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Medium-chain fatty acid nanoliposomes for easy energy supply

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ABSTRACT

Objective: Developing a nanoliposome delivery system for an easy energy supply of medium-chain fatty acids (MCFAs) to improve oral doses and bioavailability.
Methods: Bangham's method and high-pressure microfluidization were used to prepare MCFA liposomes. The easy energy-supply property of MCFA nanoliposomes was estimated by the anti-fatigue experiments of mice including a weight-loaded swimming test and its corresponding parameters (serum urea nitrogen, blood lactic acid, and hepatic glycogen). For comparison, nanoliposomes without MCFAs and MCFAs not entrapped in nanoliposomes were used throughout.
Results: Compared with crude MCFA liposomes according to Bangham's method, the MCFA nanoliposomes made by high-pressure microfluidization exhibited great advantages in their characteristics, with a small average diameter (76.2 ± 34.7 nm), narrow size distribution (polydispersity index 0.207), high ζ-potential (−50.51 mV), great entrapment efficiency (70.5%) and drug loading (9.4%), and good stability. The high-dose group and the MCFA group (680 mg/kg) showed a longer weight-loaded swimming time (104 ± 29 min, $P = 0.087$, and 108 ± 11 min, $P = 0.047$, respectively) and significantly higher hepatic glycogen (16.40 ± 1.45 mg/g, $P < 0.001$ and 17.27 ± 2.13 mg/g, $P < 0.001$, respectively) than the control group (59 ± 11 min and 8.79 ± 2.76 mg/g, respectively). Moreover, serum urea nitrogen (891.5 ± 113.4 mg/L, $P = 0.024$, and 876.6 ± 70.8 mg/L, $P = 0.015$, respectively) and blood lactic acid (6.05 ± 1.40 mmol/L, $P = 0.001$, and 5.95 ± 1.27 mmol/L, $P < 0.001$, respectively) in the high-dose group and the group with an equivalent MCFA dose were significantly lower than those in the control group (1153.6 ± 102.5 mg/L and 12.53 ± 1.86 mmol/L, respectively).
Conclusion: Similar to MCFAs, MCFA nanoliposomes prepared by high-pressure microfluidization showed a strong easy energy-supply property, which suggested that MCFA nanoliposomes could be a potential drug candidate for an easy energy supply.
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Introduction

Medium-chain fatty acids (MCFAs) have 8 to 10 carbon atoms and are mainly found in coconut and palm kernel oils. MCFAs have shown unique nutritional characteristics that are different from those of long-chain fatty acids. Owing to the higher water solubility of MCFAs compared with long-chain fatty acids, triacylglycerol resynthesis and transport by chylomicrons can be neglected, so that MCFAs can be directly absorbed through the portal vein to the liver [1,2]. MCFAs are predominantly subject to metabolism into carbon dioxide, acetate, and ketones through β-oxidation and can be quickly utilized as energy without being stored as fat [3,4]. MCFAs have been applied as an easy energy supply in human therapy, particularly for patients with fat malabsorption, extreme athletes, retarded infants, and stressed adults, and as an antimicrobial agent [5–7]. However, an excess intake of MCFAs can lead to unwanted side effects, such as stimulation of the secretion of cholecystokinin, ketogenic and narcotic effects in neonates, nausea, and gastrointestinal discomfort [8–10].

To overcome the drawbacks of MCFAs and increase oral doses, a suitable drug carrier is required. Liposome, a self-assembling colloidal particle, in which a lipid bilayer encapsulates a fraction of the surrounding aqueous medium, is a promising drug-delivery system [11]. Nanoliposome, with its nanometric sizes (50–150 nm) during storage and application, possesses physical,

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structural, and thermodynamic properties similar to liposomes [12]. Recently, several new generations of liposomes have emerged, such as magnetic liposomes, cytoskeleton-specific immunoliposomes, and adenosine triphosphate (ATP) liposomes [13]. As "bioenergy" substrates, ATP liposomes can improve the rat liver energy state and metabolism during cold-storage preservation and protect human endothelial cells from energy failure in a cell culture model of sepsis [14,15]. Similar properties have been demonstrated for liposomal coenzyme Q10 [16], and MCFAs can be expected to be another potential material of ATP liposomes. Therefore, incorporation of MCFAs into nanoliposomes as bioenergy substrates has great potential in not only increasing their targetable properties but also improving the easy energy-supply bioavailability of MCFAs in the liver. There has been, however, no report in the literature about MCFA liposomes and nanoliposomes for an easy energy supply.

In recent years, various liposomes have been prepared by high-pressure microfluidization, such as 3β [N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) cationic liposomes, hemoglobin liposomes, and pH-sensitive polymer liposomes [17–19]. High-pressure microfluidization is a technology without toxic solvents [20] that employs the combined forces of high-velocity impact, high-frequency vibration, instantaneous pressure decrease, intense shear, cavitation, and ultrahigh pressures up to 200 MPa [21,22]. The particle size distribution of liposomes produced by high-pressure microfluidization appears to be narrower and smaller than those made by traditional methods (thin-film rehydration method, reverse-phase method, freeze-dried rehydration method, and ultrasonication method, etc.), and this technology may even increase the drug-entrapment efficiency [23]. High-pressure microfluidization can be applied for a large-scale production of liposomes in a continuous process [24].

The main aim of this study was to evaluate the physicochemical and easy energy-supply properties of MCFA nanoliposomes obtained from high-pressure microfluidization. The morphology, size distribution, ζ-potential, entrapment efficiency (EE), drug loading (DL), and storage stability were investigated. Bangham's method (thin-film rehydration method) was used to produce crude liposomes as a control. Energy deficiencies associated with fatigue were measured by changes in stamina as corresponding biochemical indicators. The easy energy-supply characteristics measured included weight-loaded swimming time, serum urea nitrogen (SUN), lactic acid (LD), and hepatic glycogen (HG) by intragastric administration of MCFA nanoliposomes to mice. Nanoliposomes without MCFAs and MCFAs not entrapped in nanoliposomes were used for comparison.

Materials and methods

Materials

Medium-chain fatty acids were kindly provided by UPMC (Pittsburgh, PA, USA). Caprylic acid methyl ester and capric acid methyl ester were purchased from AccuStandard (New Haven, CT, USA). Soybean phosphatidylcholine was provided by Merya's Lecithin Co. Ltd. (Beijing, China). Cholesterol was obtained from Tianjin Damao Chemical Reagent Co. Ltd. (Tianjin, China). SUN, LD, and HG kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and reagents used were of analytical purity or higher quality, obtained commercially.

Animals and administration

Animals

Male BALB/C mice (weighing 18–22 g) were obtained from Nanchang University (Nanchang, China) and housed for an adaption of at least 1 wk in polycarbonate cages before the experiments. Free access to water and standard mouse food (formula: corn, puffing bean, soybean, rice flour, trace element, vitamin, lysine, carrier; digestive energy 16 067 kJ/kg, metabolic energy 11 987 kJ/kg) was allowed; environmental conditions consisted of 23 ± 1°C, 60 ± 5% relative humidity, and a 12-h light/12-h dark cycle. Mice were treated in accordance with provisions in the Guidelines for Animal Care and Use by the National Institutes of Health (Bethesda, MD, USA).

Grouping and administration

Mice were randomized into three MCFA nanoliposome dose groups: MCFAs not entrapped in nanoliposomes (MCFA group), nanoliposomes without MCFAs (blank group), or normal saline (control group), with 16 mice in each group that were equal in body weight before tests. MCFA nanoliposomes were given to mice at concentrations of 170 mg (low-dose group), 340 mg (medium-dose group), or 680 mg (high-dose group) per kilogram of body weight. The blank and MCFA groups were given the same dose as the high-dose group. Thirty minutes before the experiment, all mice were administered the drug only once.

