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Omega-3 fatty acids incorporated colloidal systems for the delivery of *Angelica gigas* Nakai extract

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ABSTRACT

Omega-3 (ω-3) fish oil-enriched colloidal systems were developed for the oral delivery of *Angelica gigas* Nakai (AGN) extract (ext). By constructing a pseudo-ternary phase diagram, the composition of oil-in-water (o/w) microemulsion (ME) systems based on ω-3 (oil), Labrasol (surfactant), and water was determined. AGN ext was dissolved into the ME system and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) was added to the ME formulation in order to enhance the mucosal absorption of the pharmacologically active ingredients in the AGN ext. The droplet size of AGN-loaded MEs was 205–277 nm and their morphology was spherical. The release of major components of AGN, decursin (D) and decursinol angelate (DA), from ME formulations in pH 1.2 and 6.8 buffers was significantly greater ($P < 0.05$) than that from the AGN suspension group. The pharmacokinetic properties of AGN-loaded MEs in rats were evaluated by measuring decursinol (DOH) concentrations in plasma after oral administration. TPGS-included ME (F2) resulted in significantly greater ($P < 0.05$) systemic exposure of DOH than that with ME without TPGS (F1), AGN ext + TPGS, and AGN in suspension. Severe toxicity of F1 and F2 on the intestinal epithelium was not observed by histological staining. The colloidal carriers described herein are promising delivery systems for oral administration of AGN ext.

1. Introduction

Dang-Gui (*Angelica gigas*) is a biennial or short-lived perennial plant cultured in Northeast Asia, such as China, Japan, and Korea. Cham-Dang-Gui, the dried root of *A. gigas* Nakai (AGN), has been cultivated in Korea and used as a medicinal herb. Chemical compounds included in the roots, stems, and leaves of AGN are pyranocoumarins, simple coumarins, furocoumarins, phthalides, volatile compounds, polyacetylenes, flavonoids, organic acids, polysaccharides, and phenolics [1]. Among these, pyranocoumarins are the major chemical substances of alcoholic extracts of AGN. Decursin (D), decursinol angelate (DA), and decursinol (DOH) are abundant components of coumarins obtained from AGN extract (ext). Various therapeutic properties of these major compounds have been identified, such as analgesic, anticancer, anti-inflammatory, anti-obesity, anti-diabetic, and cognitive enhancing effects [1–6]. However, the poor aqueous solubility of these compounds can restrict dietary and medicinal applications [7,8].

Diverse approaches regarding formulation development have been tried to improve the solubility and bioavailability of pharmacologically active ingredients from natural products [8–11]. Colloidal dispersion systems, which have a nanoscale particle size, can be used in natural product formulations. Microemulsions (MEs) are clear, thermodynamically stable, and isotropic liquid mixtures based on oil, surfactant, and water. MEs have been widely used to increase the solubility and mucosal absorption of drugs [12,13].

In this investigation, omega-3 (ω-3)-based ME systems were developed for the oral delivery of an ethanol extract of AGN (AGN EtOH ext). Polyunsaturated fatty acids (PUFAs) containing a double bond located at the third carbon atom from the end of the carbon chain are called ω-3 fatty acids (FAs). The ω-3 FA types that are physiologically relevant to humans include α-linolenic acid (ALA, found in plant oils, 18:3), eicosapentaenoic acid (EPA, found in fish oils, 20:5), and docosahexaenoic acid (DHA, found in fish oils, 22:6). It is known that fish oil EPA and DHA have beneficial effects for

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atopic diseases, cancer, cardiovascular diseases, cognitive decline, depression, and inflammation [14–19]. Both AGN EtOH ext and ω-3 have cognitive enhancing effects [3,14], thus ω-3-enriched ME systems containing AGN EtOH ext can be used as dietary supplements and medicines for that purpose.

Although ω-3 FA has been used to make emulsion systems for the delivery of several therapeutics [20,21]; to the best of our knowledge, it has not been used for making ME systems. Moreover, the combination of AGN EtOH ext and ω-3 for ME formulation has not yet been investigated. D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) was also incorporated into the ME system to increase the intestinal absorption of pharmacologically active components of AGN EtOH ext. Although some extents of D and DA are known to be metabolized into DOH mainly in the liver after oral administration [8], the oral absorption of other known and unknown components of AGN ext may be enhanced by ME systems and it can attribute to the improved pharmacological efficacies of AGN ext.

Herein, an ω-3/Labrasol/water/TPGS-based ME system was constructed and AGN EtOH ext was dissolved in this system. The physicochemical properties of AGN-loaded MEs, the amounts of D and DA released from MEs, and the pharmacokinetics of DOH (the major hepatic metabolite of D and DA) after oral administration in rats were thoroughly assessed.

2. Materials and methods

2.1. Materials

Fresh *A. gigas* Nakai (AGN) was purchased from a local market in Pyeongchang (Korea). Fish-derived docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) omega-3 (ω-3) FAs were obtained from Ocean Nutrition Canada Ltd. (Dartmouth, Nova Scotia, Canada). Polyethylene glycol (PEG)-8 caprylic/capric glycerides (Labrasol®) was kindly provided from Gattefosse (Cedex, France). D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Standard samples of decursin (D), decursinol angelate (DA), and decursinol (DOH) were obtained from Korea Promotion Institute for Traditional Medicine Industry (Gyeongsan, Korea). All solvents used in this investigation were high performance liquid chromatography (HPLC) grade. All other chemicals were of analytical grade and used without further purification.

