN formulations compared with an ATRA solution.

As far as the ATRA emulsion was concerned, the results show that the oral bioavailability of ATRA was also significantly enhanced, resulting in an increase in $AUC_{0-\infty}$ of about 2.69 fold compared with ATRA solution.

In the subsequent studies, the ATRA-SLN containing different amounts of Pluronic F68 (SLN D 3.0%, SLN E 2.0%, SLN F 1.0%) and Tween 80 (SLN A 3.0%, SLN B 2.0%, SLN C 1.0%) were employed to investigate the effect of surfactant on ATRA absorption enhancement. Figure 5B–C shows the plasma concentration vs time profiles following administration of these different formulations. The oral pharmacokinetic parameters are also given in Table 4.

On increasing the concentration of Pluronic F 68 from 1.0 to 2.0%, a significant absorption enhancement occurred ($P < 0.05$). When Pluronic F 68 was further increased to 3.0%, the $AUC_{0-\infty}$ also increased. The absorption was greatly improved by increasing the amount of Pluronic F68 ($P < 0.05$). On increasing the concentration of Tween 80 from 1.0 to 2.0%, the $AUC_{0-\infty}$ increased from $24.77 \pm 3.69\,\mu g\,h\,mL^{-1}$ to $33.75 \pm 4.91\,\mu g\,h\,mL^{-1}$. The absorption was greatly improved ($P < 0.05$) but when Tween 80 was further increased to 3.0%, there was no significant difference either in the $AUC_{0-\infty}$ or the C_{max} values of ATRA compared with formulations containing 2.0% Tween 80.

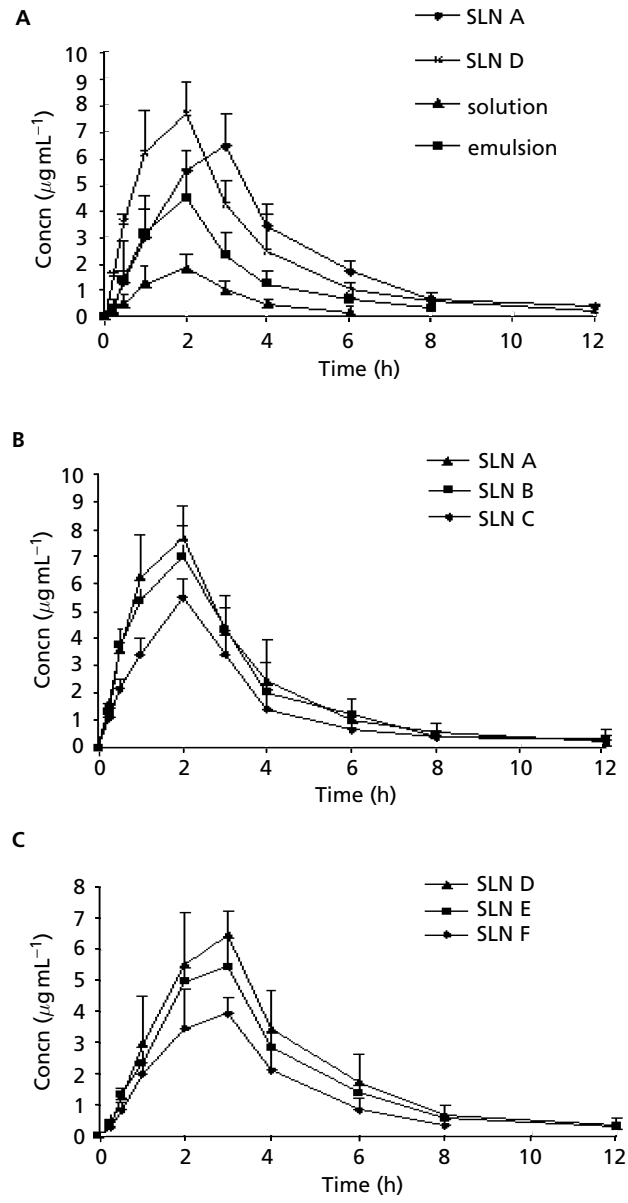

Figure 5 The mean concentration–time curve after a single oral administration of different ATRA formulations in rats ($8\,mg\,kg^{-1}$). A. SLN A, SLN D and ATRA emulsion and ATRA solution. B. ATRA-SLN containing different amount Tween 80. C. ATRA-SLN containing different amount Pluronic F68. Data are means ± s.d., n = 6.

