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Effect of sulfobutyl ether-β-cyclodextrin on bioavailability of insulin glargine and blood glucose level after subcutaneous injection to rats

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ABSTRACT

Insulin glargine is the first long-acting basal insulin analogue used for subcutaneous administration once daily in patients with type 1 or type 2 diabetes mellitus. To obtain the further bioavailability and the sustained glucose lowering effect of insulin glargine, in the present study, we investigated the effect of sulfobutyl ether-β-cyclodextrin (SBE4-β-CyD), with the degree of substitution of sulfobutyl ether group of 3.9, on pharmaceutical properties of insulin glargine and the release of insulin glargine after subcutaneous injection to rats. SBE4-β-CyD increased the solubility and suppressed aggregation of insulin glargine in phosphate buffer at pH 9.5, probably due to the interaction of SBE4-β-CyD with aromatic amino acid residues such as tyrosine of insulin glargine. In addition, SBE4-β-CyD accelerated the dissolution rate of insulin glargine from its precipitates, compared to that of insulin glargine alone. Furthermore, we revealed that subcutaneous administration of an insulin glargine solution with SBE4-β-CyD to rats enhanced the bioavailability of insulin glargine and sustained the glucose lowering effect, possibly due to the inhibitory effects of SBE4-β-CyD on the enzymatic degradation at the injection site. These results suggest that SBE4-β-CyD can be a useful excipient for sustained release of insulin glargine.

1. Introduction

Diabetes is a chronic disease wherein the pancreas does not produce enough insulin (type 1 diabetes) or the body does not respond correctly to insulin and relative insulin deficiency (type 2 diabetes). It can be a life-threatening disease and also lead to serious complications such as cardiovascular disease, kidney failure, blindness and nerve damage (Blickle et al., 2007; Patterson et al., 2009; Simo et al., 2006). The global prevalence of diabetes has been increasing in recent decades, reaching near-epidemic proportions, and is projected to more than double by 2030 (Horton, 2008). The global diabetes epidemic has devastated on not only patients and their families but also national economies.

Human insulin is a major backbone for the treatment of diabetes. Although human insulin has attributed much in clinical treatment of diabetes for long time, there are still some difficulties and challenges in hypoglycemia and short half-life. In order to overcome these drawbacks, insulin glargine (Lantus®), an insulin analogue ($C_{267}H_{404}N_{72}O_{78}S_6$, MW = 6063) with a prolonged duration of action after subcutaneous injection, was developed by replacing the asparagine at the position of 21 of the A chain with glycine, and two arginines were added to the C-terminus of the B chain in human insulin (Fig. 1) (Rolla, 2008). This alteration resulted in low aqueous solubility at neutral pH (Wang et al., 2003). Insulin glargine is supplied in an acidic solution, which becomes neutralized at the injection site, leading to a formation of microprecipitates from which insulin glargine is slowly released into the circulation (Wang et al., 2003).

Cyclodextrins (CyDs) are known to form inclusion complexes with various guest molecules (Szente and Szejtli, 1999; Uekama et al., 1998). However, the low aqueous solubility of natural CyDs, especially β-CyD, has restricted their range of applications. To improve their solubility, alkylated, hydroxyl alkylated, sulfobutyl alkylated and branched CyDs have been developed (Stella and Rajewski, 1997; Uekama, 2004; Uekama and Otagiri, 1987). Of these hydrophilic CyDs, maltosyl-β-CyD (G_2-β-CyD), 2-hydroxypropyl-β-CyD (HP-β-CyD) and sulfobutyl ether-β-CyD (SBE-β-CyD) have higher solubility in water and relatively low hemolytic activity, and thus have potential as pharmaceutical excipients for parenteral preparation (Uekama et al., 1998). In fact, natural β-CyD has a toxic effect on kidney, which is the main organ for removal of CyDs from the systemic circulation and for concentrating CyDs in the proximal convoluted tubule after glomerular filtration (Irie and Uekama, 1997). On the other hand, amorphous

