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ORIGINAL ARTICLE

N-Trimethyl chitosan-coated multivesicular liposomes for oxymatrine oral delivery

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Abstract

Background: Multivesicular liposomes (MVLs), uncoated and coated with *N*-trimethyl chitosan (TMC), have been studied for their potential use for drug delivery by the oral route. *Method*: Synthesized TMC was characterized by infrared spectroscopy, revealing the presence of trimethyl groups, and by proton nuclear magnetic resonance spectroscopy, allowing the calculation of the degree of substitution quaternization (70.2%). Oxymatrine (OM), a natural quinolizidine alkaloid used clinically for treating hepatitis B, was chosen as a model drug. The surface-modified MVLs and uncoated MVLs were characterized in vitro in terms of their shape, size, zeta potential, entrapment efficiency, coating efficiency, the stability of MVLs in polymer suspension, and the stability in simulated gastric and intestinal fluids. *Results*: In vivo, the area under the plasma concentration–time curve obtained from the pharmacokinetics study of TMC-coated MVLs was found to be about 3.26- and 1.96-fold higher than that of OM solution and uncoated MVLs, respectively. *Conclusion*: TMC-coated MVLs can be considered as a potential carrier for oral drug administration.

Key words: *Multivesicular liposome; N-trimethyl chitosan; oral delivery; oxymatrine; surface coating*

Introduction

Hepatitis B virus (HBV) can cause a variety of liver conditions including hepatitis B, chronic hepatitis, hepatic cirrhosis, and even cancer of the liver. Therefore, the prevention and treatment of HBV is a problem that requires urgent attention. Oxymatrine (OM), a major active alkaloid constituent extracted from the root of *Sophora flavescens* Ait (Kushen) and the terrestrial part of *Sophora alopecuroides* (Kudouzi), has been found to have a remarkable anti-hepatic activity[1]. OM is a weak base with a high hydrophilicity[2], and tablet and capsule formulations of OM have been approved for commercial use in China to treat HBV infection. However, the bioavailability is low when OM is administered orally[3], so an attempt has been made to develop an oral delivery system for OM.

Multivesicular liposomes (MVLs), a lipid-based carrier, are a suitable depot delivery system for encapsulating water-soluble drugs because of their high aqueous volume. MVLs are composed of nonconcentric internal aqueous chambers that are separated by lipid layers. When a drug is released from the internal vesicles, the vesicles do not break up immediately so that the drug can be released slowly. Triglycerides, the most important component of MVLs, occupy the hydrophobic spaces between the internal lipid chambers to stabilize the junctions and can be used as a tool to adjust the drug release rate[4,5]. The particle size of MVLs is about 5–50 μm, which is higher than that of conventional liposomes. MVLs have been used as a delivery vehicle for many drugs, and two products (Depocyt® and DepoDur®) have been approved for clinical applications.

In this article, we have attempted to develop MVLs as the carrier for OM administered orally, because of its high encapsulated aqueous volume, although MVLs have been studied before as a carrier for sustained release of drugs given by injection[6–12]. However, MVLs are damaged by the harsh environment of the gastrointestinal (GI) tract and fail to improve the absorption of OM. Therefore, *N*-trimethyl chitosan chloride (TMC), a quaternized chitosan derivative, has been used

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to coat MVLs containing OM because of the properties of TMC, such as the ability to enhance drug permeation through the paracellular pathway[13,14] and the ability to adhere to mucosa in the GI tract[13].

The purpose of this study is to synthesize TMC and to use it for coating MVLs containing OM. MVLs, uncoated and coated with TMC, were characterized in vitro in terms of their shape, size, zeta potential, entrapment efficiency, coating efficiency, the stability in the polymer suspension, and the stability in simulated gastric and intestinal fluids. The in vivo pharmacokinetics of TMC-coated MVLs following oral administration to rats was also investigated and compared with that of OM solution and uncoated MVLs.

Materials and methods

Materials

OM (purity >98%) was obtained from Chia Tai Tianqing Pharmaceutical Co. Ltd. (Lianyugang, China). Chitosan [with a 90% degree of deacetylation and a molecular weight (M_w) of 250 kDa] used for synthesizing TMC was purchased from Laizhou Highly Bio-products Co. Ltd. (Shandong, China). Phosphatidylcholine (PC; 92% Epikuron 200) was a gift from Degussa BioActives Deutschland GmbH & Co. KG (Freising, Germany). Phosphatidylserine (PS; purity >89.4%) was purchased from Beijing Info Ark Technology Development Co., Ltd. (Beijing, China). Glycerol trioleate (TO) was of analytical grade from Zhejiang Huangma Chemical Group Co., Ltd. (Zhejiang, China). All other materials used were of analytical or pharmaceutical grade.

