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Research paper

A novel application of α-glucosyl hesperidin for nanoparticle formation of active pharmaceutical ingredients by dry grinding

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ABSTRACT

The effectiveness of α-glucosyl hesperidin (Hsp-G) as a novel grinding aid for the preparation of drug nanoparticles by dry grinding was investigated. Poorly water-soluble drugs and Hsp-G were mixed at a weight ratio of 1/5 and ground for 60 min by a vibrational ball mill. It was evident that all poorly water-soluble drugs used in this study formed nanoparticles after the ground mixtures were dispersed into distilled water. The dissolution profile of glibenclamide from the ground mixtures of glibenclamide/Hsp-G showed dramatic improvement from that of untreated drug crystals. Administration of the ground mixture of glibenclamide/Hsp-G to rats resulted in a significantly higher rate of decrease in blood glucose levels than that of untreated glibenclamide. The area above the time-curve of plasma-glucose concentrations using the ground mixture of glibenclamide/Hsp-G was 6-fold higher than that using untreated glibenclamide. The improved dissolution rate due to nanoparticle formation of glibenclamide, induced by co-grinding with Hsp-G, was responsible for this improvement.

1. Introduction

Nanoparticulate systems have been investigated for the purpose of pharmaceutical applications such as pulmonary formulations [1,2], drug targeting [3–5], and oral administration [6,7]. Due to the difficulty of preparing nanocrystals of the drugs themselves, most researches have focused on the preparation of nanoparticulate systems using biocompatible or biodegradable materials, e.g., liposomes [8,9] and biodegradable polymers [10,11], involving drug particles. The adoption of the drug nanocrystals without additives for oral or pulmonary formulations is a simple and promising method in theory, but difficulties in actual preparations exist.

The clinical efficacy of an orally administered drug is limited when the drug molecule shows poor aqueous solubility and permeability [12–14]. Drugs with extremely low water solubility often require high doses to achieve the desired therapeutic plasma concentration after oral administration because of their poor dissolution profiles. Improvement of the aqueous solubility of poorly water-soluble drugs is one of the most essential topics in the field of pharmaceutics, because this improvement leads to increased oral bioavailability and reduction in the clinically relevant dose [15–17].

To enhance the dissolution rate and consequently the bioavailability of pharmaceutical materials, size reduction methods have been widely investigated as described by the Noyes–Whitney and/or Ostwald–Freundlich equations [18,19]. Size reduction in pharmaceutical materials is often performed by means of a dry-grinding process. Dry grinding is a simple and widely used processing in pharmaceutical technology; however, the size reduction in drug substances by dry grinding is reportedly limited to around a few micrometers due to aggregation between particles; the increased particle surface area caused by breaking micro- into nano-crystals is thermodynamically unfavorable so that nanocrystals tend to aggregate to reduce their surface area [20–22]. It was reported that downsizing a drug to the micrometer range is generally insufficient to increase the drug dissolution rate and absorption in the gastrointestinal tract when the solubility of a drug is extremely low [23]. A safe, low-cost, functional additive is desired in order to prepare submicron- or nano-range particles on dry grinding.

We previously reported that newly developed transglycosylated food additives are attractive materials for new pharmaceutical excipients. Hesperidin, a prominent constituent of citrus fruits, is well known as vitamin P. The enzyme-transglycosylated hesperidin, α-glucosyl hesperidin (Hsp-G), has unique properties in enhancing the dissolution properties of poorly water-soluble compounds [24]. When Caco-2 cells were exposed to high concentrations (10%) of Hsp-G, no cytotoxicity was observed, as the cell viability remained unchanged [25].

The application of Hsp-G as a grinding aid for nanoparticle formation of drug substance has not been previously reported. The aim of the present study was to investigate the effect of Hsp-G on the nanoparticle formation of poorly water-soluble drugs, and

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to estimate the improvement of dissolution and pharmacological effects via nanoparticle formation. Mefenamic acid (MFA), bezafibrate, furosemide, and glibenclamide (GCM) were used as examples of poorly water-soluble drugs. Of these, GCM was selected as a model drug for evaluating the improvement of pharmacological effects. The blood glucose-lowering effect of GCM after oral administration of a ground mixture of GCM/Hsp-G to rats was compared to those of untreated GCM crystals and a physical mixture of GCM/Hsp-G.

2. Materials and methods

2.1. Materials

Mefenamic acid (MFA), glibenclamide (GCM), and bezafibrate were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Furosemide was purchased from Sigma Aldrich Japan Co., Ltd. (Japan). All the drugs were used without further purification. α-Glycosyl hesperidin (Hsp-G) was a gift from Toyo Sugar Refining Co., Ltd. (Japan). Mannitol was purchased from Merck Chemicals Japan. Co., Ltd. (Japan).

