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Recent Aspects of Pharmaceutical Application of Cyclodextrins

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Abstract

Because of the multi-functional characteristics and bioadaptability, cyclodextrin (CyD) is capable of alleviating the undesirable properties of drug molecules through the formation of inclusion complexes. This paper outlines the current application of natural and chemically modified CyDs in the various pharmaceutical formulations including peptide and protein drugs. Furthermore, potential use of CyD/drug conjugates in site-specific drug delivery is discussed.

Introduction

Recently, various kinds of cyclodextrin (CyD) derivatives such as hydrophilic, hydrophobic and ionic derivatives have been successfully utilized to extend physicochemical properties and inclusion capacity of natural CyD [1–6]. The desirable attribute for the drug carrier is the ability to control the rate and/or time profile of drug release [7–9]. Hydrophilic CyDs can modify the rate of drug release, which can be used for the enhancement of drug absorption across biological barriers, serving a potent drug carrier in the immediate release formulations. Amorphous CyDs such as 2-hydroxypropyl-β-CyD (HP-β-CyD) is useful for inhibition of polymorphic transition and crystallization rates of poorly water-soluble drugs during storage, which can consequently maintain the higher dissolution characteristics and oral bioavailability of the drugs [10]. On the other hand, hydrophobic CyDs may serve as sustained release carriers for the water-soluble drugs including peptide and protein drugs [8, 11, 12]. The delayed release formulation can be obtained by the use of enteric type CyDs such as *O*-carboxymethyl-*O*-ethyl-β-CyD [13]. A combined use of different CyDs and/or pharmaceutical additives will provide more balanced oral bioavailability with prolonged therapeutic effects [14]. The most desirable attribute for the drug carrier is its ability to deliver a drug to targeted site. The CyD/drug conjugate can survive passage through stomach and small intestine, but the drug release will be triggered by enzymatic degradation of CyD ring in colon [15]. Such CyD conjugate can be a versatile means of constructing a new class of colon-targeting prodrug. Moreover, CyD/cationic polymer conjugates may be novel candidates for non-viral vectors to enhance the gene transfer of plasmid DNA [16]. On the basis of the above-mentioned knowledge, the advantages and limitations of CyDs in the design of advanced dosage forms will be discussed.

Improvement of oral bioavailability of poorly water-soluble drugs by CyD complexation

Itraconazole, an orally active triazole antifungal agent, is particularly insoluble in water at physiological pH conditions and soluble only slightly under extremely acidic media. Recently, HP-β-CyD based aqueous formulation of itraconazole was developed to improve the oral bioavailability of the solid dosage form of itraconazole. Figure 1 illustrates the proposed inclusion mode of itraconazole/HP-β-CyD complex in 1 : 2 molar ratio [17]. Cyclosporin A (CsA) and tacrolimus, immunosupresive drugs, are poorly water-soluble cyclic undecapeptide and hydrophobic macrolide lactone, respectively. These drugs are known to exhibit a low oral bioavailability and a wide range of variability in absorption. Hydrophilic CyDs such as 2,6-di-*O*-methyl-CyD (DM-CyD) and HP-CyDs significantly improved the solubility and oral bioavailability of these drugs, with the low variability in in-vivo absorption in rats [17–19]. DM-β-CyD exhibited insignificant irritation to intestinal mucosa of rats. On the other hand, the per-*O*-acetylated CyDs prolonged both plasma and lymph CsA levels. These results suggest that the relatively hydrophobic CyDs will be useful for design of the CyD-based sustained-release formulation of poorly water-soluble drugs. Interestingly, sulfobutyl ether β-CyD (SB7-β-CyD) is particularly effective to improve both the solubility and chemical stability of ON*O*-4819, prostaglandin E_1 analogue, among the hydrophilic CyDs [20].

In the case of tacrolimus-DM-β-CyD system, nonlinear pharmacokinetic behavior of the drug after oral administration was observed [21]. To gain insight into this anomalous enhancing mechanism, the effects of DM-β-

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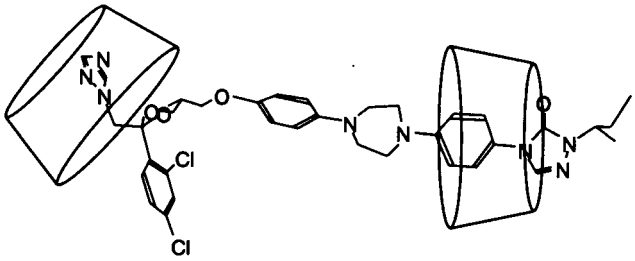

Figure 1. Proposed inclusion mode of itraconazole/HP-β-CyD complex in 1 : 2 molar ratio.

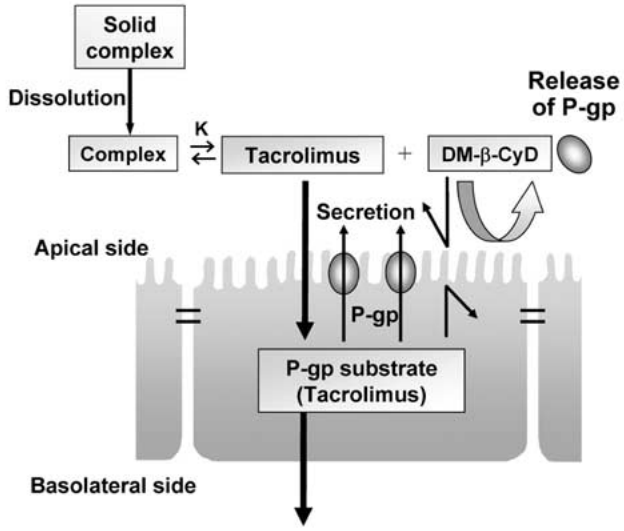

Figure 2. Possible enhancing mechanism of DM-β-CyD on oral bioavailability of tacrolimus P-gp substrate.

CyD on the efflux of tacrolimus and rhodamine 123, typical P-glycoprotein (P-gp) substrates, were examined using both Caco-2 and vinblastine-resistant Caco-2 (Caco-2R) cell monolayers. Pretreatment of the apical membranes of the monolayers with DM-β-CyD decreased the efflux of tacrolimus and rhodamine 123 without an associated cytotoxicity. DM-β-CyD decreased the P-gp level in the apical membranes of both cell monolayers, probably by allowing release of P-gp from the apical membrane into the transport buffer. DM-β-CyD, however, did not decrease the MDR1 gene expression in Caco-2 or Caco-2R cells. These results suggest that the enhancing effect of DM-β-CyD on the oral bioavailability of tacrolimus is due not only to its solubilizing effect but also, at least in part, to its inhibitory effect on the P-gp-mediated efflux of tacrolimus from intestinal epithelial cells, as shown in Figure 2.

Control of polymorphic transition and crystallization of drugs by amorphous CyDs

Crystal modifications significantly affect various pharmaceutical properties such as solubility, dissolution rate, stability and bioavailability of drugs. As a consequence, the rational control of crystal growth, habit and polymorphic transition, using pharmaceutical additives, becomes an attractive and interesting area of drug research and development. Many reports have shown that crystalline drugs such as nifedipine [22] and chloramphenicol palmitate [23] can be converted to an amorphous form by complexation with amorphous HP-β-CyD. Therefore, the effects of aging on the crystallization, dissolution and absorption of tolbutamide, oral hypoglycemic agent, from its HP-β-CyD complex were investigated, in comparison with those of polyvinylpyrrolidone (PVP) solid dispersion [10, 24]. All amorphous powders were prepared by a spray-drying method. During storage at 60 °C and 75% R.H., a stable form of tolbutamide (form I) was crystallized from the amorphous PVP dispersion, whereas a metastable form of tolbutamide (form II) was crystallized from the HP-β-CyD complex (Figure 3). The dissolution rate of tolbutamide from both HP-β-CyD complex and PVP dispersion was significantly faster than that of drug alone. However, the dissolution rate of the drug from the PVP dispersion markedly decreased with storage, because of the formation of slow dissolving form I crystals. On the other hand, the dissolution rate from the HP-β-CyD complex was only slightly decreased due to the formation of fast dissolving form II crystals. These in-vitro dissolution characteristics were clearly reflected in the in-vivo absorption of tolbutamide and the glucose plasma level after oral administration in dogs. The results suggested that HP-β-CyD is useful not only for converting crystalline tolbutamide to an amorphous substance, but also for maintaining the fast dissolution rate of the drug over a long period. Furthermore, the crystallization of drugs from CyD complexes, with storage, seemed to be different from that involving polymer excipients such as PVP.

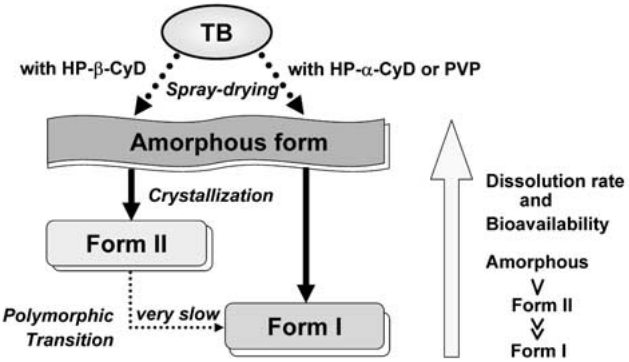

Figure 3. Proposed scheme for crystallization and polymorphic transition of spray-dried TB in HP-CyDs and PVP matrix.

Use of CyDs in peptide and protein formulation

The propensity of polypeptide and protein drugs to form reversible and irreversible aggregates in solution is of great concern as it may lead to the loss of biological potency, immunogenic reactions, unacceptable physical appearance in long-term therapeutic system. To overcome these drawbacks, several approaches have been proposed, including the use of amphiphatic excipients, chemical modification and site-directed mutation. We have recently reported the effects of hydrophilic β-CyDs on the aggregation of bovine

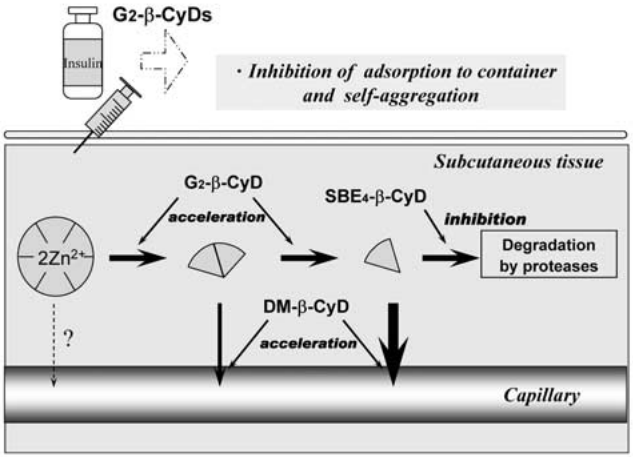

Figure 4. Preferable use of CyDs in insulin injection.

insulin in aqueous solution and its adsorption onto hydrophobic surfaces. Among the CyDs tested, maltosyl-β-CyD (G2-β-CyD), significantly inhibited insulin aggregation in neutral solution and its adsorption onto the surface of glass and polypropylene tubes by interacting with hydrophobic regions of the peptide in both concentration and time-dependent manners, whereas DM-β-CyD had only a moderate effect on the aggregation [8, 25]. In addition, SB4-β-CyD and SB7-β-CyD showed different effects on insulin aggregation, depending on the degree of substitution of sulfobutyl group. This indicates that sulfoalkyl ethers of CyDs may be a new class of parenteral drug carriers because they are highly hydrophilic and less hemolytic than parent and other hydrophilic CyDs. Then, we mainly focused on the in-vivo absorption of insulin after subcutaneous injection of insulin in the absence and presence of some hydrophilic β-CyDs [26]. When insulin solutions containing SB4-β-CyD were injected into the dorsal subcutaneous tissues of rats, the plasma immunoreactive insulin (IRI) level rapidly increased and maintained higher IRI levels for at least 8h. The bioavailability of insulin/SB4-β-CyD system was about twice that of insulin alone and approached 96%. The enhancing effects of SB4-β-CyD may be in part due to the inhibitory effect of SB4-β-CyD on the enzymatic degradation and/or the adsorption of insulin onto the subcutaneous tissue at the injection site, although this does not apparently facilitate capillary permeability. These results suggest that the hydrophilic CyDs are useful for improving the pharmaceutical properties of insulin injection, as illustrated in Figure 4.

Site specific drug delivery by CyD conjugates

CyD complex is in equilibrium with guest and host molecules in aqueous solution, with the degree of the dissociation being dependent on the magnitude of the stability constant of the complex. This property is desirable because the complex dissociates to give free CyD and drug at the absorption site, and thus only the drug in a free form enters into the systemic circulation. However, the inclusion equilibrium is sometimes disadvantageous when drug targeting is to be attempted, because the complex dissociates before it reaches the organ or tissues to which it is to be delivered. One of the methods to prevent the dissociation is to bind a drug covalently to CyD.

CyD-based colon specific delivery

CyDs are known to be barely capable of being hydrolyzed and only slightly absorbed in passage through the stomach and small intestine; however, they are fermented to small saccharides by colonic microflora. Such biological property of CyDs is useful as a source of site-specific delivery of drugs to colon and as a pro-moiety for reducing an adverse effect. In our previous studies, anti-inflammatory drugs such as biphenylylacetic acid (BPAA) and prednisolone (PD) were selectively conjugated onto the primary and secondary hydroxyl groups of CyDs through an ester-linkage, respectively, and their physicochemical properties and drug release behavior in various solutions were investigated [27]. The anti-inflammatory effect of BPAA system was evaluated using the model of carrageenan-induced acute edema in rat paw. In the case of BPAA/β-CyD complex, a rapid anti-inflammatory response was observed, compared to drug alone, because the drug was mainly absorbed from the small intestine after a fast dissolution of the complex. In sharp contrast, the BPAA/γ-CyD conjugate needed a fairly long lag time to exhibit the drug activity, because BPAA was produced after it had reached the cecum and colon. This indicates that BPAA could be released after the ring opening of CyD followed by the ester hydrolysis, and BPAA activation will take place site-specifically in the cecum and colon (Figure 5). Similarly, the anti-inflammatory effect and systemic adverse effect of the PD succinate (PDsuc)/α-CyD ester conjugate were studied using inflammatory bowel disease (IBD) model rats prepared by administration of 2,4,6-trinitrobenzenesulfonic acid [28]. Following oral administration, the anti-inflammatory effect of the PD succinate PDsuc/α-CyD ester conjugate (Figure 6) was comparable to that of PD alone. However, the conjugate exhibited the low systemic adverse effect because of low plasma level of PD. These results suggest that PDsuc/CyD conjugate may be useful as novel IBD therapeutic prodrug for colon-specific delivery owing to alleviation of the systemic side effect while maintaining the anti-inflammatory effect [29, 30].

Application of CyD in gene therapy

There are two categories of gene therapy vectors, i.e. viral vectors and nonviral vectors. The nonviral vectors have many advantages over viral vectors, such as easy to manufacture, safety, low immunogenicity, and molecular attachment of targeting ligand [31]. However, the problem is that the efficiency of nonviral vector-mediated gene transfer to cell is markedly low, compared to the viral vectors. To improve the transfection efficacy of nonviral vector, we

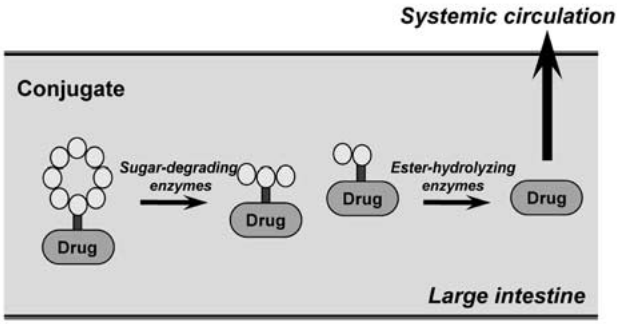

Figure 5. Proposed scheme of drug release in large intestine after oral administration of CyD/drug conjugate.

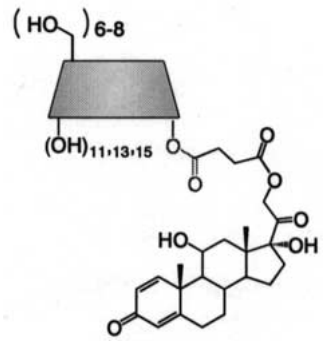

Figure 6. PDsuc/CyD conjugate.

synthesized the starburst polyamidoamine dendrimer (generation 2: G2) conjugates with α-, β-, and γ-CyDs (CDE conjugates), expecting the synergistic effect of dendrimer and CyDs [16]. The ^{1}H NMR spectroscopic data indicated that CyDs are covalently bound to dendrimer in a molar ratio of 1 : 1, as shown in Figure 7. The agarose gel electrophoretic studies revealed that CDE conjugates formed the complexes with plasmid DNA (pDNA) and protected the degradation of pDNA by DNase I in the same manner as dendrimer. The CDE conjugates showed a potent luciferase gene expression, especially in the dendrimer conjugate with α-CyD (α-CDE conjugate) which provided the greatest transfection activity (approximately 100 times higher than those of dendrimer alone and of the physical mixture of dendrimer and α-CyD) in NIH3T3 and RAW264.7 cells. Then, the gene transfection activities of three α-CyD conjugates with dendrimers of different generations (G2, G3, G4) in NIH3T3 cells were compared. The activity and stabilizing effect on the DNase I-catalyzed degradation of pDNA increased in the order of G3 > G4 > G2 conjugates, while the cytotoxicity decreased in the G4 $\approx$ G3 > G2 conjugates. In addition, the gene transfer activity of α-CDE conjugate of G3 was superior to that of Lipofectin™ and TransFast™, as shown in Figure 8. The enhanced gene transfer effect of α-CDE conjugate may be attributable to not only increasing the cellular association, but also changing the intracellular trafficking of pDNA. These findings clearly suggest that α-CDE conjugate of G3 could be a new preferable nonviral vector of pDNA. To achieve the cell specific gene transfer, we have recently designed the mannosylated α-CyD with the dendrimer as the targetable ligand to cells which express the mannose-

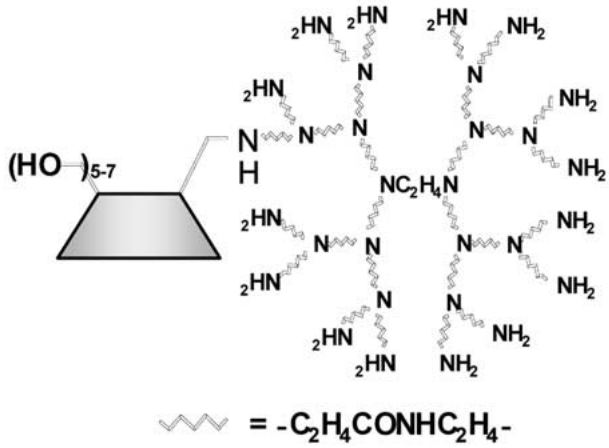

Figure 7. Non-viral vector based on polyamidoamine dendrimer (G2)/CyD conjugates for DNA delivery.

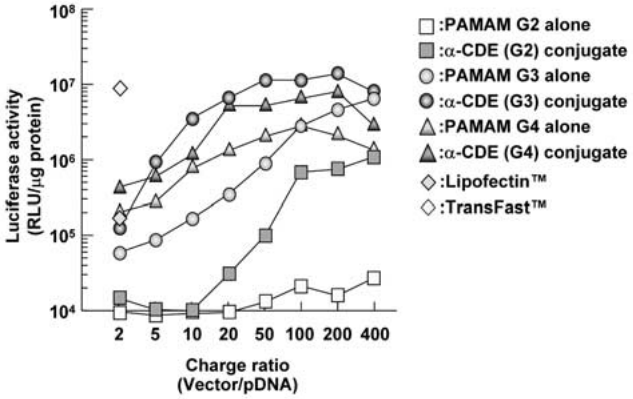

Figure 8. Transfection efficiencies of dendrimers (G2, G3, G4) and α-CDE (G2, G3, G4) conjugates for NIH3T3 cells, comparing with commercial non-viral vectors.

receptor such as macrophages, dendritic cells and epithelial cells, which will be reported elsewhere.

Perspective

A number of CyD derivatives, CyD polymers and CyD conjugates have been designed and evaluated for pharmaceutical uses [32]. The modification of CyD molecules has proceeded and become more routine. In addition, the preferable combination of CyDs and other pharmaceutical excipients or carriers such as hydrophilic polymers, nanoparticles and liposomes should foster the progress of the advanced dosage forms. Fortunately some hydrophilic β-CyDs have opened the door in practical use in the pharmaceutical formulations. Owing to the increasingly globalized nature of the CyD-related science and technology, development of the CyD-based drug formulation is also rapidly progressing. We are looking forward to seeing numerous pharmaceutical products containing CyDs in the near future.

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