Drug Development and Industrial Pharmacy, 31:551–556, 2005
Copyright © 2005 Taylor & Francis Inc.
ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500214761

Taylor & Francis
Taylor & Francis Group

Preparation and Pharmacokinetic Evaluation of Tashinone IIA Solid Lipid Nanoparticles

Jianping Liu, Jiabi Zhu, Zhiyong Du, and Bin Qin
Department of Pharmaceutics, China Pharmaceutical University, Nanjing, P.R. China

ABSTRACT Tashinone IIA loaded solid lipid nanoparticles (TA-SLN) coated with poloxamer 188 was prepared by emulsification/evaporation. The TA-SLN was characterized by transmission electron microscope and dynamic light scattering (DLS). The results showed that the TA-SLN had an average diameter of 98.7 nm with a zeta potential of −31.6 mv and the drug loading of 4.6% and entrapment efficiency of 87.7%. In vitro release experiment showed that the release of Tashinone IIA from TA-SLN was in accordance with the Weibull equation. The best model fitting experimental data was a two-compartment open model with first-order. The area under curve of plasma concentration–time (AUC) and mean residence time (MRT) of TA-SLN were much higher than those of Tashinone IIA control solution (TA-SOL). The results of pharmacokinetic studies in rabbits indicated that the formulation of TA-SLN was successful in providing a delivery of slow release of Tashinone IIA.

KEYWORDS Tashinone IIA, Solid lipid nanoparticles, In vitro release, Pharmacokinetics

INTRODUCTION

Tashinone IIA (Fig. 1), one of the liposoluble components isolated from the root of red-rooted salvia, is an effective cardiovascular agent which dilates coronary arteries and increases myocardial contractility. However it has a relatively low bioavailability due to its negligible solubility in water and short half-life (1–2 h). Tashinone IIA is prone to decompose under lighting and high temperature (>80°C) resulted readily in loss of its activity (Su et al., 1997).

Solid lipid nanoparticles (SLN) as colloidal drug carriers combine the advantages of polymeric nanoparticles, fat emulsions, and liposomes (e.g., physical stability, protection of incorporated labile drugs from degradation, controlled release, and excellent tolerability) and simultaneously avoid some of their disadvantages (Wissing et al., 2004). Solid lipid nanoparticles possess a solid lipid matrix which is consisted of biodegradable compounds that have lower toxicity compared to other synthetic biodegradable polymers. These carriers were observed to change the pharmacodynamic and pharmacokinetic fates of the active molecules when administered in vivo. The bioavailability of

Address correspondence to Jianping Liu, Department of Pharmaceutics, China Pharmaceutical University, 24 Tong Jia Xiong, Nanjing 210009, P.R. China; Fax: 0086-(25)-83301606; E-mail: liujianpingljp@hotmail.com

Order reprints of this article at www.copyright.rightslink.com

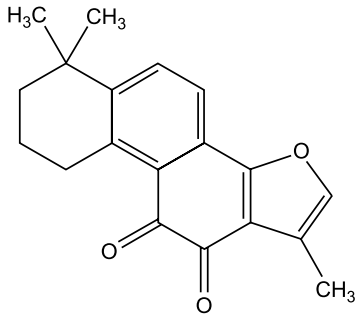

FIGURE 1 **The Structure of Tashinone IIA.**

poor water soluble drugs could be improved when these drugs are encapsulated in lipid-based vehicles via per oral route (Zara et al., 1999).

In this present work, SLN were selected to develop as Tashinone IIA sustained release vehicles. We assessed the feasibility of Tashinone IIA loaded TA-SLN to prolong release and enhance bioavailability of drug. After intravenous injection of TA-SLN, the concentrations of Tashinone IIA in plasma of rabbits were determined by high performance liquid chromatographic (HPLC). The pharmacokinetics of free and nanoparticle encapsulated Tashinone IIA were compared.

Materials

Pure soybean lecithin of medical grade was obtained from TaiWei Co. Ltd., Shanghai, China. Poloxamer 188 medical grade was purchased from BASF Company (Ludwigshafen, Germany). Tashinone IIA was purchased from Luoyang Pharmaceutical Co. Ltd., Henan, China. All reagents used were of analytical grade except methanol of chromatographic grade.