2.2. Preparation of ground mixture of drug and Hsp-G

Ground mixtures of drug/Hsp-G were prepared using a ball mill (type MM301; Retsch, Haan, Germany). A physical mixture was prepared by simple blending of the drug and Hsp-G at definite weight ratios in a glass vial using a vortex mixer for 3 min. For the preparation of ground mixtures, a physical mixture of 900 mg was ground in a mixture mill at a fixed frequency of 20 Hz for definite intervals. All prepared particles were dried in desiccators with blue silica gel under reduced pressure for 1 day before their physicochemical properties were tested.

2.3. Particle-size analysis

The particle-size distribution of the resultant particles dispersed in an aqueous phase, listed in Tables 1 and 2, was determined by a Microtrac 3300 Particle Analyzer utilizing the patented Tri-Laser Technology (MT3300EXII, Nikkiso Co., Ltd., Japan; measurement range, 0.02–2000 μm). The particle size shown in Fig. 1 was determined by the dynamic light-scattering method using FPAR-1000® (Otsuka Electronics Co., Ltd., Japan; measurement range, 0.003–5 μm).

2.4. The determination of amount of drugs existing in the four particle-size fractions

Thirty milligram of ground mixture was dispersed into 50 mL of distilled water. The total drug concentration in the suspension was 0.1 mg/mL. The drug content was divided into four fractions and expressed as follows: dissolved, ~200 nm, 200–800 nm, and coarse particles. The suspension was filtered through a 0.8-μm filter; the sample that remained in the filter comprised the "coarse" particles. The resultant suspension was filtered through a 0.2-μm filter. Since the suspension that passed through the 0.2-μm filter may include fine particle of less than 200 nm and dissolved drug molecules, it was ultracentrifuged at 100,000 rpm (414,400 g) for 45 min to separate the dissolved drug molecules. The GCM concentration was measured by HPLC under the following conditions: pump, Jasco-880-PU; detector, Jasco-875; integrator, Jasco-807-IT; column, COSMOSIL 5C_{18}-MS-II (4.6 mmφ × 150 mm; Nacalai Tesque, Tokyo, Japan); column temperature, 40 °C; wavelength, 247 nm; and flow rate, 1.0 mL/min. The GCM in the supernatant after the ultracentrifugation was estimated as dissolved drug molecules in distilled water. The mefenamic acid concentration was measured by HPLC under the following conditions: column, COSMOSIL 5C_{18}-CN-MS-II (4.6 mmφ × 150 mm; Nacalai Tesque, Tokyo, Japan); column temperature, 40 °C; wavelength, 289 nm; methanol/50 mM phosphate buffer (pH = 2.5), flow rate, 1.0 mL/min; retention time, 3.77 min. The bezafibrate and furosemide concentrations were measured by HPLC under the following conditions: column, COSMOSIL 5C_{18}-AR-II (4.6 mmφ × 150 mm; Nacalai Tesque, Tokyo, Japan); column temperature, 40 °C; wavelength, 279 nm. The retention times of bezafibrate and furosemide were

Table 1
Changes in particle size of bezafibrate with increases in the amount of Hsp-G in the bezafibrate/Hsp-G weight ratio after the ground mixture was dispersed in distilled water.

Bezafibrate/Hsp-G (w/w)	Mean diameter	D_{10}	D_{50}	D_{90} (μm)
1/0.5	18.61 ± 8.57	8.86	14.85	33.44
1/1	11.18 ± 5.53	5.09	7.72	21.77
1/2	4.91 ± 1.88	2.52	3.54	8.05
1/5	0.18 ± 0.05	0.11	0.17	0.24

Table 2
Particle size of drugs when the ground mixture of drug/Hsp-G (weight ratio of 1/5) was dispersed in distilled water.

	Mean diameter	D_{10}	D_{50}	D_{90} (μm)
Mefenamic acid	0.12 ± 0.05	0.07	0.11	0.18
Bezafibrate	0.18 ± 0.05	0.11	0.17	0.24
Glibenclamide	0.19 ± 0.07	0.10	0.16	0.28
Furosemide	0.54 ± 0.29	0.22	0.46	0.96

