Current Topics

Cutting-Edge Science of Cyclodextrin

Review

Potential Use of Cyclodextrins as Drug Carriers and Active Pharmaceutical Ingredients

Hidetoshi Arima,*a,b Keiichi Motoyama, ab and Taishi Higashiab

*Graduate School of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Chuo-ku, Kumamoto 862–0973, Japan; a Program for Leading Graduate Schools “HIGO (Health life science: Interdisciplinary and Global Oriented) Program,” Kumamoto University; 5–1 Oe-honmachi, Chuo-ku, Kumamoto 862–0973, Japan.

Received September 29, 2016

Cyclodextrins (CyDs) are extensively used in various fields, and especially have been widely utilized as pharmaceutical excipients and drug carriers in the pharmaceutical field. Owing to the multi-functional and biocompatible characteristics, CyDs can improve the undesirable properties of drug molecules. This review outlines the current application of CyDs in pharmaceutical formulations, focusing on their use as CyD-based drug carriers for several kinds of drugs. Additionally, CyDs have great potential as active pharmaceutical ingredients against various diseases with few side effects.

Key words cycloextrin (CyD); drug delivery system (DDS); active pharmaceutical ingredient; non-viral vector; supramolecule

1. Introduction

Cyclodextrins (CyDs) are cyclic oligosaccharides that possess a hydrophobic central cavity and hydrophilic outer surface.1) CyDs have been used to improve the unfavorable pharmaceutical properties of low molecular weight drugs.2) Numerous hydrophilic, hydrophobic and ionizable CyD derivatives have been developed.3) Among these CyDs, hydrophilic CyD derivatives have been widely used in the pharmaceutical field.4) These CyDs are highly water-soluble, and improve physicochemical and biopharmaceutical properties of several types of drugs.5) In addition, CyD conjugates with drugs and polymers,6) CyDs with targeting ligands,7) CyD polymers,8) CyD nanosponges,9) CyD nanogels9) and CyD supramolecules10–12) including (poly)pseudorotaxanes,13) (poly)rotaxanes14) and catenane15) have been developed to enlarge their functions for drug delivery system (DDS). Meanwhile, CyDs form inclusion complexes with not only drugs but also biomembrane components, resulting in hemolysis,16) lipid rafts malfunction17) and some pharmacological activity.18) Using these properties of CyDs, the potential of CyDs as active pharmaceutical ingredients (APIs) has been testified.19) In this review, the most recent applications of CyDs as drug carriers and APIs will be introduced.

2. CyD-Based Carriers for Prostaglandins, Anticancer Drugs and Fullerene C60

2.1. Prostaglandins

As described above, CyDs ameliorate various undesirable characteristics of low molecular weight drugs, such as water insolubility, low dissolution rate, poor bioavailability, lability, bitter taste and odor, and so on. Prostaglandins (PGs) are essentially long-chain unsaturated fatty acids containing a substituted cyclopentane ring system. The β-hydroxyketo moiety of E-type prostaglandins (PGEs) is extremely susceptible to dehydration under acidic or alkaline conditions to give A-type prostaglandins (PGAs), which are isomerized subsequently to form B-type prostaglandins (PGBs) under alkaline conditions. The biological activities of PGEs decrease with progress of these reactions. The chemical instability and low aqueous solubility of PGEs have limited dosage form design and presented a substantial challenge to pharmaceutical scientists. CyDs have successfully been applied to PGEs, and stable and soluble complexes were first marked in Japan as a PGE2-β-CyD tablet and PGE1-α-CyD injection.20,21) In aqueous solution, however, attempts to stabilize PGEs were rather disappointing because of the positive catalytic effect of natural CyDs.22)

