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RESEARCH PAPER

Sugar-Branched-Cyclodextrins as Injectable Drug Carriers in Mice

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ABSTRACT

The purpose of this study was to investigate stable complexation of drug in blood by sugar-branched-β-cyclodextrins (β-CDs) such as glucose (glu)–or galactose (gal)–branched-β-CDs and the pharmacokinetic disposition of drug in sugar-branched-β-CD complex. Complexation of steroidal drugs in sugar-branched-β-CDs and their replacement by cholesterol were measured. The complexes of dexamethasone/glucosyl-β-CDs (dexamethasone/glu-β-CD or dexamethasone/glu-glu-β-CD) were not replaced by cholesterol, which is a representative endogenous compound, whereas the complex of dexamethasone/β-CD was replaced by cholesterol. The same results were obtained in steroidal drugs such as hydrocortisone, triamcinolone, and prednisolone. Thus, the use of glu-β-CD and glu-glu-β-CD permitted the stable complexation of the drug in water. Stability constants of dexamethasone/glu-glu-β-CD and dexamethasone/gal-glu-β-CD complexes are the same, which means that the sugar moiety of the side chain in β-CD has little effect on stability constants. After the dexamethasone/gal-glu-β-CD complex or the dexamethasone/glu-glu-β-CD complex (dexamethasone: 1 mg/body) was administered intravenously to mice, dexamethasone concentrations in liver tissue and blood were measured. The dexamethasone/gal-glu-β-CD complex (66.1 ± 1.7 μg as dexamethasone/gram of liver tissue) was distributed to liver tissue significantly more than the dexamethasone/glu-glu-β-CD (β-CD) complex (59.9 ± 1.0 μg as

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dexamethasone/gram of liver) at 30 min after administration (p < .05). Sugar-branched-β-CD gave a water-soluble and stable complex for dexamethasone and changed the disposition of dexamethasone. Sugar-branched-β-CDs are potentially excellent carriers for a steroidal injectable formulation.

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides known for their ability to form inclusion complexes with many lipophilic drugs, thereby changing the physicopharmaceutical properties of these drugs. Complex formation may increase aqueous solubility (1), improve stability (2), and potentiate delayed-release behavior of the drug after oral administration (3).

This ability to increase the aqueous solubility of drugs suggested the use of CDs and their derivatives in parenteral dosage forms. A pharmacokinetic analysis of dexamethasone with hydroxypropyl-β-CD (HP-β-CD) after intravenous administration to dogs showed that the area under the curve (AUC) of dexamethasone was significantly greater than for dexamethasone without HP-β-CD (4). The delivery of estradiol through biological membranes by modified β-CD was improved (5), showing that drug/CD complexes improve the delivery of drugs through biomembranes and bioavailability. β-CD was also shown to decrease local irritation caused by intramuscular injection of chlorpromazine (6,7). Decreased vitamin A toxicity was suggested by the successful treatment with an infusion of HP-β-CD of a 2-year-old boy suffering from life-threatening hypervitaminosis A (8). Finally, the solution of propanidid in aqueous HP-β-CD complex was effective as a short acting after intravenous administration (9). Thus, drug/CD complexes increase pharmacologic effectiveness while decreasing side effects.

Although much in vivo research on CDs and their derivatives has been done, few reports have been published on the use of sugar-branched-CDs as injectable drug carriers. In the present study, sugar-branched-β-CDs were investigated as potential injectable drug carriers. To speculate on the stability of a drug/sugar-branched-β-CD complex, the stability constants of drug/sugar-branched-β-CD complexes were measured, and the pharmacokinetic disposition of dexamethasone/sugar-branched-β-CD complexes was further investigated after intravenous administration to mice.

MATERIALS AND METHODS

Materials

Chemical structures of glucose (glu)– and galactose (gal)–branched-β-CDs subjected to the study are shown in Fig. 1. Gal-branched-β-CD was synthesized by an enzymatic method using *Bacillus circulans* β-galactosidase (10,11). Gal-branched-β-CD included gal-glu-β-CD, and glu-branched-β-CDs included glu-glu-β-CD and glu-β-CD.

Other reagents were analytical grade.

