Development of Sarpogrelate External Preparation for Intractable Pain Control. I. Pre-formulation Study on Application of Modified β-Cyclodextrins

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To optimize the formulation of in-hospital sarpogrelate (SPG) preparation for external use, various cyclodextrins (CDs) were investigated for their ability to improve the aqueous solubility and chemical stability of SPG. Although hydrolysis of SPG was markedly accelerated at above pH 7.0 in aqueous solution, the addition of modified β-CD resulted in suppressed SPG hydrolysis. Addition of sulfobutylether-β-CD (SBE-β-CD, Captisol®) had the most significant stabilization effect. Phase solubility diagram and ^{1}H-NMR analyses indicated that dimethyl-β-CD and SBE-β-CD formed significantly stable inclusion complexes with SPG in aqueous solution, thereby contributing to both the increased solubility and chemical stabilization of SPG. In terms of the clinical safety of CD derivatives, SBE-β-CD was determined to be the most suitable solubilizing agent for external SPG preparation.

Key words sarpogrelate; cyclodextrin; stability; solubility; in-hospital preparation; pain control

The importance of pain control is increasing due to the increasing number of patients suffering from intractable chronic pain as the population ages. In a number of cases, pain cannot be well controlled due to obscure etiology of chronic pain or hyperalgesia. As postherpetic or neuropathic pain is difficult to cure completely, nerve block and physical therapy as supportive measures are often implemented. Nonsteroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants, Chinese herbs, systemic corticosteroids or topical steroids for use in medical treatment are also known to be effective.

Sarpogrelate [{8*R*,*S*}-1-[2-[2-(3-methoxyphenyl)ethyl]phenoxy]-3-(dimethylamino)-2-propyl hydrogen succinate hydrochloride] (SPG) is a selective 5-HT_{2A} receptor antagonist that was introduced as a therapeutic agent for thrombosis-associated ischemia. With its high affinity for 5-HT_{2A} receptor, SPG is reported to have significant analgesic effect in rats when intrathecally and systemically administered.[1—3] Topical ketanserin, another selective 5-HT_{2A} antagonist, also attenuated hyperalgesia and inflammation in arthritic rats.[4] Based on these animal studies, development of a rapidly acting treatment for patients suffering from chronic pain due to peripheral neuralgia has been the major research focus.

Recently, a transdermal ointment in which the crushed SPG tablet [containing 5% (w/w) SPG as the active ingredient; and other excipients: D-mannitol, potato starch, and partially saponified polyvinyl alcohol] is dispersed in white petrolatum has been shown to be effective against postherpetic pain.[5] However, due to its surface stickiness from white petrolatum and rough surface from the crushed SPG tablet, a reduction in compliance with drug treatment in some patients is a concern.[5—7]

In our previous studies, we prepared an aqueous gel of water-soluble polymers iota-carrageenan and polyethylene(oxide) as an external formulation with improved compliance.[8—11] Iota-carrageenan has been used as a texture modifier and a gelling agent in the food industry, and polyethylene(oxide) is obtained by the ring-opening polymerization of ethyleneoxide, giving it high viscosity and spinnability. Ointments prepared with these water-soluble polymers showed the improvement of the spreading properties compared with the ointment containing white petrolatum as the ointment base. Thus, we prepared the aqueous gel as an external formulation for administration of SPG to treat peripheral neuralgia. However, SPG is slightly water soluble (0.012 g/ml) and it can easily be hydrolyzed at the ester moiety in its chemical structure.[12] Therefore, an improvement in the solubility is required to prepare aqueous gels of SPG.

To improve the solubility and stability of SPG in the formulation, we have therefore been examining the use of additives, such as cyclodextrins (CDs). CDs can form inclusion compounds which are used for various research applications.[13—16] Applicability of CDs were investigated in order to enhance the solubility and stability of SPG in the external preparations.