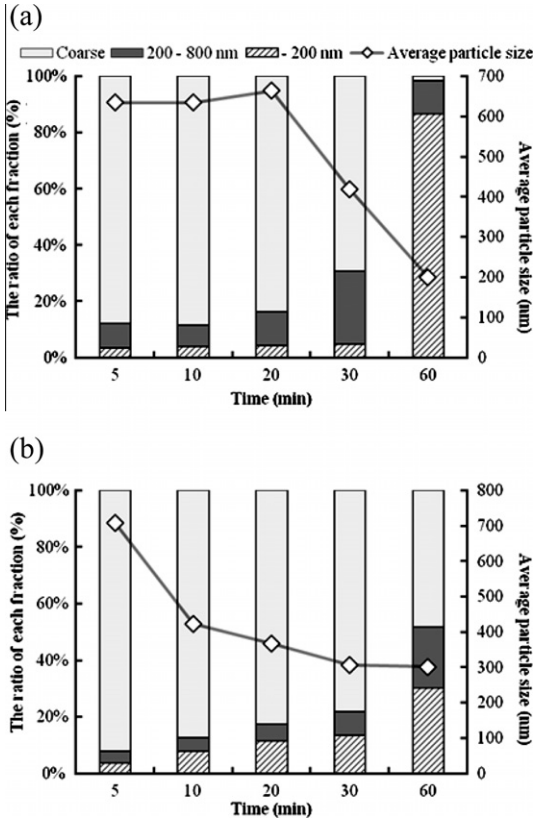

Fig. 1. Changes in average drug particle size and the ratio of drugs existing in three different particle-size fractions as a function of milling time after the ground mixture of drug/Hsp-G was dispersed in distilled water: (a) mefenamic acid (MFA), (b) glibenclamide (GCM).

5.0 and 2.8 min, respectively. Mobile phase A (solvent A) was distilled water/acetonitrile (90/10) containing 0.1% phosphate acid, and mobile phase B (solvent B) was acetonitrile. The elution profile (1.0 ml/min) was as follows: 0–2 min, linear gradient from 20% solvent A/80% solvent B to 30% solvent A/70% solvent B; 2–8 min, linear gradient from 30% solvent A/70% solvent B to 0% solvent A/100% solvent B

2.5. Scanning electron microscopy

Particle shape was observed by scanning electron microscopy (JSM-6510LV; JEOL Ltd., Japan). Prior to examination, the samples were mounted onto metal stubs and sputtered with a thin layer of gold under vacuum (JFC-1600; JEOL Ltd., Japan). The scanning electron microscope was operated at an acceleration voltage of 15 kV.

2.6. Dissolution test

A dissolution test for the commercial MFA and GCM powder and ground mixtures with Hsp-G was carried out according to the Japanese pharmacopoeia (XV), in triplicate for all samples. Physical mixtures of the drug and Hsp-G at a weight ratio of 1/5 were prepared by simple blending for 3 min. The prepared samples or the commercial drug powder (MFA: 10 mg and GCM: 2 mg) were added to 900 mL of phosphate buffer solution at pH 6.8 including 0.001% of Tween 80 at a temperature of 37 ± 0.5 °C and paddle-stirred at a rotation speed of 100 rpm. One-milliliter of each sample was withdrawn at specific time intervals (2, 5, 10, 20, 30, 60 min) and filtered through a 0.2-μm filter, and the concentrations of MFA and GCM were determined by HPLC.

2.7. Blood glucose level determination

Male Wistar rats (9 weeks, Japan SLC, Hamamatsu, Japan) were used in all *in vivo* studies. All experiments were approved and monitored by the Institutional Animal Care and Use Committee of Gifu Pharmaceutical University and were in accordance with the Japanese legislation on animal studies. Before experiments, the rats were fasted for 24 h with free access to water and were anesthetized using diethyl ether. Resulting samples were orally administered (4 mg/kg GCM) using a gelatin capsule kit for rodents (KN-346-1 mini-capsule device NATSUME SEISAKUSHO CO., LTD, Japan). Blood samples of 300 μL were taken at desired times and centrifuged for 5 min at 10,000 rpm. Serum glucose concentrations were assayed by a combination of mutase and glucose oxidase with a commercial kit (Glucose CII-Test, Wako Pure Chemicals, Osaka).

2.8. Statistical analysis

Data are presented as means ± SEM. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's test. Values of $p < 0.05$ were considered to indicate statistical significance.