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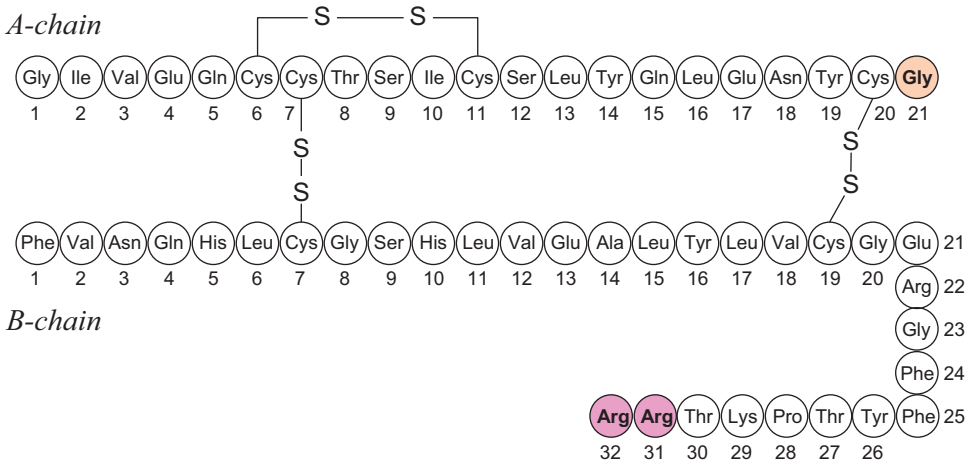

Fig. 1. Secondary structure of insulin glargine.

mixtures of highly water-soluble β-CyDs such as HP-β-CyD and SBE-β-CyD have very low systemic toxicity, compared with β-CyD.

We previously reported the effects of hydrophilic β-CyDs on the aggregation of bovine insulin in aqueous solution and its adsorption onto hydrophilic surfaces (Tokihiro et al., 1996, 1995, 1997). Of the CyDs tested, G_2-β-CyD potently inhibited insulin aggregation in a neutral solution and its adsorption onto the surfaces of glass and polypropylene tubes. In addition, SBE-β-CyDs showed the different effects on insulin aggregation, depending on the degree of substitution (DS) of the sulfobutyl ether group: SBE4-β-CyD (DS = 3.9) showed deceleration of insulin aggregation at relatively low substitution levels and SBE7-β-CyD (DS = 6.2) showed acceleration at high substitution levels (Tokihiro et al., 1997). Furthermore, we reported that subcutaneous administration of insulin solution with SBE4-β-CyD to rats rapidly increased plasma insulin level and maintained higher plasma insulin levels for at least 8 h, possibly due to the inhibitory effects of SBE4-β-CyD on the enzymatic degradation and/or the adsorption of insulin onto the subcutaneous tissue at the injection site (Tokihiro et al., 2000). However, it is still unknown whether SBE4-β-CyD shows the sustained-glucose lowering effects for insulin analogues. Of various insulin analogues, only a few experiments on pharmaceutical application of insulin glargine were performed. Therefore, in the present study, to evaluate the potential use of SBE4-β-CyD on not only bioavailability of insulin glargine but also the sustained-glucose lowering effect, we examined the effects of SBE4-β-CyD on physicochemical properties and pharmacokinetics/pharmacodynamics of insulin glargine.

2. Materials and methods

2.1. Materials

Insulin glargine was supplied by Sanofi-Aventis (Paris, France). SBE4-β-CyD was provided by CyDex (Lenexa, KS). Recombinant trypsin (EC 3.4.21.4) of proteomics grade was purchased from Roche Diagnostics (Tokyo, Japan). All other materials were of reagent grade, and deionized double-distilled water was used. Phosphate buffer (pH 9.5, $I = 0.2$) was prepared according to the U.S. pharmacopeia: 0.1 mol/L phosphoric acid solution and 0.1 mol/L sodium hydroxide solution were mixed, which followed by addition of sodium chloride.

2.2. Methods

2.2.1. Spectroscopic studies

Fluorescence and circular dichroism (CD) spectra were measured at 25 °C using a HITACHI fluorescence spectrophotometer F-2500 (Tokyo, Japan) and a JASCO J-720 polarimeter (Tokyo, Japan), respectively.

2.2.2. Solubility studies

Excess amounts of insulin glargine were shaken in phosphate buffer (pH 7.4 or 9.5, $I = 0.2$) in the absence and presence of SBE4-β-CyD for 5 days within which the solubility equilibrium was established at 25 °C. After equilibrium was attained, the solutions were filtered with Millex® GV filter 0.22 μm and insulin glargine dissolved was determined by the high performance liquid chromatography (HPLC) with Agilent 1100 series (Tokyo, Japan) under the following conditions: Merck Superspher® 100 RP-18 column (4 μm, 3 mm × 250 mm, Tokyo, Japan), a mobile phase of phosphate buffer (pH 2.5) and acetonitrile and a gradient flow, increasing the ratio of the acetonitrile (25–40%) over 30 min, a flow rate of 0.55 mL/min, a detection of UV at 214 nm.

2.2.3. Ultrafiltration studies

Ultrafiltration studies were performed using stirred ultrafiltration cells model 8010 (Millipore, Tokyo, Japan) applied with YM30 ultrafiltration discs (MWCO = 30,000) in phosphate buffer (pH 9.5, $I = 0.2$) in the absence and presence of SBE4-β-CyD at 25 °C under nitrogen current. Insulin glargine levels in filtrates were determined by HPLC as described above.