Experimental

Materials SPG was generously supplied from Mitsubishi Tanabe Pharma, Co., Ltd. (Tokyo, Japan). α-CD and β-CD (Wako Pure Chem. Ind., Ltd., Osaka, Japan), γ-CD (Kanto Chem. Co., Tokyo, Japan), hydroxypropyl-β-cyclodextrin (HP-β-CD; Nihon Shokuhin Kako Co., Tokyo, Japan), 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD; Toshin Chemical Co., Tokyo, Japan). Sulfobutyl ether-β-cyclodextrin (SBE-β-CD, Captisol®, DS 6.55) were kindly received from CyDex pharmaceuticals, Inc. Lenexa, KS, U.S.A.

Determination of SPG Concentration SPG concentration in the sample solution was determined by the internal standard method in HPLC analysis. HPLC apparatus consisted of a detector (SPD-10AVP), column oven (CTO-10AVP), and calculator (C-R8A272 nm; Shimadzu Co., Kyoto, Japan). Measurement conditions were as follows: column, Shodex C18M-4D (4.6 mm i.d.×150 mm; Showa Denko Co., Ltd., Tokyo, Japan); column temperature, 30 °C; mobile phase, methanol : 0.05 M KH_2PO_4 (70 : 30); flow rate, 0.8 ml/min; internal standard, propyl *p*-hydroxybenzoate (0.1→250 in acetonitrile).

Stability of SPG in Aqueous Solutions at Various pH A definite volume of SPG aqueous solution (1.0 ml; 100 μg SPG/1 ml H_2O) was added to 9.0 ml of 0.10 M phosphate buffer solution (pH 4.0, 6.0, 7.0, 8.0, 9.0) and incubated at 50 °C. A 250-μl aliquot of the mixture was removed at designated

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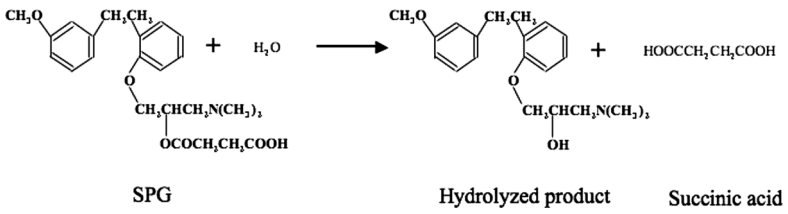

Fig. 1. Scheme of Sarpogrelate (SPG) Hydrolysis

time intervals, and the amount of remaining SPG was determined with HPLC. All experiments were performed in triplicate.

Solubility Study Excess amount of SPG (300 mg : 0.6 mmol) and successively increasing amounts of each CD were combined in 10 ml of 0.10 M HCl. The samples were shaken at 25 °C for 24 h. After equilibration, the samples were filtered with a Millipore filter (0.2 μm). The amount of SPG dissolved was analyzed by HPLC.

Stability of SPG in Aqueous Solutions by Adding Various CDs The mixture of SPG and CDs at different molar ratios (1 : 5.0, 1 : 50 or 1 : 500) was dissolved in 0.10 M phosphate buffer solution (pH 7.0) and incubated at 25, 37 or 50 °C. A 250-μl aliquot of the mixture was removed at designated time intervals, and the amount of remaining SPG was determined with HPLC. The activation energy (E_a) of SPG hydrolysis was calculated according to the Arrhenius equation.

^{1}H-NMR and 2D-ROESY Spectroscopy The formation of a complex between SPG and CDs was investigated by NMR spectroscopy. ^{1}H-NMR experiments were performed at 25 °C in D_2O on a Varian Inova 500 spectrometer (Palo Alto, CA, U.S.A.) operating at 500 MHz. ^{1}H-NMR spectra were acquired with 64 scans with a ^{1}H excitation pulse of 7.4 μs; the time between each repetition was set to 10 s to allow for full relaxation of proton magnetization. The ^{1}H-NMR spectra were acquired with a spectral width of 8000.0 Hz (≅16.0 ppm) and 64 K data points, providing a digital resolution of 0.1 Hz. The spectra were acquired with the same spectral conditions as ^{1}H-NMR. The ^{1}H spectra were processed by applying a Fourier transform with zero-filling to 64 K data points and by an exponential multiplication of the FIDs by a factor of 0.3 and 1 Hz for ^{1}H-NMR, respectively. Attributions of SPG and CDs were realized by chemical shifts observed on the NMR spectra for each mixture at various molar ratios of SPG and CDs. When the stoichiometry was assumed to be 1 : 1, the dissociation constant between SPG and CDs were given by Eq. 1[17]:

$$(\delta_{obs}-\delta_L)/(\delta_{EL}-\delta_L) = ((E_T+L_T+K_d)-\sqrt{(E_T+L_T+K_d)^2-4E_TL_T})/2L_T \quad (1)$$

where K_d is dissociation constant, E_T is total CD concentration, L_T is total SPG concentration, δ_{obs} is observed chemical shift of the SPG proton, δ_L is chemical shift of the proton in pure SPG and δ_{EL} is chemical shift of the proton in pure complex. The 2D-NMR experiments were acquired using the ROESY (rotating-frame Overhauser effect spectroscopy) pulse sequence on a Bruker Avance500 spectrometer (11.7 T) (Karlsruhe, Germany). The spin lock time, relaxation delay and temperature was set to 300 ms, 1 s and 20 °C, respectively. A spectral width was 3472 Hz (6.94 ppm) for SBE-β-CD–SPG system and 2765 Hz (5.53 ppm) for DM-β-CD–SPG system, respectively. The obtained 160×2048 K data matrix in f2 and f1 was zero filled prior to Fourier transformation, resulting in a 2048×2048 spectral data matrix. Chemical shifts were given in ppm in relation to the water residue signal at 4.824 ppm.

Results and Discussion

Hydrolysis of SPG in Aqueous Solutions at Various pH Kinetics of SPG hydrolysis (Fig. 1) was investigated in the aqueous buffer solution of various pH at 50 °C, and the semi-logarithmic plots of % SPG remaining are shown in Fig. 2a. SPG hydrolysis occurred according to first-order kinetics at all values of pH, and the apparent rate constants (k) obtained from the slope of the plots were plotted against pH (Fig. 2b). The profile showed that hydrolysis of SPG was significantly accelerated above pH 7.0. SPG hydrolysis occurred at pH between pK_{a1} (3.74) and pK_{a2} (8.45) of SPG. Therefore, the

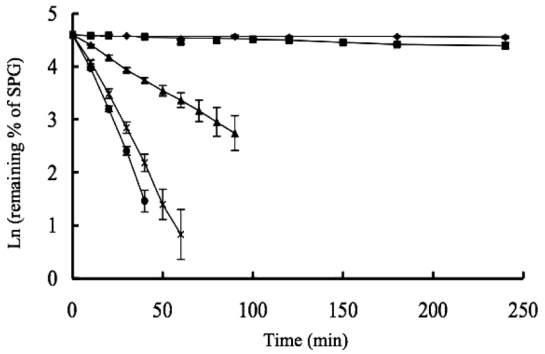

Fig. 2a. SPG Hydrolysis of in Aqueous Solutions at Various pH and 50 °C

◆, pH 4.0; ■, pH 6.0; ▲, pH 7.0; ×, pH 8.0; ●, pH 9.0. Each point represents the mean±S.D. (n=3).

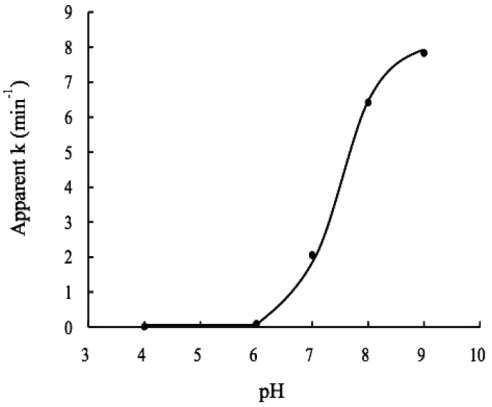

Fig. 2b. Apparent First-Order Rate Constant (k) of SPG Hydrolysis in Aqueous Solutions as a Function of pH at 50 °C

hydrolysis reaction appeared to proceed not only in the de-protonated form (–N(CH$_3$)$_2$ and –COO$^-$) but also in the zwitterionic form (–N$^+$(CH$_3$)$_2$H and –COO$^-$) of SPG.