Limaprost, a PGE1 derivative, is used for the treatment of various ischemic symptoms. It is a commercially available tablet containing the α-CyD complex, namely limaprost alfaladex.23,24) The tablet form ensures its dose uniformity and increases the aqueous solubility of limaprost. Limaprost alfaladex tablet (Opalmon) is chemically stable for 3 or more years under airtight conditions. However, once the package is opened the drug degrades relatively rapidly.24) Therefore, it is vital to warrant its stability under humid conditions, particularly in the case of single-dose packages from which tablets are removed and placed in a non-waterproof paper bag. Thus far, Inoue et al.23) reported that the stability of limaprost alfaladex including dextran was increased by addition of β-CyD. Also, the stability of limaprost alfaladex in the dextran/β-CyD composite was further increased by addition of β-CyD as an external excipient in tablets.25) The stabilization of limaprost
by both $\alpha$- and $\beta$-CyDs could be ascribed to a formation of a ternary inclusion complex\(^{26}\) (Fig. 1). Namely, the interaction between limaprost and the two CyDs was protected by inclusion of different moieties of limaprost: $\alpha$-CyD predominantly included the alkyl $\omega$-chain, whereas $\beta$-CyD included the five-membered ring.\(^{26}\) Henceforth, it is expected that use of ternary complexes of drugs with two types of CyDs will present a new trends in stabilizing and solubilizing methods.

### 2.2. Anticancer Drugs

Folic acid (FA) is one of the most popular targeting ligands due to 1) overexpression of folate receptor (FR); 2) low cost; 3) low molecular weight; 4) high safety; and 5) low immunogenicity. Therefore a number of FA-conjugated CyDs have been designed as tumor-targeting drug carriers.\(^{27,28}\) However, most of the studies have been performed only in vitro. Thereby, there are few reports that achieved targeting of anticancer drug in vivo using FA-conjugated CyDs. More recently, Okamatsu et al.\(^{29,30}\) prepared folate-appended CyD possessing a caproic acid between FA and a CyD molecule as spacer (Fol-c\(_7\)-CyD), and evaluated the inclusion complexation ability with doxorubicin (DOX) and the antitumor effects of the DOX complex in vitro and in vivo. In general, many studies report that administration in CyD solutions has no effect on drug pharmacokinetics due to low stability constant of the complexes. Meanwhile, the stability constant of the DOX/Fol-c\(_7\)-$\beta$-CyD complex showed $1.7 \times 10^6 \text{M}^{-1}$ at pH 7.3. The data suggest that after intravenous injection of drug/CyD complexes having the extremely high stability constants more than $10^6 \text{M}^{-1}$, the complexes can remain in the blood stream and distribute as the complex to the tumor tissues comparably to albumin-drug complexes. Interestingly, the stability constant of the DOX/Fol-c\(_7\)-$\beta$-CyD complex decreased approximately by one-hundredth at pH 6.8, suggesting that the complex tends to dissociate in endolysosomes after endocytosis (Fig. 2). The DOX/Fol-c\(_7\)-CyD complex showed potent antitumor activity in vitro and in vivo with negligible change of blood chemistry data after intravenous administration to Colon-26 cells-bearing mice. Remarkably, Fol-c\(_7\)-$\beta$-CyD increased antitumor activities of hydrophobic anticancer drugs such as paclitaxel and vinblastine, hydrophobic drugs, but not 5-fluorouracil, a hydrophilic drug. These findings suggest that Fol-c\(_7\)-$\beta$-CyD has the potential as a tumor-selective drug carrier.

### 2.3. Fullerene C\(_{60}\)

Fullerene C\(_{60}\) is known as a photosensitizer for photodynamic therapy, and $\gamma$-CyD forms a 2:1 complex with fullerene C\(_{60}\). Iohara et al.\(^{31}\) reported that 2-hydroxylpropyl-$\beta$-CyD (HP-$\beta$-CyD) forms a nanoparticle with fullerene C\(_{60}\), not an inclusion complex. In addition, they demonstrated the potential of fullerene C\(_{60}\)/HP-$\beta$-CyD nanoparticle as a promising photosensitizer for photodynamic therapy, and recently reported antioxidant effects of hydrophilic C\(_{60}\)(OH)\(_{10}\)/HP-$\beta$-CyD nanoparticles.\(^{32}\) The C\(_{60}\)(OH)\(_{10}\)/HP-$\beta$-CD nanoparticles had a higher scavenging activity against nitric acid and peroxynitrite (ONOO\(^-\)) than the other antioxidants such as ascorbic acid, trolox, and edaravone. Moreover, the nanoparticles had a high cytoprotective effect. Intravenous administration of C\(_{60}\)(OH)\(_{10}\)/HP-$\beta$-CyD nanoparticles to mice with a liver injury induced by acetaminophen over dose prolonged their survival rate. These results suggest that C\(_{60}\)(OH)\(_{10}\)/HP-$\beta$-CyD nanoparticles are a promising antioxidant for use in the treatment of diseases caused by oxidative stress. Overall, these findings suggest that HP-$\beta$-CyD can be a promising carriers for both fullerene C\(_{60}\) and C\(_{60}\)(OH)\(_{10}\).

### 3. CyD-Based Carriers for Protein Drugs

#### 3.1. Controlled Release for PEGylated Proteins Using CyD Polypseudorotaxanes

CyDs are known to form molecular necklaces with linear polymers, so-called polypseudorotaxanes (PPRXs).\(^{2,15}\) For example, $\alpha$-CyD forms PPRX with PEG, whereas $\beta$- and $\gamma$-CyDs, but not $\alpha$-CyD, form PPRXs.
with polypropylene glycol (PPG). PPRXs have favorable properties as DDS carriers, since they self-assemble and have high biocompatibility and inexpensive components.\textsuperscript{14} Importantly, PPRXs are poorly water-soluble, but can dissolve by dilution. On the basis of these observations, Higashi \textit{et al.}\textsuperscript{33,34} hypothesized that solubility of drugs can be adjusted by formation of PPRXs. Indeed, γ-CyD formed PPRXs with PEGylated insulin, a model pegylated protein, by including two PEG chains. When the suspension of γ-CyD PPRX was subcutaneously administrated to rats, it was gradually dissociated by dilution, and PEGylated insulin was sustainably released\textsuperscript{33} (Fig. 3). Furthermore, γ-CyD PPRX showed a prolonged hypoglycemic effect, compared with insulin and PEGylated insulin alone. These results suggest that CyD PPRXs are useful as sustained release systems for PEGylated proteins. Higashi \textit{et al.}\textsuperscript{35} also described PPRX formation of randomly-PEGylated insulin with CyDs with slow release and resistance to enzymatic degradation.

Seki \textit{et al.}\textsuperscript{36} demonstrated the formation of supramolecular structures of phenylboronic acid-modified CyDs (PBA-CyDs). PBA-α-CyD formed supramolecular polymer structures through head-to-tail interactions, whereas PBA-β-CyD formed a dimer through head-to-head interactions. The resulting supramolecular structures exhibited a low solubility in water, however, addition of sugar increased their solubility. This is a new concept for chemical-responsive materials based on the use of guest-modified CyDs, and has potential applications. Recently, Seki \textit{et al.}\textsuperscript{37} prepared a PPRX comprising 3-carboxy-5-nitrophyrinylboronic acid-modified γ-CyD (NPBA-γ-CyD) and naphthalene-modified PEG (Naph–PEG) as a sugar-responsive supramolecular structure. The Naph–PEG/NPBA-γ-CyD PPRX exhibited improved sugar responsivity. The enhanced NPBA-γ-CyD was then applied to a PPRX containing Naph–PEG–appended insulin (Naph–PEG–Ins), which showed an improved response for glucose-induced insulin release. Therefore, CyDs PPRX formation with PEGylated proteins has significant potential as sustained and stimuli-responsive protein drugs.

