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RESEARCH ARTICLE

Fabrication of Eudragit polymeric nanoparticles using ultrasonic nebulization method for enhanced oral absorption of megestrol acetate

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ABSTRACT

Megestrol acetate (MGA) is used as a progestagen to treat advanced cancers in the breast or uterus and anorexia-cachexia syndrome in cancer patients. Due to its low solubility (BCS class II), MGA bioavailability needs to be enhanced for efficacy and safety. We developed MGA-encapsulated Eudragit® L100 (EUD) nanoparticles (MGA-EUD (1:1) and MGA-EUD (2:1)) using an ultrasonic nebulization method. MGA-EUD (1:1) and MGA-EUD (2:1) consisted of MGA and EUD at the mass ratios of 1:1 and 2:1. Their physicochemical properties, i.e. particle size, loading efficiency, morphology, and crystallinity were determined. Dissolution tests were performed using USP method II. For pharmacokinetics, they were orally administered at 50 mg/kg to mice. Microcrystalline MGA suspension (MGA-MC, Megace®, BMS) was used as control. MGA-EUD (1:1) and MGA-EUD (2:1) had a smooth and spherical shape of 0.70 and 1.05 μm in diameter with loading efficiencies of 93 and 95% showing amorphous states of MGA. They significantly enhanced the dissolution potential of MGA. Oral bioavailability of MGA-EUD (1:1) and MGA-EUD (2:1) increased 2.0- and 1.7-fold compared to that of MGA-MC. It suggests that ultrasonic nebulization method for the fabrication of polymeric nanoparticles is a promising approach to improve the bioavailability of poorly soluble drugs.

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KEYWORDS
Megestrol acetate; polymeric nanoparticles; ultrasonic nebulization; Eudragit® L100; dissolution; bioavailability

Introduction

Biomedical application of ultrasound has been widely studied for decades and now substantiates its potentials into therapeutic systems, such as diagnostic imaging-combined cancer therapy, ultrasound-triggered drug release (Sirsi and Borden 2014) and ultrasound-assisted drug penetration enhancing treatments (Olbricht et al. 2013). Ultrasound technology has pioneered new areas not only in theranostics but also in manufacturing of nanomaterials (Ma et al. 2015; Stewart et al. 2017). It showed the functional capabilities in nano-crystal preparation, i.e. sonocrystallization, of pharmaceutically active drugs: aspirin (Eder et al. 2012), cloxacillin benzathine (Li et al. 2013), and acetaminophen (Bucar et al. 2015). Manish et al. (2005) reported nano-agglomerates of ibuprofen acquired from sonocrystallization method showed the improved physical properties for the tablet compressing. Metal oxide nanoparticles can be simply acquired using ultrasound technology. Sivakumar et al. generated metal oxide nanoparticles using ultrasound and a simple approach (Manickam et al. 2006). Also, they investigated the generated nanocrystals using ultrasound technology for various applications, i.e. magnetization (Manickam et al. 2006; Sivakumar et al. 2006) and incorporation of active pharmaceuticals (Tang et al. 2012; Sivakumar et al. 2014; Alzorqi et al. 2016). However, the potential of ultrasound for nanoparticle preparation, especially polymeric nanoparticles, has not been fully addressed and relatively limited than other technologies.

Among the platforms, polymeric nanoparticles have been prepared in this study using ultrasonic nebulization method in hot-air drying chamber (Figure 1(A)). Ultrasonic nebulization has been studied for the fabrication of metal nanoparticles and silicon microspheres (Burki et al. 2011; Chen et al. 2014). Nano-droplets are generated through an ultrasound driven-piezoelectric mesh membrane and gently dried to the solid phase nanoparticles by the heated laminar flowing gas. Particle size and distribution can be controlled by ultrasonic power and frequency. Scientists reported the size of particles in nano- and submicron can be easily controlled using these factors (Jia 2005; Li et al. 2010; Schmid et al. 2011).