Discussion

Although many techniques have been employed to improve the oral absorption of poorly soluble drugs, little information is available in the literature on the improvement in the oral absorption of poorly soluble drugs by

Table 4 Pharmacokinetic parameters of ATRA after oral administration of different formulations

Parameters	SLN A	SLN B	SLN C	SLN D	SLN E	SLN F	ATRA emulsion	ATRA solution
C_{max} ($\mu g\,mL^{-1}$)	7.68 ± 1.21*	6.98 ± 0.50*	5.47 ± 0.72*	6.46 ± 0.75*	5.44 ± 1.27*	3.92 ± 0.49*	4.49 ± 1.05*	1.81 ± 0.57
T_{max} (h)	2.16 ± 0.75	1.83 ± 0.41	2.33 ± 0.52	3.33 ± 0.52	3.33 ± 0.81	3.16 ± 0.75	1.83 ± 0.75	1.50 ± 0.54
K_e (h^{-1})	0.57 ± 0.09*	0.47 ± 0.10*	0.29 ± 0.08*	0.83 ± 0.09	0.68 ± 0.07*	1.03 ± 0.16	0.85 ± 0.07	0.92 ± 0.14
AUC ($\mu g\,h\,mL^{-1}$)	35.44 ± 4.07*	33.75 ± 4.91*	24.77 ± 3.69*	28.61 ± 4.34*	24.35 ± 3.37*	16.37 ± 2.66*	18.58 ± 3.18*	6.92 ± 1.21
MRT	3.22 ± 0.47	3.44 ± 0.54	3.67 ± 0.38	3.55 ± 0.32	3.58 ± 0.71	3.04 ± 0.48	3.00 ± 0.57	2.64 ± 0.48
CL ($L\,h^{-1}$)	0.055 ± 0.01*	0.06 ± 0.01*	0.08 ± 0.02*	0.07 ± 0.01*	0.083 ± 0.020*	0.12 ± 0.02*	0.108 ± 0.030*	0.543 ± 0.030
V (L)	0.10 ± 0.03*	0.126 ± 0.020*	0.278 ± 0.060*	0.084 ± 0.070*	0.121 ± 0.040*	0.119 ± 0.040*	0.127 ± 0.050*	0.314 ± 0.050
F_r	5.24 ± 1.00*	4.86 ± 1.60*	3.65 ± 0.68*	4.20 ± 0.69*	3.60 ± 0.72*	2.43 ± 0.60*	2.77 ± 0.73*	1

Data are means ± s.d., n = 6. *$P < 0.05$ compared with ATRA solution.

incorporation into SLNs. Our laboratory has carried out detailed studies of SLNs for solubilization and improving the oral absorption of poorly soluble drugs. The exciting results we obtained showed that ATRA absorption was enhanced significantly by employing SLNs. It appears that SLNs offer a promising delivery system for enhancing the oral absorption of poorly soluble drugs. We will try to summarize the possible mechanisms by which the oral absorption of ATRA is improved by SLNs.

Like other formulations, such as microemulsions or submicron emulsions, reduction in the particles size is a key factor for improving the peroral performance of poorly soluble drugs. In SLN formulations, the particle size range was reduced to less than 400 nm, resulting in an increase in surface area and saturation solubility. In-vitro release tests have confirmed that the release velocity from SLNs is significantly faster than the diffusion rate of ATRA solution. An increase in saturation solubility and, consequently, an increase in the release rate of the drug allows it to reach high concentrations in the gastrointestinal tract. Drug absorption from the gastrointestinal tract (i.e. the intestine membrane transfer) may be depicted by a passive diffusion, where the driving force for diffusion across the membrane is the concentration gradient, so that a high local concentration can increase drug absorption. While considering the ATRA solution, after preparation for about 1 h, orange microcrystals appeared in the solution with a relatively slow dissolution rate, so we predicted that the drug might precipitate at the gut wall after administration and thus result in a reduced oral absorption.

In our study, Tween 80 and Pluronic F-68 were used in the SLN formulations. Firstly, they were essential for producing SLNs of a small size and good physical stability; secondly, in in-vivo studies, we found that they also played a key role in the oral absorption of ATRA.

Due to their small particle size, SLNs may exhibit bioadhesion to the gastrointestinal tract wall or enter the intervillar spaces thus increasing their residence time in the gastrointestinal tract. This increase in adhesion will result in enhanced bioavailability. An interesting result is that SLN formulations containing Pluronic F-68 prolong the absorption time when compared with SLN formulations containing Tween 80 or ATRA solution. This may be due to the fact that SLN formulations containing Pluronic F-68 exhibit high bioadhesion and remain for a long time in the gastrointestinal tract.

