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Research Article

Glyceryl Monooleate/Poloxamer 407 Cubic Nanoparticles as Oral Drug Delivery Systems: I. *In Vitro* Evaluation and Enhanced Oral Bioavailability of the Poorly Water-Soluble Drug Simvastatin

Jie Lai,[1] Jianming Chen,[3] Yi Lu,[2] Jing Sun,[3] Fuqiang Hu,[4] Zongning Yin,[1,5] and Wei Wu[2,5]

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Abstract. Glyceryl monooleate (GMO)/poloxamer 407 cubic nanoparticles were investigated as potential oral drug delivery systems to enhance the bioavailability of the water-insoluble model drug simvastatin. The simvastatin-loaded cubic nanoparticles were prepared through fragmentation of the GMO/poloxamer 407 bulk cubic-phase gel using high-pressure homogenization. The internal structure of the cubic nanoparticles was identified by cryo-transmission electron microscopy. The mean diameter of the cubic nanoparticles varied within the range of 100–150 nm, and both GMO/poloxamer 407 ratio and theoretical drug loading had no significant effect on particle size and distribution. Almost complete entrapment with efficiency over 98% was achieved due to the high affinity of simvastatin to the hydrophobic regions of the cubic phase. Release of simvastatin from the cubic nanoparticles was limited both in 0.1 M hydrochloride solution containing 0.2% sodium lauryl sulfate and fasted-state simulated intestinal fluid with a total release of <3.0% at 10 h. Pharmacokinetic profiles in beagle dogs showed sustained plasma levels of simvastatin for cubic nanoparticles over 12 h. The relative oral bioavailability of simvastatin cubic nanoparticles calculated on the basis of area under the curve was 241% compared to simvastatin crystal powder. The enhancement of simvastatin bioavailability was possibly attributable to facilitated absorption by lipids in the formulation rather than improved release.

KEY WORDS: cubic nanoparticles; glyceryl monooleate; oral bioavailability; poloxamer 407; simvastatin.

INTRODUCTION

Self-assembling of amphiphilic molecules including some lipids in aqueous system is known to form a variety of liquid crystalline phases such as lamellar, inverted hexagonal, and inverted cubic phases (1–3). A cubic phase is composed of contorted bilayers that partition hydrophobic and hydrophilic regions into continuous but nonintersecting spaces, which is mainly known as double-diamond (*Pn3m*), gyroid (*Ia3d*), and primitive (*Im3m*) phases (4,5). Lipids such as phosphatidylcholines, phosphatidylethanolamines, PEGylated phospholipids, and various monoglycerides have been confirmed to form bicontinuous cubic phases, which usually appear as isotropic bulk gels. Those composed of surfactants of appropriate water insolubility can exist in equilibrium with excessive water and be dispersed into cubic nanoparticles, which are also named as cubosomes (6–8).

The unique structure and properties of cubic phases impart them with special interest in drug delivery (9–11). However, their potential as drug carriers has not been fully explored, especially *in vivo*, and most studies are restricted to bulk cubic-phase gels and lipid matrices capable of forming cubic phases upon hydration. Mainly owing to its bioadhesiveness, cubic-phase gel has been evaluated for potential mucosal, periodontal, local, and transdermal drug delivery with improved absorption (10–13). The cubic phase consisting of monolaurin with thermosensitive properties has been confirmed to provide sustained release of poorly water-soluble drugs (14). Liquid crystalline matrices based on oleyl glycerate (OG) was able to maintain plasma cinnarizine, a poorly water-soluble drug, level for over 100 h after oral administration with enhanced bioavailability of 344% (15). There is a demand to expand pharmaceutical applications of cubic phases including cubic nanoparticles by different administration routes.

As oral drug carriers, bulk cubic phases show potential to facilitate oral absorption possibly due to their bioadhesiveness, interaction with cell membrane, or induced secretion of physiological surfactants through lipid diges-

Jie Lai and Jianming Chen contributed equally to this work.