2.2.4. Particle size determination

Particle sizes of insulin glargine (0.1 mM) with or without SBE4-β-CyD (10 mM) in phosphate buffer (pH 9.5, $I = 0.2$) were measured by Zetasizer Nano (Malvern Instruments, Worcestershire, UK).

2.2.5. Dissolution study of insulin glargine

Insulin glargine (0.1 mM) dissolved in phosphate buffer (pH 9.5, $I = 0.2$) in the absence and presence of SBE4-β-CyD (10 mM) was precipitated by a pH shift to 7.4. After centrifugation (2500 rpm, 10 min), the supernatant was discarded and then phosphate buffer (pH 7.4, $I = 0.2$) was newly added to the precipitate at 25 °C. At appropriate intervals, an aliquot of the dissolution medium was withdrawn, centrifuged at 2500 rpm for 10 min, and analyzed for the insulin glargine by HPLC as described in Section 2.2.2.

2.2.6. Stability of insulin glargine against tryptic cleavage

Insulin glargine (0.1 mM) in phosphate buffer (pH 9.5, $I = 0.2$) was incubated with recombinant trypsin (0.02 mg/mL) in the absence and presence of SBE4-β-CyD at 37 °C. At appropriate intervals, 5 μL of sample solution was withdrawn and determined intact insulin glargine level by HPLC. The rate constants (k_c) and stability

constants (K_c) of apparent 1:1 complexes of insulin glargine/SBE4-β-CyD under the tryptic cleavage were determined by quantitative analysis according to the following equation, where k_0 and $[CyD]_t$ stands for the rate constants without CyD and the total concentration of CyD, respectively (Ikeda et al., 1975).

$$\frac{[CyD]_t}{k_0 - k_{obs}} = \frac{1}{k_0 - k_c}[CyD]_t + \frac{1}{K_c(k_0 - k_c)}$$

2.2.7. Subcutaneous administration of insulin glargine/SBE4-β-CyD solution to rats

Serum insulin glargine and glucose levels of rats were measured by the enzyme immunoassay and the mutarotase-glucose oxidase method. The solution (0.582 mL/kg) of the insulin glargine (2 IU/kg) in phosphate buffer (pH 9.5, $I = 0.2$) in the absence and presence of SBE4-β-CyD (200 mM) was subcutaneously injected in male Wistar rats (200–250 g), and at appropriate intervals blood samples were taken from the jugular veins. Serum insulin glargine and glucose were determined by Glyzyme Insulin-EIA Test Wako (Wako Pure Chemicals, Osaka, Japan) and Glucose-CII-Test Wako (Wako Pure Chemicals Ind., Osaka, Japan), respectively. Serum glucose levels after the administration of insulin glargine/SBE4-β-CyD solutions were expressed as a percentage of the initial glucose level before injection.

2.2.8. Statistical analysis

Data are given as the mean ± S.E.M. Statistical significance of means for the studies was determined by analysis of variance followed by Scheffe's test. p-Values for significance were set at 0.05.

3. Results and discussion

3.1. Spectroscopic studies

CyDs have been claimed to interact with hydrophobic residues exposed on protein surfaces and thereby to decrease aggregation of proteins (Brewster et al., 1991; Tavornvipas et al., 2006). We previously reported that SBE4-β-CyD inhibited the insulin aggregation in neutral solution, possibly due to the interaction of SBE4-β-CyD with aromatic side chain of insulin such as B26-tyrosine, A19-tyrosine, B1-phenylalanine and B25-phenylalanine (Tokihiro et al., 1997). In the present study, to reveal whether SBE4-β-CyD interacts with insulin glargine, we investigated the effects of SBE4-β-CyD (10 mM) on the fluorescence and CD spectrum of insulin glargine (0.1 mM) (Fig. 2). To obtain a clear solution of insulin glargine (0.1 mM) in the spectroscopic studies, insulin glargine with SBE4-β-CyD was dissolved in phosphate buffer (pH 9.5, $I = 0.2$) at 25 °C, because insulin glargine and SBE4-β-CyD form insoluble complex at acidic pH. In our preliminary study, we confirmed that insulin glargine dissolved in phosphate buffer (pH 9.5, $I = 0.2$) was relatively chemically stable, remaining 91.0% of insulin glargine after 6 days storage at 25 °C, in comparison with the initial concentration (data not shown). The fluorescence intensity of tyrosine of insulin glargine at 306 nm was quenched by the addition of SBE4-β-CyD (Fig. 2A). As tyrosine is a hydrophobic amino acid having a phenyl group in the molecule, SBE4-β-CyD interacts with those aromatic amino acid residues of insulin glargine. The apparent 1:1 stability constant (K_c) of the insulin glargine/SBE4-β-CyD complex was determined by the titration curves of the fluorescence intensity against a concentration of SBE4-β-CyD with the Scott's equation (Ikeda et al., 1975). The stability constant of insulin glargine/SBE4-β-CyD complex in phosphate buffer (pH 9.5, $I = 0.2$) at 25 °C was calculated to be $20 \pm 5\ M^{-1}$. The CD spectrum of insulin glargine (0.1 mM) showed negative bands at 210 nm and 220 nm in phosphate buffer (pH 9.5, $I = 0.2$) (Fig. 2B). The two negative bands assigned to α-helics (a characteristic feature of the monomer) and β-sheets (a predominant feature of dimer) (Goldman and Carpenter, 1974). However, CD spectrum of insulin glargine in the presence of SBE4-β-CyD (10 mM) was changed only very slightly, compare to that of insulin glargine alone, suggesting that SBE4-β-CyD did not induce a conformational change in insulin glargine in phosphate buffer (pH 9.5, $I = 0.2$). These results suggest that SBE4-β-CyD interacts with insulin glargine without topological change of insulin glargine in phosphate buffer (pH 9.5, $I = 0.2$).