Megestrol acetate (MGA) (Figure 1(B)) is a synthetic steroidal progestin for the treatment of anorexia-cachexia in patients with AIDS and cancer (Feliu et al. 1992; Mateen and Jatoi 2006). Since MGA has a low solubility in water (2.0 μg/mL) categorized as a biopharmaceutics classification system (BCS) class II (Li et al. 2015 #35), daily dose of MGA in oral suspension (800 mg) is a problematic issue. Thus, new platform of MGA is essential to enhance the bioavailability and decrease the dosing size (Farinha et al. 2000; Mateen and Jatoi 2006; Yeh and Schuster 2006; Li et al. 2015). MGA has been encapsulated with pH-sensitive polymer; Eudragit® L100 (EUD) in this study. EUD (Figure 1(C)) is a poly(methacrylic acid-co-methyl methacrylate) mainly used for the sustained delivery of oral drugs (Gallardo et al. 2008; Nguyen et al. 2017). Interestingly, the increased ionization of carboxylic acids (solubilization) of EUD at the intestinal pH condition induces co-solubilization of water-insoluble drug (Siepmann et al. 2008; Nadal et al. 2016). Kim et al. (2001) reported EUD nanoparticles maximized the oral absorption of cyclosporine when formulated with microemulsion pre-concentrate.

In this study, we proved the beneficial effects of ultrasonic technique for the preparation of polymethacrylate nanoparticles of water-insoluble drug, MGA. Particle size of nanoparticles was

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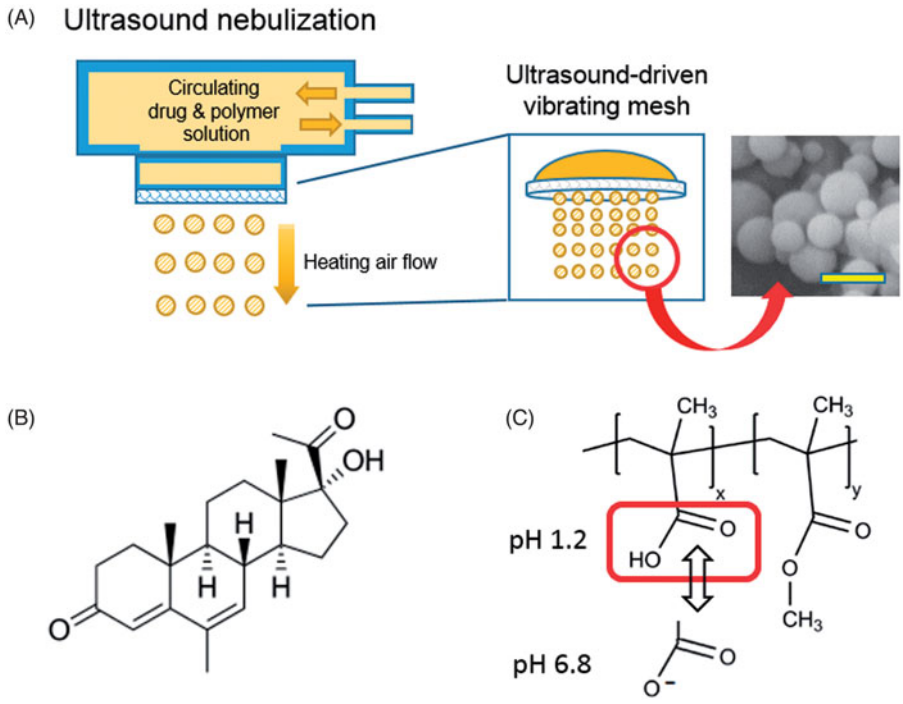

Figure 1. (A) Schematic diagram of ultrasonic nebulization technique and chemical structures of (B) MGA and (C) Eudragit® L100.

measured using ImageJ program (NIH, Bethesda, MD). Loading efficiencies of MGA into polymeric nanoparticles were evaluated. Morphology, crystallinity, and thermal characteristics were analyzed using a scanning electron microscopy (SEM), a powder X-ray diffraction (PXRD), and a differential scanning calorimetry (DSC). Dissolution profiles of nanoparticles were monitored using USP method II. For pharmacokinetic analysis, MGA nanoparticles were orally administered at 50 mg/kg into mice. The relative bioavailability in fasting states was calculated. MGA microcrystal suspension (MGA-MC, Megace®, Bristol-Myers Squibb Co., UK) was used as control.