[1] Department of Pharmaceutics, West China School of Pharmacy, Sichuan University, Chengdu 610041, China.
[2] Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai 201203, China.
[3] School of Pharmacy, Second Military Medical University, Shanghai 200433, China.
[4] School of Pharmacy, Zhejiang University, Hangzhou 310058, China.
[5] To whom correspondence should be addressed. (e-mail: yzn@scu.edu.cn; wuwei@shmu.edu.cn)

tion in the gastrointestinal tract (14–16). However, these bulk cubic phases have drawbacks that render them inappropriate for oral use; the bulk gel is difficult to be formulated as oral dosage forms, while the liquid crystalline matrices take a long time for lipid digestion to complete. Cubic nanoparticles, retaining most of the properties of their "parent" cubic-phase gel, are easier to handle and assumed to be advantageous as oral drug carriers (17). Until now, only a few references address cubic nanoparticles as percutaneous, but not oral, drug carriers (18).

The experience with cubic liquid crystalline phases, either gels or nanoparticles, sheds light on the potential use of cubic phases as oral drug delivery carriers. In fact, cubic nanoparticle is one kind of the secondary vehicles (micelles, mixed micelles, cubic nanoparticles, and vesicular carriers) formed after the digestion of lipids in the small intestine, which plays important roles in absorption enhancement of hydrophobic drugs (19). Cubic nanoparticles are small particles with lyotropic and bioadhesive properties, which provide them more opportunities to penetrate the "unstirred water layer" (20) and make contact with intestinal endothelial mucosa. Furthermore, poorly water-soluble drugs can be solubilized in the hydrophobic domains of cubic nanoparticles, which facilitate encapsulation of the drugs in the secondary vehicles during digestion and results in enhanced absorption. However, this concept has not been elucidated and needs confirmation by both *in vitro* and *in vivo* evidence.

Herein, we employed the concept of using cubic nanoparticles as oral drug delivery carriers to enhance the bioavailability of a poorly water-soluble drug in this study. The model drug simvastatin, a lipid regulator, that shows poor bioavailability due to limited dissolution rate (21,22) was incorporated into lyotropic glyceryl monooleate (GMO)/poloxamer 407 cubic nanoparticles with aims to maintain it in a solubilized state in the gastrointestinal tract and to facilitate quick transformation into mixed micelles upon digestion, which will finally lead to enhanced oral bioavailability. Cubic nanoparticles were prepared through fragmentation of the bulk cubic-phase gel and characterized *in vitro*. Pharmacokinetic study in beagle dogs was carried out to confirm the effect of enhancement in oral bioavailability of simvastatin. Since this is a preliminary study, mechanisms of enhanced absorption by cubic nanoparticles will not be addressed here and will be explored in the following series of studies.

MATERIALS AND METHODS

Materials

GMO (monoolein) DIMONANR MO 90/D was a gift from Danisco Cultor (Grindsted, Denmark). Poloxamer 407 (PEO98POP67PEO98) was provided by BASF (Ludwigshafen, Germany). Sephadex G-50 was purchased from Pharmacia. Water was prepared by Milli-Q purifying system (Millipore, USA). Micronized simvastatin crystal powder with a mean diameter of <5 μm was purchased from Apeloa Pharmaceuticals (Zhejiang, China). All other chemicals were of analytical purity.

Preparation of GMO/Poloxamer 407 Cubic Nanoparticles

Cubic nanoparticles were prepared through fragmentation of the GMO/poloxamer 407 bulk cubic gel (23). GMO and poloxamer 407 in different weight ratios were first melted at 60°C in a hot water bath, after which simvastatin was added and stirred continuously until total dissolution. Milli-Q deionized water was added gradually and vortex mixed for 1 min to achieve a homogenous state. After equilibration for 24–48 h at room temperature, an optically isotropic cubic-phase gel was formed. Subsequent fragmentation with water to form a crude dispersion was performed by intermittent probe sonication (JYD-650, Shanghai, China) in a water bath at 25°C for 5 min. The crude dispersion was finally homogenized by passing five cycles through a high-pressure homogenizer (Avestin Em-C3) at 689 bar and 25°C to obtain an opalescent dispersion of the cubic nanoparticles. The final dispersions were stored at room temperature and protected from light.

Particle Size

Particle size was determined by photon correlation spectroscopy using a Zetasizer Nano® (Malvern Instruments, Malvern, UK) at 25°C. The instrument was equipped with a 4-mW He–Ne laser (633 nm) for the size determination. Samples were diluted with deionized water prior to the measurement, and dispersant viscosity was set to 0.8872 cP at 25°C. Particle size was analyzed by the Dispersion Technology Software provided by Malvern Instruments. The polydispersity index (PDI), which was a dimensionless number indicating the width of the size distribution, was also obtained.