2.2. Development and characterization of AGN-loaded MEs

AGN was dried in an oven at 55 °C for 24 h then cooled at room temperature. Dried AGN was immersed in ethanol (EtOH) and heated at 80 °C for 2 h to extract pharmacologically active ingredients. EtOH was then completely eliminated by drying to obtain dried AGN EtOH ext.

The composition (oil, surfactant, and water) of ME systems was determined by drawing a pseudo-ternary phase diagram. The phase diagram was constructed by using a water titration method. Oil (ω-3) and surfactant (Labrasol) were thoroughly blended in different weight ratios ranging from 1:9 to 9:1. Distilled water (DW) was slowly added to the oil and surfactant mixture at each ratio and the change in color, between transparency and opaqueness, was detected. By plotting the points corresponding to the regions of transparency and opaqueness, the ME region (monophasic area) was identified [22,23].

To prepare the ME systems, the optimal ratio of oil: surfactant: water (ω-3: 8.7%; Labrasol: 52.2%; DW: 39.1%, w/w) was selected. After blank ME was prepared, AGN EtOH ext (10 mg/ml) was solubilized into the blank ME to prepare the F1 formulation (Table 1). The F2 formulation was prepared by completely dissolving TPGS (5 mg/ml) into the F1 formulation (AGN-loaded ME system).

Images of blank F1, blank F2, F1, F2, and an AGN suspension (in DW) were taken for assessing the formation of the ME systems. The particle size, polydispersity index, and zeta potential of the blank (without loading AGN EtOH ext) MEs and AGN-loaded MEs (F1 and F2) were measured using the electrophoretic light scattering (ELS) method according to the manufacturer's protocol (ELS; ELS-Z1000; Otsuka Electronics, Tokyo, Japan). Incubation time-dependent stability of the ME systems was assessed by measuring particle size after incubating up to 30 days.

The morphology of developed MEs (F1 and F2) containing AGN EtOH ext was observed using a transmission electron microscope (TEM). Aliquots of ME formulations were placed onto copper grids with film, stained with 2% (w/v) phosphotungstic acid, washed with DW, and dried under an air stream for 20 min. Dried samples were observed using TEM (JEM 1010; JEOL, Tokyo, Japan).

2.3. In vitro *release test*

The release profiles of D and DA from ME formulations were assessed by dialysis in pH 1.2 and 6.8 buffers containing 0.1% (w/v) Tween 80. AGN EtOH ext (1 mg) suspended in DW (0.1 ml) or AGN-loaded ME formulation (0.1 ml) was loaded into a mini GeBA-flex dialysis tube (14 kDa molecular weight cut-off; Gene Bio-Application Ltd., Kfar Hanagide, Israel). Dialysis tubes containing each sample were placed in each buffer (30 ml) and incubated in a shaking bath at 37 °C with an agitation speed of 100 rpm. Aliquots (0.5 ml) of release media were collected at specific times (30, 120, and 240 min) and an equivalent volume of fresh buffer was added.

The released amounts of D and DA were quantitatively determined by a liquid chromatography-tandem mass (LC-MS/MS) system according to reported methods with slight modifications [8,24]. AGN EtOH ext was dissolved in methanol at 2.5 μg/ml and this was used for measuring the contents of D and DA in AGN EtOH ext. Two μl of 2.5 μg/ml AGN EtOH ext or a 2 μl aliquot of collected release media was injected onto an LC-MS/MS system equipped with an Agilent Technologies 1260 Infinity HPLC system (Agilent Technologies, Wilmington, DE, USA) and an Agilent Technologies 6430 Triple Quad LC/MS system. The separation of peaks for D and DA was accomplished by using Kinetex 2.6 μ C18 100A column (100 × 4.6 mm; Phenomenex, Torrance, CA, USA) with a C18 guard column (4 × 2.0 mm; Phenomenex, Torrance, CA, USA). The mobile phase was prepared by mixing acetonitrile (A) and water containing 0.2% formic acid (B) and the flow rate was maintained at 0.5 ml/min. The gradient elution program was as follows: (1) 0–1 min. A:B = 20:80 (v/v), (2) 1–45 min, A:B = 20:80–70:30. (3) 45–50 min, A:B = 70:30, (4) 50–51 min, A:B = 70:30–20:80, and (5) 51–60 min, A:B = 20:80. The optimized ESI source settings were as follows; 300 °C gas temperature, 11 l/min gas flow, 15 psi nebulizer pressure, and 4000 V capillary voltage, respectively. The fragmentation transitions of D and DA were identical, with *m/z* 329.2–229.1. The fragmentor voltage and collision energy were set at 130 V and 18 eV, respectively. The retention times of D and DA were 34.3 min and 34.7 min under these analytical conditions. Data acquisition and processing were performed with MassHunter Workstation

Table 1
Composition of AGN-loaded ME formulations.

Composition (%)	F1	F2
Labrasol (w/w)	52.2	52.2
DW (w/w)	39.1	39.1
ω-3 (w/w)	8.7	8.7
TPGS (mg/ml)	–	5
AGN EtOH ext (mg/ml)	10	10