Preparation of MCFA nanoliposomes

Bangham's technique [25] was applied to prepare the crude MCFA liposome suspension. Briefly, a mixture of soybean phosphatidylcholine, cholesterol, Tween-80, MCFAs, and vitamin E (6:1:1.8:1.125:0.12, mass ratio) was solubilized in absolute alcohol and evaporated to a dry film in a rotary evaporator at 40°C. After further drying the lipid film for 4 to 6 h under a vacuum, the dried lipid film was rehydrated with phosphate buffered saline (pH 7.4) to make a crude MCFA liposome suspension.

To decrease the particle size of the crude liposomes and increase the encapsulation efficiency of MCFA liposomes, high-pressure microfluidization processing was used at room temperature. The high-pressure microfluidization treatment was carried out continuously or recycled with a microfluidizer (M-7125, Microfluidic Corp., Newton, MA, USA), which worked on the principle of dividing a pressurized stream into two parts, with each part passing through a fine orifice and impacting or colliding against each other inside the interaction chamber of the microfluidizer [26,27]. The samples, with a final concentration up to 8%, were treated with four passes under a pressure of 120 MPa.

Characterization of MCFA nanoliposomes

The morphologies of crude MCFA liposomes and nanoliposomes were examined by transmission electron microscopy (Hitachi H-600, Hitachi, Tokyo, Japan). The samples were dropped onto a copper grid and then air-dried at room temperature before being observed under transmission electron microscopy.

The average diameter and ζ-potential were determined by a dynamic laser light scattering technique at 25°C using a Nicomp 380 ZLS (PSS, Santa Barbara, CA, USA). The intensity was detected at an angle of 90°. The MCFA liposomes were diluted with distilled water before measurement.

The percentage of the drug incorporated into the liposomes was measured after free drug had been separated from the liposomes by centrifugation and extraction. About 5 mL of MCFA liposomes was centrifuged at 8000 rpm for 30 min and then a volume of 10 mL of N-hexane was added to withdraw the non-entrapped MCFAs. The rest of the suspension was mixed with 35 mL of 10% Triton X-100 methanol and then sonicated for 30 min to break down the liposomes. The entrapped MCFAs were extracted by 10 mL of *N*-hexane and then fatty acid methyl esters were prepared by direct transesterification [28]. Fatty acid methyl esters were analyzed by gas chromatography (Agilent 6890 Series GC System, Aglient Technologies, Santa Clara, CA, USA) with a flame ionization detector. The injection volume was 1 μL. The chromatographic column contained HP-innowax polyethylene glycol (30 m×0.32 mm×0.5 μm) (Agilent Technologies) and flow rates were 20 mL/min for nitrogen, 40 mL/min for hydrogen, and 450 mL/min for air. The temperature of the injection port and the detector was maintained at 280°C. The oven temperature was programmed to maintain a temperature of 150°C for 5 min, increase to 180°C at a rate of 6°C/min, and remain at 180°C for 2 min. The total run time for one cycle was 12 min. Peak areas and retention times were calculated (Agilent ChemStation B.03.01 [317], Agilent Technologies) and fatty acid methyl esters were identified by comparing retention times with those of standard caprylic acid methyl ester and capric acid methyl ester. The EE and DL values were calculated as $EE\% = (W_{in}/W_{total}) \times 100$ and $DL\% = (W_{in}/W_{lipids}) \times 100$, respectively, where W_{in} is the analyzed weight of MCFAs encapsulated in the liposomes, W_{total} represents the MCFAs present in the initial preparation, and W_{lipids} is the total weight of lipids.

Stability studies

The crude MCFA liposomes and nanoliposomes were stored at 4°C for 3 mo in a sealed condition. The average diameter and drug-entrapment efficiency were determined at fixed intervals (0, 7, 15, 30, 60, and 90 d).

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