Entrapment Efficiency

The percentage of drug incorporated in the cubic nanoparticles was determined by gel permeation chromatography. About 2 g Sephadex G-50 was packed in a 30-cm glass column with an inner diameter of 1.5 cm. After preconditioning with 30 mL water, the cubic nanoparticles containing simvastatin was mounted and eluted with 10 mL of water. The eluting curves were delineated through monitoring the turbidity at a wavelength of 450 nm by a UV-2401 spectrophotometer (Shimadzu, Japan). The eluate containing cubic nanoparticles was extracted by methanol, and simvastatin content was determined using an Agilent 1100 high-performance liquid chromatography (HPLC) system. Methanol/water (90/10, *v*/*v*) with a pH of 4.0 was used as the mobile phase at a flow rate of 1.0 mL/min. Simvastatin were separated by the C18 column (Diamonsil, 5 μm, 4.6×150 mm, Dikma, China) guarded with a refillable precolumn (C18, 2.0×20 mm, Alltech, USA) and detected at 238 nm. Entrapment efficiency (EE%) was calculated according to the following equation:

$$EE\% = W_c/W_t \times 100\%$$

where W_c and W_t denote the drug content in the cubic nanoparticles and the total drug in dispersion, respectively. Since the overall column recovery was within 95–102%, calculated as the ratio of total drug in eluate to total drug in untreated nanoparticle dispersion, the entrapment efficiencies have not been adjusted accordingly.

Cryo-TEM

A drop of 3 μL cubic nanoparticle dispersion was placed on carbon-coated holey film (Quantifoil) supported by a copper grid and gently blotted with filter paper to obtain a thin liquid film on the grid, and then the grid was plunged into liquid ethane. Excessive ethane was removed and the sample was transferred into a cryo-transmission electron microscope (cryo-TEM; JOEL JEM-2010, Japan). Samples were viewed under low-dose conditions at a constant temperature around −170°C. The images were recorded digitally with a CCD camera (Gatan 832) under low-dose conditions with an underfocus of approximately 3 μm and an acceleration voltage of 200 kV.

In Vitro Release

Dynamic dialysis method was used to evaluate *in vitro* release of simvastatin from the cubic nanoparticles in a ZRS-8G release tester (Tianjin, China) according to the Chinese Pharmacopoeia Method III (the small beaker method). One milliliter of freshly made cubic nanoparticles suspension (equivalent to 0.8 mg drug) was put in the dialysis bag (∅ 16 mm, MWCO 14,000 Da) and sealed. The dialysis bags were put, using a sinking basket, in the beaker containing 70 mL release medium maintained at 37°C and a paddle revolution speed of 100 rpm. Release medium was either simulated gastric fluids (SGF), 0.1 M hydrochloride solution containing 0.2% (*w*/*v*) sodium lauryl sulfate, or fasted-state simulated intestinal fluids (FaSSIF). Each 500 mL FaSSIF (24) contained 3 mM sodium taurocholate, 0.75 mM lecithin, 0.174 g sodium hydroxide, 2.230 g monobasic sodium phosphate, 3.093 g sodium chloride, and deionized water adding to 500 mL. The FaSSIF was adjusted to pH 6.5, and the osmolarity was about 270 mOsm/kg. Good sink condition was maintained throughout the release test. At time intervals, 1 mL release sample was withdrawn and filtered through a 0.45-μm nylon film filter. Simvastatin content in the release medium was quantified using an HPLC method similar to that in entrapment efficiency determination. The dialysis bag was validated to have little hindering effect on simvastatin release by studying the release data of saturated simvastatin solution obtained with the dialysis bag.

Determination of Simvastatin in Dog Plasma by RP-HPLC

Simvastatin in dog plasma was determined by the Agilent 1100 series HPLC system (Agilent, USA), which consisted of a quaternary pump, a degasser, an autosampler, a column heater, and a tunable ultraviolet detector. Simvastatin was separated by C18 column (Diamonsil, 5 μm, 4.6×150 mm, Dikma, China) guarded with a refillable precolumn (C18, 2.0×20 mm, Alltech, USA) and detected at 238 nm. The mobile phase was composed of acetonitrile and 0.1% phosphoric acid in a volume ratio of 75/25 adjusting the pH to 4.0. The mobile phase was pumped at a flow rate of 1.0 mL/min, and the column temperature was set to 40°C.

