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Note

α-Glucosyl hesperidin induced an improvement in the bioavailability of pranlukast hemihydrate using high-pressure homogenization

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ABSTRACT

The α-glucosyl hesperidin (Hsp-G)-induced improvement of both the dissolution and absorption properties of pranlukast hemihydrate (PLH) was achieved by means of a high-pressure homogenization (HPH) processing. The average particle size in the HPH-processed suspension was decreased significantly after 50 cycles of processing and reached a constant size of ca. 300 nm. The amount of dissolved PLH gradually increased with the pass number of HPH processing, and was extremely higher than the PLH solubility (0.8 μg/mL at 37 °C) after the HPH processing. On a dissolution study of the freeze-dried sample of HPH-processed PLH/Hsp-G (1/10), the apparent solubility of PLH was at least 2.5-fold more than that of untreated PLH crystals. The transport study showed that the amount of PLH that had permeated through the Caco-2 cell monolayers was improved in the case of HPH-processed PLH/Hsp-G (1/10). The bioavailability of PLH from HPH-processed PLH/Hsp-G (1/10) showed a 3.9- and 2.2-fold improvement over the PLH crystal in terms of C_{max} and AUC values, respectively. Hsp-G formed an associated structure in aqueous media. High-pressure homogenization provides a good opportunity for molecular-level interaction of PLH and the associated structure of Hsp-G to occur. The use of Hsp-G under HPH processing was a promising way to enhance the dissolution and absorption of PLH without using an organic solvent.

Pranlukast hemihydrate (PLH) is a cysteinyl leukotriene receptor antagonist, which is used as an anti-asthmatic agent. PLH exhibits extremely low aqueous solubility (0.8 μg/mL H_2O at 37 °C), resulting in poor absorption when administered orally and low bioavailability. In addition, the fact that PLH cannot dissolve into commonly used organic solvents such as methanol, ethanol, acetone, etc. poses a limitation to the preparation of PLH formulations via crystallization processes.

There are many techniques to improve the dissolution of poorly water-soluble drugs. High-pressure homogenization (HPH) has been extensively used to efficiently reduce the particle size (Grau et al., 2000), process highly concentrated suspensions (Müller et al., 2001) and prepares emulsions (Tian et al., 2007). HPH presents various advantages over other milling techniques as it is very simple, timesaving and an organic solvent-free process. Therefore, HPH can be used to prepare particles of poor-water-solubility drugs such as PLH for which usage of organic solvents is limited.

Hesperidin is reported to have significant anti-inflammatory, hypotensive, and analgesic effects (Gang et al., 2001). However, the applicability of hesperidin itself as a preventive medicine is limited due to its extremely low solubility in water. Its solubility has been shown to be greatly improved when transglycosylated with cyclomaltodextrin glucanotransferase (Kometani et al., 1996, 1999, 2008). The transglycosylation of hesperidin to form hesperidin glycosides led to more than 300 times higher solubility than that of the original hesperidin. We reported that spray-drying particles with α-glucosyl hesperidin (Hsp-G) enhanced the dissolution and absorption of poorly water-soluble drugs via their encapsulation in the micelle-like formation of Hsp-G (Tozuka et al., 2010; Uchiyama et al., 2010a,b). However, the spray-drying method is limited for compounds that are less soluble in relatively polar organic solvents such as ethanol and acetone. Besides, processing without an organic solvent has the advantage of avoiding residual organic solvent.

In this study, we tried to encapsulate PLH in the structure formed by Hsp-G in aqueous media via processing without an organic solvent. High-pressure homogenization was chosen for this purpose. The dissolution and absorption profile of PLH after processing with Hsp-G was compared to the untreated PLH and physical mixture of Hsp-G/PLH.

