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Improvement of digoxin oral absorption in rabbits by incorporation into solid lipid nanoparticles

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In this study, digoxin (DG)-loaded solid lipid nanoparticles (DG-SLNs) were successfully prepared by an ultrasonic and high pressure homogenization method. The particle size and distribution, drug loading capacity, drug entrapment efficiency (EE %), zeta potential, and long-term physical stability of the SLNs were characterized in detail. A pharmacokinetic study was conducted in rabbits after oral administration of 0.25 mg DG in different SLNs and it was found that the relative bioavailability of DG in the SLNs was significantly increased compared with that of a DG solution. The addition of CMC-Na in SLNs also markedly increased the oral absorption of DG. These results indicate that DG absorption is enhanced significantly by employing SLN formulations and SLNs are a potential as an oral delivery carrier for poorly water soluble drugs.

1. Introduction

Many new chemical entities identified in drug discovery programs are insufficiently soluble in aqueous media to allow for their adequate and reproducible absorption from the gastrointestinal tract (GI) following oral administration. Oral administration of highly lipophilic, poorly water-soluble drugs often results in poor and highly variable bioavailability due to poor dissolution *in vivo*. Attempts to overcome the solubility problem and improve oral absorption have been investigated in many recent studies (Yang et al. 2004; Itoh et al. 2002; Brocks et al. 2002; Balakrishnan et al. 2009; Kennedy 2008; Chen et al. 2009). One of the promising way is incorporation of the poorly water-soluble drugs in the solid lipid nanoparticles (SLNs).

SLNs have been reported as an alternative drug delivery system to traditional polymeric nanoparticles (Mehnert and Mader 2001). The nanoparticles are in submicron size range and they are composed of physiologically tolerated lipid components, at room temperature the particles are in solid state (Müller and Lucks 1996). SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes (Schwarz and Mehnert 1999). Our previous research showed that SLNs can enhance the absorption and bioavailability of all-trans retinoic acid (Hu et al. 2004). When all-trans retinoic acid was loaded into SLNs, the oral bioavailability in rats were increased four to five-fold compared to that of suspension. The mechanisms of enhanced oral absorption in ATRA-SLNs are mainly attributed to reduction in the particles size and the lipid protection of the drug from chemicals as well as enzymatic degradation. Recently, some studies reported that the bioavailability of poorly hydrophilic and lipophilic drugs can be improved when these drugs are encapsulated in SLNs (Ugazio et al. 2002; Yang et al. 2009; Li et al. 2009).

In this study, digoxin-loaded SLNs were successfully prepared and the physicochemical characteristics of SLNs were investigated. The oral bioavailability of DG-SLNs was compared with that in a solution to assess the feasibility of SLN to enhance the oral bioavailability of DG. The effect of CMC-Na in SLNs on the oral absorption enhancement of DG was studied. The absorption mechanism of the SLNs was also discussed.

2. Investigations, results and discussion

2.1. Characterization of SLNs

TEM shows that the particles had round and uniform shapes. The mean diameters of GMS-SLNs and CP-SLNs were 120.6 ± 10.4 nm, 132.5 ± 13.3 nm, respectively. The zeta potential of GMS-SLNs and CP-SLNs were -21.6 ± 2.1 mV, -25.8 ± 1.6 mV, respectively. SLNs stored at 4 °C were found to be more stable in terms of change in size and entrapment efficiency (Table 1). After 6 months of storage at 4 °C, no dramatic increase in the size of GMS-SLNs and CP-SLNs occurred.