On increasing the amount of Tween 80 and Pluronic F-68, it seems that there was not only a reduction in particle size, but also an improvement in the absorption of ATRA. The surfactants may have contributed to an increase in the permeability of the intestinal membrane or improved the affinity between lipid particles and the intestinal membrane. Some particles may be taken up into the lymphatic organs and eventually enter the systemic circulation (René et al 2001; O'Driscoll 2002; Nishimukai et al 2003). A number of studies have reported an improvement in the oral absorption of poorly soluble drugs by co-administration of various P-glycoprotein inhibitors and cytochrome P450 (CYP) 3A inhibitors (Zhang & Benet 2001). Administration of CYP3A inhibitors, such as ketoconazole and liarozole, can increase the plasma concentrations of ATRA in animal models and in man (Van Wauwee et al 1988, 1990; Muindi et al 1994). Tween 80 and Pluronic F-68 might moderately inhibit the P-glycoprotein efflux system (Seeballuck et al 2003; Zhang et al 2003), leading to the improved oral absorption of ATRA. We hypothesize that ATRA oral absorption may be further improved by co-administration of a P-glycoprotein inhibitor.

Another advantage of SLN formulations over ATRA solution is the lipid protection of the drug from chemical as well as enzymatic degradation, thereby delaying the in-vivo metabolism. ATRA is rapidly metabolized in-vivo to a variety of oxidized and conjugated metabolites (Regazzi et al 1997). Different CYP isozymes are able to catalyse the 4-hydroxylation of retinoic acid, the main pathway of ATRA metabolism. By incorporation into nanoparticles, ATRA can be embedded into a solid lipid matrix thus reducing its exposure to enzymatic degradation following absorption.

From the statistical analysis of in-vivo pharmacokinetic data, we concluded that SLNs improved the bioavailability of ATRA significantly compared with ATRA solution and the amount of surfactant also significantly enhanced the oral absorption of ATRA in SLNs.

Many reports have shown that the use of submicron emulsions was a promising way to improve oral drug absorption (Georges et al 2003). In our study, an ATRA emulsion formulation was also prepared and its oral absorption was investigated. Although the oral bioavailability was improved, it was highly unstable. The aqueous solubility of ATRA is almost nil (8×10^{-5} mg mL^{-1}) (Montassier et al 1997) and its solubility in lipophilic solvents, such as soybean oil, is also quite low and precludes the use of simple oil-in-water emulsion formulations. We made many attempts to increase its stability but the results were all unsatisfactory. In SLNs, no precipitation of ATRA was observed after one year of storage.

It is well known that ATRA is unstable under light and heat. SLNs were prepared quickly in a dark room and during their preparation, ATRA was subjected to heat exposure. After the preparation of SLNs, SLN samples were analysed by HPLC and the results showed that no ATRA decomposition occurred in any of the SLN formulations.

High-pressure homogenization has emerged as a reliable and powerful technique for the preparation of SLNs. Homogenizers of different sizes are commercially available from several manufacturers at reasonable prices. High-pressure homogenization has been used for years for the production of nanoemulsions for parenteral nutrition, and a large-scale production line has been designed, having a capacity of 50–150 kg SLN dispersion per h. In contrast to other techniques, scaling up represents no problem in most cases (Müller et al 1997, 2000; Gohla & Dingler 2001; Dingler & Gohla 2002).

The use of SLNs opens up new perspectives for the formulation of poorly soluble drugs. The poor aqueous solubility of ATRA also makes it difficult to prepare parenteral formulations. To date, only a liposomal parenteral formulation is available for clinical use (Estey et al 1996). Some studies report that SLNs also have the potential for parenteral administration (Yang et al 1999). So, the SLN formulations can not only improve the oral absorption of ATRA, but can also be formulated for parenteral use, and further studies of this field are needed.

Conclusion

In our study, a poorly aqueous-soluble drug ATRA was successfully incorporated into SLNs by a high-pressure homogenization technique. The physicochemical characterization and long-term physical stability were investigated. The in-vitro release tests showed that the release velocity of the SLNs is always fast compared with the diffusion rate of the ATRA solution. An oral pharmacokinetic study was conducted in male rats and the results showed that SLNs produced a significant improvement in the bioavailability of ATRA compared with ATRA solution. The amount of surfactant also had an important influence on the oral absorption of ATRA. Compared with the ATRA emulsion, which also produces an improvement in oral absorption, SLNs exhibit a high physical stability. The absorption mechanisms of the SLN formulations are discussed. It appears that SLNs offer a promising delivery system for the enhancement of the bioavailability of poorly soluble drugs, and are suitable for large-scale production.

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