Synthesis and characterization of TMC polymers

TMC was synthesized from chitosan in a two-step reaction according to the method described by Sieval et al.[15] The synthesized polymer was characterized by infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The IR spectrum of chitosan and TMC, in the form of KBr tablets, were run on an IFS-55 infrared spectrometer (Bruker, Fällanden, Switzerland). For the ^{1}H-NMR spectra, an AVANCE 600-MHz ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) spectrometer (Bruker) was used. TMC polymers were dissolved in D_2O in an NMR tube and the NMR spectrum of TMC was recorded. The degree of quaternization of TMC polymer was calculated from the ^{1}H-NMR spectra using Equation (1)[15,16]:

$$DQ(\%) = \frac{\left\{\frac{A\left[(CH_3)_3\right]}{A(H)}\right\}}{9}, \quad (1)$$

where DQ (%) is the degree of quaternization as percentage, $A[(CH_3)_3]$ is the integral of the peak at 3.3 ppm, and A[H] is the integral of peaks between 4.7 and 5.7 ppm.

Preparation of TMC-coated MVLs containing OM

A double emulsification method was used to prepare the MVLs containing OM[17]. A lipid solution of 1.5 mL ethyl ether containing 40 mg PC, 10 mg PS, 40 mg cholesterol, and 10 mg TO was emulsified with 1 mL of the first aqueous solution containing 40 mg/mL OM, 8% sucrose (w/v), and 50 mM arginine (pH 7.0) with a mixer (T 18 basic ULTRA-TURRAX; IKA Works, Guangzhou, China) at 10,000 rpm for 6 minutes. Then, the first emulsion was mixed with 3 mL of the second aqueous solution containing 5% (w/v) glucose and 40 mM L-lysine (pH 8.0) at 6000 rpm for 40 seconds to obtain the w/o/w double emulsion. The ethyl ether was removed by flushing nitrogen over the surface of the second emulsion at about 35°C, and the MVLs suspension was formed.

For the preparation of TMC-coated MVLs, MVLs suspension was added dropwise to the TMC solution under stirring (60 rpm at room temperature) in a volume ratio of 1:2. Excess unbound TMC and unentrapped OM were removed by centrifuging at 1000 × *g* for 5 minutes before characterization.

Coating efficiency

Coating parameters were optimized by measuring the change in coating efficiency of TMC. The coating amount of TMC was determined by means of conductivity to measure the concentration of TMC in the supernatant. A 5% glucose solution (w/v) was used to dissolve TMC to avoid interference from electrolytes. The TMC-coated MVLs were centrifuged at 3000 rpm for 5 minutes, and the supernatant was collected and diluted to a suitable concentration to measure the conductivity. The amount of TMC coating the vesicles was calculated for the reduced TMC concentration in the solution after coating.

For optimization of the concentration of TMC, formulations with different concentrations [0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.7%, 1.0%, and 1.5% (w/v)] were prepared and incubated for a fixed time period of 24 hours and the coating efficiency was measured. The optimum concentration was determined at which no significant change in coating efficiency was recorded on further increasing the concentration of TMC. Similarly, for optimization of the incubation time, formulations with the previously optimized concentration of TMC were prepared and incubated for different time periods and

the coating efficiency was measured. After completion of the coating, no significant change in coating efficiency was recorded.

Particle characterization of uncoated MVLs and TMC-coated MVLs

The morphology of uncoated MVLs and TMC-coated MVLs from an optimized formulation was observed using a light microscope (classica E220LED; Motic China Group Co. Ltd., Xiamen, China). A drop of the sample was placed on a blood counting chamber and photomicrographs were recorded using a digital camera (FinePix A345, Fujifilm China Investment Co. Ltd., Shanghai, China).

The particle size distribution was analyzed using a Laser Diffraction Particle Size Analyzer (LS 230, Beckman Coulter Inc., Fullerton, CA, USA) and a Malvern Zetasizer was used to measure the zeta potential.