Materials and methods

Chemicals

MGA was purchased from Sigma-Aldrich (St. Louis, MO). Eudragit® L100 (EUD), poly(methacrylic acid-co-methyl methacrylate) (1:1) (PubChem CID:6658), and silicon dioxide were purchased from Evonik Industries AG (Essen, Germany). Magnesium stearate was obtained from Whawon Pharmaceutical (Seoul, Korea). Distilled water was from the Milli Q (Millipore Co., Miliford, MA). All other chemicals were of analytical grade without further purification.

Preparation of MGA-loaded polymeric nanoparticles

MGA-loaded polymeric nanoparticles were prepared using ultrasonic nebulization method, composed of MGA, EUD, magnesium stearate, and silicon dioxide. Two formulations, MGA-EUD (1:1) and MGA-EUD (2:1), were designed at the mass of 500, 500, 15, and 15 mg and 666, 333, 15, and 15 mg of MGA, EUD, magnesium stearate, and silicon dioxide, respectively. After accurate weighing and mixing, they were dissolved in methanol and directly fed to a nanospray dryer (B-90, Büchi, Germany) with standard nozzle (0.7 mm in diameter) connected to an ultrasonic device at the frequency of 50–100 kHz according to the standard operating protocol. The solutions were delivered to the nanospray dryer under the following operation conditions: 120 °C inlet air temperature, 110 °C head temperature, 58 °C outlet temperature, 133 L/h feeding gas flow rate. The spray mesh hole was 4 μm in diameter. The solid nanoparticle powders were collected from the walls and bottom in the vessel after slow depressurization to atmospheric pressure.

MGA-loading efficiency

The MGA content in polymeric nanoparticles was determined using a high-performance liquid chromatography (HPLC, Agilent 1200 series, Agilent technologies, CA) with ultraviolet (UV) detection. One hundred milligram of MGA was dissolved in 100 ml of acetonitrile as a stock solution and 60% of acetone was used for further dilution. Standard solutions of MGA at 0.1, 0.2, 0.5, 1.0, 5.0, and 10 μg/mL were prepared for the calibration curves. An appropriate amount of sample was dissolved in acetonitrile, filtered and directly injected to HPLC systems connected to a column (Capcell Pak UG120, 5 μm, 4.6 × 150 mm). The mobile phase consisted of 55% acetonitrile in water (v/v). Flow rate was fixed at 1 ml/min. MGA was detected at 280 nm.

SEM observation

The morphological analysis of MGA-loaded polymeric nanoparticles was performed using SEM (Mini-SEM; S-4000 M, Hitachi High-Technologies Co., Japan). Samples were placed on a brass stub, then coated with platinum for 120 s at 15 mA. The images were obtained at 20 kV accelerating voltage.

Particles size estimation

Particle size of MGA-loaded polymeric nanoparticles was calculated by using ImageJ program (NIH). Results from SEM of MGA-EUD (1:1) and MGA-EUD (2:1) were introduced to ImageJ program. Diameter of more than 100 particles expressed in the units of pixels were converted into universal unit of length (μm) after the

calibration procedure. The acquired data were summarized and transformed into particle distribution curves.

Powder X-ray diffraction

The X-ray diffraction patterns of MGA and MGA-loaded polymeric nanoparticles were obtained using a PANanalytical X'Pert PRO diffractometer with monochromatic CuKα-radiation over a range of 2θ/deg from 5 to 40° at a scan rate of 2°/min.