Complexation of Cholesterol by Sugar-Branched-β-Cyclodextrin

Cholesterol (5 mg) was accurately weighed into 10-ml liquid scintillation vials to which 5 ml of aqueous solutions containing various concentrations of β-CD or sugar-branched-β-CDs (glu-glu-β-CD and glu-β-CD) were added (0–100 mM). The solutions were stirred at 100 cycles/min for 48 hr in a water bath at 37°C ± 0.5°C. Solids in the equilibrated solution were filtered off using a 0.4-μm polycarbonate nucleopore membrane (Millipore, Japan). Formation of the complexes was determined by phase solubility diagrams. Complexed cholesterol was measured by a

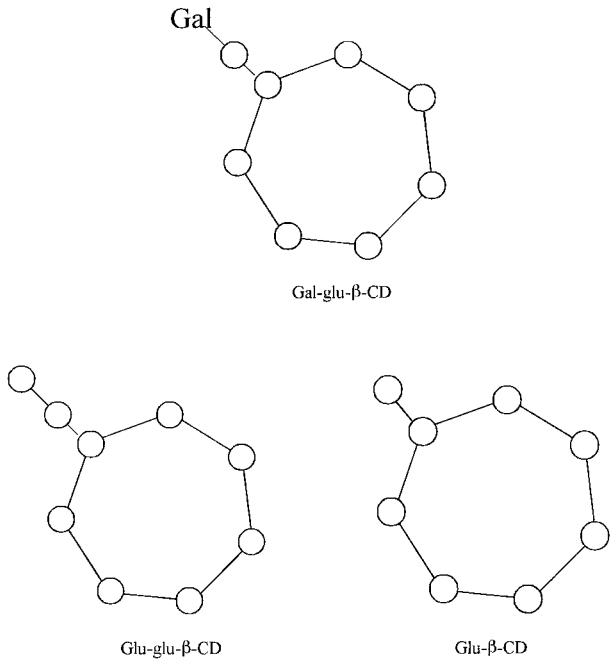

Figure 1. Chemical structures of sugar-branched-β-CDs synthesized enzymatically. Glucose (○); galactose (Gal).

commercially available test kit (Cholesterol-E-test Wako, COD-DAOS method, Wako, Japan).

Complexation of Steroidal Drugs into Sugar-Branched-β-Cyclodextrins and Their Replacement by Cholesterol

First, the effect of the concentration of β-CD or sugar-branched-β-CDs on the inclusion of steroidal drugs was investigated. The steroidal drug (dexamethasone, hydrocortisone, triamcinolone, and prednisolone) (5 mg) was weighed accurately into scintillation vials immersed in a water bath at 37°C ± 0.5°C to which 2 ml of aqueous solutions containing various concentrations of β-CD or sugar-branched-β-CDs (glu-glu-β-CD and glu-β-CD) were added (0, 2.5, 5.0, 7.5, and 10 mM), and the solutions were stirred at 100 cycles/min for 48 hr. Solids in the equilibrated solution were filtered off using a 0.4-μm polycarbonate nucleopore membrane.

Second, replacement by cholesterol of steroidal drugs in sugar-branched-β-CD complexes was investigated. β-CD and glu-β-CD (5 mM) were incubated with steroidal drugs (5 mg) at 37°C for 2 days. Cholesterol (5 mg) was then added to the solutions (2 ml), simulating normal cholesterol concentrations in human blood of 1.2–2.5 mg/ml. In another experiment, β-CD (5 mM, 2 ml of water) was incubated with cholesterol (5 mg/2 ml) at 37°C for 2 days, followed by the addition of steroidal drugs (5 mg/2 ml) to the solution.

In these experiments, the presence of steroidal drugs in samples withdrawn at fixed times was measured by high-performance liquid chromatography (HPLC) (equipment was an LC6, Shimadzu, Japan, eluent was ethanol/H_2O = 55/45; flow rate was 1.5 ml/min; detector 240 nm; column was Nucleosil C_{18} [ODS, Chemco, Japan]).