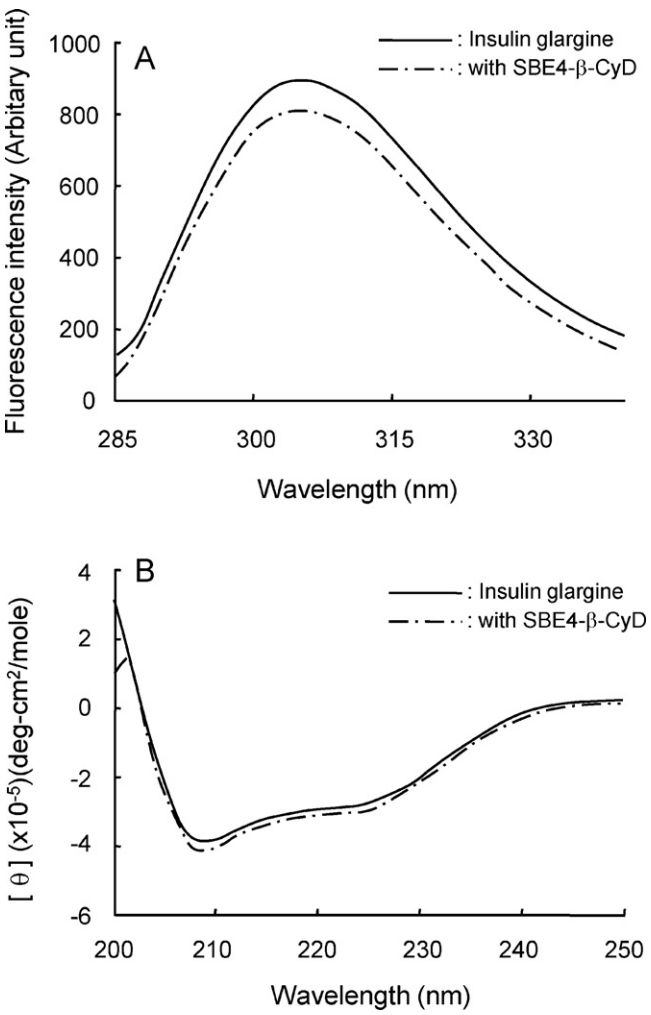

Fig. 2. Effects of SBE4-β-CyD (10 mM) on fluorescence spectrum (A) and circular dichroism spectrum (B) of insulin glargine (0.1 mM) in phosphate buffer (pH 9.5, $I = 0.2$) at 25 °C. The excitation wavelength in measurement of fluorescence spectrum was 277 nm.

3.2. Solubility studies

Currently subcutaneous injection of clear solution is the main stream for administration of insulin and its analogues. However, insulin or insulin glargine is poorly soluble in aqueous solutions, in particular around the isoelectic point (pI), approximately pH 6.7, close to the physiological pH (Brange et al., 1997). Then, the effect of SBE4-β-CyD on the solubility of insulin glargine was examined at pH 7.4 and 9.5. As shown in Fig. 3, the solubility of insulin glargine in phosphate buffer both at pH 7.4 and 9.5 was significantly increased by the addition of SBE4-β-CyD. It is estimated that the increase in the solubility of insulin glargine was caused by the complexation between SBE4-β-CyD and aromatic amino acid residues of insulin glargine such as tyrosine. These results suggest that SBE4-β-CyD potentially enhances the solubility of insulin glargine in phosphate buffer.