Determination of encapsulation efficiency

The content of OM was determined using a reversed phase high-performance liquid chromatography (HPLC) method[18]. The HPLC system consisted of a Waters 510 pump and an SPD-10A UV detector set at 220 nm. The drug was determined at 30°C on a 250 × 4.6 mm, 5 μm Kromasil ODS column (Dalian Institute of Chemical Physics, Dalian, China). The mobile phase consisted of phosphate buffer/acteonitrile (90:10, v/v, 0.1% phosphoric acid solution, adjusted to pH 3.0 with triethylamine) and this was pumped through the column at a rate of 0.8 mL/min.

Saline was added to 0.25 mL uncoated MVLs and TMC-coated MVLs suspension to obtain a volume of 5 mL and then the free OM was separated by centrifugation at 3000 rpm for 5 min. A solution of 10% Triton X-100 was used to break up the MVLs to determine the total amount of drug[18]. The encapsulation efficiency (EE%) of OM was calculated from the following Equation (2):

$$\mathrm{EE}\,(\%) = \frac{C_{\mathrm{total}} - C_{\mathrm{free}}}{C_{\mathrm{total}}} \times 100, \qquad (2)$$

where C is the concentration of OM.

Stability of MVLs in polymer suspension

The change in encapsulation efficiency of OM was used to test the stability of MVLs to TMC solution. MVLs were incubated with TMC (0.1%, 0.5%, 1.0%) for different periods (1, 3, 6, 12, and 24 hours), and the encapsulation efficiencies were determined.

Stability in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4)

To investigate the stability of TMC-coated MVLs, coated and uncoated MVLs were separately diluted 10-fold with simulated gastric fluid (SGF) or simulated intestinal fluid (SIF). After magnetic stirring for 2 hours, the OM concentration was determined in the supernatant using HPLC after centrifugation (see Section 'Determination of encapsulation efficiency'). SGF was composed of 0.2% sodium chloride and 0.7% hydrochloric acid. The final solution was adjusted to about pH 1.2. SIF was prepared by dissolving 6.8 g KH_2PO_4 in 500 mL distilled water followed by addition of 10 g pancreatin. Distilled water was then added to make up the volume up to 1 L, and the pH was adjusted to 7.4 with 0.2 M NaOH.

In vivo pharmacodynamic studies

Animals

Female Wistar rats (Experimental Animal Center of Shenyang Pharmaceutical University) weighing 230–250 g were used throughout this study. The rats underwent jugular vein cannulation 3 days before the experiment in order to carry out the in vivo experiment while the animals were in conscious condition. The rats were fasted overnight before the study, but were allowed water ad libitum.

Drug administration and blood sampling

OM solution, uncoated MVLs, and TMC-coated MVLs were administered orally to three groups, each of six rats as a single dose of 50 mg/kg. Blood samples (0.7 mL) were periodically taken from the heparinized polyethylene cannula placed in the left jugular vein after administration. The heparinized blood was immediately centrifuged at 9500 × *g* for 5 minutes and plasma obtained was stored at −20°C until analysis.

Sample preparation

Aliquots of 100 μL acetparaphenetidine solution (internal standard solution, 20 μg/mL) and 400 μL perchloric acid (6%) were added to a 300 μL aliquot of rat plasma. The mixture was mixed for 30 seconds and centrifuged at 3000 rpm for 5 minutes. Then, 400 μL 20% NaOH was added to the supernatant, and the mixture was extracted with 4 mL of chloroform-*n*-butanol (98/2, v/v) by shaking for 1 minute. The organic phases were separated by centrifugation at 3000 rpm for 10 minutes, transferred to a 10 mL tube, and evaporated to dryness under a nitrogen stream at 50°C. The residue was dissolved in 100 μL mobile phase and 20 μL of this solution was injected into the HPLC system for analysis.

Data analysis

Plasma concentration versus time data were analyzed by a two-compartment model using the DAS 2.1.1 computer program. The elimination half-life ($t_{1/2}$) was determined by linear regression using at least three data points from the terminal portion of the plasma concentration–time plots. The maximum plasma concentration (C_{max}) and the time to reach this maximum (T_{max}) were obtained directly from the concentration–time profiles. The area under the plasma concentration–time curve up to the last time (AUC_{0-t}) was calculated by the trapezoidal rule with extrapolation to infinity ($AUC_{0-\infty}$) using the equation $AUC_{0-t} + C_{last}/ke$. Statistical analysis was performed using Student's *t*-test with $P < 0.05$ as the level of significance.

Results and discussion

Synthesis and characterization of TMC polymers

TMC was characterized using IR spectrometry (Table 1) to identify the presence of *N*-trimethyl groups. The IR spectra of chitosan and TMC were similar, but a new deformation vibration of C−H at 1486 cm^{-1} was observed in the TMC IR spectrum, which proved that the primary amino groups of chitosan had been substituted by methyl groups, suggesting the trimethylation of chitosan.