Differential scanning calorimeter

The thermal properties of MGA and MGA-loaded polymeric nanoparticles were determined by a DSC (DSC Q-1000, TA Instrument, Leatherhead, UK). Aliquots of samples (10 mg) were placed in an aluminum pan and crimped with an aluminum lid. DSC analyses were carried out with a nitrogen flow of 20 ml/min at a temperature ramp speed of 10 °C/min and the heat flow was set from 50 to 300 °C. The instrument was calibrated before starting experiments.

In vitro *release of MGA from polymeric nanoparticles*

In vitro release study was carried out using a USP dissolution apparatus II (708-DS Dissolution Apparatus, Agilent Technologies, CA) at 50 rpm. Polymeric nanoparticles containing 10 mg of MGA were filled into hard gelatin capsules and dropped into 500 ml of simulated gastric juice without pepsin (pH 1.2, first dissolution medium) at 37 ± 0.5 °C. In two hours after the incubation in simulated gastric juice, 400 ml of 0.235 M sodium phosphate monobasic solution was added into the dissolution medium to adjust the pH to 6.8. Aliquots of 3 ml samples were taken at predetermined time interval, collected, filtered (0.45 μm) and analyzed using the HPLC method with UV detection as mentioned above. An equal volume of fresh dissolution medium was added to compensate.

Pharmacokinetic analysis

All animal care and experiments were carried out following the protocol and guidance approved by the Animal Care and Use Committee in Inha University. Male Balb/c mice weighing about 23–26 g were used and quarantined for one week prior to use. The mice were distributed in five animals per group, and had free access to water. The animals fasted for 12 h before the experiments. MGA-loaded polymeric nanoparticles were orally administered into the mice at 50 mg/kg as free MGA. MGA-MC was used as control. Blood samples were collected at 0.33, 0.66, 1, 2, 4, 6, 12, and 24 h. Plasma samples were obtained after centrifugation of blood samples at 13,000 rpm. for 10 min at 4 °C and stored at −20 °C for further analysis. The samples were analyzed using LC-tandem mass spectrometry (MS/MS) (3200 Q TRAP, AB SCIEX, Applied Biosystems/MDS SCIEX) (Li et al. 2015). The pharmacokinetic parameters of area under the curve (AUC_{last}), peak concentration (C_{max}), peak time (T_{max}), and half-life ($T_{1/2}$) and relative oral bioavailability of MGA-loaded polymeric nanoparticles were calculated using WinNonlin™ (ver. 3.0, Pharsight).

Statistical analysis

All data were presented as the mean ± standard deviation (SD). Differences between groups were tested for statistical significance was determined by Student's *t*-test. Null hypotheses of no difference were rejected if *p* values were less than 0.05.

Results

Morphology and size distribution of MGA-loaded polymeric nanoparticles

Ultrasonic nebulization-generated polymeric nanoparticles had a smooth surface and spherical shape (Figure 2(A,B)). No aggregates were observed in MGA-EUD (1:1) and MGA-EUD (2:1). The particle