Pharmacokinetic Disposition of Dexamethasone/Sugar-Branched-β-Cyclodextrin Complexes in Mice

The concentration of dexamethasone in blood and liver tissue after intravenous administration of dexamethasone/sugar-branched-β-CD complexes to mice was measured. Dexamethasone (1 mg/ml) was incubated with gal-glu-β-CD or glu-glu-β-CD (5.0 mM) at 37°C for 2 days with continuous stirring (100 cycles/min). A dexamethasone/sugar-branched-β-CD complex solution (dexamethasone: 1 mg/body) were administered intravenously into the tail veins of mice (18–21 g, aged 4 weeks, SLC, Japan). At fixed times (10 or 30 min) after administration, mice were sacrificed by chloroform overdose, and blood and livers were removed. The blood samples, with a few drops of heparin used as an anticoagulant, were immediately placed on ice after collection. To assay dexamethasone in blood, water (0.2 ml) and internal standard in methanol (1 ml; butylparaben 0.5 μg/ml) were added to the blood sample (0.2 ml). The sample was then mixed with a vortex mixer, centrifuged (3000 rpm, 10 min), and filtered (0.5 μm). For assay of dexamethasone in liver tissue, the internal standard solution (3 ml; butylparaben 5 μg/ml) was added to a liver tissue sample. The sample was then homogenized with a homogenizer (Polytron, Kinematica AG Littau, Switzerland), centrifuged (3000 rpm, 10 min), and filtered. The concentrations of dexamethasone in the blood and liver tissue were measured by HPLC.

RESULTS AND DISCUSSION

Complexation of Steroidal Drugs in Sugar-Branched-β-Cyclodextrins and Their Replacement by Cholesterol

It was reported that cholesterol is easily complexed by β-CD (12) and by dansyl-modified β-CD (13). In addition, cholesterol is complexed by HP-β-CD in blood after intravenous administration of HP-β-CD (14). Therefore, following intravenous administration of steroidal drug/sugar-branched-CD complexes, the guest drug has to compete with cholesterol, which naturally exists in blood, and replacement of the steroidal drug in the complex by cholesterol may occur, eventually releasing the drug from the complex.

Figure 2 shows solubility diagrams of cholesterol complexed in β-CD, glu-β-CD and glu-glu-β-CD as a function of CD concentration. The solubility of cholesterol was 0.3–0.4 mM under the existence of 10 mM sugar-branched-β-CD, while cholesterol itself is insoluble in water. Under the existence of glu-β-CD or glu-glu-β-CD, the concentration of cholesterol was remarkably increased, as shown in Fig. 2, suggesting complexation of cholesterol in the sugar-branched-β-CD. The phase solubility diagrams (i.e., plots of guest solubility as a function of the CDs concentration) are generally classified as either type A (a freely soluble complex is formed) or type B (a complex with definite solubility is formed). The B type includes the B_I type and the Bs type. In Fig. 2, adding 50 mM glu-β-CD, the solubility of cholesterol in the solution reached a peak and declined, indicating that glu-β-CD complexed all solid-state cholesterol. At glu-β-CD concentrations greater than 50 mM, dissolved cholesterol reached a peak and then decreased gradually, suggesting glu-β-CD complexed liquid-state cholesterol,

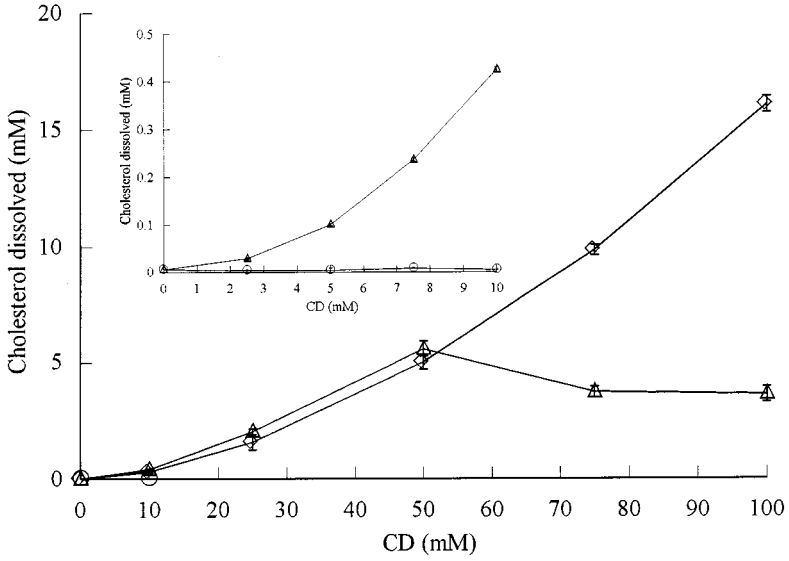

Figure 2. Solubility diagrams of cholesterol/β-CD and cholesterol/sugar-branched-β-CD complexes. Cholesterol/β-CD complex (○); cholesterol/glu-β-CD complex (△); cholesterol/glu-glu-β-CD complex (◇). Each point and vertical bar represent the mean ± SD (n = 4).

presumably followed by microcrystalline precipitation of the complex, as reported by Uekama, Hirayama, and Irie (15).

Figure 3 shows a solubility diagram of dexamethasone/β-CD complex, dexamethasone/glu-β-CD, and dexamethasone/glu-glu-β-CD complexes, in the presence and absence of cholesterol, as a function of CD concentration. No reduction of dexamethasone solubility in either the dexamethasone/glu-β-CD complex or the dexamethasone/glu-glu-β-CD complex was observed in the presence of 2.5 mg/ml of cholesterol. In contrast, solubility was reduced for the dexamethasone/β-CD complex in the presence of cholesterol, which strongly suggests replacement of the guest compound, dexamethasone, in the presence of cholesterol. The cholesterol itself is almost insoluble in water; therefore, we could not determine stability constants of complexes containing cholesterol. The results shown in Fig. 3, however, indicate that cholesterol competes with dexamethasone in the complex formation, but not with sugar-branched-β-CDs, probably caused by steric hindrance between cholesterol and sugar moieties of the sugar-branched-β-CD.

Figures 4A–C show solubility diagrams for other steroidal drugs (e.g., hydrocortisone, triamcinolone, and prednisolone). The solubility of hydrocortisone leveled off for hydrocortisone/β-CD complex in the presence of cholesterol at the concentration of more than 5 mM β-

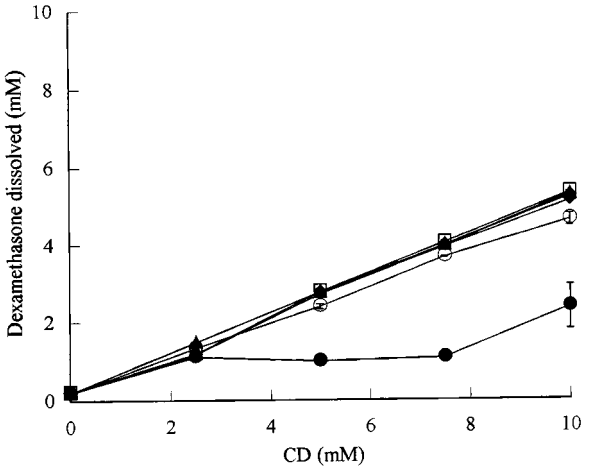

Figure 3. Solubility diagrams of dexamethasone/sugar-branched-β-CD complexes and of dexamethasone/sugar-branched-β-CD complexes in the presence of cholesterol as a function of CD concentration. Dexamethasone/gal-glu-β-CD complex (□); dexamethasone/β-CD complex (○); dexamethasone/glu-β-CD complex (△); dexamethasone/glu-glu-β-CD complex (◇); dexamethasone-β-CD complex in the presence of cholesterol (●); dexamethasone/glu-β-CD complex in the presence of cholestersol (▲); dexamethasone/glu-glu-β-CD complex in the presence of cholesterol (◆). Each point and vertical bar represent the mean ± SD (n = 4).

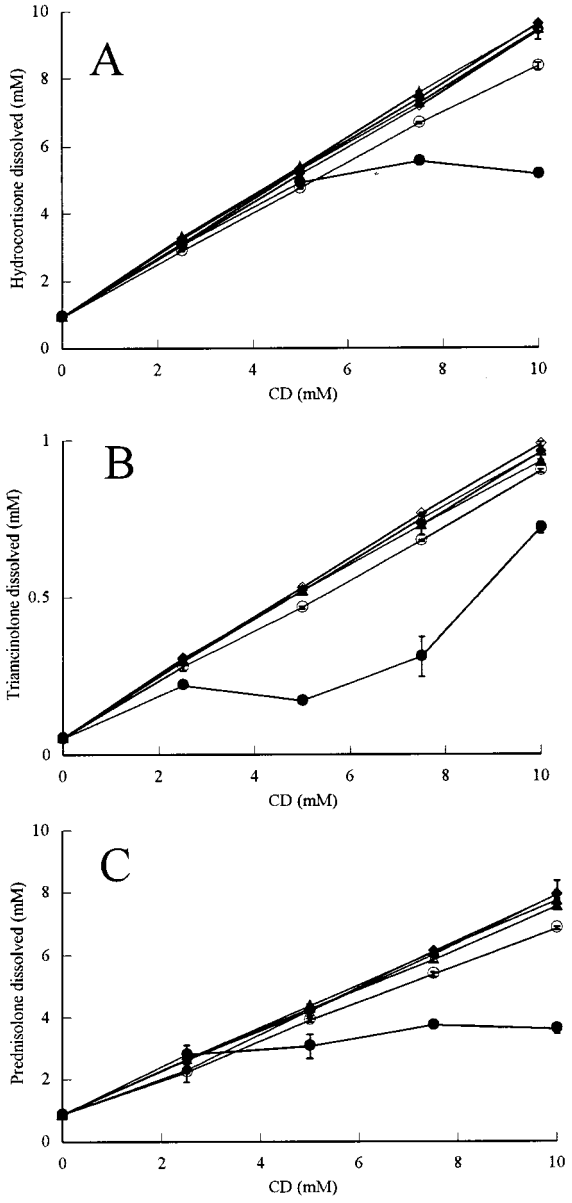

Figure 4. Solubility diagrams of steroidal drug (hydrocortisone (A), triamcinolone (B), prednisolone (C))/sugar-branched-β-CD complexes and of steroidal drug/sugar-branched-β-CD complexes in the presence of cholesterol as a function of CD concentration. Steroidal drug/β-CD complex (○); steroidal drug/glu-β-CD complex (△); steroidal drug/glu-glu-β-CD complex (◇); steroidal drug/β-CD complex in the presence of cholesterol (●); steroidal drug/glu-β-CD complex in the presence of cholesterol (▲); steroidal drug/glu-glu-β-CD complex in the presence of cholesterol (◆). Each point and vertical bar represent the mean ± SD (n = 4).

CD (Fig. 4A). The solubility of triamcinolone or prednisolone/β-CD complex also leveled off at the beginning of 2.5 mM. Solubility of triamcinolone in triamcinolone/β-CD complex increased at the beginning of 5.0 mM (Figs. 4B and 4C). Dissolution amounts of hydrocortisone, triamcinolone, and prednisolone in either the glu-β-CD or the glu-glu-β-CD complexes were linearly increased with an increase of CD concentration, and no reversed effect was observed in the presence of 2.5 mg/ml of cholesterol (Figs. 4A–4C). Under the existence of cholesterol, solubility diagrams have a similar pattern for hydrocortisone-β-CD and prednisolone-β-CD complexes, while the solubility of triamcinolone increased with an increase of β-CD concentration after a solubility decrease. This is probably because cholesterol forms the Bs type of complex (15).With the increase of β-CD, triamcinolone continues to form a soluble complex with β-CD, but the solubility of cholesterol-β-CD reaches a maximum and levels off due to its lower aqueous solubility.

Figure 5 shows solubility diagrams of the dexamethasone/β-CD and the dexamethasone/glu-β-CD complexes in the presence of a cholesterol and cholesterol/β-CD as a function of time. The amounts of dexamethasone dissolved for the dexamethasone/glu-β-CD in the absence of cholesterol and the dexamethasone/

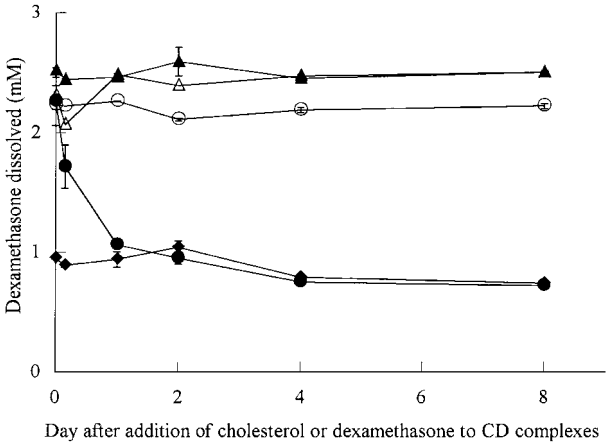

Figure 5. Solubility diagrams of dexamethasone/sugar-branched-β-CD complexes in the presence of cholesterol and of cholesterol/sugar-branched-β-CD complexes in the presence of dexamethasone as a function of time. Dexamethasone/glu-β-CD complex (△); dexamethasone/glu-β-CD complex in the presence of cholesterol (▲); dexamethasone/β-CD complex (○); dexamethasone/β-CD complex in the presence of cholesterol (●); cholesterol/β-CD complex in the presence of dexamethasone (◆). Each point and vertical bar represent the mean ± SD (n = 4).

glu-β-CD complexes in the presence of cholesterol remained constant. These results suggest that no replacement by cholesterol occurred. In contrast, replacement did occur when cholesterol was added to the dexamethasone/β-CD complex solution. It took 1 day to reach plateaus of solubility for dexamethasone/glu-β-CD complex and cholesterol/β-CD complex in the presence of cholesterol and dexamethasone, respectively. After 1 day, the drug solubility of these complexes reached a plateau (Fig. 5). The time to reach equilibrium was 1–2 days in this study. This was consistent with results of a previous study (16).

The above results strongly support an assumption that introduction of glu-glu- or glu- side chains to β-CD gave a very water soluble and stable complex for dexamethasone. Figures 4A–4C support the above assumption for other steroidal drugs. This suggests sugar-branched-β-CDs are potentially excellent carriers for a steroidal injectable formulation.

Complexation of Steroidal Drugs by Gal- or Glu-Branched-β-Cyclodextrins

The apparent stability constant of the complex K was calculated according to Fig. 3 as follows: K = slope/intercept/(1 − slope), where slope is a slope of the phase solubility diagram, and intercept is the solubility of a drug (17).

The results showed no significant differences in apparent stability constants among the dexamethasone/branched-β-CD complexes listed in Table 1. The stability constant of the dexamethasone/gal-glu-β-CD complex (5410/M) was almost the same as that of the dexamethasone/glu-glu-β-CD complex (5130/M). Similar stability constants were obtained for β-CD, glu-β-CD, and glu-glu-β-CD in dexamethasone, hydrocortisone, triamcinolone, and prednisolone. In addition, there was no significant stability constant difference between sugars, gal or glu, which occupied the end position of branched CDs, as measured by each stability constant.

Pharmacokinetic Disposition of Dexamethasone/Sugar-Branched-β-Cyclodextrin Complexes in Mice

The concentrations of dexamethasone in blood and liver after intravenous administration of either a dexamethasone/gal-glu-β-CD complex or a dexamethasone/glu-glu-β-CD complex to mice were measured (Figs. 6A and 6 B). Despite having nearly equal complex stability constants (Table 1), dexamethasone given as the dexamethasone/gal-glu-β-CD complex decreased more rapidly in blood than the dexamethasone/glu-glu-β-CD complex when measured at 10 and 30 min after administration (Fig. 6A). Dexamethasone concentration in liver tissue was 15–29 times higher than that in blood for both a gal-glu-β-CD and a glu-glu-β-CD complexes (Figs. 6A and 6B). At 10 min after administration, no significant difference in dexamethasone level was observed in liver tissue regardless of whether the gal-glu-β-CD complex or the glu-glu-β-CD complex was the carrier. However, at 30 min, dexamethasone levels from the dexamethasone/gal-glu-β-CD complex was significantly higher than that in the dexamethasone/glu-glu-β-CD complex in liver tissue ($p < .05$, Fig. 6B). Frijlink and coworkers showed that flurbiprofen concentrations in liver, brain, kidney, and spleen tissues at 10 min after intravenous administration increased significantly when coadministered with a HP-β-CD complex compared with no HP-β-CD coadministration in rats (18). This result supports an assumption that the drug was not completely released from the drug/CD complex by 10–30 min after intravenous administration. Calculation of the ratio of free dexamethasone in liver tissue versus in blood showed that dexamethasone released from a dexamethasone/gal-glu-β-CD complex was higher, but not

Table 1

Stability Constants of Steroidal Drug/Sugar-Branched-β-CD and Steroidal Drug/β-CD Complexes

	Stability Constant (M^{-1})			
	Dexamethasone	Hydrocortisone	Triamcinolone	Prednisolone
Gal-glu-β-CD	5410			
Glu-glu-β-CD	5130	5620	1640	2890
Glu-β-CD	4920	5610	1580	2160
β-CD	4030	2910	1570	1920

