European Journal of Pharmaceutics and Biopharmaceutics 76 (2010) 238–244

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Transglycosylated stevia and hesperidin as pharmaceutical excipients: Dramatic improvement in drug dissolution and bioavailability

Hiromasa Uchiyama, Yuichi Tozuka, Masaaki Imono, Hirofumi Takeuchi *

Laboratory of Pharmaceutical Engineering, Gifu Pharmaceutical University, Gifu, Japan

ARTICLE INFO

Article history:
Received 16 April 2010
Accepted in revised form 8 July 2010
Available online 15 July 2010

Keywords:
α-glucosyl hesperidin
α-glycosyltransferase treated stevia
Flurbiprofen
Probucol
Dissolution enhancement
Absorption enhancement

ABSTRACT

The capability of transglycosylated materials, α-glycosyltransferase-treated stevia (Stevia-G) and α-glycosyl hesperidin (Hsp-G), to enhance the bioavailability of poorly water-soluble drugs was investigated. Spray-dried particles (SDPs) of drug/transglycosylated material, such as, flurbiprofen (FP)/Stevia-G, probucol (PRO)/Stevia-G, FP/Hsp-G, and PRO/Hsp-G were prepared. All SDPs showed pronounced improvement in both dissolution rate and apparent drug solubility. The amount of dissolved PRO was significantly improved to that of untreated PRO crystals when prepared as SDPs of PRO/Stevia-G or PRO/Hsp-G. There was no cytotoxicity to Caco-2 cells at levels of 10% Stevia-G or Hsp-G solution. Values for the area under the plasma concentration–time curve (AUC) of untreated PRO, SDPs of PRO/Hsp-G and PRO/Stevia-G after oral administration to rats were 4.94 ± 2.06, 26.08 ± 4.52 and 48.79 ± 9.97 μg h/mL, respectively. Interestingly, AUC values in cases of the FP system were in the order of untreated FP < SDPs of FP/Stevia-G < SDPs of FP/Hsp-G. The effect on drug absorption enhancement may depend on the type of transglycosylated materials used. Stevia-G, a newly investigated material for this purpose, was found to have good potential for use as a pharmaceutical excipient.

1. Introduction

Poor water solubility of drugs results in low bioavailability and limited clinical efficacy. Dissolution plays an important role in the absorption of low-solubility and high-permeability drugs. Various methods to improve the dissolution of poorly water-soluble drugs have been reported [1–3]. The preparation of solid dispersions in pharmaceutically acceptable water-soluble polymers has been shown to be particularly effective in enhancing the rate of dissolution and the oral bioavailability [4–7]. This is a result of the higher aqueous solubility of the amorphous drug in a solid molecular dispersion, which in turn enhances absorption [8]. Functional food additives are potentially useful candidates for preparing solid dispersions, since those materials are relatively safe and inexpensive. Among them, transglycosylated food additives are attractive materials for new pharmaceutical excipients. We have already focused on an α-glucosyl hesperidin (Hsp-G). Hesperidin, a common constituent of citrus fruits, is well known as vitamin P. It possesses significant anti-inflammatory, hypotensive, and analgesic effects. Nevertheless, the use of hesperidin by the industry is limited because of its insolubility in aqueous solutions. Kometani et al. reported that the solubility of hesperidin was greatly improved by transglycosylation, with Hsp-G having a solubility about 300 times greater than that of hesperidin [9–11]. We previously reported that spray-dried particles (SDPs) of a water-insoluble drug and Hsp-G showed a pronounced enhancement of dissolution and absorption when compared to solid dispersions of the drugs with hydrophilic polymers. In addition, a direct relationship between drug solubility and the ratio of Hsp-G loaded was observed [12,13].

Stevia is a herb belonging to the Compositae family estimated to comprise 150–300 species [14,15]. Stevia rebaudiana, commonly known as sweet leaf, sugarleaf, or simply stevia, is widely grown for its sweet leaves. As a sweetener and sugar substitute, stevia's taste has a slower onset and longer duration than that of sugar, although some of its extracts may have a bitter or licorice-like aftertaste at high concentrations. Chan et al. reported that intravenous administration of stevioside resulted in a significant hypotensive effect in spontaneously hypertensive rats without adverse effects on heart rate or serum catecholamine levels [16]. Chen et al. reported that stevioside can regulate blood glucose levels by enhancing not only insulin secretion, but also by decreasing PEPCK gene expression in rat livers [17]. Since stevioside has been used as a natural sweetener for 20 years, and no significant adverse effects have been reported, this helps to establish its safety in long-term human usage. Alpha-glucosyl stevia (Stevia-G) is the transglycosylated material that has a sweeter taste and higher solubility than Stevia. The sweetness of Stevia-G may have the potential to mask the bitter taste of many drugs. However, there have been no reports on the utility of Stevia-G for the improvement of

* Corresponding author. Address: Laboratory of Pharmaceutical Engineering, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu 501-1196, Japan. Tel.: +81 58 230 8100; fax: +81 58 230 1022.
E-mail address: takeuchi@gifu-pu.ac.jp (H. Takeuchi).

0939-6411/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2010.07.006

dissolution and absorption of water-insoluble drugs. The purpose of this study was to evaluate the potential of Stevia-G to enhance the dissolution and absorption of poorly water-soluble drugs and to evaluate its utility when compared to Hsp-G.