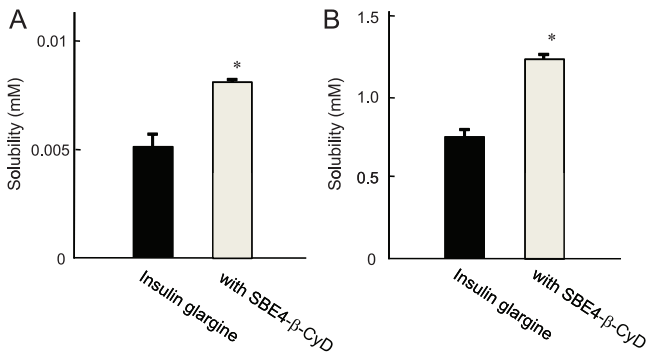

Fig. 3. Effect of SBE4-β-CyD (10 mM) on solubility of insulin glargine in phosphate buffers ((A) pH 7.4 and (B) pH 9.5, $I = 0.2$) at 25 °C. The concentration of insulin glargine was determined by HPLC. Each value represents the mean ± S.E.M. of 3 experiments. $^*p < 0.05$, compared to insulin glargine.

3.3. Ultrafiltration studies

The aggregation of insulin and its analogue are elicited by many kinds of factors such as the concentration of insulin, pH, temperature, shaking and so on (Rolla, 2008; Wang et al., 2003). Insulin glargine forms dimer, tetramer, hexamer and further soluble multimer by non-covalent interaction as proceeding in self-association (Havelund et al., 2004, Kurtzhals, 2004). Therefore, we performed ultrafiltration studies to estimate the effects of SBE4-β-CyD on aggregation of insulin glargine using the membrane YM30 (MWCO = 30,000) in phosphate buffer (pH 9.5, $I = 0.2$). As shown in Fig. 4, insulin glargine permeated the ultrafiltration membrane by approximately 50%. SBE4-β-CyD significantly enhanced the permeation of insulin glargine up to almost 70%. These results suggest that SBE4-β-CyD leads to dissociation of soluble multimer of insulin glargine.

Following ultrafiltration experiment, particle sizes of insulin glargine were determined in the absence and presence of the SBE4-β-CyD (Table 1). Particle size of insulin glargine alone in phosphate buffer (pH 9.5, $I = 0.2$) was 744 ± 82 nm. On the other hand, the addition of SBE4-β-CyD significantly increased in the particle size to 1724 ± 275 nm. The sulfobutyl groups of SBE4-β-CyD are known to be strongly hydrated in aqueous solution. Therefore, the hydrodynamic diameter of insulin glargine in the presence of SBE4-β-CyD is supposed to include not only insulin glargine and SBE4-β-CyD but also water molecules hydrated with SBE4-β-CyD. With these reasons, the increased particle size may be observed in the solution of insulin glargine with SBE4-β-CyD. These results suggest the potential use of SBE4-β-CyD for an aggregation-inhibitor for insulin glargine, resulting from maintaining of the soluble form of insulin glargine through the formation of complex with insulin glargine.

Table 1
Particle size of insulin glargine with or without SBE4-β-CyD (10 mM) in phosphate buffer (pH 9.5). The particle size was measured by Zetasizer Nano. The concentration of insulin glargine and SBE4-β-CyD were 0.1 mM and 10 mM, respectively.

	Particle size (nm)
Insulin glargine	744 ± 82
With SBE4-β-CyD	1724 ± 275

3.4. Dissolution study of insulin glargine

Insulin glargine is believed to precipitate at the physiological pH after subcutaneous injection of the solution due to p*I* (about pH 6.7), which is followed by a sustained release of insulin glargine over 24 h from injection site because of an extremely low solubility in aqueous solution at pH of around p*I* (Wang et al., 2003). In order to investigate the effects of SBE4-β-CyD on the sustained release of insulin glargine, the dissolution rate of insulin glargine from isoelectric precipitates formed in the absence and presence of SBE4-β-CyD was determined (Fig. 5). Insulin glargine (0.1 mM) was dissolved in phosphate buffer (pH 9.5) in the presence and absence of SBE4-β-CyD (10 mM), and then isoelectric precipitation of insulin glargine was obtained after pH shift from 9.5 to 7.4. Then, the release of insulin glargine was determined in phosphate buffer (pH7.4) in the absence of SBE4-β-CyD. SBE4-β-CyD significantly increased the dissolution rate of insulin glargine after 3 h through