Phase Solubility Diagrams of SPG with Various CDs Interaction between SPG and various CDs in 0.10 M HCl was studied using their phase solubility diagrams (Fig. 3). In all the systems, apparent solubility of SPG increased linearly with an increase in the amount of CD added, demonstrating an A_L type phase solubility diagram.[18] By assuming 1 : 1 stoichiometry in the solution, stability constants of the complexes were calculated according to Eq. 2 (Table 1). Among unmodified CDs (α-, β-, and γ-CD), the complex with β-CD was the most stable with a stability constant of 877 M^{-1}.

$$\text{stability constant (M}^{-1}\text{): } K' = \text{slope/intercept} \times (1-\text{slope}) \quad (2)$$

In the complexes with modified β-CDs, the stability constant with HP-β-CD was found to be comparable (911 M^{-1}) to that with β-CD, while the SPG/DM-β-CD complex demonstrated a remarkably high stability constant of

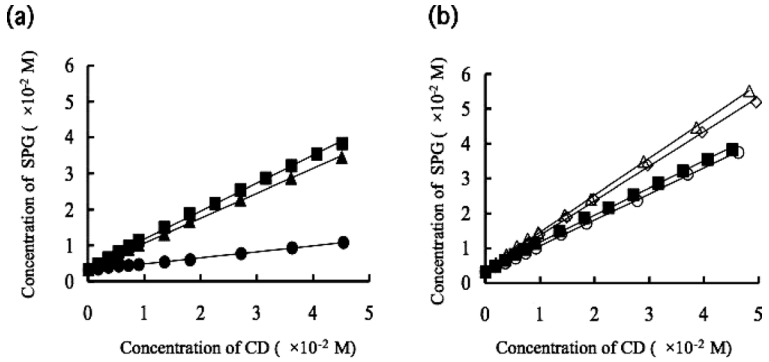

Fig. 3. Phase Solubility Diagram of SPG/CD Systems at 25 °C

(a) ●, α-CD; ■, β-CD; ▲, γ-CD. (b) ■, β-CD; ○, HP-β-CD; ◇, DM-β-CD; △, SBE-β-CD.

Table 1. Stability Constants (K') of SPG/CD Complexes in 0.10 M HCl at 25 °C

CD	K' (M^{-1})
α-CD	62.8
β-CD	877
γ-CD	612
HP-β-CD	911
DM-β-CD	30700
SBE-β-CD	—

30700 M^{-1} (Table 1). SBE-β-CD also markedly increased SPG solubility, but the stability constant could not be calculated by Eq. 2 because the slope of the graph was greater than unity. This suggested contribution of the SBE moiety for SPG solubilization or the formation of a higher order complex between SBE-β-CD and SPG.

Variation in SPG Hydrolysis Behavior Relative to β-CD Additions Hydrolysis behavior of SPG at 50 °C was studied in a buffer solution (pH 7.0) containing different amounts of modified β-CDs (SPG : CD = 1 : 5.0, 1 : 50, and 1 : 500 molar ratios) (Fig. 4). For β-CDs at 1 : 500 ratio, the reaction was not completed due to low aqueous solubility of β-CD.

Among the CDs, SBE-β-CD or DM-β-CD demonstrated an excellent stabilizing effect against SPG hydrolysis. Especially when excess amounts (1 : 500) were added, the hydrolysis rate in the system containing SBE-β-CD and DM-β-CD was found to be about 1/6 and 1/3 that without CD addition, respectively. Taken together with the phase solubility results showing a large stability constant (30700 M^{-1}) for the SPG/DM-β-CD complex, the stable molecular interaction of SPG with CDs appear to play an important role in the chemical stabilization of SPG in solution.

To investigate the effect of temperature, apparent first-order rate constants of SPG hydrolysis at pH 7.0 were evaluated at 25, 37 and 50 °C for 1 : 5.0 and 1 : 50 molar ratios (SPG : CD) (Table 2). While the activation energies of the SPG/DM-β-CD (1 : 5.0 and 1 : 50) and SPG/SBE-β-CD (1 : 5.0) systems were comparable with that without CD addition, only the SPG : SBE-β-CD system at 1 : 50 ratio showed a relatively high activation energy (108.9 kJ/mol). This result seems to be due to the difference in interacting mode of SPG with DM-β-CD and SBE-β-CD from that with the other CDs. Figure 5 shows the Arrhenius plots for each system and activation energies (E_a) listed in Table 3 were calculated with

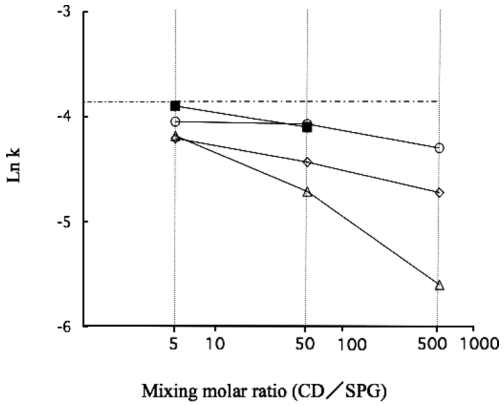

Fig. 4. Effect of CD/SPG Molar Ratio on Apparent First-Order Rate Constant of SPG Hydrolysis at 50 °C and pH 7.0

■, β-CD; ○, HP-β-CD; ◇, DM-β-CD; △, SBE-β-CD. The broken line represents the level for the CD free system.

Table 2. Apparent First-Order Rate Constants for SPG Hydrolysis in the Presence of CDs at Various Temperatures and pH 7.0

	Apparent rate constant ($\times 10^{-3}$ min^{-1})					
	at 25 °C		at 37 °C		at 50 °C	
SPG : CD ratio	1 : 5.0	1 : 50	1 : 5.0	1 : 50	1 : 5.0	1 : 50
β-CD	0.89	0.91	4.1	3.9	20	17
HP-β-CD	0.81	0.79	3.8	3.2	18	17
DM-β-CD	0.72	0.49	3.5	2.7	15	12
SBE-β-CD	0.72	0.32	3.4	1.5	15	9.0

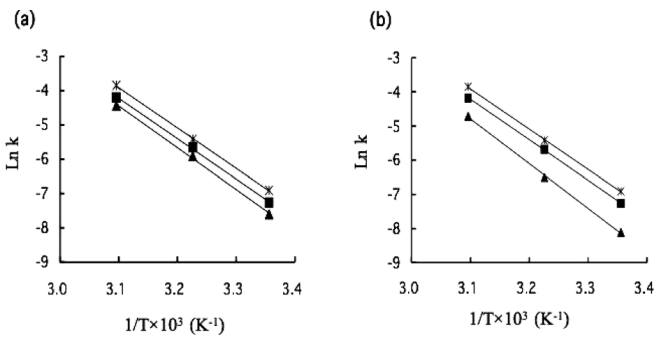

Fig. 5. Arrhenius Plot for SPG Hydrolysis at Various CD/SPG Molar Ratios

(a) DM-β-CD, (b) SBE-β-CD. Molar ratio: ×, 0; ■, 5.0; ▲, 50.

$R^2>0.997$.