Flurbiprofen (FP) and probucol (PRO) were used as poorly water-soluble model drugs. SDPs of FP and PRO were prepared with Hsp-G or Stevia-G and evaluated by scanning electron microscopy (SEM) and powder X-ray diffractometry (PXRD). The dissolution profiles of drugs from the SDPs with Hsp-G or Stevia-G were compared to those of the untreated drugs. The pharmacokinetic of the drugs after their oral administration to rats as SDPs with Hsp-G or Stevia-G were compared to those of the untreated drugs and to those of physical mixtures with Hsp-G or Stevia-G.

2. Materials and methods

2.1. Materials

Flurubiprofen (FP) was purchased from Tokyo Kasei Co., Ltd., and used without further purification. Probucol (PRO) was purchased from Wako Pure Chemical Industries, Ltd. (Japan) and used without further purification. α-Glucosyl hesperidin (Hsp-G: α-G Hesperidin PAT) was gifts from Ezaki Glico Co., Ltd., Hsp-G was prepared as described [18]. The ratio of transglycosylated Hsp-G in synthesized powders is more than 85% and the solubility of Hsp-G in water is ca. 20 g/100 mL. α-glycosyltransferase-treated stevia (Stevia-G) were gifts from Toyo Sugar Refining Co., Ltd. Stevia-G is obtained by glucosylating stevia extract with α-glucosyltransferase; thereafter, the resultant was separated and then purified using ion exchange resin. Stevia-G consists mainly of α-glucosylstevioside (more than 90%). The solubility of Stevia-G in water is estimated as ca. 320 g/100 mL. The chemical structure of Stevia is depicted in Fig. 1. The symbols R_1 and R_2 in the chemical structure of stevia indicate the presence of glucose units. Further α-glycosylation of these glucose residues using α-glucosyltransferase results in the formation of Stevia-G. All other chemicals and solvents were of reagent grade.

2.2. Preparation of spray-dried particles (SDPs)

Particles containing the drug and additive (FP/Hsp-G, FP/Stevia-G, PRO/Hsp-G, and PRO/Stevia-G) were prepared by the spray-drying method. For the system containing Hsp-G, 5 g of Hsp-G and 500 mg of FP or PRO were dissolved in an ethanol/water solution (8:2 v/v) prior to the spray-drying. For the system containing Stevia-G, 5 g of Stevia-G and 500 mg of FP or PRO were dissolved in an ethanol/water solution (6:4 v/v). Those solutions were fed to a spray dryer (GS31; Yamato, Japan) at rate of 10 mL/min and sprayed into a drying chamber from a nozzle with a diameter of 406 μm at a pressure of 0.13 MPa. The inlet and outlet temperatures of the drying chamber were fixed at 120 and 70 °C, respectively. All SDPs were dried in desiccators with blue silica gel under reduced pressure for 1 day before testing their physicochemical properties.

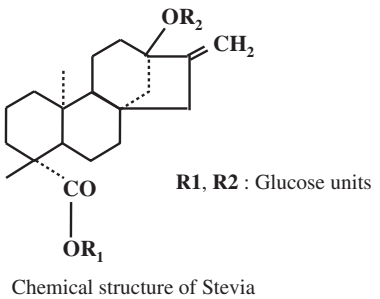

Fig. 1. Chemical structure of Stevia.

2.3. Physicochemical property of SDPs

Particle shape was observed by scanning electron microscopy (JSM-T 330A; Nihon Denshi, Japan). Prior to examination, the samples were mounted onto metal stubs and sputtered with a thin layer of gold under vacuum. The scanning electron microscope was operated at an acceleration voltage of 15 kV. The crystalline form of FP and PRO in SDPs was measured by the powder X-ray diffraction method (RAD-IC; Rigaku Denki, Japan). The scanning rate was 4°/min over a 2-theta range of 5–30°.

2.4. Dissolution test

A dissolution test for the commercial FP and PRO powder and SDPs with Hsp-G or Stevia-G was carried out according to the Japanese pharmacopoeia (XV). The physical mixtures (PMs) of drug and Hsp-G or Stevia-G at a weight ratio of 1:10 were prepared by simple blending for 3 min. The prepared samples or the commercial drug powder (50 mg) were added to 900 ml of distilled water at a temperature of 37 ± 0.5 °C and paddle stirred at a rotation speed of 50 rpm. Three-milliliter samples were withdrawn at specific time intervals and filtered through a 0.2-μm filter, and the concentrations of FP and PRO were determined by HPLC.

2.5. Measurement of surface tension

Surface tension was measured by an online tensiometer, SITA Science Line t60 (SITA Messtechnik GmbH, Dresden, Germany). This tensiometer measures the whole dynamic ranges of measuring tasks of surface tension by measuring bubble pressure. In this study, a long bubble lifetime (1000 msec) was selected to measure a semi-static condition in order to detect low concentrations of additives. Triplicate measurements were done for each experiment under controlled conditions at 37.0 °C. Ultrapure water was used for this experiment (Milli-Q® Academic A10, Millipore, Bedford, MA, USA).

2.6. Cytotoxicity test using Caco-2 cell monolayer

The cytotoxicities of Hsp-G and Stevia-G were determined by measuring the production of the yellow formazan product upon cleavage of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) by mitochondrial dehydrogenases in viable cells. The Caco-2 cells were seeded at 3.15×10^4 cells/cm^2 onto 96-well plates (Becton Dickinson, Franklin Lakes, NJ, USA). Cells were cultured for 4 days, and the culture medium was changed on alternate days (total 7 days). The culture medium was removed and washed twice with 200 μL of Hank's Buffered Salt Solution (HBSS). The cells were then exposed to 100 μL of each sample. After 120-min incubation, the cells were washed three times with 150 μL HBSS. The cells were incubated with 20 μl of CellTiter 96® AQueous One Solution Reagent (Promega, Madison, WI, USA) composed of 317 μg/ml MTS in 100 μL of culture medium. After incubation in a CO_2 incubator for 2 h, absorbance values were measured with a microplate reader (MTP 120, Corona Electric, Tokyo, Japan) at a wavelength of 492 nm. Background absorbance in cell-free wells was measured and subtracted from the measurement absorbance. A solution of 0.1% of sodium dodecyl sulfate in HBSS-MES buffer served as a positive control, and HBSS-Mes buffer served as a negative control. The percentage of cell viability was expressed as the percentage calculated by the following equation:

$$\% \text{ Cell viability} = ABS_{samples}/ABS_{control} \times 100,$$

where $ABS_{samples}$ was the absorbance values of those wells exposed to the Hsp-G and Stevia-G and $ABS_{control}$ was the absorbance values of those wells treated with HBSS-HEPES buffer.

