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REVIEW ARTICLE

Cyclodextrins, Blood–Brain Barrier, and Treatment of Neurological Diseases

Miklós Vecsernyés,[a] Ferenc Fenyvesi,[a] Ildikó Bácskay,[a] Mária A. Deli,[b] Lajos Szente,[c] and Éva Fenyvesi[c]

[a]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary*
[b]*Department of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary*
[c]*Cyclolab Cyclodextrin Research and Development Laboratory Ltd., Budapest, Hungary*

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Biological barriers are the main defense systems of the homeostasis of the organism and protected organs. The blood–brain barrier (BBB), formed by the endothelial cells of brain capillaries, not only provides nutrients and protection to the central nervous system but also restricts the entry of drugs, emphasizing its importance in the treatment of neurological diseases. Cyclodextrins are increasingly used in human pharmacotherapy. Due to their favorable profile to form hydrophilic inclusion complexes with poorly soluble active pharmaceutical ingredients, they are present as excipients in many marketed drugs. Application of cyclodextrins is widespread in formulations for oral, parenteral, nasal, pulmonary, and skin delivery of drugs. Experimental and clinical data suggest that cyclodextrins can be used not only as excipients for centrally acting marketed drugs like antiepileptics, but also as active pharmaceutical ingredients to treat neurological diseases. Hydroxypropyl-β-cyclodextrin received orphan drug designation for the treatment of Niemann-Pick type C disease. In addition to this rare lysosomal storage disease with neurological symptoms, experimental research revealed the potential therapeutic use of cyclodextrins and cyclodextrin nanoparticles in neurodegenerative diseases, stroke, neuroinfections and brain tumors. In this context, the biological effects of cyclodextrins, their interaction with plasma membranes and extraction of different lipids are highly relevant at the level of the BBB.

Key Words: Cyclodextrins, Blood–brain barrier, Tight junctions, CNS diseases, Drug delivery.

Introduction

The blood–brain barrier (BBB) constitutes a permeability barrier for systemic drugs and most of the newly developed neurotherapeutic drug candidates (1), making the treatment of neurological diseases very difficult. Different strategies based on BBB physiology and anatomy were developed to enhance the penetration of molecules across the BBB, which is a prerequisite of their central nervous system (CNS) efficacy (1,2). Cyclodextrins (CDs), as excipients and adsorption enhancers, have been extensively investigated on different biological barriers including nasal, intestinal and skin barriers (3,4), but their effects on the BBB are much less investigated and have not yet been reviewed. Considering the increasing use of CD-based systems including nanoparticles for drug delivery to brain and the recent interest in CDs as drugs to treat CNS diseases, this topic is essential from the viewpoint of neuropharmacology. This review presents an overview on the basic characteristics of CDs and their biological effects with an emphasis on barriers relevant for drug delivery to the brain. The use of CDs and CD-based formulations to treat neurological diseases is discussed, whereas other therapeutic applications are also briefly summarized.

Structure and Properties of Cyclodextrins

CDs are cyclic oligosaccharides prepared from starch by enzymatic cleavage of the amylose helix (5). The three most studied representatives consist of 6, 7 and 8 glucopyranose units called α-, β- and γ-CDs, respectively.

Address reprint requests to: Miklós Vecsernyés, Department of Pharmaceutical Technology, University of Debrecen, Egyetem Square 1, Debrecen 4010, Hungary; Phone/Fax: (+36) 52-512900/22630; E-mail: vecsernyes.miklos@pharm.unideb.hu

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These ring-shaped molecules have numerous hydroxyl moieties (18, 21 and 24, respectively) all facing outside, which makes them highly hydrophilic. On the other hand, the inner side of the cavity is less hydrophilic because of the glucosidic oxygen bonds (Figure 1). This structure enables CDs to include other less hydrophilic compounds (guests) into the cavity, forming in this way the so-called host–⊖ guest inclusion complexes.

The main driving force of the complex formation is the replacement of high-energy water molecules in the cavity with a less polar guest compound, thus creating hydrophobic interactions between the host and the guest. Hydrogen bonds might contribute. These weak interactions result in dynamic equilibrium between the complex and the free CD and guest. This equilibrium is characterized by the association (binding) constant showing the ratio of the components in dissociated and complex form:

$$K_a = \frac{[G/CD]}{[G] * [CD]}$$

where [G], [CD] and [G/CD] represent the concentrations of the free guest, free CD and of the complex, respectively.

The higher K_a means more stable inclusion and less dissociation. K_a helps to understand what happens in a mixture (e.g., in a biological system containing various lipophilic compounds to be entrapped). There is always a competition and the guest molecules characterized with a higher K_a will be preferentially included.

In addition to the lipophilic character, the geometric fit (key and hole) is a prerequisite of the complex formation. At least a part of the guest molecule should fit into the CD cavity. A tight fit is better than too large a space for a molecule. It is often the task to find the optimal cavity for a guest molecule. The molecular dimensions of the β-CD cavity (diameter 0.60–0.65 nm and height 0.78 nm) make it the best host among the three native CDs for molecular encapsulation of most of the drugs, flavors, cosmetic ingredients, pesticides, etc. (5). The stoichiometry of the complex depends on the size of the guest; even two small molecules can be hosted in a cavity or the large molecules can be entrapped by two or more CDs (2:1 and 1:2, 1:3, etc. molar ratios). For instance, cholesterol forms a 2:1 complex with randomly methylated β-CD (RAMEB) (Figure 2).

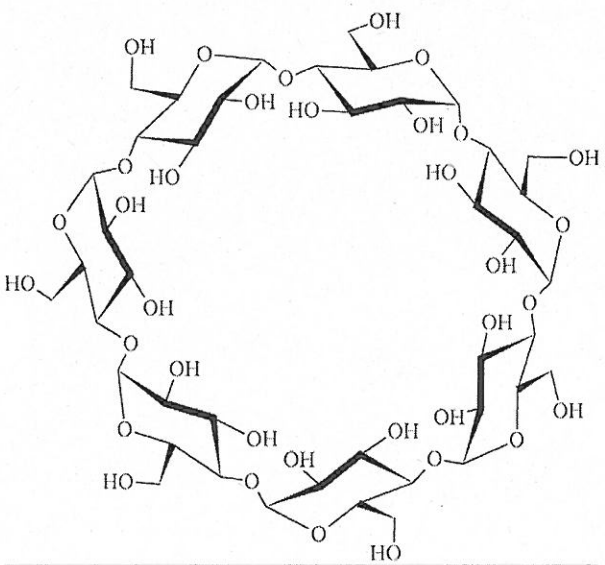

Figure 1. Chemical formula of β-cyclodextrin.