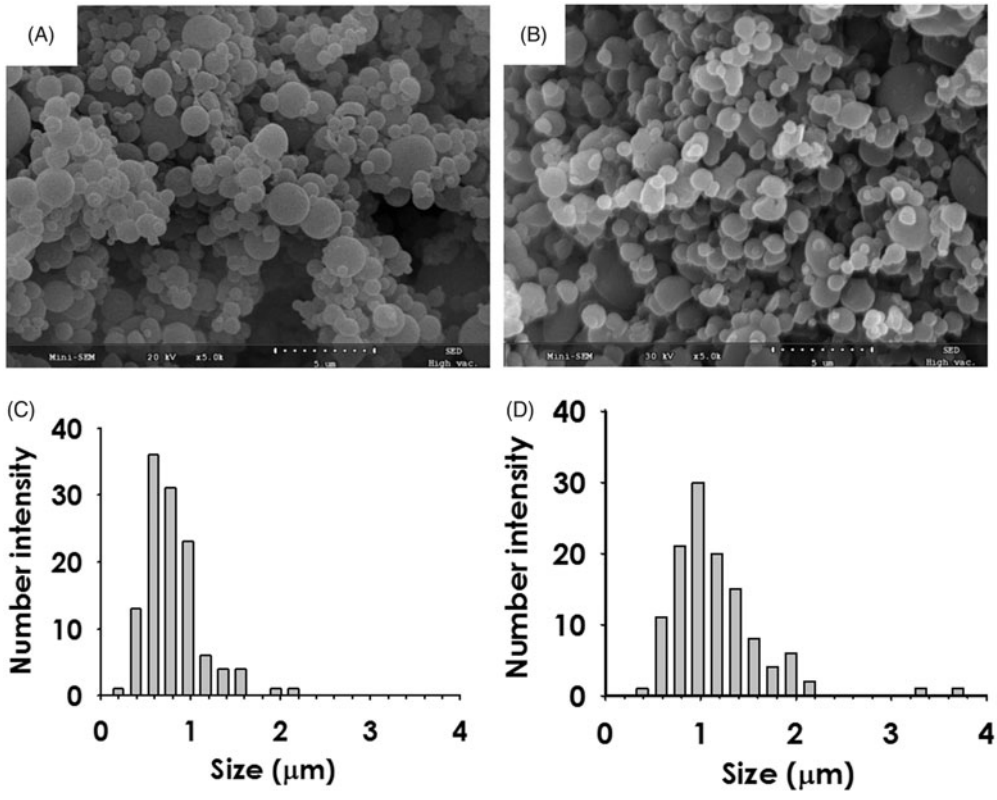

Figure 2. Representative SEM images and size distributions of MGA-loaded polymeric nanoparticles: (A and C) MGA-EUD (1:1) and (B and D) MGA-EUD (2:1).

size of MGA-EUD (1:1) and MGA-EUD (2:1) was 700 ± 322 and 1054 ± 479 nm in diameter. Polydispersity index values of MGA-EUD (1:1) and MGA-EUD (2:1) were 0.211 and 0.206, respectively.

Loading efficiencies of MGA-loaded polymeric nanoparticles

MGA-loading efficiencies on all nanoparticles showed over 90%; MGA-EUD (1:1) and MGA-EUD (2:1) had loading efficiencies of 93.48 ± 2.68% and 95.27 ± 3.24%, respectively. No significance was determined in MGA-loading efficiency based on the mass ratio of MGA to EUD from MGA-EUD (1:1) to MGA-EUD (2:1).

XRD patterns of MGA-loaded polymeric nanoparticles

The XRD pattern of crystalline MGA exhibited major peaks at 14.63 and 16.73° (Figure 3). However, there were no crystalline peaks in MGA-loaded polymeric nanoparticles. It suggested that MGA formulations were successfully developed showing in amorphous state.

Phase transition profiles of MGA-loaded polymeric nanoparticles

MGA-loaded polymeric nanoparticles were thermally analyzed and the resultant thermogram of heat flow versus temperature was plotted in Figure 4. In the thermogram, a raw MGA powder showed an endothermic peak of melting at 223.79 °C. On the other hand, MGA-loaded polymeric nanoparticles had no endothermic peaks in the thermograms. From the results, MGA-loaded polymeric nanoparticles in the amorphous states were successfully formulated. The results are comparable to the PXRD results.

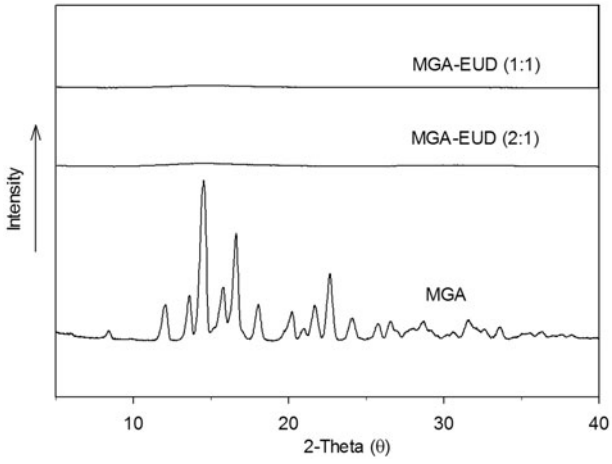

Figure 3. PXRD patterns of MGA-loaded polymeric nanoparticles. Raw MGA powder was used as control.

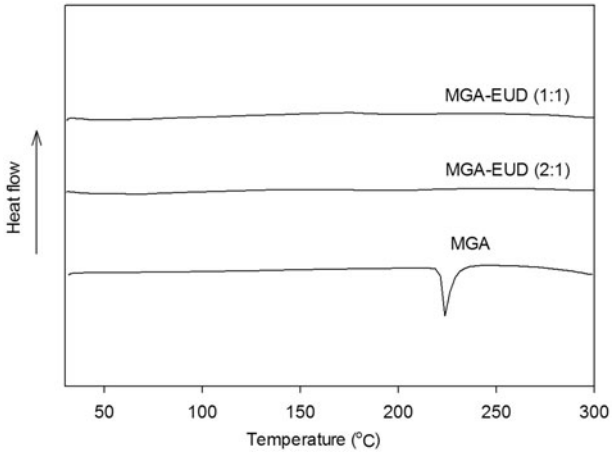

Figure 4. DSC thermograms of MGA-loaded polymeric nanoparticles. Raw MGA was used as control.

In vitro *release of MGA from polymeric nanoparticles*

Dissolution profiles of MGA from polymeric nanoparticles were obtained compared to MGA-MC (Figure 5). MGA-MC was initially dissolved to 3.93% for 0.25 h and its dissolution level was maintained to 4.04% at 5 h. The release profile of MGA from MGA-MC was not affected by the pH of dissolution media while each release profile from MGA-EUD (1:1) and MGA-EUD (2:1) was affected by the pH of dissolution media. The released MGA contents of MGA-EUD (1:1) and MGA-EUD (2:1) were 12.12 and 7.64%, respectively, at simulated gastric juice (pH 1.2) for 2 h. After changing the pH of media to 6.8, the released MGA contents increased up to 21.98 and 9.39% of MGA-EUD (1:1) and MGA-EUD (2:1), respectively. The rapid release of MGA from nanoparticles might be attributed by the strong pH-dependent solubility enhancement of EUD.

Pharmacokinetics of MGA-loaded polymeric nanoparticles

Plasma concentration versus time profiles were analyzed after single oral administration of MGA-loaded polymeric nanoparticles (Figure 6). Table 1 shows the pharmacokinetic parameters of MGA-EUD (1:1) and MGA-EUD (2:1) compared to MGA-MC. In particular, after the oral administration of MGA-MC, MGA-EUD (1:1), and MGA-EUD (2:1), AUC_{last} was 7531.7, 15,426.2 and 12,649.9 ng·h/mL, respectively. MGA-EUD (1:1) and MGA-EUD (2:1) exhibited 2.0- and 1.7-fold higher relative bioavailability comparing with MGA-MC.

Discussion

Here we report MGA-loaded polymeric nanoparticles fabricated using ultrasonic nebulization technique (Figure 1(A)) which can be defined as a combined technique of ultrasonic generation module and spray drying module to control particle size using a piezoelectronic vibrating mesh. MGA (Figure 1(B)) is a methylprogesterone-derived steroidal progestin developed for the treatment of breast