PLH particles with Hsp-G were prepared by a high-pressure homogenizer system (Nanomaizer, NV-200-D, Yoshida Kikai Co., Ltd, Japan) under an air pressure of 100 MPa. To prepare particles by HPH, 50 mg of PLH and 50 or 500 mg of Hsp-G were suspended in 50 mL-distilled water, and the suspension was homogenized. Fig. 1 shows the effect of the pass number of homogenization on the particle size and amount of dissolved PLH. The particle size of PLH

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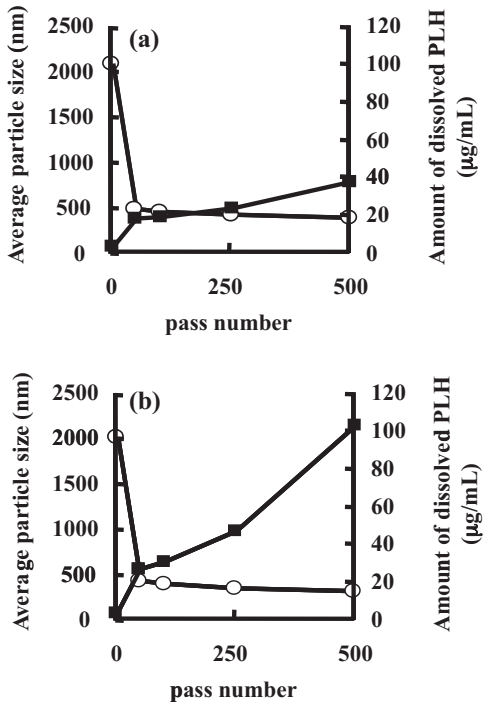

Fig. 1. Effect of pass number of high-pressure homogenization (HPH) on particle size and dissolved amount of PLH. (a) PLH/Hsp-G(1/1), (b) PLH/Hsp-G(1/10) (○, particle size; ■, amount of dissolved PLH).

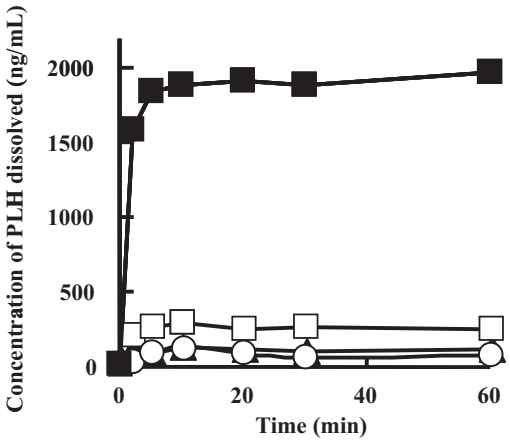

Fig. 2. Dissolution profiles of PLH in distilled water: (○, untreated PLH; ▲, physical mixture of PLH/Hsp-G(1/10); □, HPH-processed particles of PLH/Hsp-G(1/1); ■, HPH-processed particles of PLH/Hsp-G(1/10)). Each point represents the mean ± S.D. (n = 3).

was reduced as the number of homogenization cycles increased, while the particle size of PLH was almost unchanged after 50 homogenization cycles. On the other hand, the amount of dissolved PLH in the solution with Hsp-G significantly increased with the number of homogenization cycles. Interestingly, HPH-processed PLH/Hsp-G (1/10 w/w) showed a dramatic increase of dissolved PLH (100 μg/mL) to 100 times that of the pure PLH crystals.

We have reported that Hsp-G can form a micelle-like structure in an aqueous medium, resulting in a solubilizing effect of hydrophobic drug molecules via formation of a complex (Tozuka et al., 2010; Uchiyama et al., 2010a,b). The improvement of apparent solubility of PLH may be explained by the solubilization of PLH in the micelle-like structure formed by Hsp-G during the homogenization process.

To obtain particles for pharmaceutical use, the suspension resulting from HPH processing was subjected to freeze-drying at −120 °C for 72 h (FD-81TS, Tokyo Rikakikai Co. Ltd., Japan). Table 1 shows the changes of particle size and dissolved amount of PLH before/after freeze-drying. All samples show a sharp particle size distribution of PLH. When the freeze-dried sample was dispersed into distilled water, the particle size of PLH was not so different from that in the suspension, indicating the good redispersibility of the freeze-dried sample. Hsp-G is synthesized by the addition of glucose units to hesperidin. Saccharides such as mannitol and torehalose are used as cryoprotectants during the process of freeze-drying (Date et al., 2010; Shahgaldian et al., 2003). Cryoprotectants maintain the particulate nature of nanoparticles and the recoverability of the rehydrated nanoparticle suspension during the freeze-drying process (Konan et al., 2002). Hsp-G may act as a cryoprotectant in this system. On the other hand, the dissolved amount of PLH decreased after the freeze-drying process. Leakage of PLH from Hsp-G's structure through the freeze-drying process may have contributed to this result.