The properties of the included guest molecules are usually different from the free (not included) ones. They are characterized by increased/decreased solubility, enhanced/reduced stability against heat, light, hydrolysis or microbial attack, changed thermal and spectral properties (thermogravimetry, differential scanning calorimetry, ultraviolet-visible, infrared, nuclear magnetic resonance, circular dichroism, etc.) and altered mobility in the chromatographic and electrophoretic systems (6–12). In addition to these mostly beneficial changes, the complexation might possess further advantages including taste masking, odor absorption, controlled release, and enhanced bioavailability utilized by various industries such as pharmaceutical, cosmetic, food industry, biotechnology, agriculture and environmental protection, to mention only the most important fields of application (12–19).

The numerous hydroxyl groups can be readily modified into various CD derivatives via specific synthetic routes. Some of the derivatives such as hydroxypropyl-β-CD (HP-β-CD) and sulfobutyl ether β-CD (SBE-β-CD) have been thoroughly studied and registered in the U.S. and European Pharmacopoeias. Also, the methylated derivatives of β-CD, the one with methyl groups on all of the C-2 and C-6 positions (DIMEB) and those with methyl groups at random positions (CRYSMEB and RAMEB) are produced

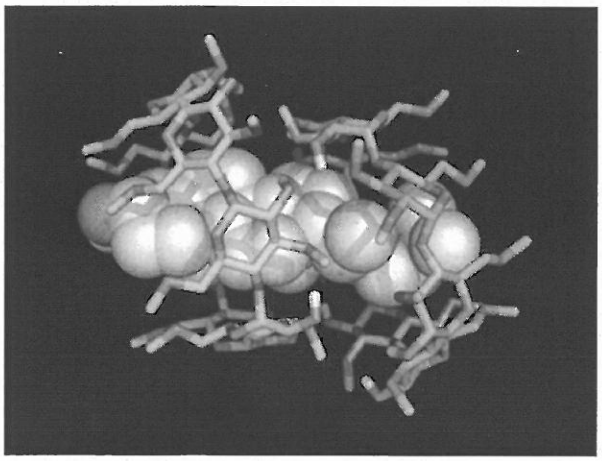

Figure 2. The molecular model of cholesterol/randomly methylated β-cyclodextrin (RAMEB) 1:2 inclusion complex. (courtesy of Virtua Drug).

on industrial scale. The maltosyl β-CD (Ma-β-CD) is preferred by Japanese manufacturers. These β-CD derivatives are all well soluble compared to the unmodified β-CD, which has low solubility in water (1.8 g/100 mL at 25 C) (5). Among the γ-CD derivatives only hydroxypropyl-γ-CD (HP-γ-CD) is produced on a large scale. It has the advantage of low aggregation over the unmodified γ-CD. The latter cannot be used in parenteral formulations due to the aggregative behavior (20). The α-CD derivatives are prepared at laboratory scale for research purposes. In addition to the hydrophilic derivatives including all of these industrially produced ones, several amphiphilic derivatives have also been described (21). At present these derivatives are primarily of academic interest.

Whereas the inclusion complexes of the native (unmodified) CDs often precipitate from aqueous solution, the hydrophilic derivatives are good solubilizers of poorly soluble compounds (22–26). The solubilizing effect is usually characterized by solubility isotherms plotting the concentration of the guest compound as the function of the concentration of the host. The typical solubility isotherms for the hydrophilic derivatives show increasing solubility at increasing CD concentration (Type A), whereas the native CDs give Type B solubility isotherms (27). Data show that the solubility of cholesterol can be enhanced by various β-CD derivatives, especially methylated ones (Me-β-CDs). The affinity of Me-β-CDs toward cholesterol depends on the number of methyl groups in a CD molecule (degree of substitution, DS) (26). However, the various Me-β-CDs are often not identified although their properties including the solubilizing effect are quite different. The native (unmodified) β-CD forms insoluble complexes with cholesterol, a phenomenon utilized by the food industry to produce various dairy products with reduced cholesterol content (28).

One of the methods of determination of the association constant (K_a) is based on the slope of the linear part of the solubility isotherm. The K_a for cholesterol/DIMEB complexes of 1:1 and 1:2 molar ratio were calculated 109 M^{-1} and 56800 M^{-2}, respectively (29). These values show that one cholesterol molecule preferentially interacts with 2 β-CD cavities.

Biological Effects of Cyclodextrins

Cellular Effects

The excellent review of Dreyfuss and Oppenheimer on cellular interactions of cyclodextrins summarizes the effects of CDs on bacterial and viral cells as well as on mammalian cells of the immune, nervous, endocrine and cardiovascular systems (26). Most of the cellular effects are based on the interaction of CDs with the cell membrane rich in lipids such as cholesterol and sphingolipids (lipid rafts). CDs also affect the cholesterol-associated membrane-bound proteins and receptors.

Numerous studies have been carried out on cell toxicity of various CDs using different cells and assays. The simplest assay uses red blood cells and measures the color intensity of the hemoglobin escaped from the disrupted cells into the medium. The hemolytic activity of native CDs increased in the order of β-CD > α-CD > γ-CD (30). Cytotoxic effects on human erythrocytes are explained by the extraction of various lipid constituents from cell membranes increasing their fluidity and permeability (31). The potencies of CDs for solubilizing various components of erythrocytes were α- > β- >> γ-CD for phospholipids, and β- >> γ- > α-CD for cholesterol and proteins.

Comparing the CD derivatives the hydrophilic derivatives, HP-β-CD, SBE-β-CD and Ma-β-CD were less hemolytic compared to β-CD, whereas Me-β-CDs even caused morphological changes in rabbit red blood cells (32). Similar results, reduced and enhanced hemolysis, were obtained for HP-α-CD and dimethyl α-CD (DIMEA), respectively. The hemolytic effect of β-CD derivatives correlated well with their affinity to cholesterol (33). A strong correlation was found between the cholesterol solubilizing effect of the β-CD derivatives and their cytotoxicity in colorimetric end-point viability test on Caco-2 human intestinal epithelial cells (33,34).