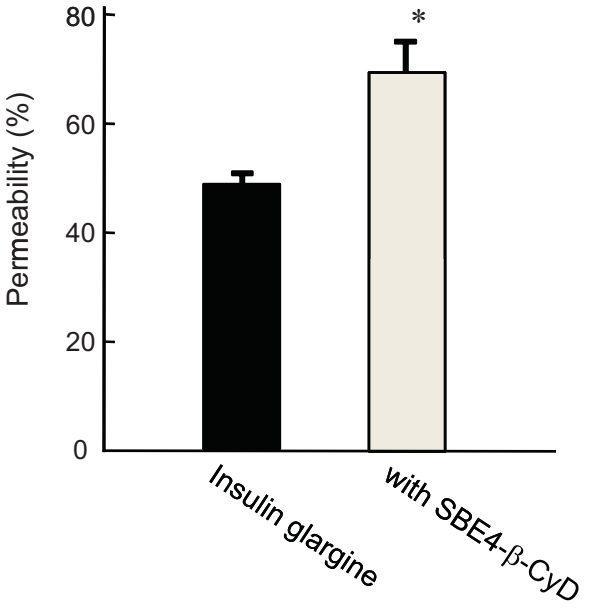

Fig. 4. Effect of SBE4-β-CyD (10 mM) on permeation of insulin glargine (0.1 mM) through ultrafiltration membrane having nominal molecular weight limit of 30,000 in phosphate buffer (pH 9.5, $I = 0.2$) at 25 °C. The concentration of insulin glargine was determined by HPLC. Each value represents the mean ± S.E.M. of 17 and 5 experiments for insulin glargine and with SBE4-β-CyD, respectively. $^*p < 0.05$, compared to insulin glargine.

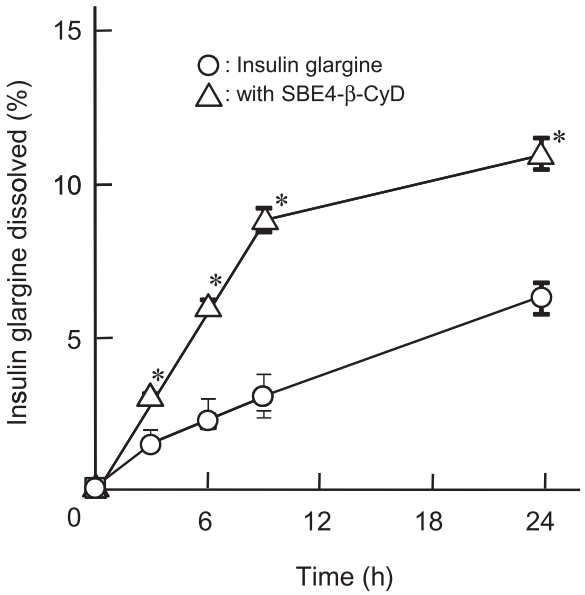

Fig. 5. Effect of SBE4-β-CyD (10 mM) on the dissolution rate from isoelectric precipitation of insulin glargine in phosphate buffer (pH 9.5, $I = 0.2$) at 25 °C. The initial concentration of insulin glargine was 0.1 mM, and then precipitated at pH 7.4. The concentration of insulin glargine was determined by HPLC. Each point represents the mean ± S.E.M. of 3 experiments. $^*p < 0.05$, compared to insulin glargine.

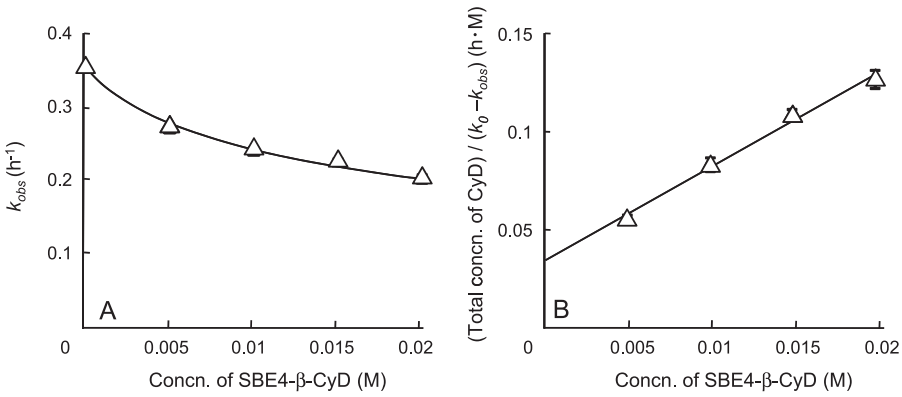

Fig. 6. Effects of SBE4-β-CyD (5–20 mM) on tryptic cleavage (2 IU) of insulin glargine (0.1 mM) in phosphate buffer (pH 9.5, I = 0.2) at 37 °C. The concentration of insulin glargine was determined by HPLC. Each point represents the mean ± S.E.M. of 3 experiments.