2.2. Pharmacokinetics studies

In this study, all rabbits were clinically healthy and no side effects were observed after oral administration. The calibration

Table 1: Stability studies of SLNs at 4 °C (mean ± S.D., n = 4)

Formulation	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
GMS-SLNs			
0 day	120.6 ± 10.4	-21.6 ± 2.1	93.9 ± 0.53
3 months	124.4 ± 11.8	-24.3 ± 1.7	94.5 ± 0.46
6 months	126.1 ± 14.2	-23.8 ± 2.5	93.7 ± 0.48
CP-SLNs			
0 day	132.5 ± 13.3	-25.8 ± 1.6	96.4 ± 0.42
3 months	131.3 ± 9.7	-27.4 ± 0.9	95.8 ± 0.36
6 months	135.8 ± 10.4	$-24.\ 6 \pm 1.2$	95.4 ± 0.44

Table 2: Pharmacokinetic parameters after after oral administration of DG to rabbits (a) GMS-SLNs (b) CP-SLNs (c) DG-solution (n = 6)

Parameter	GMS-SLNs			CP-SLNs			DG-solution
	0% CMC-Na	0.3% CMC-Na	0.5% CMC-Na	0% CMC-Na	0.3% CMC-Na	0.5% CMC-Na	
Keh^{-1}	0.069 ± 0.03	0.080 ± 0.03	0.075 ± 0.04	0.062 ± 0.03	0.081 ± 0.04	0.075 ± 0.04	0.028 ± 0.02
T1/2h	10.07 ± 1.20	8.61 ± 0.73	9.23 ± 1.04	12.42 ± 1.46	8.47 ± 1.12	9.12 ± 1.24	23.99 ± 2.37
Tmax(h)	1.75 ± 0.27	1.67 ± 0.41	2 ± 0.54	1.50 ± 0.32	1.83 ± 0.68	2.08 ± 0.49	1.58 ± 0.37
Cmax (μg/ml)	12.86 ± 1.71	21.91 ± 3.68	25.81 ± 3.22	14.99 ± 2.19	24.94 ± 2.55	26.74 ± 3.71	4.39 ± 0.58
MRTh	12.78 ± 0.87	11.04 ± 0.58	11.70 ± 0.71	14.05 ± 0.63	10.28 ± 0.58	10.65 ± 0.47	22.92 ± 0.73
AUC0-∞(μg h· mL^{-1})	114.1 ± 12.9	157.2 ± 9.5	178.9 ± 13.4	124.9 ± 13.6	190.7 ± 17.3	222.3 ± 15.1	51.1 ± 18.1
Fr(%)	224 ± 17.6	296 ± 17.6	337 ± 21.6	230 ± 22.5	371 ± 25.8	425 ± 32.5	

curves found to be linear over the range of 0.3–5 ng·mL^{-1} could be described by the equation $A = -0.1411c + 5.4741$, (r = 0.995, n = 6),where A was the logarithm value of polarization; c was the concentration of digoxin in plasma. The limit of quantification was 0.2 ng/mL. The extraction recoveries in plasma ranged from 94.8 to 105.6%. The pharmacokinetic parameters of the two formulations could both be described using non-compartmental model (Table 2) and the pharmacokinetic profiles are shown in Fig. 1. At all time points, the DG plasma concentrations were significantly higher ($p < 0.05$) for rabbits treated with DG-SLNs than for those treated with DG-solution. The C_{max} values of DG in GMS-SLNs and CP-SLNs were higher (12.86 μg/ml and 14.99 μg/ml, respectively) than those obtained with the solution (4.39 μg/ml). Twelve hours after oral administration, the DG plasma concentrations were still 2.24 μg/ml and 2.91 μg/ml, compared to 0.64 μg/ml after administration of DG-solution. Incorporation into SLN resulted in increased absorption of DG. The AUC0$-\infty$ of DG after oral administration of GMS-SLNs and CP-SLNs were 2.24- and 2.30 times higher than those obtained with the DG-solution. These results showed that DG absorption was enhanced significantly by employing the SLN formulations compared with a DG solution.

After the preparation of SLNs, SLN samples were analyzed by HPLC, and the results showed that no DG decomposition or precipitation occurred in any of the SLN formulations after six months of storage. The apparent drug concentration in the SLNs (1000 mg/L) was about 50-fold higher than the aqueous solubility of DG (about 22 mg/L). So in SLNs, drug exists in a supersaturatable state, supersaturatable SLNs are thermodynamically stable dosage forms and no drug crystallized out of the dispersion during storage. An increase in solubility made it reach high concentrations in the GI tract. The intestine membrane transfer of DG can be mostly depicted by a passive diffusion, with the concentration gradient as the driving force for diffusion across the membrane, the increased DG concentration increases drug absorption from the GI tract. While DG from solution might precipitate at the gut wall after administration and thus result in a reduced oral absorption.

The surfactants used in SLNs, such as Tween-80 and poloxamer 188, also contributed to both stability and improved bioavalability to SLNs. They could increase the permeability of the intestinal membrane and improved the affinity between lipid particles and the intestinal membrane. Recent reports suggested that P-glycoprotein can limit the intestinal absorption of digoxin by active transport back into the intestinal lumen (Westphal et al. 2000). An improvement of oral absorption of poorly soluble drugs could be achived by coadministration of various P-glycoprotein inhibitors (Zhang et al. 2001). In this study, therefore, the increase in digoxin AUC and C_{max} after oral administration is most likely due to P-gp inhibition by Tween 80 and poloxamer 188 in the GI tract. Our studies are in accordance with the previous reports by Seeballuck et al. (2003) and Zhang et al. (2003).

In this study, an interesting result is that SLN formulations containing cabroxypropyl methyl cellulose sodium (CMC-Na) improve the absorption when compared with SLNs without it. The difference in the pharmacokinetic profiles exhibited in Figs. 2 and 3 is impressive because these SLNs differ only in

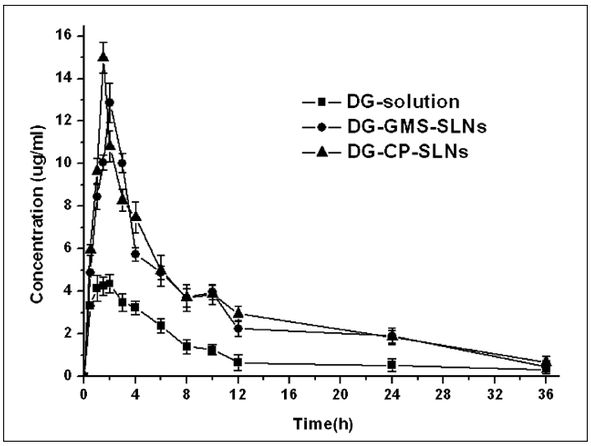

Fig. 1: Mean plasma concentration-time curves (mean ± SD) after oral administration of DG to rabbits (a) GMS-SLNs (without CMC-Na) (b) CP-SLNs (without CMC-Na) (c) DG-solution (n = 6)

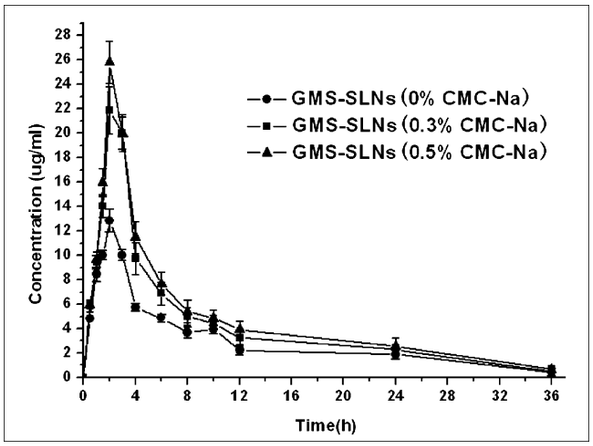

Fig. 2: Mean plasma concentration-time curves (mean ± SD) after oral administration of DG-GMS-SLNs containing different amount of CMC-Na (n = 6)

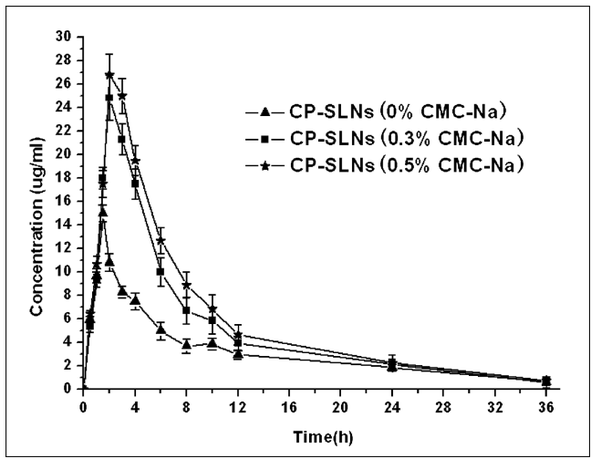

Fig. 3: Mean plasma concentration-time curves (mean ± SD) after oral administration of DG-CP-SLNs containing different amount of CMC-Na (n = 6)

