The formation of an inclusion complex between a metabolite of ginsenoside, compound K and γ-cyclodextrin and its dissolution characteristics

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Keywords
bioavailability; compound K; ginsenoside; inclusion complex; γ-cyclodextrin

Abstract

Objectives 20S-protopanaxadiol 20-O-β-D-glucopyranoside (compound K), a metabolite of ginsenoside, is only sparingly soluble in water. The aim of this study was to improve the low solubility, slow dissolution rate and low oral bioavailability of compound K by forming an inclusion complex with γ-cyclodextrin (γ-CyD), and to compare the results with those of β-CyD complex.

Methods The interactions of compound K with β and γ-CyDs were studied by the solubility method and proton nuclear magnetic resonance spectroscopy. Solid forms of compound K/CyD complexes with different molar ratios were prepared by the kneading method, and the resulting complex was characterized by powder X-ray diffractometry. The dissolution rate of the complexes was measured by the rotary disk method. In-vivo absorption studies in rats were carried out, and the serum level of compound K, after its oral administration, was measured by a liquid chromatography-tandem mass spectrometry system.

Key findings γ-CyD markedly improved the low solubility of compound K at lower CyD concentrations (<0.03 M), whereas the solubility was decreased at higher concentrations (>0.06 M). The enhancement in solubility by γ-CyD at a lower concentration was much higher than the corresponding values for β-CyD complex. The apparent 1:1 stability constant (1.5 \times 10^5 \text{M}^{-1}) for the γ-CyD complex was 18-fold larger than that (8.2 \times 10^3 \text{M}^{-1}) of the β-CyD complex. The dissolution rate of the 1:1 compound K/γ-CyD complex was faster than that for the 1:3 (guest: host) complex. These results suggest that the dissolution rate of the 1:1 complex, in which the drug is partially included, was faster than that of the 1:3 complex, in which the drug was completely included, due to the higher solubility and amorphous property of the former complex compared with the properties of the latter complex. The fast dissolution of the γ-CyD complex was reflected in the maximum plasma level (C_{\text{max}}) of the drug and the time (T_{\text{max}}) to reach the maximum plasma level after its oral administration to rats.

Conclusions The effect of γ-CyD on enhancing the solubility of compound K is much higher than that for the β-CyD complex, and the dissolution rate of the guest when it is partially included in the γ-CyD is faster the corresponding value when it is completely included in the cavity.

Introduction

Cyclodextrins (CyDs) are known to form inclusion complexes with various drugs in the solid state and in solution, and this has been successfully utilized to improve the physicochemical and biological properties of certain types of drugs. These changes in the properties of a drug as the result of CyD complexation are dependent on the stability constant and stoichiometry of the complex and the physicochemical properties of the complex itself, because
At a constant CyD concentration, a simple interaction of compound K (7.0 mg) with CyD was examined by 1H-NMR spectroscopy. Compound K and γ-CyD were dissolved in D2O at various molar ratios. 1H-NMR spectra were collected at 25 °C on a Jeol JNM-ECA500 Delta spectrometer (Tokyo, Japan) operating at 500 MHz, using a 5-mm sample tube.

**Materials and Methods**

**Materials**

Compound K was obtained from Nagase Co., Ltd (Tokyo, Japan). β- and γ-CyDs were supplied from Nihon Shokuhin Kako Co. (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

**Interaction of compound K with γ-CyD**

Interaction of compound K and γ-CyD was examined by 1H-NMR spectroscopy. Compound K and γ-CyD were dissolved in D2O at various molar ratios. 1H-NMR spectra were collected at 25 °C on a Jeol JNM-ECA500 Delta spectrometer (Tokyo, Japan) operating at 500 MHz, using a 5-mm sample tube.