24 h, compare to insulin glargine alone. This enhancing effect of SBE4-β-CyD was consistent with its solubilizing effect as shown in Fig. 3. These results suggest that SBE4-β-CyD increases dissolution rate of insulin glargine from its precipitate.

3.5. Stability of insulin glargine against tryptic cleavage

Insulin and its analogues are digested by proteinase such as trypsin, which cleaves insulin at the carboxyl side of residues B29-lysine and B22-arginine, at injection site and systemic circulation (Schilling and Mitra, 1991). Therefore, a resistance toward enzymatic degradation is required for insulin or its analogues formulation to improve their bioavailability. Next, we investigated the effects of the SBE4-β-CyD on stability of insulin glargine against trypsin digestion. In this study, insulin glargine was digested by trypsin at 2 IU of the initial concentration at pH 9.5 at 37 °C in the absence and presence of SBE4-β-CyD. As shown in Fig. 6A, the apparent degradation rate constant of insulin glargine in the absence of the SBE4-β-CyD (k_0) was $0.357 \pm 0.004\,h^{-1}$. Furthermore, the apparent rate constant (k_{obs}) in the presence of the SBE4-β-CyD decreased with the increase in the concentration of SBE4-β-CyD. The rate constant (k_c) and stability constant (K_c) of an apparent 1:1 complex calculated with the regression lines shown in Fig. 6B were $0.145 \pm 0.012\,h^{-1}$ and $144 \pm 18\,M^{-1}$, respectively. The K_c of an apparent 1:1 complex of insulin glargine and SBE4-β-CyD determined by the tryptic cleavage study was inconsistent with that by the fluorescence study. The reason for the difference in the K_c value may be due to the difference in the experimental conditions such as temperature of the solutions (25 °C for the spectroscopic study and 37 °C for the tryptic cleavage study) and the presence of trypsin. These results suggest that the inhibition of tryptic cleavage of insulin glargine by SBE4-β-CyD was caused by a formation of complex with insulin glargine, resulting from decreasing the free insulin glargine to be easily digested by trypsin. Recently, it was reported that the aspartic acid residue existing in the catalytic pocket of trypsin is responsible for attracting and stabilizing positively charged lysine and/or arginine (Leiros et al., 2004). Therefore, the insulin glargine/SBE4-β-CyD complex is speculated to ameliorate the interaction between the negatively charged aspartic acid in the catalytic pocket of trypsin and positively charged lysine and/or arginine mentioned, since SBE4-β-CyD has negative charge originated from sulfobutyl groups. This hypothesis which the insulin glargine/SBE4-β-CyD complex ameliorates the interaction between the aspartic acid and lysine and/or arginine is supported by the report that the aromatic amino acid residues in insulin glargine are capable of interacting with β-CyDs (at B24-, B25-phenylalanines, B26-tyrosine and B28-proline) locate near the three digestive sites by trypsin (B22-B23, B29-B30 and B31-B32) (Tokihiro et al., 1997). Furthermore, the interaction between SBE4-β-CyD and trypsin could be attributed to the inhibition of enzymatic degradation of insulin glargine by trypsin. These results suggest that SBE4-β-CyD acts as a stabilizer of insulin glargine against enzymatic degradation due to interaction with insulin glargine.

3.6. Subcutaneous administration of insulin glargine/SBE4-β-CyD solution to rats

To confirm whether SBE4-β-CyD is useful enhancer for insulin glargine *in vivo*, we evaluated the effects of SBE4-β-CyD on pharmacokinetics and pharmacodynamics of insulin glargine after subcutaneous injection to rats. Fig. 7A and Table 2 show the serum insulin glargine level–time profiles and pharmacokinetic parameters, respectively, after subcutaneous administration of insulin glargine (2 IU/kg) with or without SBE4-β-CyD (200 mM) in phosphate buffer (pH 9.5) to rats. When insulin glargine was injected, the time (T_{max}) required to reach maximum level (C_{max}) of insulin glargine was at 1 h after injection, and then the serum insulin glargine level decreased to the basal level. Although T_{max} in the SBE4-β-CyD system was the same as that of insulin glargine alone, SBE4-β-CyD significantly sustained the serum insulin glargine level. The area under the serum insulin glargine level–time curve (AUC) of the SBE4-β-CyD system (AUC = 934.39 (μU/mL) h) was significantly increased, compared to those of insulin glargine alone (AUC = 786.31 (μU/mL) h).

Fig. 7B and Table 3 show the serum glucose level–time profiles and pharmacodynamics parameters after subcutaneous administration of insulin glargine (2 IU/kg) with or without SBE4-β-CyD (200 mM) in the phosphate buffer (pH 9.5) to rats. When insulin glargine alone was injected, the minimal glucose level occurred at about 2 h after injection and then the serum glucose levels recovered within 6 h to basal level. On the other hand, insulin glargine administered with SBE4-β-CyD significantly sustained the

Table 2
In vivo pharmacokinetics parameters of insulin glargine with or without SBE4-β-CyD (200 mM).