2.7. Animal study

All animal experiments in the present study were performed in compliance with the regulations of Gifu Pharmaceutical University (Gifu, Japan) in line with the Japanese legislation on animal studies. Sprague–Dawley male rats (9 weeks; 200–220 g; Japan SLC Inc., Shizuoka, Japan) were used. The rats were fasted for 1 day before the experiments. Rats were anesthetized using diethyl ether. SDPs were orally administrated to rats (2 mg/mL FP and 200 mg/kg PRO) using an oral dosing syringe after dispersing in distilled water. Blood samples (400 μL) were taken from the jugular vein after predetermined time intervals following administration. Plasma was obtained from the blood samples by centrifugation for 10 min at 9730 × g. Ethanol (400 μL) was added to plasma (100 μL). The mixture was then vortexed and centrifuged at 9730 × g for 5 min to separate the plasma proteins. The supernatant was evaporated to dryness. The residue was dissolved in 100 μL of ethanol. The FP and PRO concentration in 20 μL of the solution 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). The area under the plasma concentration–time curve (AUC) was determined by the trapezoidal method.

2.8. HPLC assay

A PU-980 was used to analyze the FP and PRO concentrations. In the HPLC assay, a COSMOSIL $5C_{18}$-MS-II column (4.6 mm × 150 mm; Nacalai Tesque, Inc., Japan) was used. In the case of FP and PRO, the mobile phase consisted of 80% (v/v) methanol, 20% (v/v) water and 1% (v/v) 1 M acetic acid and 90% (v/v) acetonitrile and 10% (v/v) distilled water, respectively. The flow rate was controlled at 0.6 mL/min (FP) and 1.0 mL/min (PRO) with 20 μL of injection volume. The FP was eluted at 40 °C and quantitated at a wavelength of 254 nm, and PRO was eluted at 50 °C and quantified at a wavelength of 242 nm.

3. Results

Particles of probucol (PRO) with Stevia-G or Hsp-G were produced using the spray dryer, and the physicochemical properties were compared to those of the untreated drug and their physical mixtures (PMs). The contents of PRO in the SDPs with Hsp-G or Stevia-G as measured by HPLC were 8.5 ± 0.6% and 9.2 ± 0.3%, respectively, showing good correspondence to the theoretical content of 9.1%. The morphology of the prepared particles was examined by SEM (Fig. 2). While untreated PRO, PMs (data not shown), untreated Hsp-G (data not shown) and untreated Stevia-G (data not shown) appeared as irregularly shaped particles, a change in the morphology and shape was observed for the SDPs. SDPs showed spherical-shaped aggregates with an average particle size of about 2 μm.

Powder X-ray diffractometry (PXRD) is one of the most common techniques used to identify the crystalline structure of drugs. The XRD patterns for SDPs are shown in Fig. 3. Characteristic diffraction peaks of PRO were observed in the case of PMs with Hsp-G or Stevia-G. On the other hand, SDPs of PRO/Hsp-G and PRO/Stevia-G were characterized by the complete absence of any diffraction peak corresponding to crystalline PRO, indicating that PRO is no longer present in the crystalline form when processed using spray-drying, but exists in the amorphous state or the monomolecular dispersed state. Similar results were observed in the case of FP: the characteristic crystalline peaks of the drug disappeared after processing with Hsp-G and Stevia-G using the spray dryer (data not shown).

The dissolution profiles of the drugs in distilled water from SDPs with Hsp-G or Stevia-G were determined with reference to those of untreated PRO or FP and their corresponding PMs. (Figs. 4 and 5). The average particle size of untreated Hsp-G and Stevia-G was 10.8 μm and 12.5 μm, respectively. On the other hand, the average particle size of SDPs with Hsp-G or Stevia-G was about 2 μm (data not shown). PRO is a highly lipophilic, non-ionizable compound with extremely poor water solubility in the range of 2–5 ng/mL at 25 °C [19]. Therefore, the concentration of PRO dissolved from the untreated PRO or PM of Hsp-G or Stevia-G was under the detection limit of the described HPLC condition (50 ng/mL). However, SDPs of PRO/Hsp-G and PRO/Stevia-G showed dramatic improvement in the dissolution profile of PRO. The concentrations of probucol following dissolution from SDPs with Hsp-G and Stevia-G were about 5 μg/mL and 6.7 μg/mL, respectively, indicating the significant improvement in apparent solubility. The solubility of FP was estimated as ca. 35 μg/mL in distilled water after incubation at 37 °C for 1 week. In the case of FP, we have already reported the solubility- and dissolution rate-enhancing effects of Hsp-G [13]. Comparable results were obtained with Stevia-G, where 100% of the drug dissolved within 5 min with a maximum solubility of more than 55 μg/mL.