Preparation of TA-SLN

An optimized result of orthogonal design experiments based on the effects of single factor was described as follows. Tashinone IIA (10 mg) and stearic acid (15%) were dissolved in 10 ml of acetone absolutely by sonication (DL-720, Shanghai, China), then soybean lecithin (43%) dissolved in absolute ethanol was added to form a lipid phase. An aqueous phase was prepared by dissolving poloxamer 188 (250 mg) and glycerin (3.7 g) in distilled water. The lipid phase was injected slowly into the aqueous phase which was previously heated to (65 ± 2)°C, forming a semi-transparent presuspension under magnetic agitation. The organic solvent was evaporated at 65°C under reduced pressure. The dispersions were filtered through 0.45 μm filters and allowed to cool down to 0 ~ 2°C quickly to form a TA-SLN suspension.

Tashinone IIA control solution (TA-SOL) were prepared by dissolving Tashinone IIA (10 mg) in an aqueous solution containing Tween-80 and ethanol.

TA-SLN Characterization

Visualization by Transmission Electron Microscopy (TEM)

TA-SLN was examined by negative stain method. A drop of the sample was applied to a filmed-coated copper grid. Phosphotungstic acid (PTA) solutions were then dropped onto the grid. The stained sample was examined by transmission electron microscopy (H-7000, Hitachi, Japan).

TA-SLN Size and Zeta Potential

Particle size and zeta potential were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern zetamaster, 3000HSa, Malven, UK).

Determination of Drug Loading and Entrapment Efficiency

The TA-SLN was separated from free drug by Sephadex-G50 column (1.5 × 20 cm) to assay the entrapment efficiency of TA-SLN. Suspension of 1 ml TA-SLN was eluted by distilled water in Sephadex-G50 column. The opalescence part of the eluate was collected. Both concentration of Tashinone IIA in eluate collected and in the suspension were assayed by HPLC respectively after dilution with anhydrous ethanol. Entrapment efficiency can be calculated by the following formula:

$$E\ (\%) = C/C_0 \times 100\%$$

where E is entrapment efficiency, C is amount of drug encapsulated, and C_0 is the total amount of drug in the TA-SLN suspension.

Drug Release of TA-SLN

In vitro Tashinone IIA release from TA-SLN was evaluated using dialysis bag diffusion technique. Dialysis bags with a molecular weight cut off of 12,000 (Sigma) were filled with 4 ml of TA-SLN suspension, and then placed into 400 ml 0.5% Tween-80 aqueous

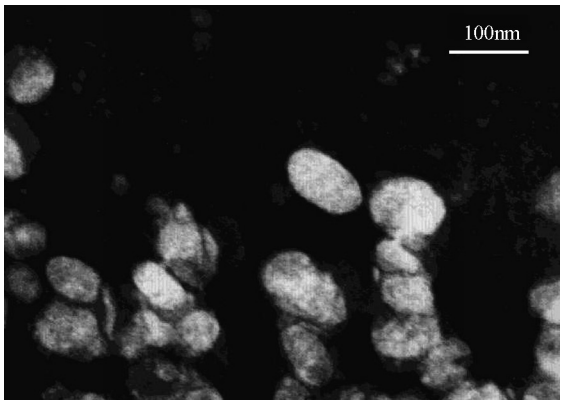

FIGURE 2 **Microphotograph of TA-SLN by Transmission Electron Microscope (× 100,000).**

solution. In vitro Tashinone IIA release was performed at 37°C using a RC Drug Dissolution Tester (Tianjin Medical Instrumental Factory, Tianjin, China) with the paddle rotation at 100 rpm. Samples were taken from the outer solution, then added with the same volume of fresh dissolution medium each time. The sample was analyzed by HPLC for Tashinone IIA.

Pharmacokinetic Study in Rabbits

New Zealand rabbits (2.0 ± 0.9 kg, $n=10$) were purchased from Experimental Animal Center of China Pharmaceutical University and approved by the Ethic Committee by the university. The rabbits were randomly divided into two groups and fasted overnight. The TA-SLN suspension and TA-SOL were administered by auricular vein injection (1 mg/kg). Plasma samples (1.5 ml) were collected in auricular vein initially and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h after dosing. Plasma was separated by centrifugation at 2500 rpm for 10 min and analyzed for Tashinone IIA.