Simvastatin in plasma samples was extracted by solid-phase extraction procedures. Briefly, to a 5-mL polypropylene screw-capped conical tube, 1.2 mL of plasma was added followed by 100 μL of internal standard (0.88 μg/mL lovastatin in methanol). The plasma samples were vortex mixed and eluted through a Waters OASIS® HLB cartridge, which was preconditioned with 1 mL methanol and 1 mL water. The cartridge was further washed with 1 mL 5% (*v*/*v*) methanol, after which simvastatin retained in the cartridge was washed out using 1 mL acetonitrile. The eluate was collected and evaporated under a light stream of nitrogen. The residue was dissolved by 100 μL acetonitrile, vortex mixed for 2 min, and centrifuged at 12,000×*g* for 10 min. Fifty milliliters of the supernatant was injected for HPLC analysis.

The concentration of simvastatin (C) was linearly correlated to the peak area ratio of simvastatin to that of lovastatin (R) within the range of 6.25 to 200 ng/mL. Typical calibration equation was as follows: $R = 0.025C + 0.058$ ($r = 0.995$). The interday precision was 8.07%, 9.18%, and 3.02% at simvastatin concentrations of 6.25, 25.0, and 200 μg/mL, respectively, whereas the intraday precision was 6.92%, 6.86%, and 4.64%, respectively. The method recovery was between 98% and 102%, and the extraction recovery was 75.06±3.18%.

Pharmacokinetic Study

The pharmacokinetics of the simvastatin cubic nanoparticles dispersion was compared to simvastatin crystal powder in beagle dogs in a randomized two-period crossover study after an oral dose of 40 mg equivalent of simvastatin. The washout period between administrations was 1 week. Six male beagle dogs weighing 8–10 kg were kept in an environmentally controlled breeding room for 1 week before the start of the experiment. The dogs were fed standard laboratory chow with water and fasted overnight before the experiment. Guidelines on experiments involving the use of animals issued by the Ethical Committee of Fudan University were strictly followed.

The simvastatin cubic nanoparticle dispersion (F4 in Table I) was given directly without dilution, while the simvastatin crystal powder was first suspended in 1% (*w*/*v*) carboxyl methylcellulose before administration. All of the two formulations were gavage administered followed by 20 mL of water. At time intervals, 4 mL of blood samples were withdrawn into heparinized test tubes and centrifuged at 3,000×*g* for 10 min using a high-speed centrifuging machine (TGL-16, Shanghai, China). Plasma was collected and stored at −20°C until analysis.

Pharmacokinetic analysis was performed by a model-independent method using the 3p97 computer program (issued by the State Food and Drug Administration of China for pharmacokinetic study). C_{max} and T_{max} were observed as raw data. Area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. Area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t}+C_t/k$ where C_t and k are the last measurable concentration and the elimination rate constant, respectively.

Statistical Analysis

Raw data were analyzed using the SPSS statistical software (version 11.0, SPSS, Inc.). Analysis of variance

Table I. Particle Size, Distribution, and Simvastatin Entrapment Efficiency of Cubic Nanoparticles Prepared Under Different Experimental Conditions

Formulation no.	GMO/poloxamer 407 ratio	Theoretical simvastatin loading (%)[a]	Particle size (nm)	Polydispersity	Entrapment efficiency (%)
Effect of GMO/poloxamer 407 ratio					
F1	100/8	3.57	135.2	0.260	100.55
F2	100/9	3.54	132.5	0.247	98.92
F3	100/10	3.51	136.4	0.291	101.74
F4	100/12	3.45	122.9	0.263	101.4
F5	100/15	3.36	113.4	0.262	100.08
F6	100/20	3.23	146.2	0.419	100.73
Effect of drug loading					
F7	100/12	0	140.6	0.184	–
F8	100/12	1.75	132.4	0.258	101.25
F9	100/12	3.45	148.8	0.285	103.77
F10	100/12	4.27	136.2	0.239	99.42
F11	100/12	5.08	140.2	0.247	96.80