The surface tension of Stevia-G or Hsp-G solutions at the air/solution interface was measured as a function of their concentration. The results are presented in Fig. 6. From the data, it is clear that the two compounds exhibited surface-active properties in aqueous solutions. Their surface tensions decreased gradually when the concentration of Hsp-G or Stevia-G was increased and reached a break point, which would correspond to the saturation

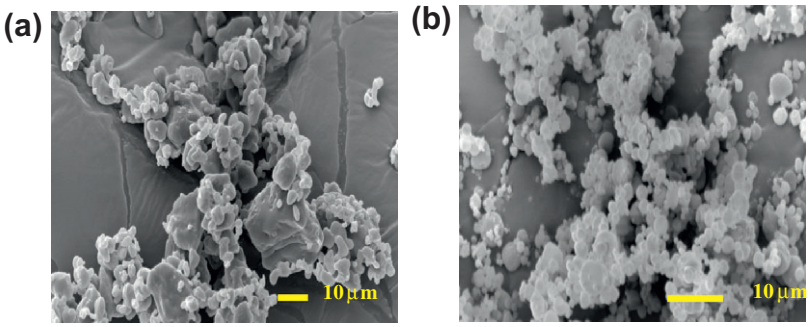

Fig. 2. SEM photographs of SDPs of PRO/Stevia-G: (a) PRO crystals and (b) SDPs of PRO/Stevia-G(1/10). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

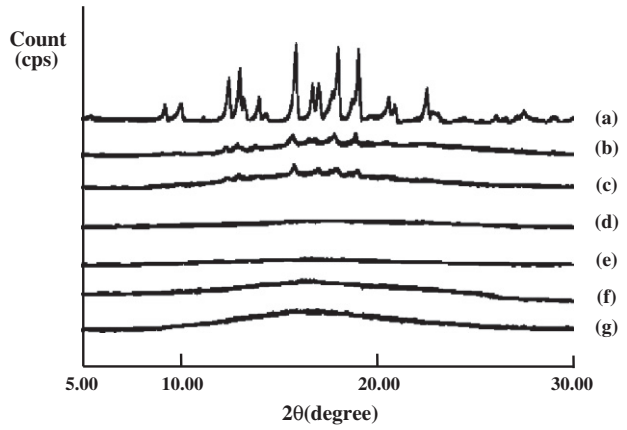

Fig. 3. Powder X-ray diffraction (PXRD) patterns of the PRO: (a) PRO crystal, (b) PM of PRO/Hsp-G(1/10), (c) PM of PRO/Stevia-G(1/10), (d) SDPs of PRO/Hsp-G(1/10), (e) SDPs of PRO/Stevia-G(1/10), (f) untreated Hsp-G, (g) untreated Stevia-G.

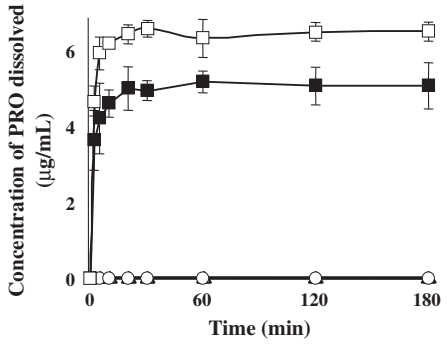

Fig. 4. Dissolution profiles of PRO in distilled water: (○, untreated PRO; ▲, PM of PRO/Hsp-G(1/10); △, PM of PRO/Stevia-G(1/10); ■, SDPs of PRO/Hsp-G(1/10)); □, SDPs of PRO/Stevia-G(1/10)). Each point represents the mean ± SD (n = 3).

of the interface by the adsorbed Hsp-G or Stevia-G molecules. At the break points, the Hsp-G and Stevia-G concentrations were about 5 mg/mL and 15 mg/mL, respectively. At the highest studied concentration, the surface tension was close to 65.5 mN/m for Hsp-G but did not reach values below 52.1 mN/m for Stevia-G, indicating that Stevia-G has higher surface-active properties than Hsp-G.

Changes in the viability of Caco-2 cells in the presence of Hsp-G or Stevia-G solutions are shown in Fig. 7. The viability of Caco-2 cells remained unchanged when the cells came into contact with high concentrations (10%) of Hsp-G or Stevia-G, indicating the very low cytotoxicity of these food additives. In the case of the sodium lauryl sulfate solution, which is an ionic surfactant, a significant decrease in viability was observed even for a 0.1% solution.

The samples for oral administration were prepared by suspension in distilled water. Fig. 8 shows the plasma concentration–time profiles of PRO in rats after oral administration of untreated PRO and SDPs with Hsp-G or Stevia-G. The maximum drug concentration (C_{max}) of untreated PRO was 0.18 ± 0.03 μg/mL. On the other hand, the C_{max} values of SDPs with Hsp-G or Stevia-G were 0.89 ± 0.06 and 1.61 ± 0.16 μg/mL, respectively, obviously higher than those of the untreated PRO. In addition, absorption from SDPs with Stevia-G was significantly improved compared to that of SDPs with Hsp-G. The AUCs until 48 h for untreated PRO and SDPs with Hsp-G or Stevia-G were 4.94 ± 2.06, 26.08 ± 4.52, and 48.79 ± 9.97 μg h/mL, respectively (Table 1). The AUC of the SDPs with Stevia-G was 9.8-fold that of untreated PRO and 1.8-fold that of SDPs with Hsp-G.