HPLC Analysis of Tashinone IIA

The level of Tashinone IIA in plasma samples was determined by a sensitive and specific HPLC (Shimadzu LC-10A, Kyoto, Japan) with ultraviolet (UV) detection. The mobile phase was a mixture (85:15 v/v) of methanol and double distilled water with a flow rate of 1 ml min^{-1}. The detection wavelength was fixed at 270 nm. Plasma samples for analysis (0.5 ml) were mixed with 5 ml ethyl acetate and then vortexed for 1 min. The solution was centrifuged at 2500 rpm for 10 min. The supernatant was collected and evaporated to remove the solvent under N_2. The residual was dissolved in 200 μl of methanol and vortexed for 1 min. The resulting supernatant was injected into the HPLC.

The blank plasma samples spiked with triplicate concentrations of Tashinone IIA reference solutions were analyzed with HPLC as described above. The mean relative recovery was evaluated by comparing the calculated concentration and measured concentration.

The chromatograms of Tashinone IIA showed a stable baseline and good resolution between Tashinone IIA and endogenous material in plasma. The lowest detectable limit of Tashinone IIA was 0.015 μg/ml. The assay was linear in range 0.031–1.24 μg/ml. The mean regression equation for five replicate calibration curves on different days was $C=0.00001777\ A+0.006604$ ($r=0.9997$). The mean relative recovery of Tashinone IIA in rabbit plasma was 101.2%. Intra- and inter-day variabilities were $<5\%$.

Data Analysis

The release profiles of Tashinone IIA from SLN were fitted with weibull equation. The pharmacokinetic parameters associated with each rabbit were estimated by compartment and non-compartment methods. Nonlinear regression analysis showed that the best model fitting experimental data was a two-compartment open model with first-order input and output from the central compartment. Pharmacokinetic parameters in plasma were obtained from the pooled concentration-time data of each experiment with statistical software. The area under the concentration-time curve ($AUC_{0\rightarrow\infty}$) was calculated using the linear trapezoidal rule, and extrapolated to infinity by dividing the last measurable concentration by the elimination rate constant. The area under the first moment curve (AUCM) was calculated by the trapezoidal rule for infinity time. Mean residence time (MRT) was determined by dividing the AUMC by the AUC.

RESULTS AND DISCUSSION

TA-SLN Characterization

Visualization by Transmission Electron Microscopy (TEM)

Micrograph of TA-SLN negatively stained by PTA and observed by TEM showed round vesicles (Fig. 2).

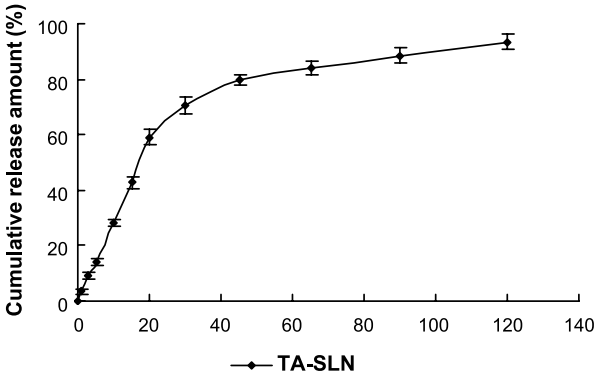

FIGURE 3 TA-SLN Release Curve In Vitro ($n=5$).

TA-SLN Size and Zeta Potential

The size distribution of TA-SLN as measured by DLS shows one narrow peak indicating that the nanoparticles population is relatively homogenous in size. The calculated mean size of TA-SLN, based on three separate measurements, is 98.7 ± 26.7 nm. The surface carried a negative charge with a zeta potential of -31.6 ± 5.3 mv ($n=3$). Drug loading was $4.6 \pm 0.5\%$ ($n=3$), and entrapment efficiency was $87.73 \pm 0.3\%$ ($n=3$).

In Vitro Release

In the present experiments, dynamic dialysis was chosen for separation of free drug from drug loaded nanoparticles. Figure 3 showed the Tashinone IIA release from SLN in 0.5% Tween-80 aqueous solution. It can be seen that the release was best described by the Weibull equation and the following regression equation was given: $\ln [-\ln (1-Q)] = 0.7908 \ln t - 2.7408$ ($r=0.9799$). The release profile was biphasic. The initial fast release was observed in the beginning of 20 h, due to the large surface area of the nanoparticles and drug enrichment in the outer shell of the particles. In the latter stage, drug release constantly and slowly was attributed to lipophilic Tashinone IIA solubilized or dispersed in lipid matrices, and therefore released mainly by dissolution and diffusion of drug from the matrices (Grassi et al., 2003). Similar results have been obtained for etomidate SLN (Zur Mühlen et al., 1998) and indomethacin nanospheres (Niwa et al., 1993).