### 3.2. CyD PPRXs as Stabilizing Carriers of Antibody Drugs

To achieve potent therapeutic effects of human immunoglobulin G (IgG), highly-concentrated formulations are required. However, stabilization for highly-concentrated human IgG is a laborious work. Higashi \textit{et al.}\textsuperscript{38} demonstrated the potentials of CyD PPRX hydrogels as stabilizers for highly-concentrated human IgG (Fig. 4). In addition, these researchers recently prepared CyD PPRX hydrogels including antibody-based drugs, omalizumab (Xolair\textsuperscript{®}), palivizumab (Synagis\textsuperscript{®}), panitumumab (Vectibix\textsuperscript{®}) and ranibizumab (Lucentis\textsuperscript{®}), and evaluated their physicochemical stabilities (unpublished data). Remarkable improvement in the stability of human IgG against thermal and shaking stresses was provided by CyD PPRX hydrogels. These results suggest that PEG/CyDs PPRX hydrogels could be a stabilizing material for highly-concentrated human antibodies.

### 3.3. Self-assembly PEGylation Retaining Activity Technique for Protein Delivery

PEG modification (PEGylation) is one of the best approaches to improve the stability and blood half-life of protein drugs; however, PEGylation may significantly reduce the bioactivities of protein drugs. Recently, Arima \textit{et al.}\textsuperscript{2} revealed Self-assembly PEGylation retaining activity (SPRA) technology through an inclusion interaction between PEGylated β-CyD (PEG-β-CyD) and adamantane-appended (Ad)-proteins (Fig. 5). PEG-β-CyD formed stable complexes with Ad-insulin and Ad-lysozyme to yield SPRA-insulin and SPRA-lysozyme, respectively. Both SPRA-proteins showed high stability against heat and trypsin digest, comparable with that of covalently PEGylated protein equivalents. It should be noted that the SPRA-lysozyme possessed almost 100% lytic activity, whereas the activity of the covalently PEGylated lysozyme was only 23%. Additionally, SPRA-insulin provided a prolonged and peakless blood glucose profile when compared with insulin glargine injection (Lantus\textsuperscript{®}). In contrast, covalently PEGylated insulin showed a negligible hypoglycemic effect. These findings indicate that SPRA technology has the potential as a generic method, sur-

---

Fig. 3. Proposed Scheme for Release of PEGylated Protein from CyD Polypseudorotaxanes

Fig. 4. CyD PPRX Hydrogels Including Human IgG with Thermal and Shaking Stabilizing Effects
passing conventional PEGylation methods for proteins.

4. CyD Conjugates with Dendrimer for Decoy DNA and Small Interfering RNA (siRNA) Delivery

Arima and colleagues\(^4\) reported that α-CyD/dendrimer conjugate (α-CDE) may be useful as DNA and oligonucleotide carriers, because of the following properties; 1) high transfer activity; 2) unique endosomal escaping mechanism; 3) negligible cytotoxicity; 4) very low hematoxicity; 5) no aggregation in serum; 6) easy preparation of polyplex; and 7) simple components. These researchers proposed a mechanism by which α-CDE exerts efficient RNA interference (RNAi) effects in cells. α-CDE/siRNA complex adsorbs on the cell surface through electrostatic interaction, then enters the cells through clathrin- and rafts-dependent endocytosis. In endolysosomes, the proton sponge effect of dendrimer as well as the lipid extraction effect of α-CyD work together to enhance endosomal escaping of siRNA. In cytoplasm, siRNA is released from the complex, then enters RNA-induced silencing complex (RISC). Finally, a potent RNAi effect is exerted.

4.1. Fucose-Appended α-CDE as Kupffer Cell-Selective Decay DNA Carriers

Kupffer cells express fucose and mannose receptors, and fucose and mannose are known as promising ligands for drug delivery to Kupffer cells.\(^4\) It has been reported that delivery efficiency of fucose-appended liposome/nuclear factor-kappaB (NF-κB) decoy DNA complex to non-parenchymal cells is two-fold higher than that of mannose-appended liposome/NF-κB decoy complex, suggesting that fucose is likely preferred over mannose as ligand for drug delivery to Kupffer cells.\(^4\) Akao et al.\(^4\) prepared fucose-appended α-CDE (Fuc-S-α-CDE (G2)) and evaluated its potential as Kupffer cells-selective decoy DNA carrier (Fig. 6). Upon lipopolysaccharide (LPS) stimulation Fuc-S-α-CDE (G2)/NF-κB decoy complex evidently inhibited inflammatory cytokine production in NR8383 cells. The survival rate of fulminant hepatitis model mice was significantly improved by intravenous injection of Fuc-S-α-CDE (G2)/NF-κB decoy complex. These results suggest potential of Fuc-S-α-CDE (G2)/NF-κB decoy complex as oligonucleotide therapy against a fulminant hepatitis. Arima and colleagues\(^4\) demonstrated the use of mannosylated dendrimer (G2, G3) conjugates with α-CyD for siRNA carriers to Kupffer cells.

4.2. Lactose-PEG-Appended α-CDE as Hepatocyte-selective Gene and siRNA Carriers

It is well-known that asialoglycoprotein receptor (ASGPR) is highly expressed on hepatocyte surface. Therefore, a galactose-appended carrier, which may be recognized by ASGPR, was used as a hepatocyte-selective carrier. Previously, Arima et al. prepared lactosylated α-CDE (Lac-α-CDE) as a gene and siRNA carrier. Lac-α-CDE showed hepatocyte-selective gene and siRNA transfer activity \textit{in vitro} and \textit{in vivo}. Recently, Hayashi et al.\(^4\) prepared PEGylated Lac-α-CDE (PEG-LacC, G3) (Fig. 6). PEG-LacC (G3) elicited a much higher RNAi effect than other PEG-LacCs (G3) in HepG2 cells, indicating that PEG-LacC (G3) has the greatest RNAi effect. Additionally, the RNAi effects of the siRNAs directed against transthyretin (siTTR) complex with PEG-LacC (G3) increased as the siTTR concentration increased, and a potent RNAi effect was observed. Also, the PEG-LacC (G3)/siRNA complex significantly suppressed TTR mRNA expression in the liver \textit{versus} the PEG-LacC (G3, DSP2)/siCont complex. Furthermore, the PEG-LacC (G3, DSP2)/siRNA complex suppressed approximately 50% of TTR mRNA expression in BALB/c mice after two intravenous administrations. The higher RNAi effect of PEG-LacC (G3)/siTTR complex could be attributed to increase in the complex stability and half-life in blood. These results suggest some potential of the PEG-LacC (G3, DSP2)/siRNA complex as a treatment against TTR-familial amyloidotic polyneuropathy (FAP) \textit{via} intravenous administration.

4.3. Folate-PEG-appended α-CDE as Tumor-Selective siRNA Carriers

Arima et al.\(^4\) prepared folate-PEG-appended α-CDE (G3) (Fol-PoC (G3)) as cancer cell-selective gene and siRNA carriers (Fig. 6). Fol-PoC (G3) showed higher gene and siRNA transfer activity, compared with α-CDE (G3) in KB cells (FR-α (+)), but not in A549 cells (FR-α (−)). Additionally, the siRNA/Fol-PoC (G3) complex indicated \textit{in vivo} RNAi effects following systemic injection in tumor cells-bear-
ing mice.\textsuperscript{47} However, little RNAi effect was observed. Thus, Ohyama \textit{et al.}\textsuperscript{48} prepared Fol-PzC (G4) to increase the \textit{in vivo} siRNA delivery efficiency of Fol-PzC (G3). Fol-PzC (G4)/siRNA complex showed highly the potent RNAi effect following systemic administration in tumor cells-bearing mice. Besides, when Fol-PzC (G4)/siRNAs directed against polo-like kinase 1 (siPLK1) complex was intravenously administrated to BALB/c mice bearing Colon-26 cells twice weekly, tumor growth was significantly suppressed compared with Fol-PzC (G4)/siCont complex. Additionally, Fol-PaC (G4)/siPLK1 complex significantly repressed \textit{PLK1} mRNA levels versus control, indicating that the antitumor activity of Fol-PzC (G4)/siPLK1 complex was based on the RNAi effect derived from siPLK1. Meanwhile, body weight of the mice slightly increased comparably to control, suggesting that Fol-PzC (G4)/siPLK1 complex does not exert the severe side effect under the experimental conditions. Taken together, these results suggest that Fol-PzC (G4)/siPLK1 complex confers antitumor activity \textit{in vivo}. Hence, targeting ligand-appended CDEs are the promising carriers for siRNA and decoy DNA.

5. Cyclodextrin Derivatives as APIs

The first use of CyD as APIs is Bridion\textsuperscript{®}, a well-tolerated agent for the reversal of neuromuscular blockade.\textsuperscript{49} This was a paradigm shift in the study of CyDs. Thereafter, many attempts to prepare novel APIs based on CyDs have been expanded. The pharmacological effects of CyDs against several diseases are shown in Fig. 7.

5.1. HP-\textbeta-CyD for Treatment of Niemann–Pick Disease Type C (NPC)

NPC is an intractable disease presenting as free form cholesterol accumulation in endosomes caused by blockade of intracellular transport of cholesterol taken in by endocytosis for lysosomes. Several reports have shown that HP-\textbeta-CyD improves cholesterol accumulation to lysosomes, and may extend survival by several weeks.\textsuperscript{50–53} In American twin NPC patients, infusion of HP-\textbeta-CyD was promptly performed as a physician-led humanitarian clinical trial. In Japan, HP-\textbeta-CyD infusion for NPC was performed by Matsuo \textit{et al.}\textsuperscript{53} Some reduction of hepatosplenomegaly and effects on neurologic symptoms were observed, but the effect was transient. Recently, intrathecal infusion of HP-\textbeta-CyD to NPC patients has been performed to reduce the risk of adverse events in lung.\textsuperscript{54} Meanwhile, Kondo \textit{et al.}\textsuperscript{55} reported effects of hydroxylalkylated \textbeta-CyDs with different cholesterol-solubilizing abilities, such as 2-hydroxyethyl-\textbeta-CyD (HE-\textbeta-CyD) and 2-hydroxybutyl-\textbeta-CyD (HB-\textbeta-CyD). Based on the results regarding cholesterol solubilizing potential, attenuating the effects against NPC abnormalities and cytotoxicity induction, HB-\textbeta-CyD may be superior in terms of safety and efficacy in Npc1 null cells compared with HE-\textbeta-CyD and HB-\textbeta-CyD. On the other hand, Soga \textit{et al.}\textsuperscript{56} reported that 2-hydroxyproplyl-\textg-CyD (HP-\textg-CyD) could reduce cholesterol accumulation and restore functional and molecular abnormalities in NPC patient-derived cells, more effectively than HP-\textbeta-CyD treatment. In addition, NPC model mice showed improved liver status and prolonged survival with HP-\textg-CyD. Thus, HP-\textg-CyD is a potential new drug candidate for future treatment of this disease. Davidson \textit{et al.}\textsuperscript{57} demonstrated that four HP-\textbeta-CyDs varying in degrees of substitution, including one currently in clinical trial, showed equivalent storage reduction, whereas other CyDs showed significant differences in relative ototoxicity and efficacy, with reductions similar for the brain and liver. Importantly, HP-\textg-CyD and two sulfoethyl ether-\textbeta-CyDs showed efficacy with reduced ototoxicity. Hence, CyDs other than HP-\textbeta-CyD may provide disease amelioration without ototoxicity and merit long-term treatment studies. While direct interactions of CyD-unesterified cholesterol are thought central to the mechanism of correction, the data show that this does not strictly correlate with complexation ability and suggest interactions with other NPC disease-relevant substrates should be considered.

Arima and colleagues\textsuperscript{58} reported cell-penetrating CyD derivatives, namely, mono-lactose-appended \textbeta-CyD (Lac-\textbeta-CyD) to deliver \textbeta-CyD selectively to hepatocytes. Lac-\textbeta-CyD having a lactose molecule significantly decreased intracellular cholesterol content in a concentration-dependent manner.
Cellular association of tetramethyl rhodamine isothiocyanate (TRITC)-labeled Lac-β-Cyd (TRITC-Lac-β-Cyd) with NPC-like HepG2 cells was greater than that of TRITC-β-Cyd. Hence, Lac-β-Cyd was transported into NPC-like HepG2 cells through ASGPR-mediated endocytosis and reduced cholesterol levels in NPC-like HepG2 cells. In addition, Motoyama et al.\textsuperscript{59} reported that octaarginine (R8)-appended β-Cyd with a spacer of γ-amino Butyric acid (R8-β-Cyd) efficiently delivered β-Cyd to various types of NPC-like cells. R8-β-Cyd significantly decreased intracellular cholesterol content compared with HP-β-Cyd with negligible cytotoxicity. These results suggest that Lac-β-Cyd and R8-β-Cyd may have potential as a drug for the treatment of hepatosplenomegaly in NPC disease. Recently, a polyrotaxane of HP-β-Cyd has been used for against NPC. For example, Thompson and colleagues\textsuperscript{60,61} demonstrated that pluronic-based β-Cyd polyrotaxanes as well as HP-β-Cyd polyrotaxanes may be promising vehicles for delivery of β-Cyd and HP-β-Cyd for mobilization of accumulated cholesterol from NPC fibroblasts. Moreover, Tamura and Yu\textsuperscript{62,63} reported that β-Cyd-threaded biodegradable polyrotaxanes ameliorate impaired autophagic flux in NPC. Taken together, these supramolecules may have potentials as novel drugs for the treatment of NPC.

5.2. CyDs for Treatment of GM1-Gangliosidosis

GM1-gangliosidosis is an inherited disease exerted by GM1-gangliosides accumulation in many tissues and organs, predominantly in the brain. Currently, there are no effective treatments against this entity, despite earnest research efforts. Maeda et al.\textsuperscript{64} reported that among several kinds of CyD derivatives, dimethyl-α-Cyd (DM-α-Cyd) decreased GM1-ganglioside levels in EA1 cells, fibroblasts from patients with GM1-gangliosidosis, and after single intraventricular injection to GM1-gangliosidosis model mice. Additionally, DM-α-Cyd decreased GM1-ganglioside levels in the brain in GM1-ganglioside model mice. Fortunately, the efflux of cholesterol or phospholipids from the cells upon treatment with CyDs was not observed. As a result, CyDs may possess potential as API for GM1-gangliosidosis.

5.3. HP-β-Cyd as Antileukemic Drug

Yokoo et al.\textsuperscript{65} demonstrated that HP-β-Cyd itself has anticancer effects against various leukemic cell lines derived from acute myeloid leukemia (AML), acute lymphoblastic leukemia and chronic myeloid leukemia (CML). HP-β-Cyd decreased cholesterol level in the cells, and lead to obvious G2/M cell-cycle arrest and apoptosis. In addition, HP-β-Cyd significantly improved survival in leukemia mouse models. Importantly, HP-β-Cyd increased survival rate and provided anticancer effects against CML cells expressing a T315I BCR-ABL mutation, and hypoxia-adapted CML cells possessing features of leukemic stem cells. Meanwhile, HP-β-Cyd had negligible adverse effects in vivo. These findings expand the possibility that HP-β-Cyd will be developed as an auspicious anticancer agent.

5.4. Folate-Appended Methyl-β-Cyd

Grosse et al.\textsuperscript{66} reported that methyl-β-Cyd (M-β-Cyd) exerts antitumor activity, but does not have selectivity to tumor cells. Onodera et al.\textsuperscript{67} demonstrated that folate-append M-β-Cyd (FA-M-β-Cyd) shows excellent and tumor-selective antitumor activity both in vitro and in vivo. It should be noted that following intravenous injection of FA-M-β-Cyd, in tumor-bearing mice, all animals were alive for more than 4 months with negligible side effects. Most interestingly, FA-M-β-Cyd induced autophagy-mediated antitumor effect, not apoptosis.\textsuperscript{68} These data suggest that FA-M-β-Cyd has potential as a unique antitumor API.