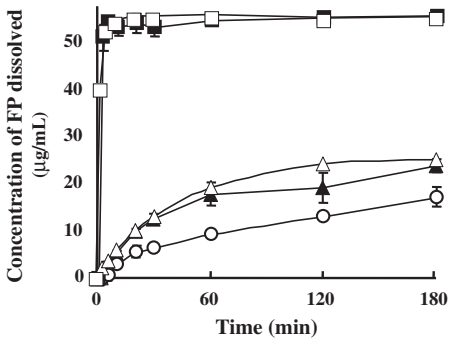

Fig. 5. Dissolution profiles of FP in distilled water: (○, untreated FP; ▲, PM of FP/Hsp-G(1/10); △, PM of FP/Stevia-G(1/10); ■, SDPs of FP/Hsp-G(1/10)); □, SDPs of FP/Sstevia-G(1/10)). Each point represents the mean ± SD (n = 3).

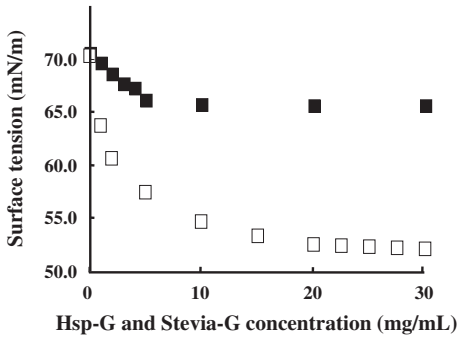

Fig. 6. Surface tension according to concentration of Hsp-G and Stevia-G: (■, Hsp-G; □, Stevia-G).

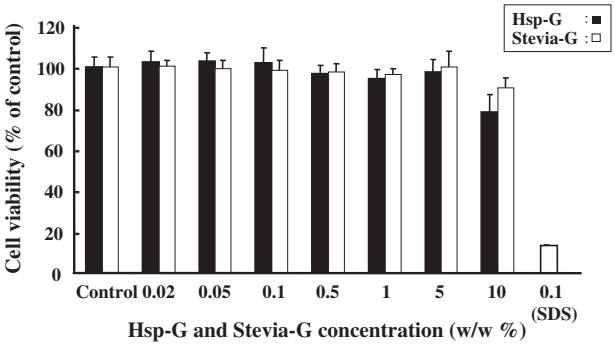

Fig. 7. Cytotoxicity of Hsp-G and Stevia-G to Caco-2 cells (n = 8).

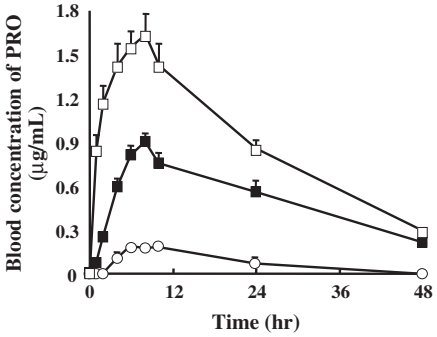

Fig. 8. Plasma concentration–time profiles of PRO in rats after oral administration of untreated PRO and spray-dried particles: (○, untreated PRO; ■, SDPs of PRO/Hsp-G(1/10); □, SDPs of PRO/Stevia-G(1/10)). Each point represents the mean ± SE (n = 6).

Table 1
Pharmacokinetic parameters of PRO after oral administration of SDPs of PRO/Hsp-G or PRO/Stevia-G in rats.

	T_{max} (h)	C_{max} (μg/mL)	AUC_0.48 h (μg h/mL)
Untreated PRO	10.0	0.18 ± 0.03	4.94 ± 2.06
SDPs of PRO/Hsp-G(1/10)	8.0	0.89 ± 0.06	26.08 ± 4.52***
SDPs of PRO/Stevia-G(1/10)	8.0	1.61 ± 0.16	48.79 ± 9.97***,###

$p < 0.001$, compared to the SDPs of PRO/Hsp-G.
*** $p < 0.001$, compared to untreated PRO.

Table 2
Pharmacokinetic parameters of FP after oral administration of SDPs of FP/Hsp-G or FP/Stevia-G in rats.

	T_{max} (h)	C_{max} (μg/mL)	$AUC_{0-12\ h}$ (μg h/mL)
Untreated FP	1.0	3.73 ± 0.43	22.06 ± 2.54
PMofFP/Hsp-G(1/10)	2.0	4.82 ± 0.64	32.09 ± 3.98*
PMofFP/Stevia-G(1/10)	2.0	5.71 ± 0.31	41.26 ± 2.03**
SDPs of FP/Hsp-G(1/10)	0.5	10.22 ± 0.14	62.65 ± 2.82***,###
SDPs of FP/Stevia-G(1/10)	0.5	8.34 ± 0.39	57.14 ± 2.27***,##

* $p < 0.05$, compared to untreated FP.
** $p < 0.01$, compared to untreated FP.
*** $p < 0.001$, compared to untreated FP.
$p < 0.001$, compared to the corresponding PMs.
$p < 0.01$, compared to the corresponding PMs.

Fig. 9 shows the plasma concentration–time profiles of FP in rats after oral administration of untreated FP, PMs with Hsp-G or Stevia-G and SDPs with Hsp-G or Stevia-G. C_{max} values of untreated FP and PMs with Hsp-G or Stevia-G were 3.73 ± 0.43, 4.82 ± 0.64 and 5.71 ± 0.31, respectively. Meanwhile, SDPs had higher C_{max} values than that of the untreated FP powder: 10.22 ± 0.14 and 8.39 ± 0.34 for SDPs with Hsp-G and Stevia-G, respectively. As shown in Table 2, the administration of 2 mg/kg of FP formulated by the spray-drying method using Hsp-G or Stevia-G resulted in a significant enhancement of the AUC compared with untreated FP and PMs. The increase in AUC of SDPs with Hsp-G or Stevia-G was nearly 2.8- and 2.2-fold, respectively compared with untreated FP.

