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Research paper

Supermolecular evodiamine loaded water-in-oil nanoemulsions: Enhanced physicochemical and biological characteristics

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ABSTRACT

The purpose of this study was to develop and evaluate the supermolecular evodiamine (EVO) loaded water-in-oil nanoemulsions containing brucea javanica oil (NESEB) with enhanced physicochemical and biological characteristics. NESEB was fabricated by applying supermolecular phytosome nanotechnology and nanoemulsification technology together, in addition to using synergistic plant essential oil as a basic composition. Preferred physicochemical and biological characteristics of NESEB were investigated and compared with free EVO and other nanoemulsive EVO carriers. The possible explanations for improved absorption and bioavailability were put forward here. NESEB had high absorption and bioavailability, for example: the absorption rate constants and permeabilities of NESEB in different intestinal segments were 3.65–6.76 times that of free EVO; the relative bioavailability of NESEB to free EVO was 846.97%. NESEB markedly improved the oral bioavailability of EVO, which was most likely due to the increased gastrointestinal absorption. The development of nanoemulsion-based supermolecular EVO nanocarriers provides valuable tactics in insoluble natural antitumor drug delivering.

1. Introduction

In clinical practice, *Evodia rutaecarpa* or "Wu-zhu-yu" in Chinese has been traditionally administered orally together with some other traditional Chinese medicines to treat inflammatory diseases so far [2]. Evodiamine (EVO) is one of the most important active ingredients of *E. rutaecarpa* [1]. Recently, EVO has been drawing great interest of scientists due to its potent antitumor activities against a variety of tumors (such as gastric cancer and colorectal tumor) [3,4]. The action mechanism of EVO has been deeply investigated. On the other hand, in order to achieve the clinical translation of EVO, the primary consideration is to fabricate a suitable formulation with greatly enhanced oral bioavailability, since water insoluble EVO shows definite antitumor activity but simultaneously exhibits poor oral bioavailability. During the fabricating process, the biomaterials approved by national agency for drug administration are considered to be the first choice. These biomaterials are expected to form new amazing nanocarriers by using cutting edge technologies, such as supermolecular phytosome nanotechnology and nanoemulsification technology. The development of such smart nanocarriers made of the approved biomaterials may ease pressure on developing new biomaterials for effectively delivering an insoluble EVO [5–8].

There has been progress in the development of supermolecular phytosome nanotechnology and nanoemulsification drug technology over the past decade. Supermolecular phytosomes refer to noncovalently bonded complexes of a natural active ingredient and phospholipids. A drug–phospholipid complex is one supermolecular drug. Distinguishing from ordinary emulsions, nanoemulsions refer to emulsive drug nanosystems with some distinctive physiochemical properties [9,10]. There are a few supermolecular phytosomal drug or emulsive drug nanosystem products currently on the market, including Meriva® (supermolecular phytosomal curcumin for the treatment of diabetes related oxidative stress) by Thorne Research Inc. (USA), Siliphos® (supermolecular phytosomal silybin for the treatment of hepatitis associated with reduction in serum ferritin) by Indena S.p.A. Inc. (Italy), NORVIR (emulsive ritonavir nanosystem for the treatment of HIV-1 infection) by Abbott Laboratories (USA), and Neoral® (emulsive ciclosporin nanosystem for suppressing the immune system) [11–14].

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Supermolecular phytosomal EVO (SEVO, or EVO-phospholipid nanocomplex) had been developed in our lab [15]. The relative oral bioavailability of SEVO was increased to 218.82% compared with free EVO. Besides SEVO, only a few EVO delivery systems (such as microemulsion for transdermal delivery, superparamagnetic Fe_3O_4-loaded polymeric nanocarrier for intravenous delivery) have been reported so far [16,17]. Obviously, it is of great clinical significance to investigate some more effective EVO delivery systems with much higher bioavailabilities than EVO and SEVO.

Since some drug–phospholipid complex based formulations, such as hydroxysafflor yellow A-phospholipid complex oil solution and salvianolic acid-phospholipid complex loaded nanoparticles, exhibited improved oral bioavailability over the simple drug–phospholipid complex [18,19], we would like to further package a SEVO in a nanoemulsive system to achieve the added effectiveness. The nanoemulsive and supermolecular phytosomal EVO was formulated by applying these two above-mentioned nanotechnologies simultaneously instead of separately. Furthermore, brucea javanica oil is a Chinese drug widely used in clinic to treat a broad spectrum of tumors, and is known for the effectiveness and safety of it plus other chemotherapy drugs [20,21]. Since oil phase is essential during the nanoemulsification process, it may be a good idea to entrap brucea javanica oil into the novel EVO delivery nanosystem, and then brucea javanica oil can provide a synergistic antitumor effect with EVO in addition to being the basic composition of the new nanoemulsive and supermolecular EVO nanocarrier. Although it is currently unavailable in either clinical practice or the research field, a nanoemulsified supermolecular drug nanocarriers composed of only biomaterials approved by national drug administration agency by applying supermolecular phytosome nanotechnology and nanoemulsification drug technology together, in addition to using synergistic plant essential oil as a basic composition, may be theoretically expected to deliver an insoluble natural drug effectively and exhibit greatly improved oral bioavailability. Here we reported some encouraging experimental data to support this hypothesis.