The cytotoxicity of β-CD derivatives based on cholesterol efflux was also proven on various other cell types (35–37). On the other hand, cellular cholesterol content was altered by incubating cells with solutions of CDs complexed with increasing levels of cholesterol (38). Recently, methylated CDs RAMEB and DIMEB became a common tool for researchers in the field of biochemistry and molecular biology for studying lipid rafts (39,40). The cholesterol content of the cell membrane can be controlled by treatment with Me-β-CDs. By removal of cholesterol the lipid rafts can be disrupted and by subsequently applying cholesterol/Me-β-CD complex the cholesterol can be rebuilt into the cell membrane and the lipid rafts are reorganized (41,42). With these techniques the role of the lipid rafts in various cell processes such as signal transduction, apoptosis, and activity of transporter proteins can be clarified (43–47). The advantage of using the reversible host–guest inclusion complex formation for capturing and release of cholesterol is just this reversibility. Another option for decreasing the cholesterol content in the cell membrane is the inhibition of the cholesterol biosynthesis by statins, but this is a unidirectional process (48).

It is well known that cholesterol forms preferentially 1:2 (guest:host) complexes with β-CDs (29). Computer simulation showed that the self-organization of β-CD into dimers is necessary for removal of cholesterol from the cell membrane (49). β-CDs rapidly bind to the membrane surface in a dimeric form and, provided that the CD dimers are in a suitable orientation, cholesterol molecules are spontaneously extracted.

A practical utilization of the high affinity of Me-β-CD to membrane cholesterol is in the artificial insemination in animal husbandry. The poor fertility rates of the sperm after freezing and thawing can be remarkably improved by pretreating the semen with cholesterol-loaded Me-β-CD prior to cryopreservation (50). Mammalian spermatozoa are sensitive to cold shock, and freezing damage is due to changes in membrane lipid composition, particularly cholesterol depletion in plasma membrane during cryopreservation (51). Supplementing cholesterol with either Me-β-CD or HP-β-CD as carrier, the vitality, motility and zona-binding capability of sperm cells are enhanced (52).

Effects of Cyclodextrins on Biological Barriers

Biological barriers are crucial to preserve the homeostasis of the organism or separate organs like the CNS. The BBB and the intestinal barrier determine the entry of drugs to the CNS and the systemic circulation, respectively; therefore, they are of utmost importance for the treatment of neurological or systemic diseases. The effect of CDs on three major elements of these endothelial and epithelial barriers restricting drug penetration, tight intercellular junctions (53), active efflux pumps and low level of nonspecific endo- and transcytosis will be summarized.

Effects of Cyclodextrins on the BBB

Paracellular Permeability and Tight Intercellular Junctions

There are several observations on the effects of various CDs on functional and morphological integrity of the BBB using *in vitro* and *in vivo* models. The most detailed investigations were performed on *in vitro* reconstituted BBB model developed and characterized in the Cecchelli laboratory (54,55) (Table 1). This setup consists of cloned bovine brain endothelial cells (BCECs) co-cultured with rat astrocytes. The model shows high transendothelial electrical resistance and low permeability values. Using this well-characterized and tight culture model of the BBB all three types of CDs were studied in native, methylated, and hydroxypropylated forms (73). The cellular toxicity of CDs was determined by the permeability of sucrose, a marker of paracellular flux across bovine brain endothelial cell monolayers co-cultured with rat glial cells. Native CDs (1—20 mM) increased the permeability of endothelial cells in the following order: α-CD > β-CD >> γ-CD. Methylation, but not hydroxypropylation, decreased the cell layer damaging effect for α-CD, whereas only hydroxypropylation, but not methylation, of β-CD and γ-CD had an attenuating effect on toxicity. In parallel, a decrease in the expression and localization of tight junction protein occludin was seen at the cell borders indicating that CDs in the millimolar (mM) concentration range damage the barrier integrity of brain endothelial cells. The potencies of CDs for solubilizing various lipids of brain endothelial cells were β- >> γ- > α-CD for cholesterol, α- >> γ- > β-CD for phosphatidylcholine, and α- > β- >> γ-CD for sphingomyelin (73). Because interendothelial junctions are associated with lipid rafts membrane microdomains in brain endothelial cells (74), the effect of CDs on barrier integrity is probably due to their lipid extraction properties. The importance of lipids in the control of paracellular barrier integrity is underlined by recent findings that short-chain alkylglycerols can quickly and reversibly open the tight junctions of brain endothelial cells (75). In red blood cells β-CD was the most toxic, whereas α-CDs were the most toxic in bovine brain endothelial cells. The difference between the toxicity of CDs in different cell types can be linked to the different lipid composition of plasma membranes. Phosphatidylcholine is enriched in the apical membrane of cultured brain endothelial cells (76), which may explain their sensitivity for α-CDs. Two CDs, γ-CD and HP-γ-CD, showing the least damaging effect on brain endothelial cells were further studied on the same culture model (57). These γ-CDs do not increase the penetration of doxorubicin across the BBB model, only in concentrations that disrupt brain endothelial junctions (>15 mM for γ-CD and 35 mM for HP-γ-CD), which was confirmed by increased penetration of the paracellular marker inulin and decreased junctional staining for occluding (Table 1).

Among monosubstituted n-alkyldimethylammonium-β-CDs (DMA-C(n)-CD with n = 2, 4 and 12), DMA-C(12)-CD was non-toxic on cultured bovine brain endothelial cells at concentrations <10 mM due to the self-inclusion of the alkyl chain in the CD cavity. A high percentage of passage (30%) of DMA-C(12)-CD through brain endothelial cells was reported (77).

Active Drug Efflux Transporters

Several members of the ATP binding cassette transporter and solute carrier families are present at the level of the BBB and actively involved in the vectorial transport of endogenous CNS metabolites and a large number of drugs from the CNS to the circulation. The two most abundant drug efflux transporters in humans are the ABCG2 or breast cancer resistance protein and ABCB1 or P-glycoprotein (78). Their largely overlapping substrate sets include important neuropharmaceuticals like antiepileptics, antineoplastic agents for brain tumor and antiretroviral drugs for neuroAIDS treatment (78). RAMEB and CRYSMEB, but not β-CD, increased the transport of doxorubicin, a P-glycoprotein substrate in bovine brain endothelial cells (56). This increase was attributed to cholesterol extraction from brain capillary endothelial cells by CDs leading to modulation of P-glycoprotein activity. Indeed, intact lipid rafts in brain endothelial cells are crucial for pathological upregulation of P-glycoprotein

Table 1. Effects of cyclodextrins on drug penetration across the blood–brain barrier

CD (concentration)	Model/BBB site of action	Toxic *in vitro* CD dose	Effects on drug permeability	Reference
In vitro studies				
β-CD (1 mM)	Bovine BCEC co-cultured with rat AC	2.5 mM	Doxorubicin transport ≈	(56)
RAMEB (1 mM)	Bovine BCEC co-cultured with rat AC	2.5 mM	2×↑ doxorubicin transport	(56)
CRYSMEB (2.5 mM)	Bovine BCEC co-cultured with rat AC	5 mM	3.7×↑ doxorubicin transport	(56)
CRYSMEB (2.5 mM)	Bovine BCEC co-cultured with rat AC	5 mM	2×↑ vincristine transport	(56)
γ-CD (1 mM)	Bovine BCEC co-cultured with rat AC	20 mM	Doxorubicin transport ≈	(57)
HP-γ-CD (1 mM)	Bovine BCEC co-cultured with rat AC	50 mM	Doxorubicin transport ≈	(57)
QA-β-CD nanoparticle	Bovine BCEC	500 μg/mL	2.2×↑ doxorubicin transport	(58)
In vivo studies				
α-CD–galanin-like peptide	Brain uptake (intranasal in mice)	N.A.	3× ↑uptake	(59)
α-CD–ribavirin	Measles encephalitis (i.p. in mice)	N.A.	Viral load↓	(60)
α-CD–ribavirin	Brain uptake (i.p. in mice)	N.A.	↑uptake	(61)
β-CD–ribavirin	Measles encephalitis in mice	N.A.	Viral load↓	(62)
DIMEB–galanin-like peptide	Brain uptake (intranasal in mice)	N.A.	3× ↑uptake	(59)
EDA-β-CD lactoferrin	Brain uptake (i.v. in mice)	N.A.	6.9×↑ in AUC of IR-977	(63)
EDA-β-CD transferrin	Brain uptake (i.v. in mice)	N.A.	3.5× ↑ in AUC of IR-977	(63)
HP-β-CD–estradiol	i.v. in ovariectomized rats	N.A.	Luteinizing hormone secretion ↓, weight ↓	(64)
HP-β-CD–testosterone	i.v. in orchidectomized rats	N.A.	Serum luteinizing hormone↑	(65)
HP-β-CD–testosterone	Intracerebral injection in rats	N.A.	Rapid efflux from brain	(66)
HP-β-CD–cholesterol	Intracerebral injection in rats	N.A.	Slow efflux from brain	(66)
HP-β-CD–dexamethasone	i.v. in rats	N.A.	Stress-induced ACTH & corticosterone↓	(67)
HP-β-CD–cyclic opioid peptides	Intrathecal (spinal) injection in rats	N.A.	Antinociception ↑	(68)
HP-β-CD–opioids	Intrathecal (spinal) injection in rats	N.A.	Prolonged spinal antinociception	(69)
HP-β-CD–chloralose	i.v. in cats	N.A.	Anesthesia ≈	
HP-β-CD–melarsoprol	Trypanosomiasis in mice	N.A.	Parasitic load↓, BBB integrity↑	(70)
Mono-6-amino-permethyl-β-CD–DPDPE	i.v. in mice	N.A.	Prolonged antinociception	(71)
RAMEB–melarsoprol	Trypanosomiasis in mice	N.A.	Parasitic load↓, BBB integrity↑	(70)
SBE_7-β-CD–carbamazepine	Pentylenetetrazole-induced seizure (p.o. in mice)	N.A.	Anti-epileptic effect↑	(72)

AC, astrocytes; ACTH, adrenocorticotropic hormone; α-CD, α-cyclodextrin; AUC, area under curve; BBB, blood–brain barrier; β-CD, β-cyclodextrin; CD, cyclodextrin; CRISMEB, crystalline methylated-β-cyclodextrin; DPDPE, 2,5-Pen-enkephalin; DIMEB, 2,6-di-O-methly-β-cyclodextrin; EDA-β-CD, mono-6-deoxy-(6-aminoethylamino)-β-CD; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; HP-γ-CD, 2-hydroxypropyl-γ-cyclodextrin; i.p., intraperitoneal injection; i.v., intravenous injection; N.A., not applicable; QA-β-CD, 3-trimethylammonium-(2-hydroxy)propyl-β-cyclodextrin; RAMEB, randomly-methylated-β-cyclodextrin; SBE_7-β-CD, sulfobutyl $ether_7$-β-CD.

by HIV-1 Tat protein, which could be blocked by Me-β-CD treatment of cells depleting membrane cholesterol and thus disrupting lipid rafts (79).

Binding and Transmonolayer Flux of Cyclodextrins

To reveal the exact mechanism of the CNS effects of CDs it is important to know the extent of their brain penetration. Cultured brain endothelial cells were used to study the transmonolayer flux of different types of CDs in non-toxic (0.5–1 mM) concentrations (73). The highest passage among CDs was observed for native β-CD, α-CDs, and HP-γ-CD, which is still low compared to BBB penetrating small molecules. No data are available on the uptake or transcellular transport of these CDs in brain endothelial cells. The flux of HP-β-CD and Me-β-CD across brain endothelial cells was the lowest and at the same level as that of efflux pump ligands. These data on the very low flux of HP-β-CD across the BBB obtained on a culture model were confirmed by *in vivo* experiments (80). No significant time-dependent crossing of HP-β-CD into the brain parenchyma was found in adult or neonatal mice measured by two separate techniques, *in situ* brain perfusion and intraperitoneal injection followed by multi-time-point regression analysis (80). Because the volume of distribution of HP-β-CD was nearly three times larger than that of the vascular space

marker sucrose, this study indicates binding of HP-β-CD to the luminal surface of cerebral endothelium (80).