**Preparation of compound K/CyD complexes**

Compound K, at molar ratios of 1:1, 1:2, 1:3 and 1:4 (guest: host), was thoroughly kneaded with small amounts of aqueous γ-CyD solutions. The compound K/β-CyD complex was prepared in the same way as that for the γ-CyD complex. Each complex was dried for 24 h at 25 °C. Starch was also kneaded with compound K in a similar manner as that for the CyDs as a control. The resulting powders were subjected to powder X-ray diffractometry (PXRD) and scanning electron microscopy (SEM) to confirm the complexation. PXRD was performed by means of a Rigaku-RINT Ultima + diffractometer (Tokyo, Japan) under the following conditions: Ni-filtered Cu-Kα radiation (1.542 Å), 40 kV, 40 mA, divergent slit of 1.74 mm (1°), scanning slit of 0.94 mm (1°), receiving slit of 0.15 mm and goniometer angular increment of 5°/min. SEM observations were carried out with a Hitachi TM-3000 (Tokyo, Japan).
Dissolution studies

The dissolution rate was measured by the rotary disk method.\(^{[11]}\) Compound K/CyDs complexes (50 mg) were compressed into 10 mm diameter tablets at a pressure of 20 MPa. The tablets were attached in a disk holder, and then immersed in 50 ml of dissolution medium (pH 1.2 or pH 6.8), which was then stirred at 50 rpm. At appropriate intervals, an aliquot was withdrawn and compound K was analysed by the HPLC method described above. Compound K/starch (weight ratio = 1:1) was also examined as control.

Pharmacokinetics studies

Rats (SD, SPF male), obtained from Charles River Laboratories Japan, were maintained under a 12-h photoperiod with free access to standard laboratory chow (CRF-1) and water. Seven-week-old rats (231–271 g) were divided into five groups with 12 rats in each group, and four blood samples were collected from one rat. Compound K, compound K/γ-CyD complexes and compound K/β-CyD complex were suspended in a 0.5% methyl cellulose solution and orally administered at a dose of 30 mg/kg for compound K via a catheter. Compound K was also intravenously administered once a day at a dose of 3 mg/kg to calculate bioavailability. Blood samples were collected at 5 min, 15 min, 30 min and 45 min and 1 h, 1.5 h, 2 h, 4 h, 8 h and 12 h after administration. The samples were mixed with prednisolone, an internal standard, and centrifuged to obtain plasma. The plasma samples were applied to liquid chromatography-tandem mass spectrometry (LC-MS/MS:Agilent 1100/API 4000 systems) to determine the concentrations of compound K. Pharmacokinetics parameters, including the maximum drug concentration (C\(_{\text{max}}\)), the time to reach a maximum drug concentration (T\(_{\text{max}}\)), the area under the curve from time zero to infinity (AUC), the mean residence time from time zero to infinity (MRT), were analysed using WinNonlin ver 6.1 (Pharsight: Certara USA, Inc.). Bioavailability (BA) was calculated according to the following expressions: \(\text{BA} = (\text{AUC p.o.}/\text{dose p.o.})/(\text{AUC i.v.}/\text{dose i.v.}) \times 100\).

Animal studies were conducted in accordance with the guidelines for animal studies in Kamakura Techno-science (Kanagawa, Japan) based on Guidelines for the Proper Conduct of Animal Experiments of the Science Council of Japan (Test No. KTN-12104).

Statistical analysis

Data are presented as the median values from \(n\) samples, and the results are reported as the mean ± SE. Kruskal–Wallis analysis of variance was performed followed by Dunn’s post-hoc tests for multiple comparisons. For all analyses, values of \(P < 0.05\) were regarded as statistically significant.