the content of CMC-Na (0% vs. 0.3%, 0.5%). The AUC0−∞ of DG after oral administration of GMS-SLNs and CP-SLNs were 2.24- and 2.30 times higher than those obtained with the DG-solution. The SLNs (with CMC-Na) resulted in a relatively high C_{max} and oral bioavailability (shown in Table 2). This result suggests that incorporation of CMC-Na in the SLNs can sustain the supersaturatable state by preventing precipitation of the drug for a long time or enter the intervillar spaces thus increasing their residence time in the GI tract. This increase in adhesion will result in enhanced bioavalability.

The specific characteristics of the lipid excipients (e.g., degree of esterification of glycerides or fatty acid chain length) chosen for SLNs can also have a important effect on the stability and oral bioavailability of DG and this should be taken into consideration during formulation screening and development. To investigate the effect of the degree of lipid material on drug absorption, SLNs of similar fractional composition, differing only in the lipid excipient used. The results indicated that enhanced bioavailability of DG (2.24 and 2.30 times) was observed with GMS-SLNs and CP-SLNs respectively, in fact, we also prepared DG-SLNs using steric acid as lipid, the SLN are unstable with flocculation and drug precipitation observed after 5 days of storage. The relatively bioavailability of freshly prepared steric acid SLNs was only 124.5% compared with DG solution. The results indicate that the higher oral bioavailability generated by the GMS-SLNs and CP-SLNs as a result of lipid excipients are responsible for the enhanced oral bioavailability of DG from the SLNs and further studies of this field are needed.

In conclusions, in the present study, digoxin-loaded SLN were successfully prepared by ultrasonication followed by homogenization method using different lipids. The DG-SLNs presented as spherically shaped under TEM. The mean diameters and zeta potential of GMS-SLNs and CP-SLNs were 120.6 ± 10.4 nm, 132.5 ± 13.3 nm, and −21.6 ± 2.1 mV, −25.8 ± 1.6 mV, respectively. The surfactant (Tween80/poloxamer) concentration was optimized at 1%/1% based on the particle size and entrapment efficiency. The stability data indicated excellent physical long-term stability in SLNs. Enhanced bioavailability of DG was observed with the two SLN formulations. The addition of CMC-Na in SLNs greatly improves the absorption of DG compared with SLNs without it. The current investigation illustrates that SLNs can be used as a powerful oral delivery approach for improving the oral absorption of digoxin.

3. Experimental

3.1. Materials

Digoxin was supplied by Shijiazhuang Pharmaceutical Co. (Shijiazhuang, China). Glyceryl monostearate and stearic acid was purchased from Tianjin kemio Chemical Reagent Exploitation Center (Tianjin, China). Compritol 888 ATO was bought from Gattefosse Co. (Shanghai, China). Soy lecithin was provided by TaiWei Pharmaceutical Co. (Shanghai, China). Tween 80 was bought from Beijing Yili Fine Chemicals Co. Ltd. (Beijing, China). Poloxamer 188 was obtained from Shanghai XieTai Chemical Industry Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade.

3.2. Preparation of DG–SLNs

DG-SLNs were prepared with an ultrasonic and high pressure homogenization (HPH) method. The desired amounts of drug, lipid matrix and soya lecithin were dissolved completely in a 25 ml mixture of absolute alcohol and acetone (3; 2, v/v) in a water bath at 80 °C. The mixture was sonicated and warmed to obtain a clear melting organic phase. Tween 80 and Pluronic F 68 were dissolved in 50 ml water and then heated to 80 °C to be used as water phase. The organic phase was added dropwise to the water phase at 80 °C followed by magnetic stirring for a few minutes, then 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. After the organic solvent had completely evaporated, 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, stored at 4 °C. DG-SLNs obtained using glyceryl monostearate and Compritol 888 ATO as lipid matrix were abbreviated as GMS-SLNs and CP-SLNs respectively.