The ^{1}H-NMR spectra of the TMC polymers are shown in Figure 1. The degree of quaternization of the polymer was 70.2% calculated from the ^{1}H-NMR spectra using Equation (1). The number of methylation steps and the base used during the process was shown to affect the degree of quaternization[19]. Highly substituted polymers (degree of quaternization 70.2%) were obtained in the study following two methylation steps using sodium hydroxide, because TMC with a high degree of quaternization (60%) has been proved to enhance absorption of the drug[20,21].

Preparation of TMC-coated MVLs containing OM

The factors influencing the stability of TMC-coated MVLs were investigated, and these were chosen based on previous experimental results. The effect of the concentration of TMC was found to be important for the resulting aggregation. When a low-concentration TMC solution was used for coating, liposomal clusters of MVLs were formed. The agglomeration of MVLs in low-concentration TMC solutions could be explained by the mechanism of polymer bridging. MVLs were negatively charged, because of the addition of the negatively charged lipid PS. The mixture of oppositely charged MVLs and TMC may result in particle aggregation because of the interaction between partly coated MVLs and uncoated MVLs. However, when excess polymer was present, restabilization occurred because of charge reversal of the polymer-coated particles. In addition, attention must be paid to the method of mixing MVLs and TMC and the volumetric ratio of MVLs and TMC.

Table 1. The relevant vibration of the chemical bond for the IR absorption peak (cm^{-1}) of chitosan and *N*-trimethyl chitosan chloride (TMC).

	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$	$\delta_{C-H}(CH_3)$	δ_{C-H}	ν_{C-O}
Chitosan	3435	2923	1651		1380	1010
TMC	3437	2927	1634	1486	1381	1066

Coating efficiency

Figure 2 shows that the coating efficiency of MVLs coated with TMC increased as the concentration of TMC increased from 0% to 0.5% (w/v), then, the amount of TMC on the surface of MVLs remained nearly constant. This indicates that saturation was reached, when MVLs were coated with 0.5% of TMC (w/v). Similar results were obtained in the experiment examining the effect of the concentration of TMC on the stability of TMC-coated MVLs, indicating that the MVLs coated with more than 0.5% (w/v) TMC did not aggregate. Therefore, 0.5% (w/v) TMC was selected as optimum.

For optimization of the incubation time, formulations using the previously optimized concentration of TMC (0.5, w/v) were prepared and incubated for different incubation periods (0, 1, 2, 3, 4, 6, 8, 12, and 24 hours), and the change in coating efficiency was recorded (Figure 3). The coating efficiency increased from initial values and approached a maximum value at 3 hours. With a longer incubation time, the change in coating efficiency was not significant, suggesting that the TMC coating was complete.

Particle characterization of uncoated MVLs and TMC-coated MVLs

The particle size of MVLs is about 5–50 μm, so the morphology of MVLs, uncoated and coated with TMC, was examined using a light microscope at 400× magnification. The morphology of the uncoated MVLs and TMC-coated MVLs is shown in Figure 4A–B, respectively. MVLs were nonconcentric spheroids in which there were many small vesicles and TMC-coated MVLs were similar to MVLs in appearance, they did not aggregate, but the small vesicles in MVLs were not obvious under the microscope probably because they were coated with TMC.

The formation of a TMC layer on the surface of MVLs was verified by comparing the particle size and zeta