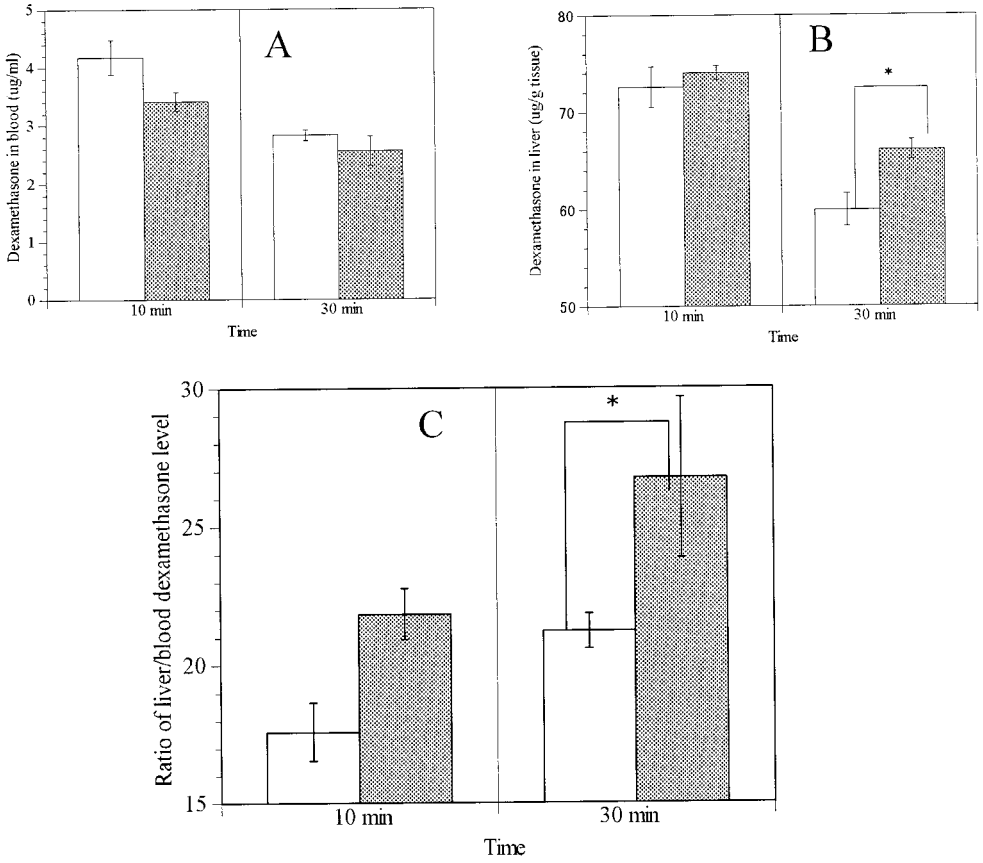

Figure 6. Dexamethasone levels (in blood (A), in liver (B), Ratio of liver versus blood (C)) at 10 and 30 min after intravenous administration of dexamethasone/sugar-branched-β-CD complexes (dexamethasone: 1 mg/body) in mice. Dexamethasone/glu-glu-β-CD complex (□); dexamethasone/gal-glu-β-CD complex (▨). Each column and horizontal bar represent the mean ± SD. Significant difference between glu-glu-β-CD complex and gal-glu-β-CD complex at $p < 0.05$ (*).

significantly higher, than from a dexamethasone/glu-glu-β-CD complex at 10 min after administration. However, the dexamethasone/gal-glu-β-CD complex showed a significantly higher free dexamethasone ratio than that from the glu-glu-β-CD complex at 30 min after administration ($p < .05$, Fig. 6C). These results suggest that dexamethasone was not easily replaced by cholesterol in blood and stayed within the sugar-branched-CD complex, and the gal-branched-CD complex was likely to have a specific interaction with hepatocytes.

Efficient liver targeting of drugs requires both ''active targeting'' and ''passive targeting.'' Active targeting means increasing the specific affinity of a molecule for its target tissues for liver (hepatocytes), while passive targeting means reducing specific interactions with nontarget tissues. A previous in vitro study demonstrated that gal-branched-CDs specifically interact with hepatocytes via the asialoglycoprotein receptor, demonstrating active targeting (19). The pharmacokinetics of β-CD and HP-β-CD in rats after intravenous administration showed that a large percentage of the β-CD and HP-β-CD doses were eliminated through the kidney (20), indicating CDs and their derivatives escaped hepatic elimination. CDs and their derivatives did not have affinity for kidney tissue, but did for liver tissue, probably due to the passive targeting mechanism. These observations suggest that the gal-branched-β-CD can possibly have both active and passive targeting characteristics for liver, which further studies should clarify.