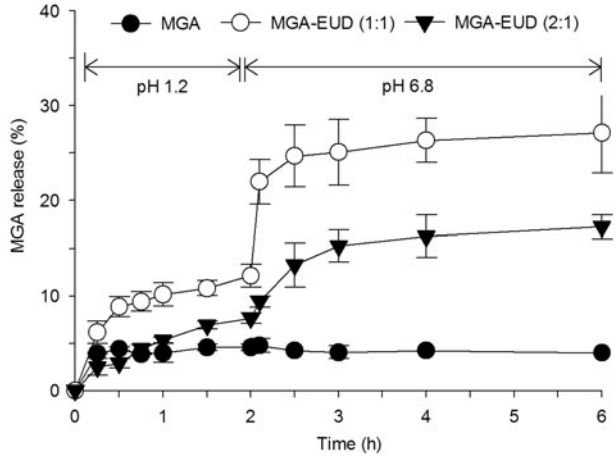

Figure 5. Dissolution profiles of MGA from polymeric nanoparticles. MGA-MC was used as control. The results represent the mean ± SD ($n = 3$).

and uterus cancer (Femia and Goyette 2005). It is also administered *via* oral route for the treatment of anorexia and cachexia in AIDS and cancer patients (Feliu et al. 1992; Mateen and Jatoi 2006; Aoyagi et al. 2015). MGA-MC is an extensively used oral suspension of microcrystalline MGA (Megace®) from Bristol-Myers Squibb Co approved by FDA. However, it has limited oral absorption due to its low solubility (BCS class II) and food effect (Li et al. 2015). Thus, the platforms for MGA are necessary to develop. MGA-loaded polymeric nanoparticles were mainly composed by pH sensitive polymer; Eudragit® L100 (Figure 1(C)) in this study. Two types of MGA-loaded polymeric nanoparticles were designed based on the mass ratios of MGA to EUD, MGA-EUD (1:1), and MGA-EUD (2:1).

To overcome the technical challenges in preparation of nanoparticles such as particle size control and encapsulation efficiency, we applied ultrasonic nebulization technique in fabrication of MGA-loaded polymeric nanoparticles (Burki et al. 2011). MGA polymeric nanoparticles processed by ultrasonic nebulization displayed nano- to submicron-sized particle with a spherical shape (Figure 2). Particle size of MGA-loaded nanoparticles increased by 1.5-fold (MGA-EUD (1:1) $\ll$ MGA-EUD (2:1)) as the drug-loading ratio increased from 1:1 to 2:1 (MGA to EUD, w/w) in the formulations. Their particle size values were superior to those of conventional spray dried nanoparticles composed by chitosan (Diaz et al. 2016). In addition, MGA-EUD (1:1) and MGA-EUD (2:1) showed high-loading efficiencies over 90% with no significant difference.

For MGA as a poorly soluble drug, an encapsulated drug into EUD in polymeric nanoparticles was in an amorphous state based on the molecular interaction of MGA in nanoparticles (Thakral et al. 2013). MGA encapsulated into EUD showed no crystalline peaks in the diffractogram suggesting the amorphous state of MGA (Figure 3). In thermogram, MGA-loaded polymeric nanoparticles had no endothermic peaks suggesting the stably and successfully manufactured state of MGA (Figure 4). The amorphous state of MGA in nanoparticles would lead to show an enhanced dissolution potential of MGA in a controlled release manner (Thakral et al. 2013).

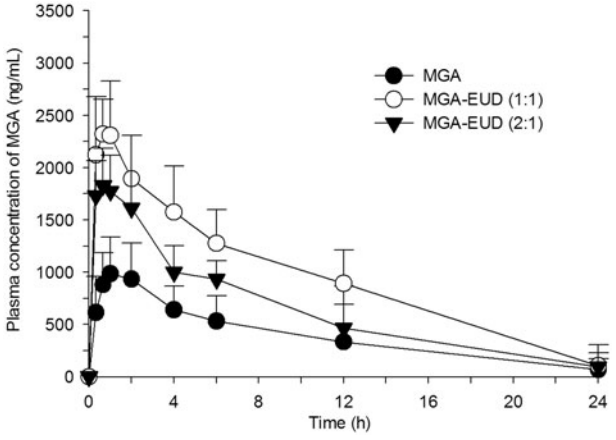

Figure 6. Plasma concentration versus time profiles of MGA in mice after a single administration of MGA-loaded polymeric nanoparticles (50 mg/kg as free MGA). MGA-MC was used as control to compare with the absorption of MGA and to calculate the relative bioavailability of MGA-loaded polymeric nanoparticles. The results represent the mean ± SD ($n = 5$).