Effects of Cyclodextrins on the Intestinal Barrier

Paracellular Permeability and Tight Intercellular Junctions

As discussed previously, CDs extract cholesterol from cell membrane. This process has several further effects that were studied on both epithelial cell layers and gut tissue. Cholesterol depletion of Caco-2 human intestinal epithelial monolayers by Me-β-CD influences the distribution of specific tight junction proteins like claudin 3, claudin 4 and occludin, and these changes affect the integrity of the epithelial barrier. As a consequence, transepithelial electrical resistance significantly decreased and the paracellular permeability of Caco-2 cell layer increased (81). A similar effect was observed on Madin-Darby canine kidney cells. After a long (2 h) Me-β-CD incubation, resistance decreased and paracellular permeability increased, whereas the tight junction network was physically disrupted (82). On the other hand, 10% (w/v) 2-HP-β-CD solution was tested on rat intestinal membrane using *in vitro* diffusion chamber method and its effect on paracellular absorption was also examined by *in situ* closed-loop technique in rat jejunum. No significant effect on membrane integrity and paracellular permeability was observed (83). Despite the safety of 2-HP-β-CD, 0.08 and 0.8% (w/v) Me-β-CD caused increased paracellular permeability in rat jejunum, but not in ileum, using the *in vitro* sac method (84).

Active Drug Efflux Transporters

Plasma membrane cholesterol depletion can also influence important efflux pumps, which limit the bioavailability of drugs. Inhibition of the transporter P-glycoprotein and multidrug resistance-associated protein 2 by 2,6-di-O-Me-β-CD can be observed on Caco-2 monolayers (85). Inhibition of P-glycoprotein by CD treatments arises through modulation of its membrane microenvironment as observed for DIMEB treated cells where changes in membrane cholesterol level, alterations in the overall lipid packing and changes in the raft association of the P-glycoprotein were described (46). Inhibition of these efflux pumps in the intestinal barrier can increase plasma concentration of their substrates. Clinically important P-glycoprotein substrates include anticancer agents, cardiovascular drugs, and immunosuppressants (86,87).

Cellular Uptake: Endocytosis

Recently a new mechanism was observed for the interaction of CDs and cells. Fluid-phase endocytosis of CDs was detected in Caco-2 intestinal cells (88). The role of this mechanism in drug absorption is not revealed, but endocytosis of CD complexes can contribute to overcome intestinal barrier for poorly absorbed drugs.

Cyclodextrins in Drug Delivery

Effect of Cyclodextrins on the Unstirred Water Layer

Water molecules are bound on the surfaces of biological membranes and form an unstirred water layer (UWL) (89). The thickness of the UWL ranges from nanometer scale to >100 μm depending on the presence of a mucus layer. On the other hand, the measured thickness of the UWL also depends on the physicochemical properties of the permeating drug molecules (90). For rapidly penetrating drugs, UWL can act as a diffusion barrier and can be the rate determining factor of the overall permeability (91,92). CDs are able to enhance permeation of lipophilic drug molecules through the UWL (93). Hydrophilic CDs such as 2-HP-β-CD improve drug permeation only if UWL significantly contributes to the barrier function of the membrane. Complexation is required for this mechanism, but extremely high complexation affinity reduces free drug availability and permeation (92).

Drug Delivery to the Brain

Data on culture models of the BBB prove the CDs can increase the transendothelial permeability of lipophilic drugs that are substrates of active efflux pumps (Table 1). RAMEB and CRYSMEB, but not β-CD, increase several fold the flux of doxorubicin across bovine brain endothelial cell monolayers (56). This increase in doxorubicin transport can be linked to their efficacy in cholesterol mobilization from brain endothelial cells. The effect is mediated by a decrease in P-glycoprotein activity because co-incubation of the efflux pump ligand vincristine with CRYSMEB also leads to increased transport. The ineffectiveness of CRYSMEB to enhance the flux of the hydrophilic paracellular marker urea indicates that the effect is not due to opening of tight junctions and increase of the paracellular pathway (56). γ-CD and HP-γ-CD, which are less effective in cholesterol release from plasma membranes, do not increase the transport of doxorubicin across the *in vitro* BBB model in concentrations not modifying barrier integrity (15 and 35 mM, respectively) (57). This result further supports the hypothesis that CRYSMEB decreases P-glycoprotein activity in brain endothelial cells by cholesterol extraction and disturbance of the lipid raft associated to transporters (74). Doxorubicin transport across cultured brain endothelial cells was also increased by quaternary ammonium β-CD (QA-β-CD) nanoparticle carriers (58). These cationic CD nanoparticles at a concentration of 100 μg/mL did not change barrier integrity, cholesterol extraction or P-glycoprotein activity in bovine brain endothelial cells. QA-β-CD nanoparticles

are more permeable than the paracellular marker dextran, and their penetration across the BBB model is probably due to endocytosis (58). A new β-CD and poly(β-amino ester) polymeric nanoparticle was developed for doxorubicin transport by the same group (94). This nanoparticle was also described to cross brain endothelial monolayers without affecting barrier integrity.

Results from animal models also indicate that CDs enhance delivery of mostly lipophilic drugs or peptides to the CNS by measurement of either brain uptake or functional parameters (Table 1). DIMEB and α-CD increased the brain uptake of the neuropeptide galanin-like peptide about 3-fold after intranasal administration in mice (59). A difference in the regional brain distribution could be observed. The greatest uptake was seen in the hypothalamus and olfactory bulb after intranasal administration with α-CD and in the olfactory bulb after intranasal administration with DIMEB. Both α-CD (60) and β-CD (62) complexed with ribavirin significantly decreased the viral load in measles encephalitis in mice after intraperitoneal injection as compared to the free drug. The effect was due to enhanced brain penetration of α-CD-ribavirin complex (61). HP-β-CD is the most studied CD derivative. Complexes with HP-β-CD increase the CNS effects of estradiol (64), testosterone (65) and dexamethasone (65) chemical delivery systems after intravenous injection in rats. HP-β-CD also enhances or prolongs the antinociceptive effects of cyclic opioid peptides (68) or opioids morphine, lofentanil, alfentanil and sufentanil (69) after intrathecal injection in mice. The solubility of chloralose, an anesthetics used in animal studies, was greatly enhanced when complexed with HP-β-CD without side effects or loss of anesthetic potency in cats (95). Inclusion complexes of HP-β-CD or RAMEB with melarsoprol improved the solubility and reduced the toxicity of the trivalent arsenical drug and cured CNS-stage *Trypanosoma brucei* infection in mice when delivered orally (70).