NMR Studies in the SPG/DM-β-CD and SPG/SBE-β-CD Systems The molecular interaction of SPG with DM-β-CD and SBE-β-CD was investigated using ^{1}H-NMR and 2D-NMR spectroscopy. Based on Eq. 1, the dissociation constants of SPG/CD complexes were calculated from the chemical shifts on the NMR spectra of each system containing various concentrations of CD. For the DM-β-CD and SBE-β-CD systems, the dissociation constants were approximately 90 mM and 30 mM, respectively, which were converted into stability constants (K') of 11000 M^{-1} and 33000 M^{-1}, respectively. These values were determined to be sufficient for CD interaction, validating the high stability constant (30700 M^{-1}) estimated from the phase solubility diagrams of the SPG/DM-β-CD system. Moreover, the stability constant of the SBE-β-CD system was found to be larger than that of SPG/DM-β-CD, suggesting that SBE-β-CD further stabilizes SPG against hydrolysis.

Table 3. Activation Energy for SPG Hydrolysis in the Presence of CDs

	E_a (kJ/mol)
SPG only	97.8
SPG : DM-β-CD (1 : 5.0)	97.9
SPG : DM-β-CD (1 : 50)	101.5
SPG : SBE-β-CD (1 : 5.0)	98.7
SPG : SBE-β-CD (1 : 50)	108.9

Interaction modes of SPG/DM-β-CD complexes were investigated using 2D-NMR spectroscopy.[19] After signal assignment of chemical shifts, correlation signals in the ROESY spectrum were analyzed to identify the conformational relationship of two vicinal protons between SPG and DM-β-CD (Fig. 6a). In the ROESY spectrum, correlation signals were observed between the H5′ proton located in the DM-β-CD cavity and the aromatic protons (H3, H5, H6, and H7) of the benzene ring on the edge of the SPG molecule. The H3′ proton also located in the CD cavity showed strong correlation signals with the methylene chain (H8 and H9) and benzene ring (H3 and H5) protons of SPG. Furthermore, both the H6′ and 6′-*O*-methyl protons of DM-β-CD, located on the side of the narrow opening of its torus, indicated correlation signals with the H3, H6 and H7 protons of SPG for