The dissolution profile of PLH from the prepared particles was evaluated in distilled water (JPXV paddle method, 900 mL) and compared to those of untreated PLH and a physical mixture of PLH and Hsp-G (Fig. 2). The dissolution rate of untreated PLH and the physical mixture of PLH/Hsp-G was considerably slow, with a maximum solubility of 0.2 μg/mL achieved after 60 min. In the case of the freeze-dried particles of HPH-processed PLH/Hsp-G (1/1), the dissolution profile of PLH was slightly improved compared to the case with the untreated drug. On the other hand, the freeze-dried particles of HPH-processed PLH/Hsp-G (1/10) showed rapid dissolution in the early stage, and 2.5-fold more of this PLH dissolved than did the untreated PLH.

The amounts of pranlukast that permeated through the Caco-2 cell monolayers after application of untreated PLH and HPH-processed particles are shown in Fig. 3. With respect to the statistical analysis, the one-way analysis of variance followed by Tukey–Kramer test was used in the case of multiple comparisons. We have already shown that there was no cytotoxicity to Caco-2 cells at levels of 10% Hsp-G solution (Uchiyama et al., 2010b). This amount was greater for PLH from HPH-processed particles than for untreated PLH at 1 h (Fig. 3(a)). The transepithelial electrical resistance (TEER) values of Caco-2 cell monolayers before and after permeation experiments are shown in Fig. 3(b). No significant differences in TEER were observed for application of untreated PLH, the physical mixture of PLH/Hsp-G, and HPH-treated particles. The particles prepared by HPH-processing showed pronounced enhancement of the dissolution properties of PLH. The dissolution-enhancement effect of HPH-treated particles with Hsp-G probably

Table 1
Changes in particle size and dissolved amount of PLH treated by HPH processing together with Hsp-G before/after freeze drying.

		Average particle size (nm)	Dissolved PLH (μg/mL)
HPH-processed particles of PLH/Hsp-G(1/1)	B.F.D	327.7 ± 48.2	34.9
	A.F.D	455.1 ± 72.5	6.6
HPH-processed particles of PLH/Hsp-G(1/10)	B.F.D	316.6 ± 83.8	103.0
	A.F.D	297.9 ± 57.5	25.2

B.F.D.: before freeze drying; A.F.D.: after freeze drying.