3. Results and discussion

3.1. Formation of drug nanoparticles by co-grinding with Hsp-G

Poorly water-soluble drugs were co-ground with Hsp-G, and the resulting powder was dispersed in distilled water. Table 1 shows the particle size changes of bezafibrate crystals in distilled water. When the ground mixture of bezafibrate/Hsp-G was dispersed in distilled water, the mean particle size decreased with an increase in the amount of Hsp-G. Finally, when the weight ratio of bezafibrate to Hsp-G was 1/5, the mean particle diameter reached the submicron range; the particle size remained almost constant as the amount of Hsp-G increased up to 20 times that of the drug (weight ratio 1/20). Table 2 shows the mean particle size of drugs when the ground mixtures of drug/Hsp-G (weight ratio 1/5) were dispersed in distilled water. When the drug/Hsp-G weight ratio was 1/0.5, the mean particle size of the ground-mixture suspensions did not reach the submicron range. Under this condition, the mean particle diameters of glibenclamide and furosemide were 2.98 and 3.98, respectively (data not shown). On the contrary, all the samples listed in Table 2 exhibited the formation of submicron particles; these results indicate that an adequate amount of Hsp-G was necessary for nanoparticle formation on grinding.

Fig. 1 shows the difference in average particle size of MFA and GCM and their ratio in three different particle-size fractions as a function of grinding time, when the ground mixture of MFA/Hsp-G was dispersed into distilled water. The average particle size was almost unchanged for grinding for 5–20 min, and most of the MFA particles existed as coarse particles. Grinding for 60 min resulted in submicron-size MFA particles of less than 200 nm. The mean MFA particle size for 60 min was almost identical to the sample ground for more than 60 min. On the other hand, the average particle size of GCM decreased gradually as a function of grinding time, and finally reached a constant value of around 300 nm. Based on the results of these preliminary investigations, we chose the weight ratio of 1/5 (drug/Hsp-G) and the grinding time of 60 min as an appropriate condition for our system. We performed a long-term stability test on the MFA/Hsp-G system as a first step to ensuring Hsp-G formulation stability. After the storage of the ground mixture of MFA/Hsp-G (1/5) for 3 months in a vial at room temperature, the stored sample showed nano-sized particles with a mean particle size of 220 nm when dispersed in distilled water. After the ground mixture of MFA/Hsp-G (1/5) was dispersed into distilled water, the average particle size of MFA was increased from 110 to 220 nm and the mean particle size was maintained around 220 nm for at least 6 h (data not shown).

Fig. 2 shows the drug content existing in the four different particle-size fractions. The suspension from the 0.2-μm-pore filtration was ultracentrifuged to separate the two components of the transparent solution, and the component existing as nanoparticles less than 200-nm diameter from the fraction. The contents of drug molecules involved in the supernatant fraction differed among the drugs used. Relatively high amounts of drug were found in this fraction for bezafibrate and furosemide, while the MFA and GCM exhibited low values. This difference is related strongly to the solubility of the drugs in distilled water, since the amount of dissolved drugs (the black column) in the supernatant corresponded roughly to the solubility of the drugs used in this study.

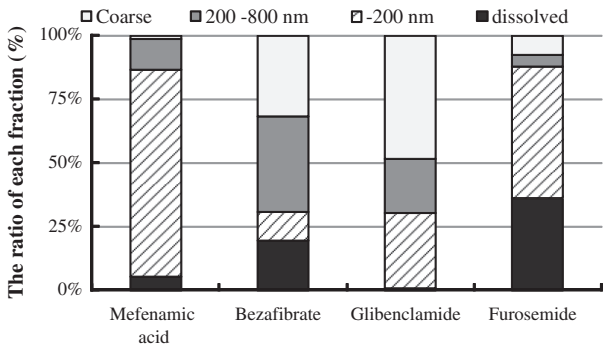

Fig. 2. The drug content existing in four different particle-size fractions after the ground mixture of drug/Hsp-G was dispersed in distilled water.