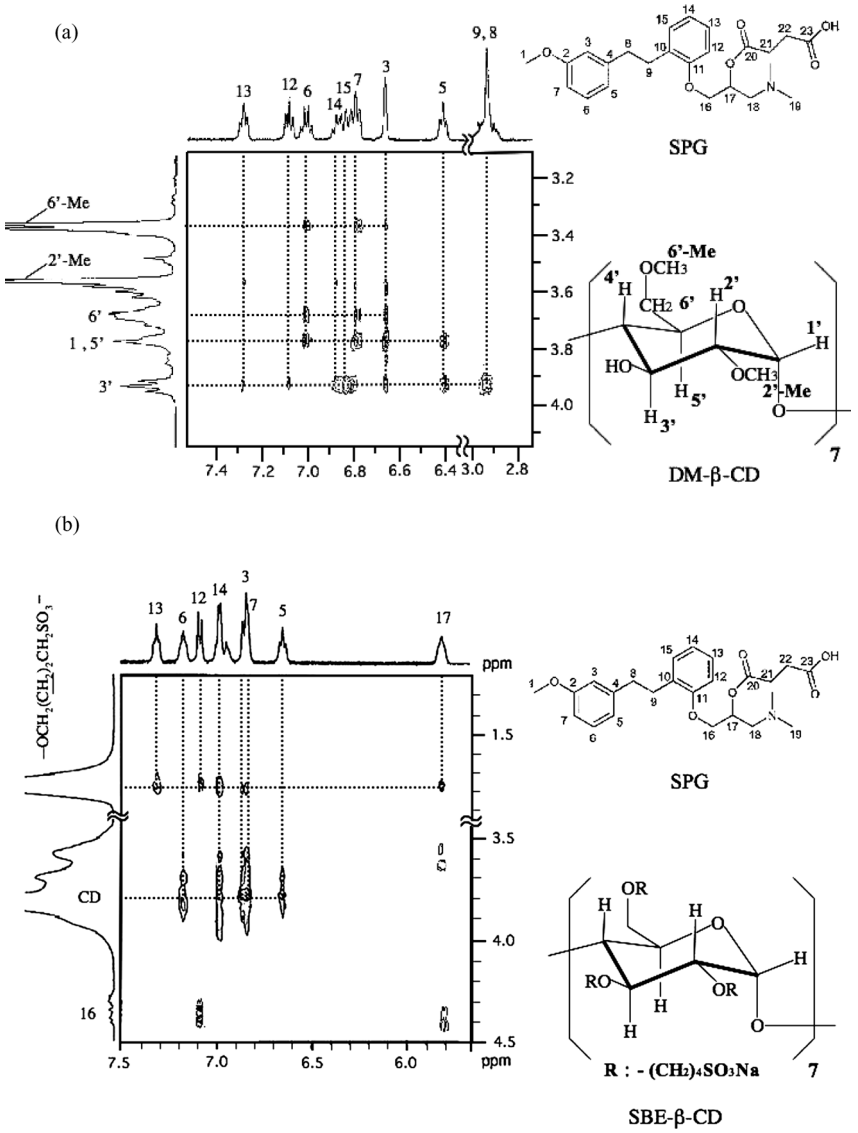

Fig. 6. 2D-ROESY Spectra of SPG/CD Complexes in D_2O at 20 °C
(a) SPG/DM-β-CD complex. (b) SPG/SBE-β-CD complex.

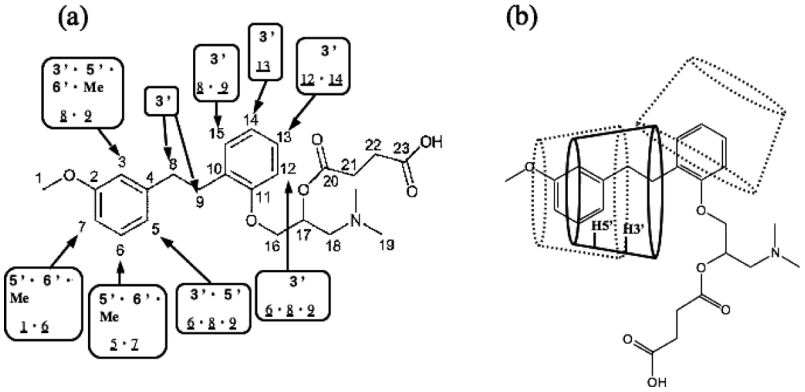

Fig. 7. Interaction Mode between SPG and CDs Based on Cross Peak Signals on the ROESY Spectra

(a) Protons considered to be close in space to SPG protons based on correlation signals. Bold and underlined letters represent protons derived from the CD and SPG molecule, respectively. (b) Possible interaction mode with DM-β-CD.

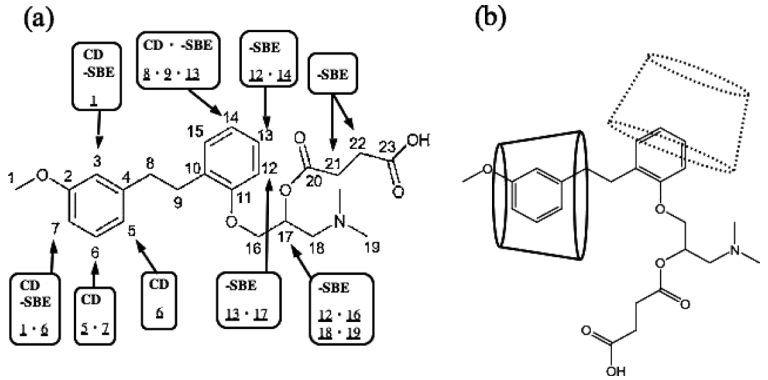

Fig. 8. Interaction Mode between SPG and CDs Based on Cross Peak Signals on the ROESY Spectra

(a) Protons considered to be close in space to SPG protons based on correlation signals. Bold and underlined letters represent protons derived from the CD and SPG molecule, respectively. (b) Possible interaction mode with SBE-β-CD.