Next, the enhanced release profiles of MGA from nanoparticles were confirmed (Figure 5). Dissolution of MGA from nanoparticles was explained by a phenomenon of interaction between MGA and EUD matrix in hydrodynamic environment (e.g. swelling and diffusion) surrounded by biological fluids like enzymatic juices. EUD is a synthetic and anionic co-polymer of methacrylic acid and methyl methacrylic acid (1:1) for oral dosage forms (Ammar et al. 2016; Nadal et al. 2016). It has functions with a controlled release and targeting in jejunum, due to pH-sensitive dissolving property over pH 6 (Thakral et al. 2013). The mechanism of enhancement of dissolution potential by EUD was generally explained by an increased saturation solubility of drug based on molecular or amorphous interaction, and an increased drug stability in gastrointestinal (GI) tract due to its pH sensitivity (Wang and Zhang 2012). Nadal et al. (2016) described ferulic acid-loaded EUD microspheres after spray drying, which showed the loading efficiencies up to 102% and controlled the drug release for 2 h. Ammar et al. (2016) also described EUD-based cinnazarine delivery system using a solvent evaporation method. Cinnazarine coated by EUD was released at pH 6.8 from 2 to 8 h for intestine targeting.

Finally, we confirmed the improved pharmacokinetic parameters of MGA-loaded polymeric nanoparticles compared to MGA-MC at the same dose (Figure 6). MGA-loaded polymeric nanoparticles at 50 mg/kg of MGA were orally administered into mice. MGA-MC had low bioavailability due to solubility problems. However, MGA-loaded polymeric nanoparticles enhanced the bioavailability no matter which MGA mass ratios to EUD. MGA-EUD (1:1) and MGA-EUD (2:1) of nanoparticles had 2.0- and 1.7-fold increases of relative bioavailability compared to MGA-MC (Table 1). It suggested that EUD can increase a drug muco-adhesiveness in GI tract while it can increase a saturation solubility of drug and a drug stability in all over the GI tract as mentioned above (Wang and Zhang 2012). However, we could not observe the pH-dependent absorption profiles of MGA. It was predicted in *in vitro* dissolution experiments, but could not be confirmed in animal experiments.

Conclusion

MGA-loaded polymeric nanoparticles were successfully manufactured using an ultrasonic nebulization technique. They showed nano-scale to submicron size and spherical shape. MGA showed high-loading efficiencies into nanoparticles over 90% suggesting its amorphous state after encapsulation into EUD. Their dissolution potentials were enhanced in a pH dependent and controlled release manner. For pharmacokinetics, the relative bioavailability of nanoparticles to MGA-MC increased in a mouse model. From the results, polymeric nanoparticles improved the dissolution potential and relative bioavailability of MGA compared to MGA-MC, a conventional microcrystalline suspension. In addition, ultrasonic spray dried polymeric nanoparticles can be a next generation platform for solubilization of poorly soluble drugs.

Table 1. Pharmacokinetic parameters of MGA-loaded polymeric nanoparticles in a single oral administration of 50 mg/kg into mice ($n = 5$).

	Pharmacokinetic parameters				
Formulation	$AUC_{(last)}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	Relative bioavailability
MGA-MC	7531.7 ± 1174.0	1117.3 ± 287.3	1.5 ± 0.5	3.5 ± 1.5	–
MGA-EUD (1:1)	15426.2 ± 7422.0	2389.7 ± 401.4	0.9 ± 0.6	5.6 ± 1.0	2.0
MGA-EUD (2:1)	12649.9 ± 2881.1	1930.5 ± 311.6	0.6 ± 0.3	4.8 ± 1.2	1.7

Disclosure statement

No conflict of interests was reported by the authors.

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