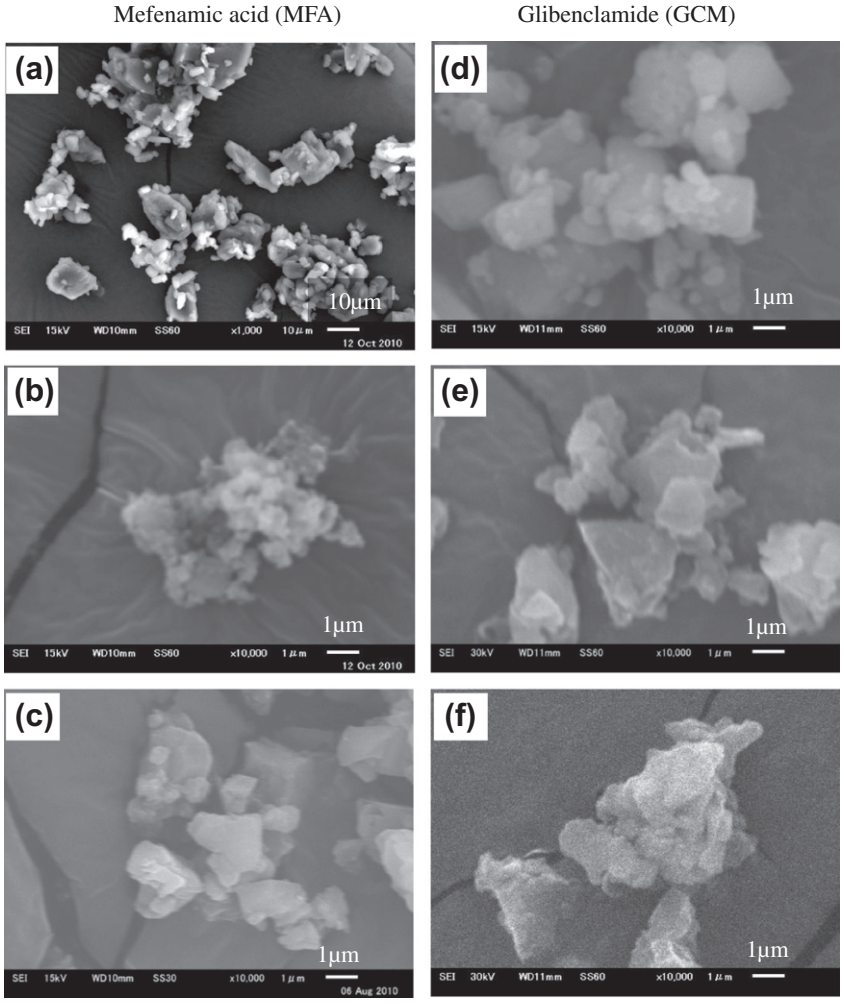

Fig. 3. Scanning electron micrograph images of: (a) untreated MFA, (b) ball-milled MFA, (c) ground mixture of MFA/Hsp-G(1/10), (d) untreated GCM, (e) ball-milled GCM, (f) ground mixture of GCM/Hsp-G(1/10); scale bar indicates the length of 1 μm.

In a previous study, we reported that a nano-sized (2–3 nm) association, formed by 4 Hsp-G molecules, could be obtained when the Hsp-G concentration was more than 5 mg/mL in aqueous medium [24]. With respect to the apparent solubility enhancement, we reported the possibility of the formation of a nano-sized complex between poorly water-soluble drugs and Hsp-G by effective incorporation of the poorly water-soluble drugs into the Hsp-G hydrophobic core through hydrophobic interactions. [25–27]. Since the investigation in Fig. 2 was performed under the Hsp-G concentration of 0.5 mg/mL, the associated tetramer form of Hsp-G in aqueous media could not occur due to the low concentration. Therefore, the enhancement of the apparent solubility of drugs by the existence of Hsp-G was not observed under low-concentration conditions.

3.2. Morphological investigation of drugs and their dispersibility in water

Morphological investigation of MFA and GCM was performed by scanning electron microscopy. Fig. 3 depicts SEM images of untreated drug crystals, ball-milled drugs, and ground mixtures of drug/Hsp-G. All were irregularly shaped particles and were interestingly composed of submicron-sized primary particles. During the grinding process, particle-size reduction occurred continuously along with the aggregation of drug particles; nevertheless, the particle size could not ultimately be reduced to a submicron level. The crystal properties of commercial drug powders and ground mixtures with Hsp-G were evaluated by powder X-ray diffraction analysis in Fig. 4. Weak X-ray diffractions of the MFA crystal and an almost halo-like pattern for GCM were observed in ground mixtures with Hsp-G. This indicates that most of the crystals may convert partially to an amorphous state or the intensity of the crystals may decrease by grinding. Two monotropic polymorphs of GCM having melting points of 180 and 210 °C were reported [28]. Although the intensity of X-ray diffraction of GCM decreased when the commercial GCM crystal itself was ground (data not shown), we did not detect any diffraction peaks of another form of GCM. Therefore, the polymorphic conversion may not occur during the grinding process. Besides, we confirmed that when the ground mixture of MFA/Hsp-G and GCM/Hsp-G was stored in a vial at 25 °C for 3 months, the X-ray diffraction patterns were almost unchanged. Fig. 5 shows the drug content of MFA and GCM existing in the four different particle-size fractions after the ground mixtures of drug/Hsp-G were dispersed in distilled water. The ground mixtures of drug/mannitol and ball-milled samples of drugs produced very small fractions of submicron-size drug particles, indicating that the dry grinding of the drug itself or the drug with mannitol is not sufficient for the formation of nanoparticles.

Among additives having relatively low molecular weight, mannitol has been reported to be a good grinding aid for size reduction in drugs [29]; however, no significant effects of the addition of mannitol have been found for the nanoparticle formation of drugs.