4. Discussion

Drugs with poor aqueous solubility will typically exhibit dissolution rate-limited absorption because a drug must first dissolve in gastric and/or intestinal fluids before it can permeate the membranes of the GI tract to reach systemic circulation. Therefore, enhancing the solubility and dissolution rate of poorly water-soluble drugs is an important issue in pharmaceutical research. Solid dispersion technologies have been reported to improve the dissolution characteristics of poorly water-soluble drugs and, in turn, their oral bioavailability. In this context, many materials have been investigated for the preparation of solid dispersions, such as PVP and PEG [20,21]. We have focused on transglycosylated food additives as drug carriers, since such materials are relatively safe and inexpensive. We previously reported that the SDPs of drugs with Hsp-G showed pronouncedly improved drug solubility [12,13]. In this study, we investigated the potential of Stevia-G as carrier material and compared its solubility- and absorption-enhancing effects to those of Hsp-G.

We have already reported that SDPs of hydrophobic drugs with Hsp-G resulted in a dramatic increase in FP solubility when compared to untreated drugs and solid dispersions with hydroxypropylmethyl cellulose (HPMC) [13]. In addition, the apparent solubility of FP in hydrochloric acid solution (pH 1.2) was improved 10-fold when compared to untreated FP crystals when prepared as SDPs with Hsp-G. Solid dispersions with stabilizing polymers such as HPMC, known to form hydrogen bonds with hydrophobic drugs, have been reported as maintaining the supersaturated state and preventing the precipitation of such drugs out of solution [22,23]. In addition, Hancock and Parks reported that solubility enhancement could be achieved by the use of amorphous systems [24].

As shown in Fig. 4, the apparent solubility of PRO from SDPs was extremely high compared to that from untreated PRO. A higher enhancement effect of dissolution properties was found for PRO/Stevia-G than that of PRO/Hsp-G. These effects were not observed in the case of the PM of PRO and hydrophilic polymers such as PVP and HPMC (data not shown). Similarly, SDPs of FP/Stevia-G exhibited a marked improvement of FP dissolution compared to untreated FP (Fig. 5). These results suggested that Stevia-G or Hsp-G may form a particular complex with hydrophobic drugs, by which drugs are solubilized in a special structure. The structure of the complex that can dissolve in aqueous media appears to be similar in the case of Stevia-G and Hsp-G. However, it is possible that Stevia-G may form more stabilized interaction with PRO compared with Hsp-G, resulting in its higher solubility enhancement of PRO.

To investigate the detailed mechanism for the solubilizing effect of transglycosylated materials, we measured the surface tension as a function of additive concentration (Fig. 6). The surface tension values of aqueous Hsp-G and Stevia-G solutions were found to decrease when the concentration of Hsp-G or Stevia-G was increased, indicating surface activity at the water–air interface. The micellization process in water results from a delicate balance of intermolecular forces. The transglycosylation of hesperidin (Hsp-G), hesperidin glycosides, led to more than 300 times higher solubility than original hesperidin [9]. Hsp-G and Stevia-G would have a good balance between hydrophilic and hydrophobic moieties by transglycosylation and may form the micelle-like structure in an aqueous medium. As a result, Hsp-G and Stevia-G may improve the dissolution profile of poorly water-soluble drugs.

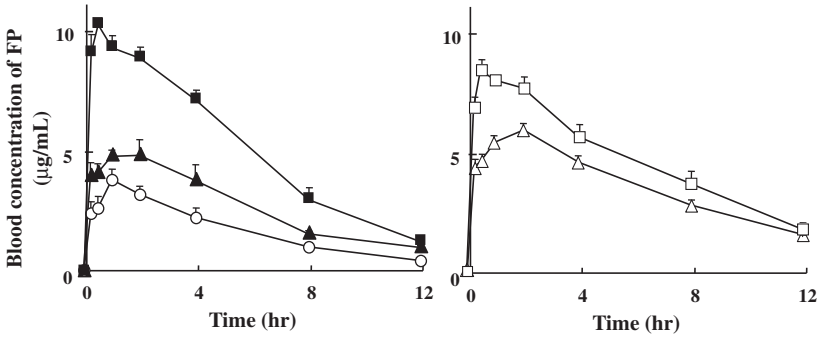

Fig. 9. Plasma concentration–time profiles of FP in rats after oral administration of untreated FP and spray-dried particles: (○, untreated FP; ▲, PM of FP/Hsp-G(1/10); △, PM of FP/Stevia-G(1/10); ■, SDPs of FP/Hsp-G(1/10); □, SDPs of FP/Stevia-G(1/10)). Each point represents the mean ± SE ($n = 6$).

The cytotoxicities of Hsp-G and Stevia-G were examined in a Caco-2 cell monolayer using SDS as a positive control. While 10% concentrations of Stevia-G and Hsp-G solution did not have any toxic effect to Caco-2 cells, a 0.1% concentration of SDS solution had high cytotoxicity. Surfactants may conveniently be divided into ionics and non-ionics, depending on the charge of the hydrophilic head group. Ionic surfactants such as SDS are generally considered more highly potent cell-toxic and irritant materials than non-ionic ones [25]. As a result, it is difficult to apply ionic surfactants to formulation design. In general, surface-active materials play an important role in contemporary pharmaceutical biotechnology, since they are largely utilized in various drug dosage forms to control wetting, stability, and bioavailability, among other properties [26,27]. Stevia-G and Hsp-G are surface-active materials that showed no cytotoxicity to a Caco-2 monolayer. This result indicates that Hsp-G and Stevia-G are potentially safe materials, and that it would be quite reasonable to use them for drug formulations.