[a] Theoretical simvastatin loading was calculated as the ratio of simvastatin to total solids (simvastatin + GMO + poloxamer 407)

was done to determine the significance of the differences between groups; a P value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Preparation of GMO Cubic Nanoparticles

Several approaches have been employed to prepare cubic liquid crystalline nanoparticles. Fragmentation of cubic bulk gel by high-energy dispersion like sonication and homogenization was the most common method used to process cubic nanoparticles (8,23). Using ethanol as a hydrotrope, cubic nanoparticles could be produced spontaneously by dilution of a monoolein–ethanol–water system (25). The spray-drying technique has also been used to produce dry powder precursors of cubic nanoparticles (17,26). Until now, knowledge about the preparation and characterization of cubic liquid crystalline nanoparticles was based on study with blank cubic phases, and only a few addressed systems loaded with drugs. Although the fragmentation method was endowed with several problems when scaling up, the starting-up bulk gel provided a nice tool to study the effect of drug loading on the physicochemical properties of the cubic phase.

After equilibrating for 24 h, clear and viscous gels formed for the GMO/poloxamer 407 binary systems with a poloxamer 407 weight percentage over 4% (*w*/*w*). Observed under polarized light microscopy, diffraction of a dark isotropy region confirmed the formation of the liquid crystalline phase. Cubic-phase gel formed more rapidly when the ratio of poloxamer 407 increased up to 20%. At low simvastatin doses, no significant change in the appearance of the bulk gel was observed. However, when theoretical simvastatin loading increased to over 8% (*w*/*w*), calculated as the ratio of simvastatin to total solids, the bulk gel began to turn opalescent, indicating reduced solubility of simvastatin in the GMO/poloxamer cubic phase. Over simvastatin theoretical loading of 15%, the cubic-phase gel could not form.

Since it was soft, the bulk gel could be easily disrupted by sonication. After homogenization for several cycles, the crude dispersion turned to a homogenous milky white suspension. Upon storage at ambient temperature, no obvious sedimentation or aggregation of the dispersed cubic nanoparticles could be observed for a few days.

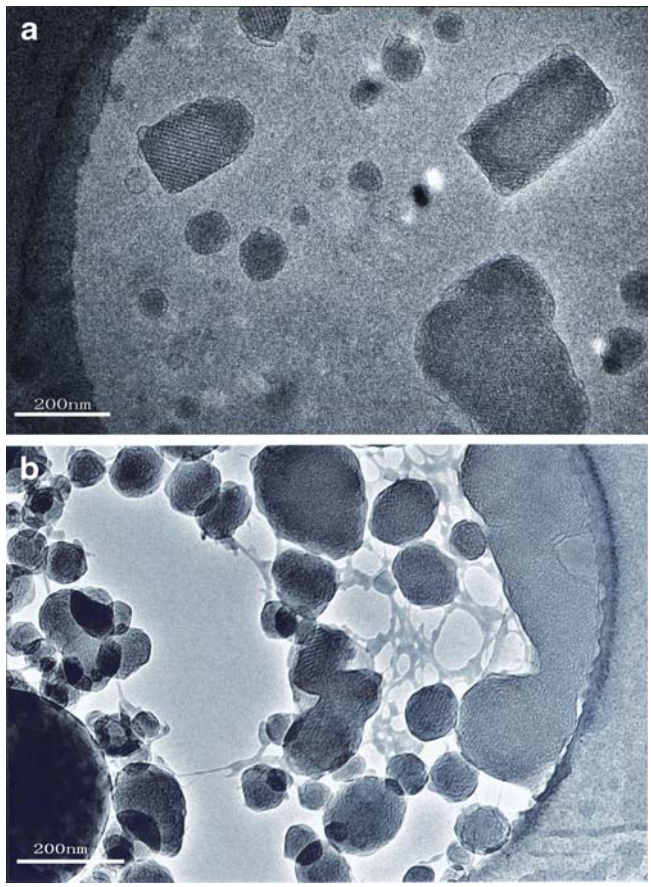

Fig. 1. Cryo-TEM micrographs of poloxamer 407/GMO (12/100) cubic nanoparticles: **a** blank; **b** simvastatin-loaded at 3.45% (*w*/*w*) level. The bar equals to 100 nm

