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Research paper

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

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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

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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.

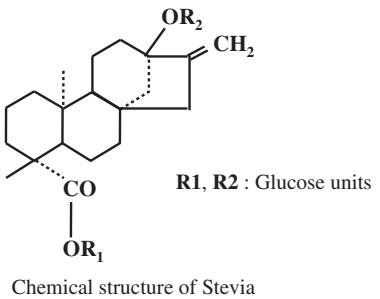

Fig. 1. Chemical structure of Stevia.

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: