

Inhibitory Effect of Cyclodextrin on Complexation of Risperidone with Tea Catechin

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Risperidone (RISP) is an antipsychotic agent indicated for the acute and maintenance treatment of schizophrenia. Taking an oral solution containing RISP with the green tea, the efficacy was surely decreased by forming the complexes between RISP and the tea catechin. In this study, the inhibitory effect of cyclodextrin (CD) on complexation of RISP with (-)-epigallocatechin gallate (EGCg) in the solution was clarified by isothermal titration microcalorimetry (ITC). Both solutions of RISP (2.4 mM) and EGCg (2.4 mM) at pH 3.5 were mixed, then RISP in filtrate was reduced to 64%. In the mixed solution adding β -CD, however, RISP in filtrate was recovered to 100% approximately. According to the measurement at pH 3.5 and 298K by ITC, EGCg formed the insoluble complex with RISP at associate constant (K) of $8.45 \times 10^2 \text{ M}^{-1}$ exothermically, $\Delta H = -14.8 \text{ kJ}\cdot\text{mol}^{-1}$, and formed the complex with β -CD ($K = 2.10 \times 10^4 \text{ M}^{-1}$, $\Delta H = -29.6 \text{ kJ}\cdot\text{mol}^{-1}$). While, little heat of reaction between RISP and β -CD was observed and the calorimetric titration curve for the mixed solutions of RISP and EGCg with β -CD was almost same as that for EGCg- β -CD system. From the results, it was indicated that the complexation between RISP and EGCg in oral solution could be inhibited by adding β -CD to include EGCg into the β -CD cavity.

Keywords: Calorimetry; Risperidone; Catechin; Cyclodextrin; Insoluble Complex

1. Introduction

Risperidone (RISP) belongs to the drug group of the second generation antipsychotic. In recent medical therapy for schizophrenia, oral medication treatment with second generation antipsychotic is recommended as first choice. The RISP oral solution was developed for the purpose of the compliance improvement of the patient of dysphagia and the patient in disfavor with increase of the number of times to take the tablet. The RISP oral solution is used frequently because of immediate effect in comparison with the tablet. Because the RISP oral solution has bitterness, the pharmacist instructs the patient to take it diluted with a glass of water, juice or soup. On the other hand, the dilution with the tea leaf extraction drink such as green tea, oolong tea or black tea reduces the amount of RISP in the solution. The factor of the amount decline of RISP above-mentioned was found to be insoluble complexation with the catechin in the tea leaf extraction drink.^{1), 2)} Japanese drinks considerably a lot green tea than oolong tea or black tea. A patient of schizophrenia is more likely to take RISP oral solution diluted with green tea. Thus, it is important for medical treatment of schizophrenia to find a method of obstructing the amount decline of RISP with the tea catechin. We found that the residual rate of RISP in the solution was increased when the cyclodextrin (CD) was added in the mixture of RISP and (-)-epigallocatechin gallate (EGCg). In this study, the inhibitory effect of CD on complexation between RISP and EGCg in the aqueous solution and the mechanism were clarified.

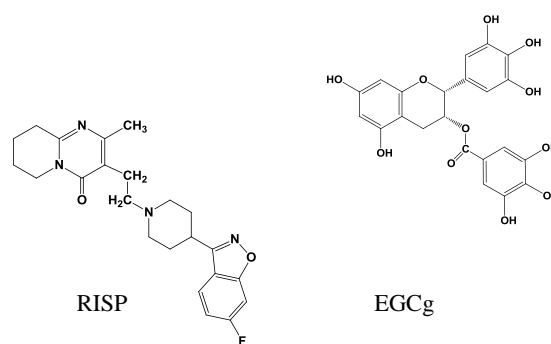


Fig. 1 Chemical Structures of Risperidone and EGCg

2. Materials and methods

2.1 Materials

Risperidone (RISP) was purchased from LKT Laboratories, Inc. (St Paul, Minnesota, USA). β -Cyclodextrin (β -CD) and γ -cyclodextrin (γ -CD) purchased from Kanto Kagaku corporation (Tokyo, Japan), α -Cyclodextrin (α -CD) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and 2-Hydroxypropyl- β -cyclodextrin (HP- β -CD) from Sigma-Aldrich Japan corporation (Tokyo, Japan) were used. (-)-Epigallocatechin gallate (EGCg) was obtained from Sigma-Aldrich. All reagents used in this study were analytical

grade. Experiments were done in the tartaric acid buffer solution at pH 3.5.

2.2 Addition of the Cyclodextrin to the Mixture of Risperidone and (-)-Epigallocatechin Gallate

After RISP (2.4 mM) and EGCg (2.4 mM) were dissolved in buffer solution at pH 3.5, CD was added to the solution at molar ratio 1 : 4 (RISP : CD). The mixture was stirred for 30 min. and then filtered using 0.45 μm membrane filter. The amount of RISP in filtrate was determined by HPLC (Jasco PU-2080 plus; Jasco Corporation, Tokyo, Japan) equipped with UV detector (Jasco UV-2075 plus) at 280nm. The analytical column used was Mightysil RP-18 GP (250 \times 4.6 mm i.d.) (Kanto Kagaku corporation, Tokyo, Japan). The mobile phase was composed of 0.5 w/v % K_2HPO_4 aqueous solution and acetonitrile (75 : 25 v/v). The flow rate was 1.5 ml min^{-1} and sample injection volume was 5 μl .

2.3 Microcalorimetry

All measurements were performed with Thermal Activity monitor 2277 (Thermometric, Järfälla, Sweden) at 298.15 ± 0.0001 K. Reaction cell put in the calorimeter was initially filled with a 3 ml of titrand solution. Titrant solution of 15 μl was injected into the cell with a micro syringe (Hamilton, Bonaduz, Switzerland) automatically at 16 times every ten minutes, and the heat of reaction was measured. The heat of dilution was measured for each sample to obtain the reaction heat (ΔQ) for the complexation. EGCg, RISP and CD were dissolved in tartaric acid buffer solution at pH 3.5.

In the case of measuring ΔQ for the complexation of EGCg with RISP or CD, 1 mM EGCg was used as a titrand, and RISP or 10 mM of each CD was prepared as a titrant. The calorimetric titration curves were analyzed based on the model that EGCg or RISP formed a complex with CD at molar ratio of 1:1.³⁾ The values of associate constant (K) and enthalpy change (ΔH) were determined by the same procedure as shown in the previous paper.¹⁾

In the case of measuring ΔQ for the adding effect of β -CD on EGCg-RISP complexation, the solution including each 1 mM of EGCg and RISP was used as a titrand, and 10 mM β -CD was prepared as a titrant.

3. Results and discussion

3.1 Addition of the Cyclodextrin to the Mixture of Risperidone and (-)-Epigallocatechin Gallate

According to the book written by Isemura et al.⁴⁾, the content of the catechin in the green tea leaf is EGCg (54%), (-)-epigallocatechin (22%), (-)-epicatechin gallate (11%), (-)-epicatechin (10%), respectively. Therefore, most of the catechin included in the commercial green tea drink is EGCg, and it is more likely to interact with RISP. When the 2.4mM RISP solution and the 2.4mM EGCg solution were prepared with pH3.5 tartaric acid buffer and then were mixed at the equal volume, the residual rate of RISP in filtrate reduced to approximately 60 %.

After both solutions of RISP (2.4 mM) and EGCg (2.4 mM) at pH 3.5 were mixed, then CD was added to the mixture at molar ratio RISP : EGCg : CD = 1 : 1 : 4 (Fig. 2). When α -CD was added, as well as the case of without CD, the mixture was cloudy. On the other hand, the cloudiness disappeared when β -CD, HP- β -CD or γ -CD was added. After filtration of each mixture, the residual rate of RISP in filtrate was determined using HPLC (Fig. 3). In the mixed solution adding CD (except α -CD), RISP in filtrate was recovered to 100% approximately. However, the residual rate of RISP in the solution which α -CD was added was almost same as without CD. It was revealed that β -CD, HP- β -CD and γ -CD could inhibit interaction between RISP and

EGCg, but α -CD could not inhibit it. From these results, the inhibitory effect of CD on insoluble complexation of RISP and EGCg depended on the diameter of the CD's cavity. In other words, the cavity diameter of β -CD, HP- β -CD or γ -CD was enough size for inclusion of RISP and/or EGCg, but α -CD's cavity diameter was too small.

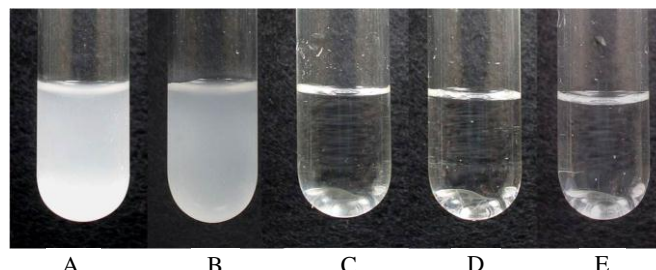


Fig. 2 Aqueous Solutions of the Mixture at Molar Ratio EGCg : RISP : CD = 1 : 1 : 4

- A : without CD
- B : with α -CD
- C : with β -CD
- D : with HP- β -CD
- E : with γ -CD

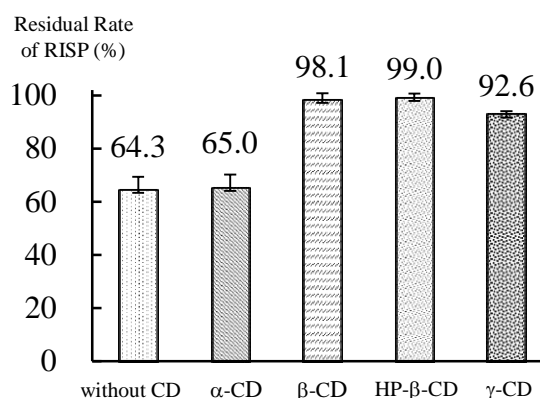


Fig. 3 Residual Rate (%) of RISP in the Mixture of EGCg, RISP and CD

3.2 Reaction Heat for Complexation of EGCg with RISP and EGCg with CD

Figure 4 shows the calorimetric titration curves of EGCg with RISP or CD at pH3.5 and 298K. The estimated K and thermodynamic parameters (ΔG , ΔH , ΔS) for the complexation were listed in Table 1. The values of ΔQ increased exothermically as the concentration of CD or RISP increased. However, ΔQ for the complexation between EGCg and α -CD was too small to calculate thermodynamic parameters. It seems that α -CD was hard to form complex with EGCg. The ΔQ values for the complexation of EGCg with other CDs were bigger than those with α -CD. Especially, β -CD and HP- β -CD strongly formed complexes (inclusion complexes) with EGCg ($K = 2.10 \times 10^3 \text{ M}^{-1}$ and $K = 9.72 \times 10^3 \text{ M}^{-1}$, respectively). The large negative values of ΔH and the small negative values of ΔS reflected the hydrogen bonding formation in the complexes. While, the values of K and ΔH for the complexation between EGCg and RISP were obtained to be $K = 8.45 \times 10^2 \text{ M}^{-1}$ and $\Delta H = -14.8 \text{ kJ/mol}$. It was suggested that EGCg tended to interact with CD rather than RISP.

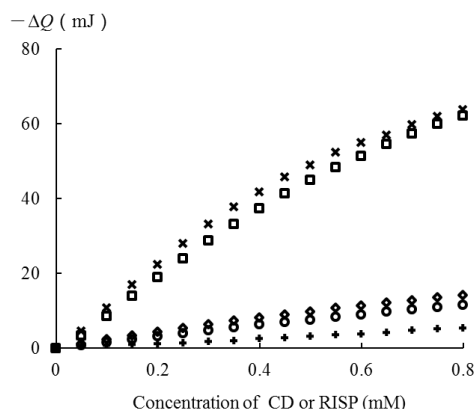


Fig. 4 Calorimetric Titration Curves of EGCg with CD and RISP

- + : α -CD
- : β -CD
- × : HP- β -CD
- ◇ : γ -CD
- : RISP

Table1 Associate Constants and Thermodynamic Parameters for the Complex between EGCg and CD

	K (1/M)	$-\Delta G$ (kJ/mol)	$-\Delta H$ (kJ/mol)	$-\Delta S$ (J/mol K)
α -CD	—	—	—	—
β -CD	2.10×10^4	24.7	29.6	-16.4
HP- β -CD	9.72×10^3	22.7	38.8	-54.0
γ -CD	1.27×10^3	17.7	15.2	8.70

3.5 Effect of β -CD on the Complex Formation between RISP and EGCg

In Fig.5, a calorimetric titration curve of RISP/EGCg mixed solution with β -CD and another one of only EGCg solution with β -CD as a comparison were shown. There was seen little difference in the both titration curves. On the other hand, the heat of reaction between RISP with β -CD could not be observed in the calorimetric titration (not presented), indicating that β -CD hardly interacted with RISP. From these results, it is clarified that β -CD interacted with EGCg rather than RISP in the mixture of RISP and EGCg. The similar results were obtained for other CDs

In conclusion, the formation of insoluble complex between RISP and EGCg was inhibited by adding β -CD and HP- β -CD. Because CD could strongly form inclusion complexes with EGCg but not interact with RISP in aqueous solution at pH 3.5. Therefore, RISP oral solution including β -CD or HP- β -CD could be taken with soft-drink extracted from tea leaf. This would be useful for the development of a new formulation of RISP oral solution.

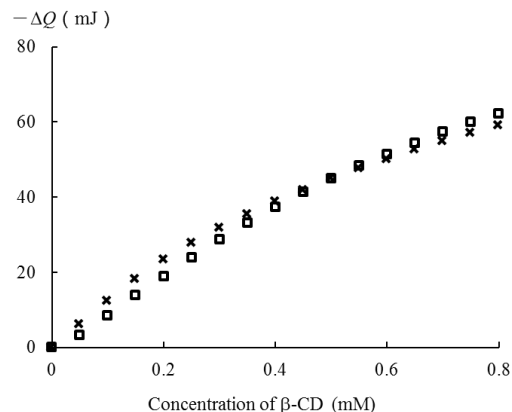


Fig. 5 Calorimetric Titration Curves of RISP/EGCg Mixture and EGCg with β -CD

- × : RISP/EGCg mixture
- : EGCg

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