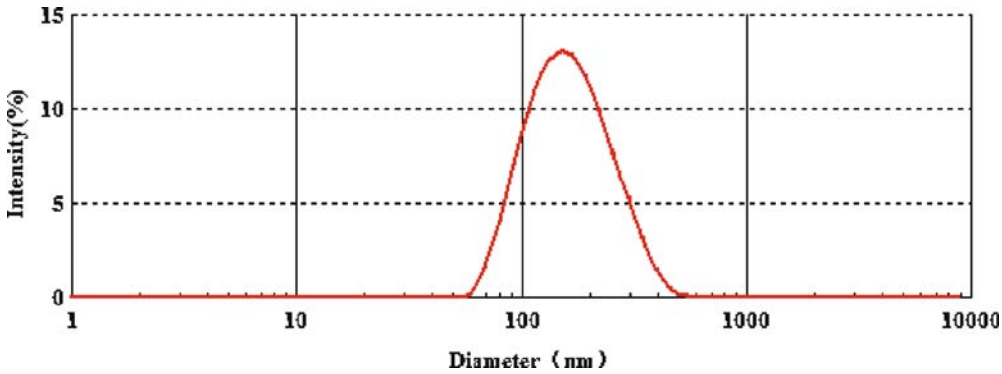

Fig. 2. Size distribution of simvastatin-loaded (3.45%, *w/w*) poloxamer 407/GMO (12/100) cubic nanoparticles determined by photon correlation spectroscopy

Cryo-TEM

Cryo-TEM is a useful tool to identify the internal structure of dispersed cubic nanoparticles. In this study, both blank and simvastatin-loaded GMO/poloxamer 407 cubic nanoparticles were observed by cryo-TEM. Figure 1a shows the images of cubic nanoparticles with diameters ranging from tens to over 100 nm. Nanoparticles with large diameters (>100 nm) exhibited ordered texture with near-square, rectangle, and irregular geometry. Since the drug loading was quite low, the ordered texture suggested the possible formation of cubic liquid crystalline phase of the lipids rather than the crystalline phase of the drug, which was consistent with the cryo-TEM observations by other researchers (17,18). For nanoparticles of smaller diameter, the ordered texture could also be observed, while their geometry appeared as somewhat spherical or oval. The cubic texture of nanoparticles with diameters <50 nm could not be clearly identified and vesicular structure could be proposed for them.

Figure 1b shows the image of the cubic nanoparticles loaded with simvastatin. There was obvious difference in the texture and shape of the cubic nanoparticles as a result of simvastatin loading. Most of the nanoparticles were near spherical, and those with much bigger diameters had irregular shape contrasting with blank cubic nanoparticles that had square or rectangular image projections. The texture of the cubic nanoparticles became somewhat untypical upon drug loading (Fig. 1b). As a hydrophobic drug, simvastatin molecules had a tendency to partition into the hydrophobic domains of the cubic phase. It was assumed that simvastatin molecules at low levels only affect the lipid bilayer slightly, which could not disturb the whole cubic phase. At much higher levels, the effect of drug loading became more obvious and the cubic phase lost its regular cubic texture as observed in this study. It was assumed that incorporation of drug into cubic phases would alter the lipid bilayer contortion and thus affected the cubic texture and the geometry of the dispersed nanoparticles.

Particle Size and Distribution

The bulk cubic-phase gels could be easily disintegrated into cubic nanoparticles of tens to a few hundreds of nanometers. Figure 2 shows a typical lognormal distribution of the cubic nanoparticles with diameters ranging from 60 to 500 nm. Since the cubic nanoparticles were prepared through fragmentation of the bulk cubic-phase gel, the particle size was largely dependent on the homogenization pressure and cycles. The mean diameter of the cubic nanoparticles varied within the range of 100–150 nm (Table I). However, GMO/poloxamer 407 ratio and theoretical simvastatin loading seemed to have little effect on particle size. Although a decreasing tendency was observed when poloxamer 407 content increased to GMO/poloxamer 407 ratio of 100/12 and 100/15, the difference between different formulations was not significant because of the relatively large particle size distribution. The cubic nanoparticles at GMO/poloxamer ratio of 100/20 showed larger particle size and polydispersity for which the reason was still unclear and might be attributable to the effect of overly large quantity of poloxamer 407 in the formulation. Blank nanoparticles seemed to have smaller PDI, while GMO/poloxamer 407 and theoretical simvastatin loading had no significant effect on size distribution.