In contrast to peripheral administration of drug-CD complexes, when HP-β-CD is injected to brain a rapid clearance from the CNS (within <24 h) and excretion to urine is observed in rats (66). Efflux from CNS via bulk flow of interstitial and cerebrospinal fluids was supposed as a potential mechanism but was not investigated. An even more rapid brain efflux was described for testosterone complexed with HP-β-CD after intracerebral injection. The authors presumed that testosterone crosses the BBB, binds to specific carrier proteins in serum and is excreted by the liver (66). It is tempting to speculate that multidrug resistance-associated protein 4, which is present at the BBB, and transport conjugated steroids (96) may participate in the brain efflux of testosterone observed in this experiment. The brain clearance of cholesterol injected in the form of a complex with HP-β-CD to brain is very slow. Cholesterol released from the HP-β-CD complex is largely retained in the brain with uneven distribution after 3 days postinjection (66).

Intravenous injection of DPDPE, a cyclic opioid pentapeptide conjugated to mono-6-amino-permethyl-β-CD results in improved bioavailability and prolonged antinociceptive activity (71). A novel nano-drug delivery system for brain-targeting was developed in which lactoferrin and transferrin were selected as targeting ligands and conjugated via a polyethylene glycol linker to mono-6-deoxy-(6-aminoethylamino)-β-CD (63). Several-fold increase in brain uptake of the cargo, an infrared dye (IR-977), was obtained in mice after intravenous administration (63). Complex formation of sulfobutyl ether (7)-β-CD with carbamazepine resulted in significantly higher anti-epileptic activity in pentylenetetrazole-induced convulsion model in mice as compared with the effect of orally administered carbamazepine suspension indicating higher penetration to CNS (72).

Intestinal Drug Delivery

The potential of CDs to be used as penetration enhancers for drugs has been widely investigated on intestinal barrier models as reviewed by Loftsson (3). CDs can increase the intestinal delivery even for large biomolecules like peptides. DIMEB is a potent enhancer of intestinal absorption of insulin *in vivo* (97,98). DIMEB was found to be more effective to enhance bioavailability and absorption of insulin than all other CDs tested: HP-β-CD, α-, β- γ-CDs. This effect seems to correlate with the cholesterol depleting efficacy of CDs (3). In addition to other well-characterized effects of CDs on drug complexation and unstirred water layer (93), cholesterol depletion from epithelial cell membrane (99), especially from lipid rafts, and subsequent loss of TJ integrity, displacement of TJ proteins (81) can explain the absorption enhancing effect of DIMEB for peptides.

Nasal Drug Delivery

The nasal mucosa offers a novel approach for systemic administration of biologically active drugs (e.g., estrogen) by avoiding first pass metabolism or degradation in the liver and gastrointestinal tract. It is true that CDs are able to enhance the drug bioavailability, but free CDs can also affect the barrier function of the nasal mucosa or may have an influence on the nasal mucociliary function. Therefore, the concentration and application circumstances of CDs should be considered before nasal administration. Me-β-CDs were primarily shown to be useful excipients on nasal drug delivery systems (100–103). Observations of drug bioavailabilities in humans showed that CDs can improve the nasal absorption of lipophilic drugs (100–102) and some oligopeptides. CDs are able to increase the bioavailability of peptides such as calcitonin (103). The absorption increasing effect of CDs is less effective in human subjects in the case of polypeptides and proteins. Al Omaria et al. (104) demonstrated that the inclusion complex of ibuprofen masks the irritant effect caused by ibuprofen nasal spray on

the oral cavity, throat, and pharynx. In oophorectomized women, α-CD containing estradiol nasal spray was found to be well tolerated by patients applied over a 6-month period (105). On the other hand, RAMEBs have irritative and inflammatory effects on epithelial cells of the nasal mucosa, depending on the exposure time (106,107). Their possible effects on mucociliary functions can be considered in nasal preparations.

Pulmonary Drug Delivery

Similar to the nasal pathway, pulmonary drug delivery is a promising way for systemic drug application. The lungs have a large surface area, good blood supply and low degradation activity of enzymes; therefore, the absorption process from the pulmonary area is very effective. In addition, first-pass metabolism and drug degradation in the gastrointestinal tract can be eliminated by choosing pulmonary drug delivery (108–110). CDs can mostly be used in pulmonary applications through their complexation capability with an active ingredient by mixing compatible drugs in dry powder formulation (111). CDs can reduce the bad smell and taste and local irritation in the lungs. The effect of CDs on drug release profile in the lungs can be another goal of their pulmonary application (112–116). The absorption profiles of various CDs were studied in animal pharmacokinetic experiments in order to reveal safety properties of the CDs after pulmonary administration (109,110). Interestingly, a relatively high bioavailability of DIMEB and HP-β-CD was found in rabbits, and it was higher than CD absorption rate observed using other routes of administration. Based on this observation, pulmonary CD application can be considered as the future choice for increased systemic absorption with acceptable safety profiles.

Cyclodextrins in Topical Skin Formulations

Bioavailability of topically administered drugs is very low due to their poor penetration into the skin, which limits not only the topical treatment of skin diseases but also transdermal therapy. The barrier function of human skin is mainly based on the specific attributes of the stratum corneum. Lipids in the stratum corneum form bilayer surrounding the corneocytes and hinder the permeability of active pharmaceutical ingredients (117,118). Consequently, many investigations aim to develop optimal formulations with high efficacy and low side effects or irritation (119). Skin penetration can be enhanced by increasing either drug solubility in the skin or drug permeability into/through the skin. Drug saturation in the topical formulation is also a crucial point (117). Conventional chemical enhancers like fatty acids, alcohol and propylene glycol improve cutaneous drug delivery, but at the same time lipid structure within the barrier may be damaged (120). There are several attempts to avoid membrane disruptions either by developing novel and combined vehicle systems (microemulsions, liposomes, niosomes, nanoparticles) (121) or by introduction of modern devices (iontophoresis, sonophoresis and electroporation) (122).

CDs are able to influence both drug solubility and permeability into/through the skin, but some other important factors may be considered. Aqueous medium is the first criterion to apply them as penetration enhancers (3). It means that a cream base with hydrophilic characteristics such as gels or oil in water ointments need to be chosen (3,120). The optimal concentration of CDs can be calculated in the ointment base by the help of critical micelle concentration (20). The stratum corneum is also the main barrier for CDs because hydrophilic CDs cannot penetrate intact skin. It is thought that some types of CDs (β-CD, RAMEB, HP-β-CD) can extract skin lipids under specific conditions. Pretreatment by CDs does not usually increase skin permeability, and reduced permeability was observed if CDs were used in very high concentrations (3). The cosmetic industry focuses on the smell or odor-masking effects of CDs to improve patient acceptance of skin products (123).

When novel drug delivery systems like nanoparticles and liposomes (121,124), modern penetration enhancers (non-ionic amphiphilic tensides, i.e., sucrose esters) (125) and CDs are combined, there is an additive or synergistic effect on drug delivery through the skin (126). The mechanisms of drug delivery from aqueous CD solutions might be both diffusion and membrane controlled. In some cases, CDs can hinder the absorption of lipophilic drugs into or through the skin and increase the active pharmaceutical ingredient retention time in the stratum corneum. Hence, they have a wide potential in the development of sunscreen formulations (127,128).

Cyclodextrins in Marketed Drugs

The first pharmaceutical product containing CD, prostaglandin E2/β-CD sublingual tablets (Prostarmon E, Ono), was marketed in Japan in 1976. Nowadays there are numerous examples for the application of CDs in pharmaceutical technology. In 2008, ~600 published patents and patent applications were found in which drug formulations contained CDs (129).

Dissolution and absorption enhancement are the most frequent applications of these excipients. Dissolution enhancement is based on their complex formation with lipophilic guest molecules, as presented earlier. CDs can be applied to drugs belonging to Biopharmaceutics Classification System Class II (low solubility/high permeability) and Class IV (low solubility/low permeability) to increase their solubility and absorption by complex formation (130,131). Taxol, a widely used anticancer agent belonging Class IV, is a good example to demonstrate the effectiveness of CD complexation. Several publications reports on the efficiency of β-CDs to improve its solubility

Table 2. Examples of intravenously applied, cyclodextrin containing products (marketed or in clinical development)

Brand names	Active ingredient	Cyclodextrin	Indications	Company (marketing authorization)
α-Cyclodextrins				
Alprostadil®, Alprostapint®, Caverject®, Edex®	Prostaglandin E1	α-CD	Erectile impotency	Pfizer (EU)
Prostavasin®	Prostaglandin E1	α-CD	Peripheral arterial occlusive disease	Ono (Japan); Schwarz/UCB (EU)
2-Hydroxypropyl-β-cyclodextrins				
Sporanox®	Itraconazole	HP-β-CD	Fungal infections	Janssen (EU, USA)
MitoExtra®	Mitomycin C	HP-β-CD	Disseminated adenocarcinoma	Novartis (EU)
Sulfobutylether-β-cyclodextrins				
Carbella®	Carbamazepine	SBE-β-CD	Epilepsy	Lundbeck: NDA submission to FDA
Cerenia®	Maropitant	SBE-β-CD	Motion sickness in dogs	Pfizer Animal Health (USA, EU)
Kyprolis®	Carfilzomib	SBE-β-CD	Multiple myeloma	Onyx Pharmaceuticals (USA)
Nexterone®	Amiodarone	SBE-β-CD	Arrhythmia	Baxter International (USA)
Noxafil®	Posaconazole	SBE-β-CD	Fungal infections	Merck (EU)
Vfend®	Voriconazole	SBE-β-CD	Fungal infections	Pfizer (USA, EU, Japan)
N.A.	SAGE-547	SBE-β-CD	Refractory status epilepticus	Sage Therapeutics: Phase I-II
N.A.	Melphalan	SBE-β-CD	Multiple myeloma	Spectrum Pharmaceuticals: orphan
N.A.	Topiramate	SBE-β-CD	Epilepsy	CURx Pharmaceuticals: phase I, orphan
γ-Cyclodextrins				
Bridion®	Sugammadex	Sugammadex	Neuromuscular blocking agent	Merck (EU, Japan, Australia)
CardioTec®	^{99m}Tc teboroxime	HP-γ-CD	Radionuclide for cardiac imaging	Squibb (USA), Bracco (USA)

α-CD, α-cyclodextrin; β-CD, β-cyclodextrin; CD, cyclodextrin; FDA, U.S. Food and Drug Administration; γ-CD, γ-cyclodextrin; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; HP-γ-CD, hydroxypropyl-γ-CD; N.A., not available; NDA, new drug application; SBE-β-CD, sulfobutylether-β-cyclodextrin.

(132–134), on the other hand methylated β-CD derivatives are also able to improve taxol permeability through Caco-2 monolayer (135). Taxol CD complexes were incorporated in poly(anhydride) nanoparticles, which resulted elevated oral bioavailability of taxol in rats (136).

Approximately 50 different CD-containing drug products are present currently on various world markets. Selected intravenously applied, CD containing marketed products available worldwide are listed in Table 2.

Cyclodextrins as Drugs

CDs were considered as carriers of active ingredients without any physiological effects till the first concerns on the possible complexation of important compounds in the gut or in the blood. Pitha suggested that HP-β-CD administered parenterally as a solubilizer of a poorly soluble drug may influence the redistribution of lipophilic components such as hormones and vitamins within the organism after releasing their cargo (137). This author used intravenous CD treatment (DIMEB and HP-β-CD in mice and in human, respectively) to capture excess vitamin A in hypervitaminosis (138,139). DIMEB or HP-β-CD and the complex were excreted by urine and resulted in enhanced survival of rats (Figure 3). This was the first human application of an "empty" CD (CD without cargo), that is the use of CDs as drug and not as auxiliary excipient.