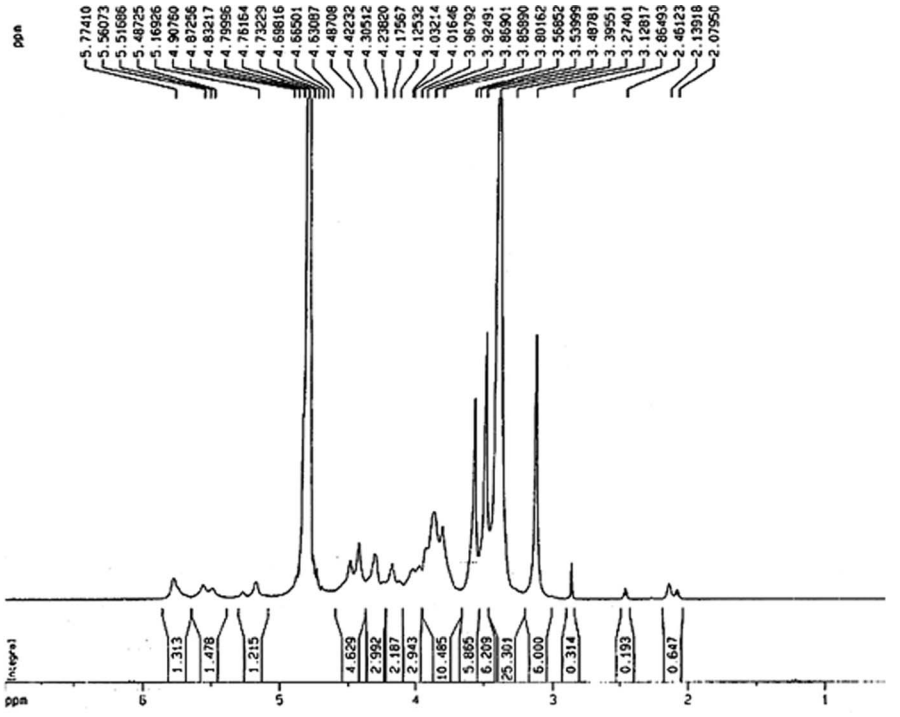

Figure 1. ^{1}H-NMR spectrum of *N*-trimethyl chitosan chloride (TMC).

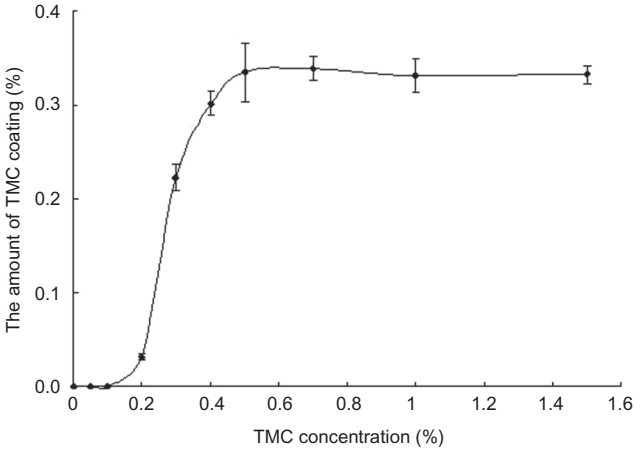

Figure 2. Optimization of the concentration of TMC coating. The formulations were incubated for 24 hours with different concentrations of TMC. Values are expressed as mean ± SD ($n = 3$).

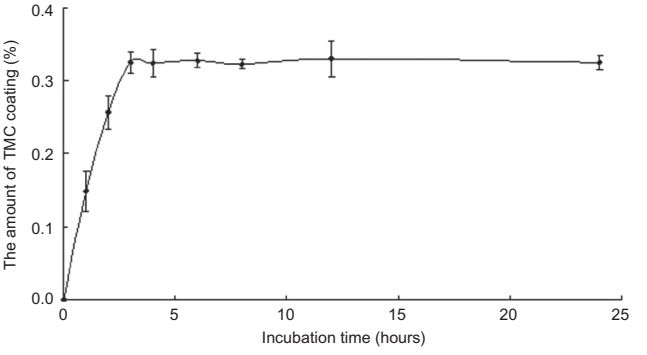

Figure 3. Optimization of the incubation time for complete coating. MVLs were incubated with 0.5% TMC for different times. Values are expressed as mean ± SD ($n = 3$).

potential of MVLs before and after TMC coating. The particle size and zeta potential of the analyzed samples are summarized in Table 2. MVLs after coating with TMC showed a nonsignificant increase in particle size compared with uncoated MVLs (Table 2). The mean diameter of MVLs was 17.04 μm and more than 90% of MVLs had a size range of 6–30 μm. Because of the large size, TMC coating did not produce a significant change in size. Therefore, the zeta potential was measured to further verify the formation of a coating on the surface of the MVLs.