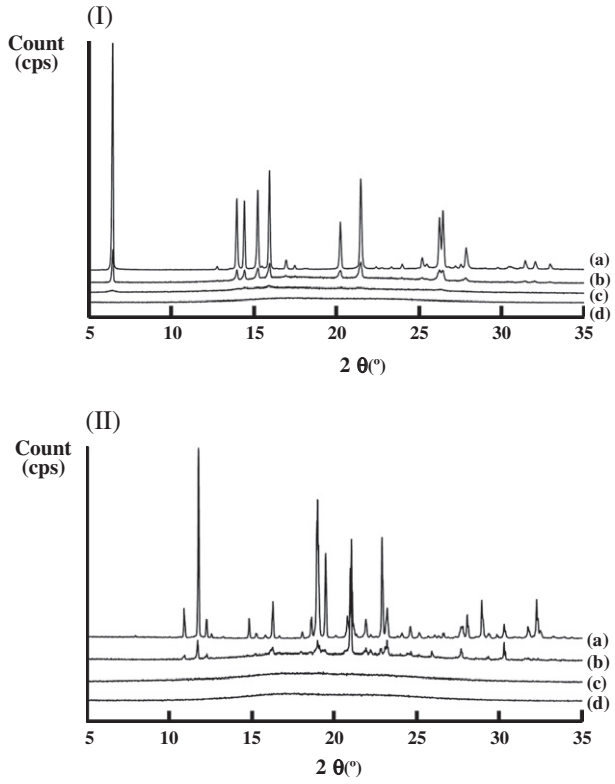

Fig. 4. Powder X-ray diffraction patterns of (I) MFA and (II) GCM systems: (a) unprocessed drug, (b) physical mixture of drug/Hsp-G (1/5), (c) ground mixture of drug/Hsp-G (1/5), (d) Hsp-G.

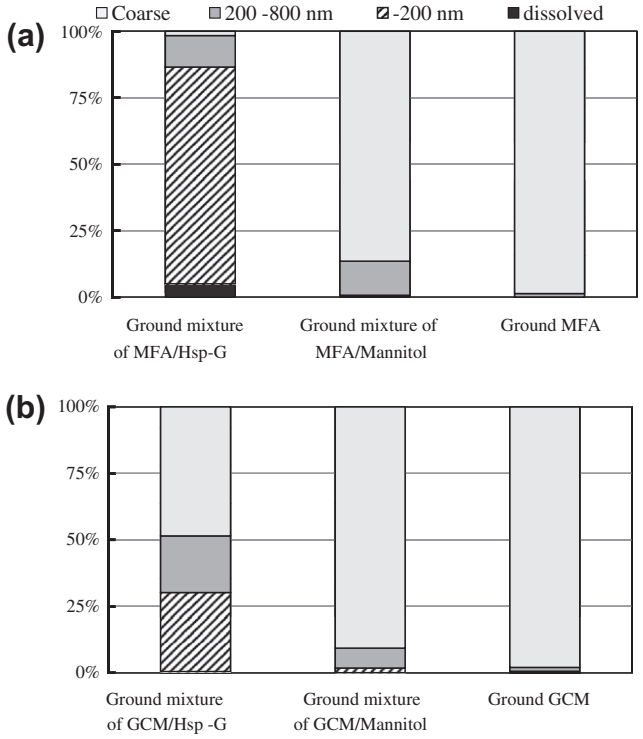

Fig. 5. Drug content existing in the four different particle-size fractions after the ball-milled drugs, and ground mixtures of drugs with mannitol or Hsp-G were dispersed in distilled water: (a) MFA, (b) GCM.

With respect to the nanoparticle formation of drugs, drug nanoparticles have been formed by co-grinding with cyclodextrins [30]. Cyclodextrins have been reported to form non-inclusion complexes as well as complex aggregates and complexes formed by interactions with the cyclodextrin surface [31]. When a drug is co-ground with cyclodextrins in a suitable moisture condition, cyclodextrin molecules appear to form a network structure with neighboring cyclodextrin molecules, covering the particle surface. Hsp-G has a lower molecular weight (Mw. 772) than typical cyclodextrins (Mw. ~970–1300) without a cavity. Although details of the mode of interaction should be investigated further, it can be conjectured that Hsp-G molecules produce some kind of interaction to prevent nanoparticle aggregation on dry grinding.