Results and Discussion

Interaction of compound K with β- and γ-CyDs

Figure 1 shows phase solubility diagrams for compound K with β- and γ-CyDs in water. The β-CyD system showed an A\(_2\) type diagram, that is, the solubility of the drug increased linearly with increasing β-CyD concentration, and no precipitation of compound K/β-CyD complex was observed within the solubility limit of β-CyD (about 0.015 M). On the other hand, the γ-CyD system showed a typical B\(_2\) type diagram, that is, the solubility of the drug increased linearly for γ-CyD concentrations of up to about 0.03 M, and then levelled off and decreased at γ-CyD concentrations above 0.05 M due to the precipitation of solid complex. The Kc values of the β- and γ-CyD complexes were determined to be \(8.2 \times 10^{3} \text{ M}^{-1}\) and \(1.5 \times 10^{4} \text{ M}^{-1}\), respectively, indicating that compound K has a higher affinity for γ-CyD than for β-CyD. The chemical analysis of the solid γ-CyD complex that precipitated at higher CyD concentrations (\(>0.08 \text{ M}\)) indicated that compound K interacted with γ-CyD in a molar ratio of 1:3 (guest : host). It is noteworthy that the solubility of compound K at a γ-CyD concentration of 0.03 M was markedly higher than that at a 0.1 M concentration of γ-CyD, indicating that compound K formed higher order complexes with γ-CyD in a consecutive fashion, and the solubility of the complexes was different depending on the stoichiometry employed. We assumed that the initial large increase (S\(_{\text{plateau}}\) – So) in the solubility was due to the formation of a 1:1 complex, while the low solubility at a γ-CyD concentration of 0.1 M corresponded to the 1:3 complex, where S\(_{\text{plateau}}\) and So are the solubility of the drug at the plateau region in the phase diagram and the intrinsic solubility of the drug, respectively. To gain additional insights into the interactions that occur in solution,
H-NMR spectroscopic studies were carried out. Figure 2 shows the effect of γ-CyD on the 1H-chemical shift of the H18, 19, 29 and 30 methyl groups of compound K in deuterium oxide. These protons were selected as an index for the shift displacements, because they gave sharp single peaks. These peaks became significantly broadened with increasing γ-CyD concentrations, suggesting that the steroid moiety of compound K was preferably included in the γ-CyD cavity, thus restricting its molecular motion. Figure 3 shows the effect of compound K on the chemical shifts of the γ-CyD protons (H1-H6). The H5 proton, which is located inside the cavity, was significantly shifted upfield, whereas the shifts for the other protons remained essentially the same, indicating that the guest is inside the γ-CyD cavity.

Figure 2 1H NMR spectra of compound K in the presence of γ-CyD in D2O. Molar ratio of compound K : γ-CyD = (a) 1:1, (b) 1:1.5, (c) 1:2, (d) 1:3, (e) 1:4.
Unfortunately, we were not able to conduct in-depth NMR studies on the \( \beta \)-CyD system because of the poor water solubility of compound K. We expected that \( \beta \)-CyD likely included the hydrophobic steroidal moiety rather than the hydrophilic glucose or the small alkyl moieties of compound K, similar to \( \beta \) and \( \gamma \)-CyD complexes with digitalis glycosides.\(^{12}\)

Solid complexes of compound K with \( \beta \)- and \( \gamma \)-CyDs in different molar ratios were prepared by the kneading method, and their powder X-ray diffraction patterns are shown in Figure 4. Compound K gave a halo pattern, indicating that it was in an amorphous state. The 1:1 \( \beta \)-CyD complex gave sharp diffraction peaks which were different from those of \( \beta \)-CyD alone. The diffraction pattern of the 1:3 \( \beta \)-CyD complex was superimposable with that for \( \beta \)-CyD alone and the 1:1 complex, for example, the peaks of \( 2\theta = 12^\circ, 17^\circ \) and \( 21^\circ \) (arrow in Figure 4g) in the 1:3 complex came from those of \( \beta \)-CyD alone. These results suggest that compound K predominantly forms a 1:1 complex. On the other hand, \( \gamma \)-CyD complexes gave different diffraction patterns depending on the stoichiometry (Figure 4a), that is, a halo pattern in the 1:1 complex, small diffraction peaks in the case of the 1:2 complex and sharp peaks in the cases of the 1:3 and 1:4 complexes. The diffraction pattern of the 1:3 and 1:4 complexes coincided with that of \( \gamma \)-CyD complexes with a head-to-head channel structure.\(^{13}\) These results suggest that compound K is deposited in the cavity of the columnar channels of linearly
aligned γ-CyD molecules. At least three γ-CyD molecules are required for the complete inclusion of compound K. In the case of complexes with a stoichiometry of more than 1:4, empty γ-CyD molecules are involved in the formation of the columnar structure. On the other hand, it is difficult for compound K to be completely included in one γ-CyD cavity, and the result is the production of an amorphous 1:1 complex. Figure 5 shows microscopic observations of compound K and its β- and γ-CyD complexes. Compound K exists in powder form, while both the 1:1 and 1:3 β-CyD complexes were in crystalline form. The appearance of the 1:1 γ-CyD complex was slightly different from that of the 1:3 γ-CyD complex, that is, the 1:3 crystalline γ-CyD complex (Figure 5e) resembled the 1:1 and 1:3 crystalline β-CyD complexes (Figures 5b, c) in appearance, showing a block form with a very rough surface. On the other hand, the amorphous 1:1 γ-CyD complex (Figure 5d) was a blockish powder with a very smooth surface, consistent with the X-ray diffraction pattern of the γ-CyD complexes (Figure 4).