3.3. Particle size and zeta potential

The morphology of DG-SLNs was examined using a transmission electron microscopy (TEM, JEM-1200EX, JEOL, Tokyo, Japan). After dilution with doubly-distilled water, the samples were negatively stained with 2% (w/v) osmium tetroxide for observation.

The particle size of DG-SLNs was measured by photon correlation spectroscopy (PCS) using a NICOMP particle sizing system (CW380, Santa Barbara, California, USA) at a fixed angle of 90 degrees and at a temperature of 25 °C. The particle size analysis data were evaluated using the volume distribution. Zeta potentials were measured using the same instrument at electrical field strength of 10 v/cm and at the same temperature. Prior to measurement, SLN dispersions were diluted 20-fold and 50-fold with the original dispersion preparation medium for size determination and zeta potential measurements respectively. All the measurements were performed in triplicate.

3.4. Drug encapsulation efficiency and drug loading

The SLNs were dissolved in methanol to preferentially precipitate the lipid. After centrifugation (4000 rpm for 15 min), the drug content in the supernatant was measured by HPLC analysis using a HPLC pump (L-7100, Hitachi) and and a UV-Vis detector (L-7420, Hitachi, Japan) with a C18 reverse phase column (Diamonsil TM 5 μ C18, 150 × 4.6 mm, Beijing, China). The mobile phase composed of acetonitrile and water (46: 54, v/v) at a flow rate of 1 mL/min, and the effluent was monitored at 230 nm.

The SLN dispersions were subjected to ultracentrifugation (Hitachi CS120GXL Micro Ultracentrifuge, Japan) at 60000 rpm for 4 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:

$$\text{Drug content (\% w/w)} = \frac{\text{amount of DG in the SLNs}}{\text{weight of SLNs}} \times 100 \quad (1)$$

$$\text{Drug Entrapment efficiency (\%)} = \frac{\text{amount of DG in the SLNs}}{\text{amount of DG used in formulation}} \times 100 \quad (2)$$

3.5. Storage stability studies

The DG-SLNs formulations were studied for stability studies at 4 °C. These formulations were determined at regular time intervals for any change in particle size, zeta potential and drug content.

3.6. Pharmacokinetic studies in rabbits

Eighteen healthy New Zealand White rabbits (2.18 ± 0.24 kg) approximately 10 months old were used in this study. All animal experiments complied with the requirements of the National Act of the People's Republic of China on the use of experimental animals.

The rabbits were divided into 3 groups comprising six animals each. All animals were kept for overnight fasting but allowed free access to water. Before oral administration, the restrain devices were placed in the marginal vein each animal. Blood (1 mL) samples were collected from the restrain devices for control (at 0 min). Two types of SLNs and DG solution were orally administered (25 mg DG dispersed in 100 ml mixture of absolute alcohol, propylene glycol and water (1:1:2, v/v)) at a DG dose of 0.25 mg/rabbit. Blood samples were taken from restrain devices of each rabbit and collected in tubes containing heparin as anticoagulant at 0.5 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 h after administration. Samples were centrifuged within 0.5 h after collection and plasma samples were stored at $-20\,^{\circ}$C until analysis.

Rabbit plasma (500 μL) was added to 3% 5-salicylsulfonic acid 50% methanol aqueous solution (500 μL), then vortexed for 30 s, and then centrifuged (4,000 rpm for 10 min). The supernatant were measured by fluorescence polarization immunoassay (TDxFLx; Abbott Laboratories). The area under the concentration-time curve from time zero to time t (AUC0−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: AUC0−∞ = AUC0−t + Ct /Ke, where Ct is the DG concentration observed at last time, and Ke 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 Fr at infinity at the same dose was calculated as: Fr = AUCsln, 0−∞/ AUCsol, 0−∞.

3.7. Statistical analysis

The data obtained from the release rate and pharmacokinetic parameters were analyzed statistically using Student's t-test with $p < 0.05$ as the minimal level of significance.

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