As shown in Fig. 5, untreated drug crystals and ball-milled drugs did not create submicron-size particles in distilled water, while the ground mixtures of drug/Hsp-G clearly show the existence of submicron-size particles. A plausible explanation was that ground mixtures of drugs with Hsp-G consisted of drug crystallites in an amorphous matrix of Hsp-G. When the ground mixtures of drugs with Hsp-G were dispersed into distilled water, the Hsp-G matrix dissolved and acted as a good dispersing agent by preventing the aggregation of the drug particles. We have reported that the Hsp-G showed weak surface activity (65 mN/m) when the Hsp-G concentration was more than 5 mg/mL [26]; however, according to the negligible surface activity of Hsp-G in the 0.5-mg/mL experimental condition, the surface activity of Hsp-G may not contribute to the prevention of nanoparticle aggregation. Although the mechanism of Hsp-G in preventing the aggregation of drug particles should be investigated in more detail, Hsp-G at sufficient concentrations appears to be an effective dispersing agent of drug nanoparticles in aqueous media.

3.3. Dissolution profiles of MFA and GCM from the ground mixture with Hsp-G

Fig. 6 shows the dissolution profiles of MFA and GCM from the obtained particles. The dissolution profiles of the drugs in distilled water from ground mixtures with Hsp-G or mannitol were determined with reference to those of untreated and ground drugs and their corresponding physical mixtures. The total amount of MFA and GCM dispersed in the water was 10 and 2 μg/mL, respectively. On the dissolution profile of MFA, the ground mixture of MFA/Hsp-G dissolved rapidly in phosphate buffer solution (pH 6.8) in comparison with untreated MFA, the physical mixture of MFA/Hsp-G, and the ground mixture of MFA/mannitol. The ground mixture of MFA/Hsp-G resulted in improvement in the dissolution rate of MFA.

In the GCM system, the ground mixture of GCM/Hsp-G showed a pronounced increase in dissolution rate compared with untreated GCM, the physical mixture of GCM/Hsp-G, and the ground mixture of GCM/mannitol. More than 50% of the GCM loaded was dissolved within 2 min in the case of the GCM/Hsp-G weight ratio of 1/5. We confirmed that the final dissolved amounts after 24 h were 1.6, 1.4, and 1.3 μg/mL for the ground mixture of GCM/Hsp-G, the physical mixture of GCM/Hsp-G, and the GCM crystal, respectively. Therefore, almost all the GCM loaded was dissolved after the 24 h experiment, and no significant further enhancement of solubility of GCM was observed. Although the intensity of GCM diffraction almost disappeared, a polymorph conversion was not observed because there was no new peak in powder X-ray diffraction compared to those in untreated GCM Fig. 4b. The dramatic improvement in the dissolution rate of GCM may be chiefly explained as effects of Hsp-G molecules: namely, the downsizing effect of forming nanoparticles of GCM together with the drug-particle-dispersing effect of Hsp-G, preventing the aggregation of GCM nanoparticles when dispersed in water. Partial amorphization of GCM in the presence of Hsp-G upon dry grinding may also have contributed.

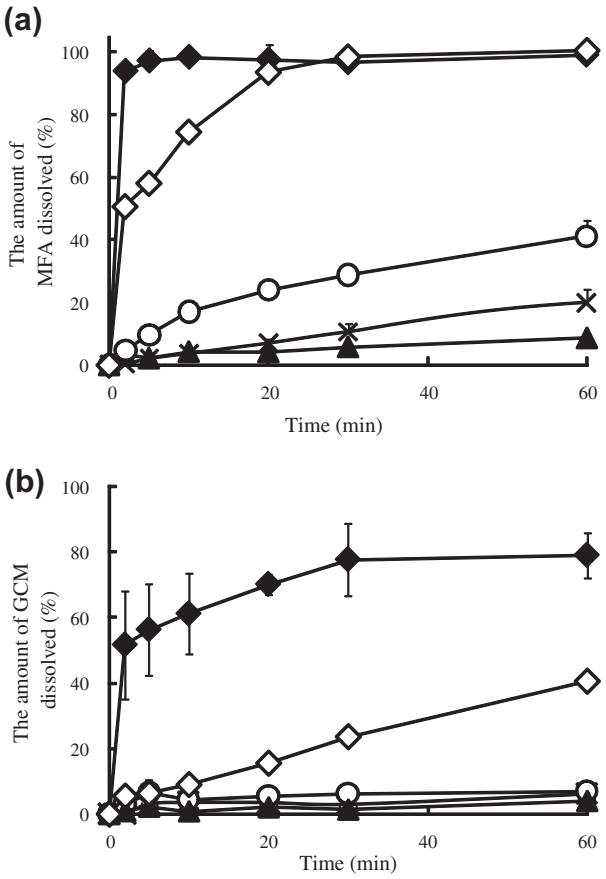

Fig. 6. Dissolution profile of drugs from the ground mixtures of drug/Hsp-G systems in JPXV second fluid (pH 6.8) containing 0.001% Tween 80: × untreated drug crystals ▲, ball-milled drugs ○, physical mixture of drug/Hsp-G (1/5); ◇, ground mixture of drug/mannitol (1/5); ♦, ground mixture of drug/Hsp-G (1/5) (mean ± SD, $n = 3$); (a) MFA, (b) GCM.