In the experiments outlined below, a nanoemulsified supermolecular evodiamine containing brucea javanica oil (NESEB) only using biomaterials approved by national drug administration agency were designed and prepared, and then the *in vivo* pharmacokinetics and the *in situ* absorption of NESEB were evaluated. Compared with EVO, SEVO, conventional nanoemulsified evodiamine (NEE), nanoemulsified supermolecular evodiamine (NESE), and nano-emulsified evodiamine containing brucea javanica oil (NEEB), NESEB markedly improved the oral bioavailability of EVO, which was probably due to the increased gastrointestinal absorption. As far as we know, this was the first time to load a drug–phospholipid nanocomplex into a nanoemulsive delivering system. It was the first time that a preferred nanoemulsified supermolecular nanosystem with high oral bioavailability has been produced by combining phytosomal nanotechnology with nanoemulsifying technology, in addition to using synergistic antitumor oil as a basic composition, to deliver an insoluble antitumor natural drug. Our studies focus on the greatly improved oral bioavailability *in vivo* and the possible reason (obviously enhanced gastrointestinal absorption) of this delivery nanosystem. The development of such a novel nanosystem represented a valuable tactic in new medical application of commonly used biomaterials approved by national drug administration agency for effectively delivering insoluble antitumor natural drugs.

2. Materials and methods

2.1. Materials

EVO was obtained from Yuancheng Technology Development Co., Ltd. (Wuhan, China), purity 99.13%. Ethyl oleate was purchased from Shanghai Chemical Reagent Co. (Shanghai, China), analytical grade. Polyethylene glycol 400 was purchased from Tianjin Guangfu Fine Chemical Co., Ltd. (Tianjin, China). Cremorphor EL 35 was purchased from BASF Corporation (Ludwigshafen, Germany). Brucea javanica oil was purchased from Shanghai Kexin Biology Engineering Co., Ltd. Soybean phospholipid (Lipoid S 75) was purchased from Phospholipid GmbH (Nattermannllee, Germany). All other chemicals and reagents used were of analytical or chromatographic grade. Male Sprague-Dawely rats obtained from the Animal Center of Chongqing Medical University (Chonqing, China) were all specific pathogen free animals. Their weights were 200–250 g. The animal studies were conducted in accordance with the protocol approved by the Laboratory Animal Committee, Chongqing Medical University. The animals were raised under controlled conditions with free access to diet and water, while fasted at least 18 h before drug administration in the gastrointestinal absorption study and 12 h in the pharmacokinetical study, respectively.

2.2. Fabrication and characterization of NESEB

Firstly, SEVO was produced using a modified solvent evaporation method [15]. Briefly, a mixture of phospholipid and EVO at a molar ratio of 2:1 was dissolved in 50 mL ethanol, and magnetically stirred at 60 °C for 3 h. The solvent was evaporated to dryness under hypobaric conditions; the resulting SEVO was stored until use. Secondly, SEVO was loaded into the NESEB using a nanoemulsifying technique. An appropriate amount of SEVO was added into an isotropic mixture of ethyl oleate, brucea javanica oil, cremorphor EL 35 and polyethylene glycol 400 (at a mass ratio of 24:12:13:10) and the resulting mixture was then magnetically stirred at 60 °C for 6 h, cooled to 30 °C, added dropwise by 5 mL of distilled water under continuous stirring with a magnetic stirrer. NEEB was prepared in a similar way to prepare NESEB, and the only difference was that EVO instead of SEVO was added in the preparing process. NESE was prepared in a similar way to prepare NESEB, and the only difference was that ethyl oleate instead of a blend oil (ethyl oleate and brucea javanica oil) was added in the preparing process, i.e., no brucea javanica oil was added in the preparing process. NEE was prepared in a similar way to prepare NESE, and the only difference was that EVO instead of SEVO was added in the preparing process.

The conductivity of NESEB and its dilution (5 times with ethyl oleate) were determined at 25 °C by an electric conductivity analyzer (DDB-303A, Shanghai Precision & Scientific Instrument Co. Ltd, Shanghai, China), respectively. The size and zeta potential of NESEB were determined at 25 °C by dynamic light scattering (Zeta-Sizer Nano-ZS90, Malvern, UK). These studies were performed at refractive index of 1.45 because the refractive index for all formulation was around this value. The sample cell was made of quartz (Malvern, UK). The sample was prepared by diluting 4 mL of NESEB with 16 mL of ethyl oleate. In the *in vitro* release tests, NESEB was placed in a dialysis tube and then immersed into the release media of phosphate buffer system (PBS, pH 6.8). A HPLC method was used to determine the free EVO.

2.3. Gastrointestinal absorption of NESEB

The *in situ* gastric absorption test was carried out as previously described with some modifications [22,23]. Briefly, the experimental rats under intraperitoneal anesthesia with chloral hydrate were fixed supinely. After a 3 cm incision was made in the abdominal midline, the pylorus (the stomach outlet) was cannulated with a flexible 2 mm internal diameter tubing and then ligated. A small incision was made in the cardia (the stomach inlet). After the stomach was rinsed with the artificial gastric juice, the cardia was ligated and 4 mL of NESEB (or EVO, CNEE, NESE, and NEEB) at the