5.5. M-β-Cyd as the Fertilization Promotion Agent

In vitro fertilization technology is widely available for infertility treatment, stock raising and laboratory animal domains. Takeo et al.\textsuperscript{69} reported that M-β-Cyd dramatically improves the fertilization rate of mouse sperm by facilitating cholesterol efflux from sperm plasma membrane. As well as frozen semen, fertility rate was improved using M-β-Cyd. These results suggest that M-β-Cyd may be useful as a fertilization promotion compound.

6. Conclusion

In this review, we introduced the potential use of CyDs as agents for drug delivery carriers and API. CyDs have significant potential as promising drug carriers for PGs, antitumor drugs, proteins, antibodies, siRNA and decoy DNA. In addition, CyDs may be useful as APIs against various diseases such as lysosomal storage diseases, leukemia and solid tumors. Additionally, M-β-Cyd promotes in vitro fertilization of mouse sperm.\textsuperscript{70} Hence, we expect that medical supplies including CyD-based molecules will be developed and contribute to improvement of quality of life of patients and families of those with a broad spectrum of diseases.

Acknowledgments

We would like to express sincere thanks to K. Uekama and F. Hirayama, Faculty of Pharmaceutical Sciences, Sojo University and T. Irie, Graduate School of Pharmaceutical Sciences, Kumamoto University, for their valuable advice, warm support and kind help. We are most proud of all of the students who have engaged in CyD research in our laboratory. This work was partially supported by Grant-in-Aid for Scientific Research (C) (16590114, 18590144, 20590037), Grant-in-Aid for Scientific Research (B) (16H05223) and Grant-in-Aid for Challenging Exploratory Research (16K15322) from Japan, Society for the Promotion of Science and The Uehara Memorial Foundation.

Conflict of Interest

Hidetoshi Arima is a chief technology officer of CyDing Co., Ltd.

References

1. Lofsson T., Jarho P., Masson M., Jarvinen T., Expert Opin. Drug Deliv., 2, 335–351 (2005).
2. Arima H., Hayashi Y., Higashi T., Motoyama K., Expert Opin. Drug Deliv., 12, 1425–1441 (2015).
3. Uekama K., Chem. Pharm. Bull., 52, 900–915 (2004).
4. Uekama K., Hirayama F., Irie T., Chem. Rev., 98, 2045–2076 (1998).
5. Arima H., Motoyama K., Higashi T., Adv. Drug Deliv. Rev., 65, 1204–1214 (2013).
6. Martinez A., Ortize Mellet C., Garcia Fernandez J. M., Chem. Soc. Rev., 42, 4746–4773 (2013).
7. Davis M. E., Brewster M. E., Nat. Rev. Drug Discov., 3, 1023–1035 (2004).
8. Swaminathan S., Cavalli R., Trotta F., Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol., 8, 579–601 (2016).
9. Moya-Ortega M. D., Alvarez-Lorenzo C., Concheiro A., Lofsson T., Int. J. Pharm., 428, 152–163 (2012).
10. Harada A., Acc. Chem. Res., 34, 456–464 (2001).
11. Harada A., Li J., Kamachi M., Nature (London), 364, 516–518
66) Grosse P. Y., Bressolle F., Pinguet F., Br. J. Cancer, 78, 1165–1169 (1998).

67) Onodera R., Motoyama K., Okamatsu A., Higashi T., Arima H., Sci. Rep., 3, 1104 (2013).

68) Onodera R., Motoyama K., Tanaka N., Ohyama A., Okamatsu A., Higashi T., Kariya R., Okada S., Arima H., Sci. Rep., 4, 4417 (2014).

69) Takeo T., Hoshii T., Kondo Y., Toyodome H., Arima H., Yamamura K., Irie T., Nakagata N., Biol. Reprod., 78, 546–551 (2008).

70) Aguila L., Arias M. E., Vargas T., Zambrano F., Felmer R., Reprod. Domest. Anim., 50, 931–938 (2015).