The zeta potential is a useful parameter for monitoring liposome stability, and particles with zeta potentials more positive than +30 mV or more negative than −30 mV are normally considered stable. The zeta potentials of TMC-coated MVLs and uncoated MVLs were +36.8 ± 4.36 mV and −85.8 ± 10.60 mV (Table 2),

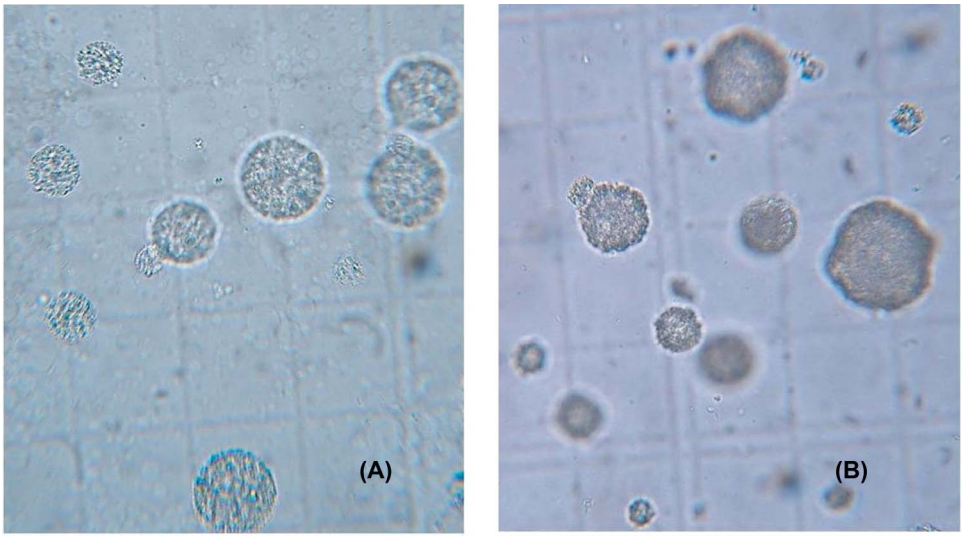

Figure 4. Microscopic pictures of uncoated MVLs and TMC-coated MVLs at 400× magnification taken by a light microscope. (A) Uncoated MVLs. (B) TMC-coated MVLs (the square on blood counting chamber = 20 × 20 μm).

Table 2. Particle size (μm), zeta potential (mV), and encapsulation efficiency (%) of analyzed samples. (The concentration of TMC was 0.5%, w/v, the incubation time was 3 hours.)

Samples	Size (μm)	Zeta potential (mV)	EE (%)
MVLs	17.04 ± 4.99	−85.8 ± 10.60	65.07 ± 1.01
TMC-MVLs	21.74 ± 11.05	+36.8 ± 4.36	53.31 ± 2.74

respectively, which showed that 0.5% TMC (w/v) was not only enough to coat MVLs but also rendered the particles stable. TMC carried a high positive charge, so the main interaction between MVLs and TMC was an electrostatic attraction and the adsorption of TMC increased the density of the positive charges and rendered the zeta potential positive.

Oxymatrine encapsulation efficiency

Although MVLs have a large enough aqueous volume to be suitable for encapsulating water-soluble drugs, the OM encapsulation efficiency of MVLs was only 65.07 ± 1.01% (Table 2). This lower encapsulation efficiency may be due to the interaction between drug and liposomal bilayer. OM is a weakly basic drug with a pK_a of 6.7[2], which suggested that both charged and uncharged species would be present in solution. Negatively charged membranes were present in an aqueous solution because of PS, which would attract positively charged OM by electrostatic interaction. However, there was little interaction between uncharged drug and negative-charged lipid, so it is likely that the uncharged form would be able to cross membranes, which would explain the low encapsulation efficiency of MVLs containing OM when prepared by passive trapping techniques. However, OM, entrapped in MVLs, has a high drug-loading capacity (1 mg OM/mg PC) compared with other passive loading methods[18,22].

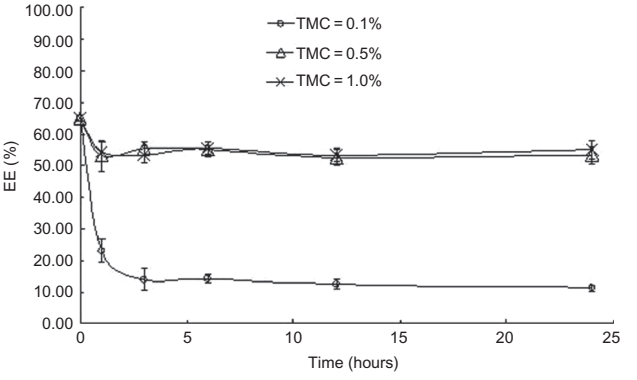

Figure 5. The effect of TMC concentration and incubation time on the encapsulation efficiency of OM. Values are expressed as mean ± SD (n = 3).