**Dissolution and absorption behaviour of the complexes**

Figure 6 shows the dissolution profiles of compound K from tablets of its β- and γ-CyD complexes in the JP XVI first (pH 1.2) and second (pH 6.8) dissolution fluids. The dissolution of compound K from its starch tablet was negligible in both the pH 1.2 and 6.8 media. The dissolution of the 1:1 β-CyD complex was also negligible in case of the pH
1.2 medium and was very slow in the pH 6.8 medium. On the other hand, the dissolution of compound K was significantly improved by complexation with γ-CyD, especially in the pH 6.8 medium, for example, the 1:1 γ-CyD complex was 100% dissolved within 60 min in the pH 6.8 medium. It is noteworthy that the dissolution of the 1:1 γ-CyD complex was faster than that of the 1:3 γ-CyD complex, which can be attributed to the high solubility of the former complex compared with the latter complex, and, additionally, to the amorphous property of the former complex.

Figure 7 shows the plasma concentrations of compound K after the oral administration of the 1:1 and 1:3 γ-CyD complexes to rats, in comparison with those of compound K/starch and the 1:1 β-CyD complex. The pharmacokinetic parameters are summarized in Table 1. The fast-dissolving property of the γ-CyD complexes was reflected in the absorption, that is, the C_{max} value for compound K was increased more than twofold in the case of the administration of the γ-CyD complexes and a faster T_{max} value compared with the control. The AUC value was significantly increased as the result of the γ-CyD complexation, and the BA of the γ-CyD complex was 1.5–2-fold larger than that for the drug alone, although the absolute values were low. Regarding pharmacokinetic parameters, there were no significant differences between the β-CyD complex and compound K alone. Thus, the γ-CyD complexes may be useful as a fast-dissolving form of compound K when compared with the β-CyD complex. The plasma concentrations of the 1:3 γ-CyD complex at the initial stage of absorption were higher than those of the 1:1 γ-CyD complex, which is inconsistent with the dissolution results (Figure 6). It is likely that factors other than the dissolution may be at play.

**Figure 6** Dissolution profile of compound K/CyDs complexes in pH 1.2 (a) and pH 6.8 (b) dissolution media. Each point represents the mean ± SE of three experiments. *P < 0.05 versus compound K/γ-CyD (1:3). ○: compound K, ◆: compound K/β-CyD (1:1), ▲: compound K/γ-CyD (1:1), ■: compound K/γ-CyD (1:3).
in the in-vivo absorption, such as the small amounts of gastrointestinal fluids, the inhibition of precipitation of compound K and competitive inclusion by bile acids at higher γ-CyD concentrations, low plasma concentrations and the enterohepatic circulation of compound K and so on. Further studies will be necessary to elucidate these differences in the absorption between the 1:1 and 1:3 γ-CyD complexes.

Conclusions

Compound K formed a 1:1 inclusion complex with β-CyD and high order complexes with γ-CyD. The solubility enhancing effect of γ-CyD on compound K was much higher than that for the β-CyD complex. The increase in solubility as the result of the formation of a 1:1 γ-CyD complex was higher than that for the case of 1:3 complexation. The dissolution rate of the former complex from tablets was faster than that of the latter complex due to the higher solubility and amorphous property of the former. These dissolution behaviours were reflected in the Cmax and Tmax values after oral administration to rats. The present results suggest that partial inclusion complexes, in which the guest is partially included in the γ-CyD cavity may be more beneficial than the completely included complex in terms of improving the low solubility of drugs.

References


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