We investigated the potential effect of SDPs with Stevia-G and Hsp-G to improve the bioavailability of poorly water-soluble drugs. FP and PRO were selected as model drugs to represent Class II compounds according to the Biopharmaceutical Classification System. Class II compounds are known to have poor solubility and high permeability. Therefore, improvement of the dissolution profile of Class II compounds results in significant enhancement of their bioavailability. FP is a weakly acidic drug possessing very low aqueous solubility, resulting in slower dissolution and absorption rates after oral administration. Mizoe et al. reported that FP-containing microparticles prepared by the four-fluid nozzle-spray drier enhance the dissolution and absorption of FP [28]. Govindarajan et al. reported that a solid dispersion with FP and hydroxypropyl β-cyclodextrin improved the bioavailability of the drug in humans by solid dispersion system with hydroxypropyl β-cyclodextrin [29]. In the case of Stevia-G and Hsp-G, SDPs showed pronounced improvement of the dissolution profile of FP. SDPs dramatically enhanced the dissolution of FP compared with untreated FP in water. Hsp-G and Stevia-G must be accompanied by a significant enhancement in the bioavailability of FP. PRO may be one of the most insoluble drugs available today; its bioavailability through oral administration is, at maximum, only 6% [30]. Some increasingly popular approaches to overcome this problem are the use of a self-emulsifying drug delivery system, nanoparticles prepared by co-grinding, and solid dispersion systems with PVP [31–33]. These approaches have succeeded in increasing the absorption as a result of enhancing the solubility and dissolution characteristics of PRO. In animal studies, the improvement in C_{max} and AUC values after oral administration of SDPs significantly reflects the results of dissolution enhancement. The concentration of dissolved PRO from SDPs with Stevia-G was 6.5 μg/mL compared to 5.1 μg/mL with Hsp-G. These *in vitro* dissolution enhancement results were well correlated with results of our *in vivo* absorption study. As for the PRO system, C_{max} and AUC from SDPs with Stevia-G were considerably high compared with those of Hsp-G. On the other hand, for the FP system, SDPs with Hsp-G showed higher improvement in absorption compared with Stevia-G. These results are related to the differences in the solubilizing effect of transglycosylated materials towards hydrophobic drugs, and may arise from the interaction mode between the hydrophobic drug and transglycosylated material. Therefore, an appropriate selection of transglycosylated material leads to higher absorption of poorly water-soluble drugs. Consequently, preparation of SDPs with Hsp-G or Stevia-G was confirmed as a promising method for the improvement of dissolution and enhancement of bioavailability of water-insoluble drugs.

Acknowledgement

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (22790041). We thank Ezaki Glico Co., Ltd., and Toyo Sugar Refining Co., Ltd., for the kind gift of α-Glucosyl Hesperidin (Hsp-G: α-G Hesperidin PAT) and α-Glucosyl Stevia, respectively.