3.4. Improvement in pharmacological effect by co-grinding with GCM/Hsp-G

To investigate the effect of particle-size reduction in GCM on the enhancement of the pharmacological effect, the blood glucose-lowering effect after oral administration in rats was investigated. Fig. 7 shows the percent reduction in blood glucose and the area above the plasma-glucose-concentration time curve (AAC) after oral administration of capsules. Results suggest that the ground mixture of GCM/Hsp-G(1/5) was more efficient than the physical mixture or crystals of GCM in enhancing the absorption of GCM. The therapeutic efficacy of GCM was significantly enhanced and prolonged with administration of capsules containing ground mixture of GCM/Hsp-G(1/5). Comparison of the AAC values can provide a quantitative evaluation of the therapeutic efficiency of the different formulations with regard to reduction in blood glucose levels. Nanoparticle formation from grinding with Hsp-G resulted in more than 6- and 2-fold increases in the pharmacological efficacy of GCM when compared with untreated GCM crystals and the physical mixture, respectively.

Although several factors are involved in biological absorption, it has often been reported that particle-size reduction is effective at increasing the amount of drug absorbed, even in the case of poorly water-soluble drugs [32]. As a possible explanation of the absorption enhancement upon particle downsizing, Sugano described the particle drifting effect [33]. This is the effect of drug particles drifting into the unstirred water layer adjacent to the intestinal membrane. The particle drifting effect was reported to become significant as the particle size becomes smaller. As shown in Fig. 4b, a relatively large amount of GCM crystals were able to exist as nanoparticles. This may have contributed to the enhancement of GCM absorption.

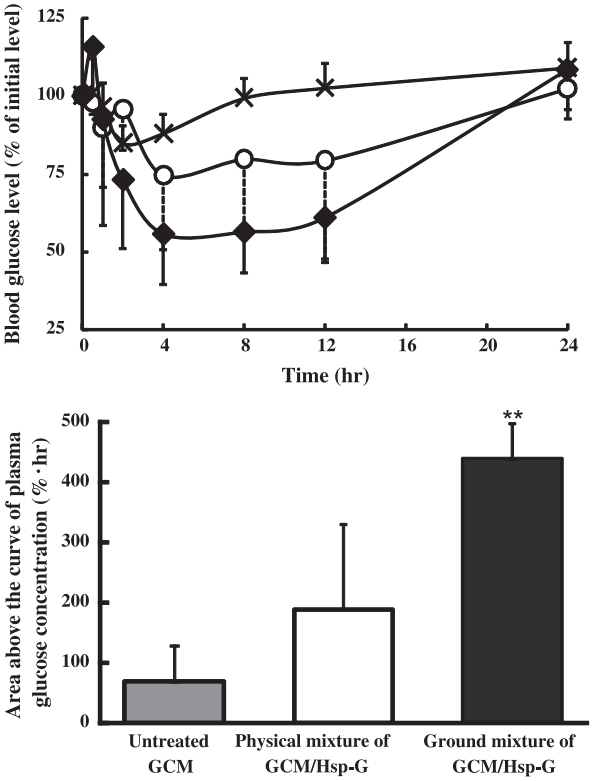

Fig. 7. Changes in (A) glucose level after intragastric administration of prepared GCM particles and (B) area above the plasma concentration time curve (AAC) values during 12 h after oral administration into rats: ×, untreated GCM; ○, physical mixture of GCM/Hsp-G (1/5); ♦, ground mixture of GCM/Hsp-G (1/5) Each point represents the mean ± SE. $^{**}p < 0.01$: significantly different from untreated GCM.

4. Conclusions

The novel grinding aid α-glucosyl hesperidin (Hsp-G) was found to be effective in the preparation of drug nanoparticles by dry grinding. Good dispersibility was found when ground mixtures of drugs and Hsp-G were dispersed into water, indicating that Hsp-G was able to act as a good dispersing agent. Although the details of the drug–Hsp-G interaction in the solid-state and solution should be investigated further, we confirmed that Hsp-G is a uniquely well-suited material for the formulation of drug nanoparticles.

Acknowledgments

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