Stability of MVLs in polymer suspension

The addition of TMC reduced the encapsulation efficiency of OM, regardless of the TMC concentration (Figure 5). The encapsulation efficiency of OM fell sharply from 65.07% to 11.22% with respect to 0.1% TMC. In fact, for lower TMC concentrations, coating MVLs with TMC would lead to a reduction in stability, which may be the reason for the low encapsulation efficiency. Although a TMC of 0.5% and 1.0% was sufficient for MVLs' coating (see Section 'Coating efficiency'), the encapsulation efficiency of OM fell to 53.31% and 55.08%, respectively, after a 24-hour incubation. This may be explained by the strong affinity of TMC for the phospholipids, which could disrupt the lipid packing.

However, TMC only affected the outside and it was unable to interfere with the bilayer, so that no significant change in the encapsulation efficiency of OM was observed on further increasing the TMC concentration and incubation time.

Stability in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4)

From Figure 6, it can be seen that the stability of uncoated MVLs in SGF was low, and high amounts of encapsulated OM (about 80%) were released in 2 hours. TMC-coated MVLs were found to exhibit significantly higher stability under the same conditions (only 10% OM released after a 2-hour incubation). However, there was little leakage of OM from MVLs in SIF (OM loss of about 10%), no matter whether MVLs were coated with TMC. Therefore, this suggests that the TMC-coated MVLs can be used to deliver drug to the intestinal tract following oral administration. The drug leakage from uncoated MVLs in SGF is probably related to vesicle disruption, and in fact, sedimentation was observed when uncoated MVLs were incubated in SGF. To prevent lipid aggregation, negatively charged lipid PS was added when MVLs were prepared. Charged lipids were distributed relatively homogeneously throughout the liposomal membrane and repelled each other electrostatically. However, an acidic solution may cause the clustering of charged lipid species[23] and because ion binding may shield the negative charges of PS, this may result in the aggregation of lipids.

In vivo pharmacokinetic studies

Pharmacokinetics studies were carried out on the TMC-coated MVLs formulation (the concentration of TMC was 0.5%, w/v, the incubation time was 3 hours) administrated to rats orally at a dose of 50 mg/kg in comparison with uncoated MVLs and OM solution. Figure 7 shows the profiles of the OM plasma concentrations versus time for the three formulations of OM, and the relevant pharmacokinetics parameters are summarized in Table 3.

Table 3 summarizes that the plasma OM concentration reached a peak at 2.1 and 2.7 hours, in the case of OM solution and uncoated MVLs ($P > 0.05$), respectively, whereas it took 4.8 hours for TMC-coated MVLs, indicating slower absorption of OM from TMC-coated MVLs than from OM solution and uncoated MVLs ($P < 0.01$). This similar T_{max} between OM solution and MVLs may be due to the instability of MVLs in acid solution [see Section 'Stability in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4)]. Our data indicate that significant disruption of MVLs occurs in the

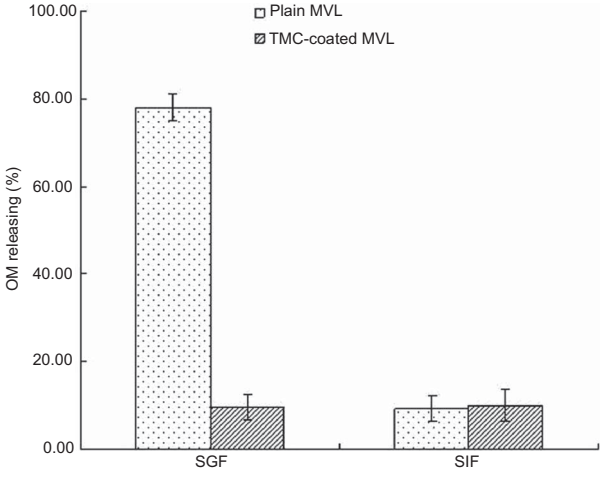

Figure 6. Stability in SGF and SIF, at 37°C, expressed as OM leakage, after 2 hours. Values are expressed as mean ± SD ($n = 3$).