System	T_{max} (h)[a]	C_{max} (μU/mL)[b]	AUC ((μU/mL) h)[c]
Insulin glargine	1	211.44 ± 11.99	786.31 ± 45.46
Insulin glargine/SBE4-β-CyD	1	186.84 ± 7.76	934.39 ± 49.11*

Each value represents the mean ± S.E.M. of 7–11 experiments.
[a] Time required to reach the maximum serum insulin glargine level.
[b] Maximum serum insulin glargine level.
[c] Area under the serum insulin glargine level–time curve up to 9 h post-administration.
* $p < 0.05$, compared to insulin glargine.

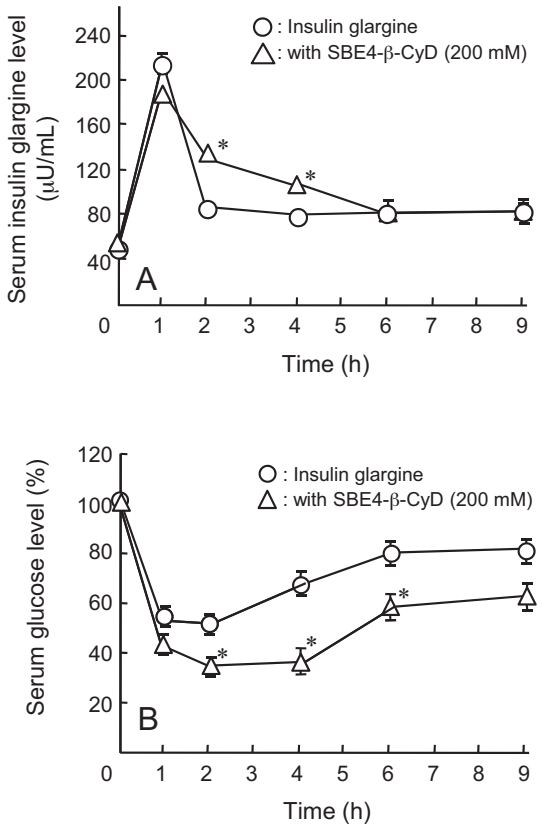

Fig. 7. Effects of SBE4-β-CyD (200 mM) on serum insulin glargine (A) and glucose (B) levels after subcutaneous administration of insulin glargine (2 IU/kg) to rats. Each point represents the mean ± S.E.M. of 7–11 experiments. *$p < 0.05$, compared to insulin glargine.

Table 3
In vivo pharmacodynamics parameters of insulin glargine with or without SBE4-β-CyD (200 mM).

System	T_{nadir} (h)[a]	C_{nadir} (%)[b]	AUC_G (% h)[c]
Insulin glargine	1.67 ± 0.18	47.78 ± 5.07	265.70 ± 39.3
Insulin glargine/SBE4-β-CyD	3.17 ± 0.40	31.93 ± 3.96	451.82 ± 38.5*

Each value represents the mean ± S.E.M. of 7–11 experiments.
[a] Time to nadir blood glucose concentration.
[b] Nadir blood glucose concentration.
[c] The cumulative percentage of change in serum glucose levels up to 9 h post-administration.
* $p < 0.05$, compared to insulin glargine.

blood–glucose lowering effect. We confirmed that this sustained glucose lowering effects of SBE4-β-CyD was in a dose-dependent manner (data not shown). Further, the area under serum glucose level–time curve (AUC_G) was significantly increased by the addition of SBE4-β-CyD (Table 3). These results may be contribute to: (1) the inhibitory effects of SBE4-β-CyD on the enzymatic degradation of insulin glargine (Fig. 6); (2) the enhancement of solubility and the dissolution rate of insulin glargine by SBE4-β-CyD (Figs. 3–5). To gain insight into the mechanism, further elaborate study on the adsorption of insulin glargine in the presence of SBE4-β-CyD onto subcutaneous tissue at injection site is under estimation. Furthermore, the improving effects of the other β-CyD derivatives including SBE7-β-CyD on pharmaceutical properties of insulin glargine are under investigation.

In conclusion, in the present study, we revealed that SBE4-β-CyD enhanced both bioavailability and a persistence of the blood–glucose lowering effect of insulin glargine after subcutaneous injection to rats, probably due to the inhibitory effects of SBE4-β-CyD on the enzymatic degradation at the injection site, resulting from the interaction with insulin glargine molecule. These findings indicate that SBE4-β-CyD can be a useful excipient for sustained release of insulin glargine.

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