References

[1] N. El-Gendy, E.M. Gorman, E.J. Munson, C. Berkland, Budesonide nanoparticle agglomerates as dry powder aerosols with rapid dissolution, J. Pharm. Sci. 98 (2009) 2731–2746.
[2] M. Fukuda, D.A. Miller, N.A. Peppas, J.W. McGinity, Influence of sulfobutyl ether beta-cyclodextrin (Captisol) on the dissolution properties of a poorly soluble drug from extrudates prepared by hot-melt extrusion, Int. J. Pharm. 28 (2008) 188–196.
[3] A. Zimmermann, A. Millqvist-Fureby, M.R. Elema, T. Hansen, A. Müllertz, L. Hovgaard, Adsorption of pharmaceutical excipients onto microcrystals of siramesine hydrochloride: effects on physicochemical properties, Eur. J. Pharm. Biopharm. 71 (2009) 109–116.
[4] S.W. Jun, M.S. Kim, G.H. Jo, S. Lee, J.S. Woo, S.J. Park, Hwang, Cefuroxime axetil solid dispersions prepared using solution enhanced dispersion by supercritical fluids, J. Pharm. Pharmacol. 57 (2005) 1529–1537.
[5] M. Valleri, P. Mura, F. Maestrelli, M. Cirri, R. Ballerini, Development and evaluation of glyburide fast dissolving tablets using solid dispersion technique, Drug. Dev. Ind. Pharm. 30 (2004) 525–534.
[6] K. Wu, J. Li, W. Wang, D.A. Winstead, Formation and characterization of solid dispersions of piroxicam and polyvinylpyrrolidone using spray drying and precipitation with compressed antisolvent, J. Pharm. Sci. 98 (2009) 2422–2431.
[7] H. Takeuchi, S. Nagira, H. Yamamoto, Y. Kawashima, Solid dispersion particles of amorphous indomethacin with fine porous silica particles by using spray-drying method, Int. J. Pharm. 293 (2005) 155–164.
[8] C. Leuner, J. Dressman, Improving drug solubility for oral delivery using solid dispersions, Eur. J. Pharm. Biopharm. 50 (2000) 47–60.
[9] T. Kometani, T. Nishimura, T. Nakae, H. Takii, S. Okada, Synthesis of neohesperidin glycosides and naringin glycosides by cyclodextrin glucanotransferase from an Alkalophilic Bacillus Species, Biosci. Biotech. Biochem. 60 (1996) 645–649.
[10] T. Kometani, T. Nishimura, T. Nakae, H. Takii, S. Okada, A method for preparation of soluble carthamin, red pigment from safflower, using glycosyl hesperidin, Food Sci. Technol. Res. 5 (1999) 265–270.
[11] T. Kometani, T. Fukuda, T. Kakuma, K. Kawaguchi, W. Tamura, Y. Kumazawa, K. Nagata, Effects of α-glucosylhesperidin, a bioactive food material, on collagen-induced arthritis in mice and rheumatoid arthritis in humans, Immunopharmacol. Immunotoxicol. 30 (2008) 117–134.
[12] Y. Tozuka, J. Kishi, H. Takeuchi, Anomalous solubility enhancement of naringenin from the solid dispersion with α-glucosylhesperidin, Adv. Powder Technol. 21 (2010) 305–309.
[13] H. Uchiyama, Y. Tozuka, M. Imono, H. Takeuchi, Improvement of dissolution and absorption properties of poorly water-soluble drug by preparing spray-dried powders with α-glucosyl hesperidin, Int. J. Pharm. 392 (2010) 101–106.
[14] L.J. Grashoff, W.M. Bierner, K.D. Northington, Chromosome numbers in North and Central American Compositae, Brittoni 24 (1972) 379–394.
[15] D. King, E. Robinson, Aspects of the development of *Moniliformis dubius*, J. Parasitol. 53 (1967) 142–149.
[16] P. Chan, D.Y. Xu, J.C. Liu, The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats, Life Sci. 63 (1998) 1679–1684.
[17] T.-H. Chen, S.-C. Chen, P. Chan, Y.-L. Chu, H.-Y. Yang, J.-T. Cheng, Mechanism of the hypoglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*, Planta Med. 71 (2005) 108–113.
[18] T. Kometani, Y. Terada, T. Nishimura, H. Takii, S. Okada, Synthesis of hesperidin glycosides by cyclodextrin glucanotranferase and stabilization of the natural pigments, J. Jpn. Soc. Food Technol. 42 (1995) 376–382.
[19] N. Yagi, Y. Terashima, H. Kenmotsu, H. Sekikawa, M. Takada, Dissolution behavior of probucol from solid dispersion system of probucol–polyvinylpyrrolidone, Chem. Pharm. Bull. 44 (1996) 241–244.
[20] N. Ahuja, O.P. Katare, B. Singh, Studies on dissolution enhancement and mathematical modeling of drug release of a poorly water-soluble drug using water-soluble carriers, Eur. J. Pharm. Biopharm. 65 (2007) 26–38.
[21] P. Gupta, A.K. Bansal, Spray drying for generation of a ternary amorphous system of celecoxib, PVP, and meglumine, Pharm. Dev. Technol. 10 (2005) 273–281.
[22] S.L. Raghavan, B. Kiepfer, A.F. Davis, S.G. Kazarian, J. Hadgraft, Membrane transport of hydrocortisone acetate from supersaturated solutions; the role of polymers, Int. J. Pharm. 221 (2001) 95–105.
[23] S.L. Raghavan, A. Trividic, A.F. Davis, J. Hadgraft, Effect of cellulose polymers on supersaturation and in vitro membrane transport of hydrocortisone acetate, Int. J. Pharm. 193 (2000) 231–237.
[24] B.C. Hancock, M. Parks, What is the true solubility advantage for amorphous pharmaceuticals?, Pharm Res. 17 (2000) 397–404.

[25] K. Ekelund, K. Osth, C. Påhlstorp, E. Björk, S. Ulvenlund, F. Johansson, Correlation between epithelial toxicity and surfactant structure as derived from the effects of polyethyleneoxide surfactants on Caco-2 cell monolayers and pig nasal mucosa, J. Pharm. Sci. 94 (2005) 730–744.
[26] Y. Tanaka, M. Inkyo, R. Yumoto, I. Nagai, M. Takano, S. Nagata, Nanoparticulation of poorly water soluble drugs using a wet mill process and physicochemical properties of the nanopowders, Chem. Pharm. Bull. 57 (2009) 1050–1057.
[27] R.S. Dhumal, S.V. Biradar, S. Aher, A.R. Paradkar, Cefuroxime axetil solid dispersion with polyglycolized glycerides for improved stability and bioavailability, J. Pharm. Pharmacol. 61 (2009) 743–751.
[28] T. Mizoe, S. Beppu, T. Ozeki, H. Okada, One-step preparation of drug-containing microparticles to enhance the dissolution and absorption of poorly water-soluble drugs using a 4-fluid nozzle spray drier, J. Control. Release 120 (2007) 205–210.
[29] S.R. Govindarajan, M.S. Nagarsenker, Formulation studies and in vivo evaluation of a flurbiprofen-hydroxypropyl β-cyclodextrin system, Pharm. Dev. Technol. 10 (2005) 105–114.
[30] J.F. Heeg, M.F. Hiser, D.K. Satonin, J.Q. Rose, Pharmacokinetics of probucol in male rats, Pharm. Sci. 73 (2006) 1758–1763.
[31] S. Flemming Nielsen, E. Gibault, H. Ljusber-Wahren, L. Arleth, Characterization of prototype self-nanoemulsifying formulations of lipophilic compounds, J. Pharm. Sci. 96 (2007) 876–892.
[32] Y. Kubo, Y. Terashima, N. Yagi, H. Nochi, K. Tamoto, H. Sekikawa, Enhanced bioavailability of probucol following the administration of solid dispersion systems of probucol–polyvinylpyrrolidone in rabbits, Biol. Pharm. Bull. 32 (2009) 1880–1884.
[33] J. Shudo, A. Pongpeerapat, C. Wanawongthai, K. Moribe, K. Yamamoto, In vivo assessment of oral administration of probucol nanoparticles in rats, Biol. Pharm. Bull. 26 (2007) 321–325.