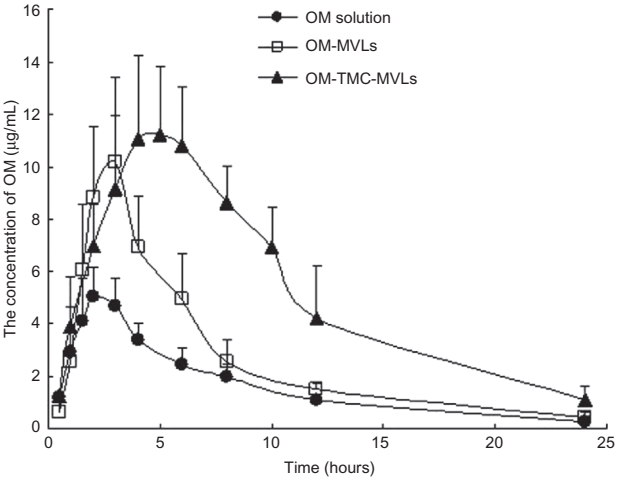

Figure 7. Mean OM plasma concentration–time profile of OM solution, uncoated MVLs, and TMC-coated MVLs (the concentration of TMC was 0.5%, w/v, the incubation time was 3 hours) and following oral administration of a dose of 50 mg/kg. Values are expressed as mean ± SD ($n = 6$).

Table 3. Pharmacokinetic parameters of OM solution, uncoated MVLs, and TMC-coated MVLs (the concentration of TMC was 0.5%, w/v, the incubation time was 3 hours) and following oral administration of a dose of 50 mg/kg. Values are expressed as mean ± SD ($n = 6$).

Parameters	OM solution	OM-MVLs	OM-TMC-MVLs
T_{max} (hours)	2.083 ± 0.801	2.750 ± 0.612	4.833*,** ± 1.169
C_{max} (μg/mL)	5.811 ± 0.555	10.76* ± 2.701	13.32*,*** ± 2.034
$t_{1/2}$ (hours)	5.287 ± 2.832	6.744 ± 2.534	5.680 ± 1.362
$AUC_{0\text{-}t}$ (μg/mL h)	38.42 ± 5.451	63.75* ± 12.12	125.3*,*** ± 18.21
$AUC_{0\text{-}\infty}$ (μg/mL h)	40.96 ± 7.231	68.46* ± 10.63	135.0*,** ± 24.15

*$P < 0.01$ versus OM solution. **$P < 0.01$ versus OM-MVLs. ***$P < 0.05$ versus OM-MVLs.

gut and most of OM was released from MVLs. Similarly, the reason for the slow absorption of OM from TMC-coated MVLs may be that the TMC coating can withstand disruption in the GI tract and release the drug slowly.

As far as the C_{max} is concerned, the values for both uncoated MVLs and TMC-coated MVLs were significantly higher than for OM solution, especially for TMC-coated MVLs ($P < 0.01$). The elimination half-lives ($t_{1/2}$) of uncoated MVLs and TMC-coated MVLs were not significantly different when compared with that of OM solution ($P > 0.05$).

In the GI tract, OM could be transformed to its active metabolite, matrine (MT), by intestinal bacteria[24], which means that the more OM metabolized, the less OM was absorbed. From the results, the AUC value was only 38.42 μg/mL h, when the OM solution was administered orally. Most of the OM was metabolized in the intestinal tract and this may be the main reason for the relatively lower OM levels in the blood. A slight improvement in the OM levels ($P < 0.01$) after feeding OM in uncoated MVLs could be observed compared with OM solution, and the AUC value was 63.75 μg/mL h. This could be explained if the MVLs that did not break up in the stomach could protect OM from metabolism in the intestinal tract and increase the OM levels in the blood. The AUC of TMC-coated MVLs was 125.3 μg/mL h, which was markedly increased by about 3.26- and 1.96-fold when compared with OM solution and uncoated MVLs, respectively, indicating that oral absorption of OM was markedly improved by its administration as TMC-coated formulations. This could be attributed to the protective effect of the TMC coating against the acid environment of the gut, so that TMC-coated MVLs remain stable even after passing through the stomach. Moreover, TMC was proved to be bioadhesive which helps delaying the intestinal transit time[13]. In addition, TMC could facilitate the paracellular transport of hydrophilic compounds by opening the tight junctions between epithelial cells[21]. Thus, TMC-coated MVLs might be a promising approach to provide efficient absorption with increased bioavailability of OM.

Conclusion

In this research, TMC was synthesized and used to coat MVLs. In vitro studies confirmed that the TMC-coated MVLs could increase the stability of MVLs in acid solution compared with uncoated MVLs. We also investigated the pharmacokinetics after oral administration of OM solution, uncoated MVLs, and TMC-coated MVLs. TMC-coated MVLs further increased the effective drug concentration compared with OM solution. Considering the significant increase in both the C_{max} and the AUC of OM, TMC-coated MVLs could be the effective carriers for improving of oral absorption of OM.

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