all combinations. Based on these results, the main molecular interaction mode in the SPG/DM-β-CD complex was determined as follows: (1) the aromatic ring on the SPG molecular edge is inserted within the DM-β-CD cavity at the wide opening of its torus, and (2) the methylene chain of SPG is inserted deeply into the DM-β-CD cavity, showing complete inclusion. Moreover, only the correlation signal between the H3′ proton of DM-β-CD cavity and the aromatic protons (H12, H13, H14, and H15) of the benzene ring in the middle of the SPG molecule were also observed, suggesting another possible interaction mode in which the benzene ring in the middle of the SPG molecule is partially inserted in the DM-β-CD cavity (Fig. 7b). Since some relatively strong correlation signals were also observed among the protons of the hydrophobic portions of SPG, molecular interactions especially hydrophobic ones may occur between SPG molecules (Fig. 7a).

As precise assignment of NMR chemical shifts could not be determined for the SPG/SBE-β-CD complex, due to non-uniform substituted positions of the SBE side chain to the CD ring, the ROESY analysis for SPG/SBE-β-CD interaction was performed by assuming that the chemical shifts of the protons located in the SBE-β-CD cavity (H3′ and H5′) were almost the same as those of DM-β-CD (Fig. 6b). In the SBE-β-CD complex, similar correlation signals between the protons in the SBE-β-CD cavity (H3′ and H5′) and the aromatic protons of SPG (benzene ring on the molecular edge, H3, H5, H6, and H7; in the middle, H14) were observed on the ROESY spectrum (Fig. 6b) as those observed in the DM-β-CD system, but no correlation signals were observed for the protons (H8 and H9) on the methylene chain of SPG. However, protons within the SBE substituted groups of SBE-β-CD showed correlation signals with several protons on the SPG molecule (H3, H5, H6, H7, H12, H13, H14, H17, H21, H22), suggesting that the SBE groups stabilize the inclusion complex through cooperative interaction. Taken together, the molecular interaction mode in the SPG/SBE-β-CD complex was determined as follows: (1) the aromatic ring on the SPG molecular edge is inserted in DM-β-CD cavity; (2) the benzene ring in the middle of SPG may also be partially inserted in the DM-β-CD cavity; and (3) the SBE groups cooperatively interact with SPG (Fig. 8b). Moreover, the ROESY spectrum of the SPG/SBE-β-CD complex also suggested molecular interactions between the SPG molecules as found in the case of the SPG/DM-β-CD complex (Fig. 8a).

While marked stability of the SPG inclusion complex with both DM-β-CD and SBE-β-CD was demonstrated, SBE-β-CD at high concentration (SPG : SBE-β-CD = 1 : 500) more effectively suppressed SPG hydrolysis compared to DM-β-CD. This greater suppression on SPG hydrolysis could be a result of the cooperative interaction of the SBE side chains with the SPG molecule inserted in the CD cavity.

Conclusion

Possible applications of CDs were investigated for improving solubility and stability of SPG. The phase solubility diagrams demonstrated that both DM-β-CD and SBE-β-CD, which can effectively solubilize SPG, may be used as solubilizing agents in the preparation of external formulations. While addition of either DM-β-CD or SBE-β-CD improves the chemical stability of SPG in aqueous solution, DM-β-CD may not be appropriate for clinical use due to its hemolytic activity.[20] Therefore, SBE-β-CD appears to be the most suitable solubilizing agent for SPG external preparation.

Phase solubility diagram and ^{1}H-NMR analyses demonstrated a quite stable inclusion complex of SPG and SBE-β-CD. 2D-NMR results suggested that the complex exists in two interaction modes: complete inclusion of the edge-side aromatic ring of SPG and partial inclusion of the middle benzene ring both within the SBE-β-CD cavity. In addition, the SBE group appears to cooperatively interact with the SPG moiety. Consequently, the marked stability of the SPG–SBE-β-CD complex was considered to result from these molecular interactions, and these interactions modes could play an important role in depression of hydrolysis for ester part of SPG molecule.

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