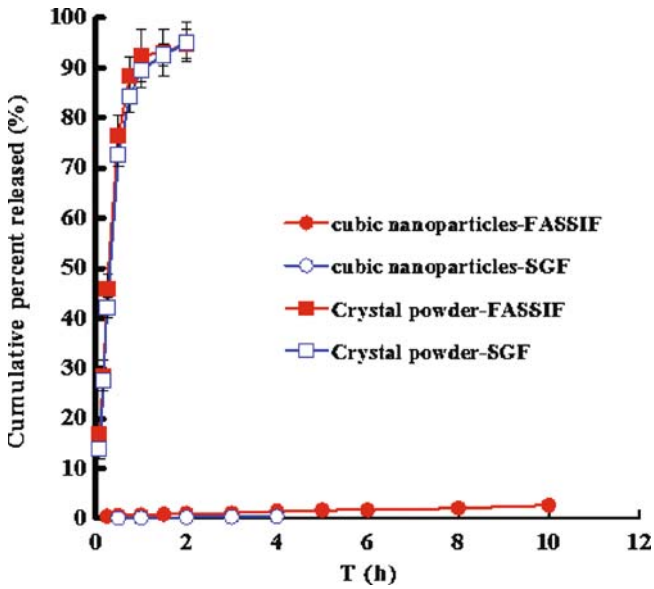

Fig. 3. Release profiles of simvastatin crystal powder and cubic nanoparticles in SGF and FaSSIF

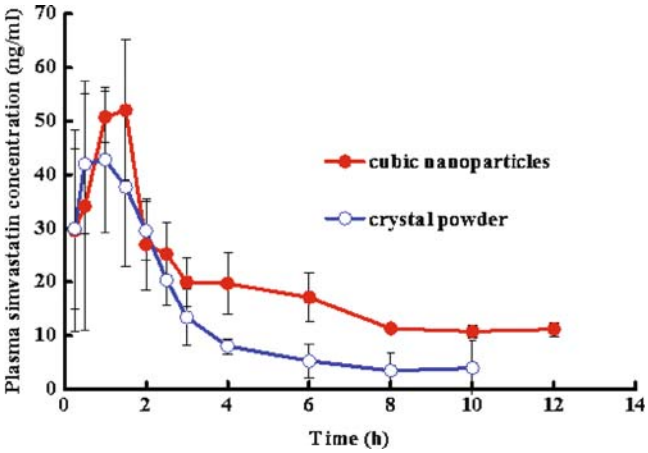

Fig. 4. Plasma simvastatin concentration–time plot after a single oral dose of simvastatin cubic nanoparticles and crystal powder

Entrapment Efficiency

Entrapment efficiency of simvastatin into the cubic nanoparticles was high and independent of processing parameters (Table I). Under different GMO/poloxamer 407 ratios and different simvastatin loading, the entrapment efficiency was as high as over 98%, showing little loss into the dispersion medium. Although the mean size and distribution of the cubic nanoparticles varied as the processing parameters changed, the entrapment efficiency of simvastatin remained stable. It was indicated that diffusion of simvastatin into the dispersion medium was negligible due to its high lipophilicity, even though more diffusion surface was exposed as a result of particle size reduction. Only at saturation levels of simvastatin, when disturbance of consistent bulk cubic phase was obvious, could a slight decrease in entrapment efficiency be observed.

In Vitro Release

Figure 3 gives the release profiles of simvastatin from crystal powder and cubic nanoparticles. Simvastatin crystal showed over 90% release at 1 h both in SGF and FaSSIF, while cubic nanoparticles showed <3% simvastatin release in the same media at 10 h. This was in coincidence with the high entrapment efficiency of simvastatin in cubic nanoparticles. The high affinity of simvastatin with the hydrophobic domain in the cubic phase made it difficult to escape from the nanoparticles. Due to detectable degradation, release of simvastatin in SGF from both simvastatin crystal powder and cubic nanoparticles was only evaluated for 4 h.

This disparity of apparent release rate between simvastatin crystal powder and cubic nanoparticles contradicted significantly with oral bioavailability results as shown below. It seemed that *in vitro* evaluation protocols of apparent release rate was not suitable for cubic nanoparticles. Further evaluation of *in vitro* release of cubic nanoparticles should take into account the mechanism of lipid digestion.