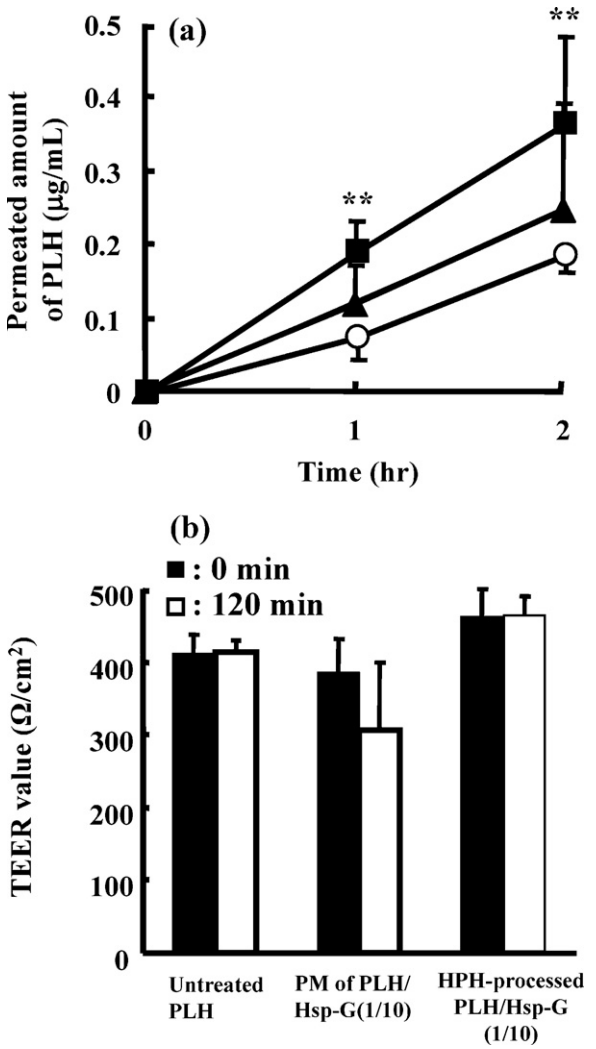

Fig. 3. The amount of pranlukast that permeated through Caco-2 cell monolayers (a) and changes of TEER after application of untreated PLH or HPH-processed particles (b). (○, untreated PLH; ▲, physical mixture of PLH/Hsp-G(1/10); ■, HPH-processed particles of PLH/Hsp-G(1/10)). Each point represents the mean ± S.D. ($n = 4$). (**$p < 0.01$, compared to untreated PLH).

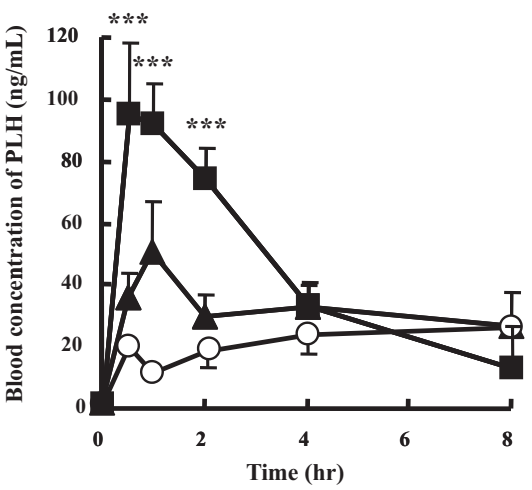

Fig. 4. Plasma concentration-time profiles of PLH in rats after oral administration of untreated PLH and HPH-processed particles: (○, untreated PLH; ▲, physical mixture of PLH/Hsp-G(1/10); ■, HPH-processed particles of PLH/Hsp-G(1/10)). Each point represents the mean ± S.E. ($n = 4$–6). (***$p < 0.001$, compared to untreated PLH).

contributed to the large amount of PLH that permeated through the Caco-2 cell monolayer.

The efficiency of the particles prepared by a high-pressure homogenizer in enhancing the intestinal absorption of PLH was evaluated in an in vivo study (Fig. 4). The suspension of untreated PLH, physical mixture of PLH/Hsp-G or particles prepared by HPH (dose = 40 mg/kg. 1.0 mL/rat) were orally administrated to the anesthetized rats. The rats were fasted 24 h prior to oral administration. Animal experiments were approved by the animal welfare commission of GIFU Pharmaceutical University. The maximum drug concentration (C_{max}) of particles prepared by HPH was 3.9-fold compared to that of the untreated PLH. The areas under the curve (AUCs) up to 8 h for untreated PLH, the physical mixture of PLH/Hsp-G, and prepared particles by HPH with PLH/Hsp-G were 159.82 ± 39.41, 236.86 ± 50.04, and 344.41 ± 40.47 μg·h/mL, respectively. AUC of the particles prepared by the high-pressure homogenizer with Hsp-G was 2.2-fold that of the untreated PLH. It was reported that the absorption site of PLH is in the upper part of the gastrointestinal tract (Ishido et al., 1993). Thus, the improvement in the initial dissolution rate is especially significant for improvement of the gastrointestinal absorption of pranlukast (Chono et al., 2008). Mizoe et al. (2007) reported that the oral absorption of PLH was obviously higher for the PLH–mannitol microparticles than for the untreated PLH. In vitro dissolution test the particles treated by HPH with Hsp-G showed more rapid dissolution and higher solubility than untreated PLH. The increase in the rats' absorption of PLH would be attributed to drug dissolution in the small intestine, and a good correlation between the in vivo bioavailability and the in vitro dissolution can be found.

In conclusion, HPH processing of PLH/Hsp-G dramatically improved the dissolution and absorption of PLH. HPH processing performed in aqueous media can overcome the problem of residual solvent in final product. Therefore, making a nano-complex structure with Hsp-G using HPH processing appears to be a promising way to improve the bioavailability of drugs with extremely poor water solubility.

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