Pharmacokinetics of TA-SLN

Figure 4 shows that the plasma drug levels versus time curves after administration of TA-SLN and TA-SOL. Compared with TA-SOL, the higher plasma concentration of TA-SLN after intravenous administration might be due to the small size of nanoparticles. Similar results were reported by Kurihara et al. (1996b) for palmitoyl rhizoxin lipid emulsion. It was found that the behavior of the emulsion particles in the body is dependent on the size of the emulsions. Small size emulsions (100–110 nm) showed the highest plasma concentrations of palmitoyl rhizoxin after intravenous injection (Kurihara et al., 1996a).

The pharmacokinetic parameters of Tashinone IIA in plasma is shown in Table 1. Compared with TA-SOL, the AUC of TA-SLN was 3.324 for the dose of 1 mg/kg. The MRT increased 3.866 times in plasma compared with the same dose of TA-SOL, which may be due to the coating of poloxamer 188 on the surface

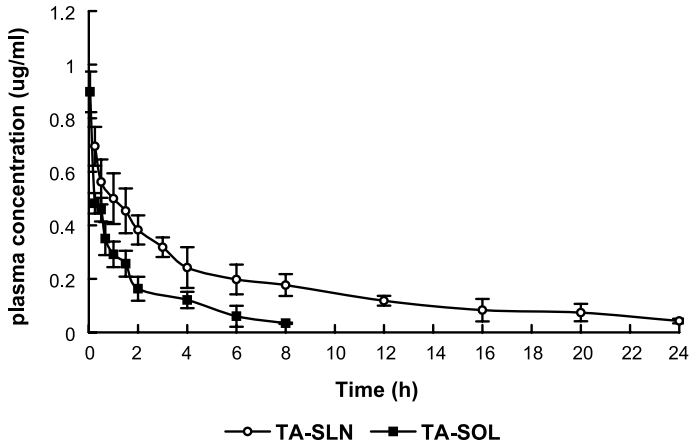

FIGURE 4 Mean Plasma Concentration-Time Curves of Tashinone IIA in 10 Rabbits After i.v. Injection of TA-SLN (1 mg kg^{-1}) and TA-SOL (1 mg kg^{-1}).

TABLE 1 Pharmacokinetic Parameters Based on Statistic Moment Theory of TA-SLN and TA-SOL

	TA-SLN		TA-SOL		
Parameter	Mean	SD	Mean	SD	*P*
AUC (mg × H/L)	4.644	0.576	1.397	0.255	< 0.001
AUMC (mg × h^2/L)	53.38	4.237	4.242	0.933	< 0.001
S2	1497	583.8	29.82	7.463	< 0.01
MRT (h)	11.70	2.215	3.026	0.270	< 0.001
VRT (h^2)	197.7	122.7	12.21	3.115	< 0.05

TABLE 2 Pharmacokinetic Parameters of TA-SLN and TA-SOL After Intravenous Administration

	TA-SLN		TA-SOL		
Parameter	Mean	SD	Mean	SD	*P*
A (mg/L)	0.408	0.123	0.689	0.154	< 0.05
B (mg/L)	0.338	0.069	0.460	0.037	< 0.05
α (1/h)	0.796	0.250	8.740	3.364	< 0.01
β (1/h)	0.173	0.169	0.408	0.091	< 0.01
Vc	1.350	0.134	0.884	0.119	< 0.01
$t_{1/2}$ α (h)	0.944	0.324	0.093	0.045	< 0.01
$t_{1/2}$ β (h)	8.071	1.185	1.766	0.404	< 0.001
k_{21} (1/h)	0.429	0.213	3.739	1.296	< 0.01
k_{10} (1/h)	0.171	0.042	0.927	0.129	< 0.001
k_{12} (1/h)	0.284	0.105	4.481	2.485	< 0.05
AUC (mg × h/L)	4.450	0.934	1.267	0.328	< 0.001
CLs (L/h)	0.234	0.051	0.827	0.200	< 0.01

of TA-SLN and the sustained release of Tashinone IIA from TA-SLN. Poloxamer 188 coated Camptothecin nanoparticles remained in the circulation and organ for an extended period of time (Yang et al., 1999). Clozapine nanoparticles coated with poloxamer 188 exhibited prolonged release time (Venkateswarlu & Manjunath, 2004). In Table 2, terminal half life (t1/2) and clearance ratio (Cls) of Tashinone IIA in plasma with free drug solution was about 1.766 h and 0.827 L/h, respectively, whereas with SLN, a 4.570 fold increase in t1/2 and 3.534 fold decrease in Cls were observed. The data indicated the elimination of Tashinone IIA was delayed when incorporated into SLN.

Tashinone IIA is prone to decompose under lighting and high temperature. Tashinone IIA also has a short half life and its conventional dosage form, such as injections and tablets, showed a short effect time and low bioavailability. Owing to the change in pharmacokinetics of Tashinone IIA, such as the prolongation of half life, mean residual time, and the enhancement of AUC, Tashinone IIA incorporated into SLN indicated that TA-SLN could protect the active Tashinone IIA from the influence of high temperature and light, and could improve the plasma concentration and treatment efficacy.

CONCLUSIONS

In vitro release test showed that TA-SLN exhibited sustained release and the data were well described by the Weibull equation. Compared with TA-SOL, TA-SLN showed a higher bioavailability, a prolonged residence time, and half life. The pharmacokinetic studies in rabbits after intravenous administration of TA-SLN and TA-SOL showed that the AUC of TA-SLN was superior to that of TA-SOL.

REFERENCES

Grassi, M., Voinovich, D., Franceschinis, E., Perissutti, B., & Filipovic-Grcic, J. (2003). Theoretical and experimental study on theophylline release from stearic acid cylindrical delivery systems. *Journal of Controlled Release*, *92*(3), 275–289.

Kurihara, A., Shibayama, Y., Mizota, A., Yasuno, A., Ikeda, M., Sasagawa, K., Kobayashi, T., & Hisaoka, M. (1996a). Enhanced tumor delivery and antitumor activity of palmitoyl rhizoxin using

stable lipid emulsions in mice. *Pharmaceutical Research*, *13*(2), 305–310.

Kurihara, A., Shibayama, Y., Mizota, A., Yasuno, A., Ikeda, M., & Hisaoka, M. (1996b). Pharmacokinetics of highly lipophilic antitumor agent palmitoyl rhizoxin incorporated in lipid emulsions in rats. *Biological & Pharmaceutical Bulletin*, *19*(2), 252–258.

Niwa, T., Takeuchi, H., Hino, T., Kunou, N., & Kawashima, Y. (1993). Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with D,L-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *Journal of Controlled Release*, *25*(1–2), 89–98.

Su, Z. R., Liu, Z. Q., & Zhou, H. (1997). Change of chemical composition of tashinone in concentration and drying technology (1)—degradation mechanism of Tashinone IIA under the heat and moisture. *Chinese Traditional Patent Medicine*, *19*(11), 5–6.

Venkateswarlu, V., & Manjunath, K. (2004). Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. *Journal of Controlled Release*, *95*(3), 627–638.

Wissing, S. A., Kayser, O., & Müller, R. H. (2004). Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews*, *56*(9), 1257–1272.

Yang, S. C., Zhu, J. B., Lu, Y., Liang, B. W., & Yang, C. Z. (1999). Body distribution of camptothecin solid lipid nanoparticles after oral administration. *Pharmaceutical Research*, *16*(5), 751–757.

Zara, G. P., Cavalli, R., Fundaro, A., Caputo, O., & Gasco, M. R. (1999). Pharmacokinetics of doxorubicin incorporated in solid lipid nanoparticles (SLN). *Pharmacological Research*, *40*(3), 281–286.

zur Mühlen, A., Schwarz, C., & Mehnert, W. (1998). Solid lipid nanoparticles (SLN) for controlled drug delivery—drug release and release mechanism. *European Journal of Pharmaceutics and Biopharmaceutics*, *45*(2), 149–155.

Copyright of Drug Development & Industrial Pharmacy is the property of Marcel Dekker Inc.. The copyright in an individual article may be maintained by the author in certain cases. Content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.