CONCLUSIONS

Dexamethasone in glu-glu-β-CD or glu-β-CD complexes as a guest molecule was not replaced by choles-

terol in aqueous solution. Sugar-branched-β-CDs can be used for an injectable carrier of low-solubility steroidal drugs. The amount of a dexamethasone/gal-glu-β-CD complex was distributed significantly higher in liver tissue than a dexamethasone/glu-glu-β-CD complex in mice. Interaction of galactose on the side chain of β-CD with asialoglycoprotein receptor on hepatocytes was suggested.

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REFERENCES

1. F. Veiga, J. J. C. Teixeira-Dias, F. Kedzierewicz, A. Sousa, and P. Maincent, Int. J. Pharm., 129, 63 (1996).
2. D. Duchene, B. Debrueres, and C. Vaution, Sci. Tech. Prat. Pharma., 1, 37 (1985).
3. K. Uekama, T. Horikawa, Y. Horiuchi, and F. Hirayama, J. Controlled Release, 25, 99 (1993).
4. K. Dietzel, M. E. Brewster, N. S. Bodor, and H. Derendorf, Int. J. Pharm., 59, 225 (1990).
5. M. E. Brewster, K. S. Estes, T. Loftsson, R. Perchalski, H. Derendorf, G. Mullersman, and N. Bodor, J. Pharm. Sci., 77, 981 (1988).
6. T. Irie, S. Kuwahara, M. Otagiri, K. Uekama, and T. Iwamasa, J. Pharmacobiodyn., 6, 790 (1983).
7. T. Irie, M. Otagiri, K. Uekama, Y. Okano, and T. Miyata, J. Incl. Phenom., 2, 637 (1984).
8. T. O. Carpentar, J. M. Pettifor, R. M. Russel, J. Pitha, S. Mobarhan, M. S. Ossip, S. Wainer, and C. S. Anast, J. Pediatr., 111, 507 (1987).
9. C. R. Mackenzie, J. P. Fawcett, D. W. Boulton, and I. G. Tucker, Int. J. Pharm., 159, 191 (1997).
10. S. Kitahata, K. Fujita, Y. Takagi, K. Hara, H. Hashimoto, T. Tanimoto, and K. Koijumi, Biosci. Biotech. Biochem., 56, 242 (1992).
11. K. Koizumi, T. Tanimoto, K. Fujita, K. Hara, N. Kuwahara, and S. Kitahata, Carbohydr. Res., 238, 75 (1993).
12. L. Fang-yu, O. K. Dane, and K. M. Ashim, Pharm. Res., 7, 869 (1990).
13. A. Ueno, S. Minato, I. Suzuki, M. Fukushima, M. Ohkubo, T. Osa, F. Hamada, and K. Murai, Chem. Lett., 605 (1990).
14. H. W. Frijlink, A. C. Eissens, N. R. Hefting, K. Poelstra, C. F. Lerk, and D. K. F. Meijer, Pharm. Res., 8, 9 (1991).
15. K. Uekama, F. Hirayama, and T. Irie, Chem. Rev., 98, 2045 (1998).
16. A. Yoshida, M. Yamamoto, F. Hirayama, and K. Uekama, Chem. Pharm. Bull., 36, 4075 (1988).
17. T. Higuchi and A. Connorsk, Adv. Anal. Chem. Instrum., 4, 117 (1965).
18. H. W. Frijlink, E. J. F. Franssen, A. C. Eissens, R. Oosting, C. F. Lerk, and D. K. F. Meijer, Pharm. Res., 8, 380 (1991).
19. T. Shinoda, A. Maeda, S. Kagatani, Y. Konno, T. Sonobe, M. Fukui, H. Hashimoto, K. Hara, and K. Fujita, Int. J. Pharm., 167, 147 (1998).
20. H. W. Frijlink, J. Visser, N. R. Hefting, R. Oosting, D. K. F. Meijer, and C. F. Lerk, Pharm. Res., 7, 1248 (1990).