Pharmacokinetic Study

Pharmacokinetic parameters of simvastatin cubic nanoparticles and powder were compared in beagle dogs. Mean plasma simvastatin concentration was plotted as a function of time and shown in Fig. 4. Pharmacokinetic parameters are shown in Table II. After oral administration, simvastatin cubic nanoparticles were absorbed much slower than simvastatin powder with T_{max} value of 1.5 and 0.84 h ($P<0.05$), respectively. The C_{max} of cubic nanoparticles (53 ng/mL) was higher than that of powder (42 ng/mL), indicating facilitated absorption of simvastatin by cubic nanoparticles. It was worth noting that the plasma simvastatin level of cubic nanoparticles sustained for over 12 h, while the simvastatin level of crystal powder dropped below the detection limit at the same time point. These results indicated that significantly enhanced bioavailability ($P<0.05$) of simvastatin has been achieved through incorporation into cubic nanoparticles. Calculated on the basis of $AUC_{0-\infty}$, the relative bioavailability of simvastatin cubic nanoparticles compared to micronized crystal powder was about 241%.

The above results indicated that the cubic nanoparticles have the potential to be used to increase the oral bioavailability of highly lipophilic drugs. The underlying mechanisms of enhancement, however, are still unclear and provoke future research interests. Looking into past experience with lipid-based and liquid crystalline phase matrix oral formulation provides some interpretation of the mechanisms of enhanced absorption and bioavailability. As a monoglyceride, GMO formulations seemed to be degraded by intestinal esterase after oral ingestion, which was clearly indicated in a previous study with GMO and OG liquid crystalline matrices (5). In that study, sustained release and enhanced bioavailability of a water-insoluble drug cinnarizine was observed after oral ingestion of OG liquid crystalline matrix. A second absorption phase of the drug from gastrointestinal tract was proposed to interpret the delayed elimination phenomenon. However, the GMO matrix seemed to degrade rapidly in simulated intestinal conditions and failed to enhance the bioavailability compared to the cinnarizine suspension. In this study, the oral bioavailability of simvastatin was significantly enhanced after incorporation into cubic nanoparticles, which indicated different absorption mechanisms for cubic nanoparticles. Although intestinal degradation cannot be excluded, cubic nanoparticles, which are lyotropic carriers with hydrophilic surface, may have more chances to contact with the

Table II. Pharmacokinetic Parameters After Oral Administration of Simvastatin Cubic Nanoparticles and Crystal Powder

Formulation	t_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC_{0-t} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)	Relative bioavailability (%)[a]
Cubic nanoparticles	1.50±0.60	55.26±6.27	164.88±130.9	231.70±21.09	371.47±64.41	241.23
Crystal powder	0.84±0.16	47.13±14.64	6.85±5.71	131.78± 5.42	153.99±91.13	

[a] Calculated on $AUC_{0-\infty}$ with micronized simvastatin powder as reference

endothelial cell membrane, overcoming the "unstirred water layer" barrier (23). Either released drug or cubic nanoparticles can be transported across the endothelial cell membrane (27), therefore achieving enhanced drug absorption.

Although cubic nanoparticles are possibly absorbed intact across the cell membrane, it is not believed as the dominating mechanism. Secondary drug carriers, like micelles, mixed micelles, niosomes, and so on (20) may be produced during the digestion process of cubic nanoparticles. It is assumed that cubic nanoparticles will be broken down in the intestinal fluid by esterase, and smaller cubic nanoparticles and other secondary carriers will form and transport the loaded drug across the unstirred water layer and result in enhanced absorption. However, the fate of cubic nanoparticles and mechanisms of enhanced absorption of water-insoluble drugs need to be elucidated in future research.

CONCLUSIONS

Simvastatin-loaded GMO/poloxamer 407 cubic nanoparticles were prepared through fragmentation of the bulk cubic-phase gel, and the inner cubic structure has been identified by cryo-TEM. The oral bioavailability of simvastatin cubic nanoparticles was enhanced significantly at 2.41-fold compared to micronized simvastatin crystal powder. Besides increased relative bioavailability, cubic nanoparticles showed sustained plasma simvastatin level for over 12 h. Mechanisms of facilitated absorption by lipid was proposed rather than improved release as cubic nanoparticles show limited release in SGF and FaSSIF.

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