The first marketed "empty" CD with pharmaceutical effect is Sugammadex, i.e., 6-per-deoxy-6per(2-carboxyethyl) thio-γ-CD, commercialized by Merck under the trade name Bridion®. It is a special γ-CD derivative developed for capturing muscle relaxants rocuronium and vecuronium used in anesthesia during surgery. The tailored modification of the γ-CD ring was so successful that extremely high binding constants (2.5×10^7 and 1.0×10^7 for rocuronium and vecuronium, respectively) were obtained showing

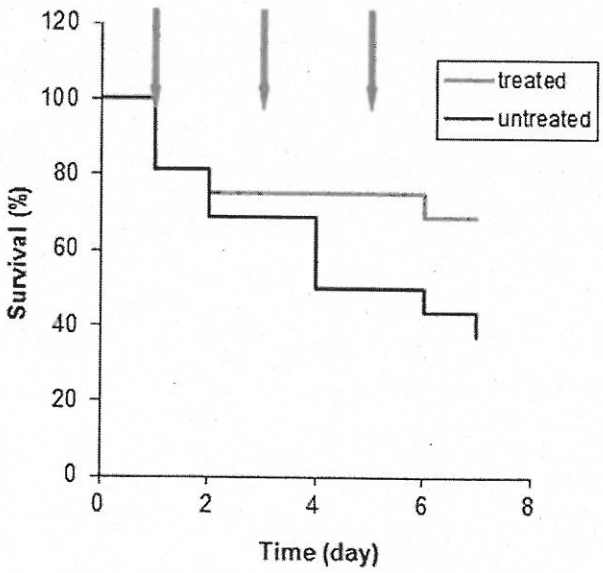

Figure 3. Survival rate of mice made hypervitaminous by s.c. injection of retinoic acid (100 mg/kg) by every other day until the first deaths were observed (day 0). Treated animals received i.p. injection of dimethyl-β-cyclodextrin (DIMEB) (480 mg/kg) on days 1, 3 and 5 (arrows). Redrawn with permission from Pitha and Szenthe (138).

enormous affinity toward these molecules (140). The binding is so specific that no other components in the blood are encapsulated. Sugammadex revolutionized anesthesia because of the fast reversal of the neuromuscular block after surgery and the absence of significant adverse effects. It has been approved in Europe, Australia, and Japan and is now available for clinical use in more than 40 countries except the U.S. FDA approval is still pending because of hypersensitivity toward Sugammadex observed in some patients. A recent *in vitro* study indicated that Sugammadex in clinically relevant concentrations (37.5–150.0 μg/mL) may cause toxicity to cultured neurons, although it practically cannot permeate through intact BBB due to its structure and high molecular weight (141). The clinical experience has been published in more than 100 scientific papers and in a few reviews in the last 10 years (142–144). Sugammadex is still expensive but the reduced recovery time, lack of side effects and enhanced patient throughput can compensate for the extra cost compared to traditional treatment with acetylcholinesterase inhibitors (145).

The other "empty CD" having regulatory approval is HP-β-CD, which received orphan drug designation for the treatment of Niemann-Pick type C (NPC) disease. It has been long included in U.S. and EU Pharmacopoeias as an excipient (drug carrier, solubilizer), but as a therapeutic agent against this rare lysosomal disease it was authorized by FDA and EMA only in 2010 and 2013, respectively. The fast granting of orphan drug designation about 10 years after the incidental discovery of the beneficial effect of HP-β-CD in 2001 (137) was due to the exceptionally good cooperation between academia, industry and government initialized by patient organizations (146).

Administration of Cyclodextrins in Neurological Diseases

Niemann-Pick Type C Disease

NPC is an autosomal recessive lipid storage disorder characterized by progressive neurodegeneration (146,147). Presenting symptoms in early childhood are ataxia, seizures, progressive deterioration of motor functions followed by reduced weight gain, cognitive decline and premature death (146,147). Owing to mutations of the genes *NPC1* or *NPC2* responsible for cholesterol trafficking, NPC patients accumulate cholesterol in their organs and also in brain, causing severe neurological symptoms. The positive effects of HP-β-CD both in animal experiments and human clinical studies (Figure 4) were explained at first by cholesterol solubilization (148–150). Camargo et al. (151) published the first evidence that intraperitoneal injection of HP-β-CDs decreases liver cholesterol storage and slightly delays neurological symptoms in $Npc1^{-/-}$ mice, although the BBB was shown to be practically non-permeable for CDs, and intrathecal

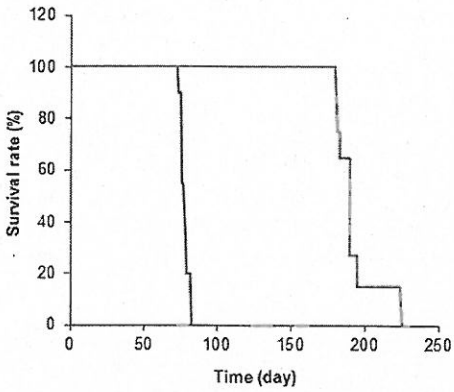

Figure 4. Survival rate of $Npc1^{-/-}$ mice with no treatment and chronic treatment with s.c. injection of 20% hydroxypropyl-β-cyclodextrin (HP-β-CD) (4000 mg/kg) every other day starting postnatal day 7. Adapted and modified with permission from Davidson et al. (148).

application did not improve the efficacy. Later on, however, it became clear that HP-β-CD can hardly, if at all, enter into cells (80,152), so the sink mechanism was hypothesized that HP-β-CD removes cholesterol from the cell membrane from outside stimulating in this way the cholesterol trafficking within the cell toward the membrane. Since then, animal studies have confirmed that HP-β-CD treatment reduces cholesterol pool in liver, brain, and other organs (148,152–155), improves pathological lysosomal enzyme activity (156), prevents neurodegeneration and reduces tau pathology (148,152–155), delays the appearance of neurological symptoms (148,152–155), and improves longevity (148,153–155) (Table 3). However, there was no evidence of increased cholesterol concentration in plasma or urine of treated $Npc1^{-/-}$ mice, suggesting that HP-β-CD does not carry cholesterol from the cells into the blood for urinary excretion (165), and the sequestered cholesterol is excreted as bile acid (166). Recent studies delved deeper into the cellular mechanisms, addressing the role of inefficient autophagy, processes to digest the cell's own components, in NPC and the stimulating effect of HP-β-CD on the autophagic processes including the enzymatic esterification of cholesterol (167,168). In spite of the fact that the mechanism is unclear, sporadic treatments of children with NPC have started in several countries. A Phase I clinical trial to prove the efficacy and to determine the proper dose has been going on in the National Institutes of Health (NIH) (169).

CDs can no longer be considered as inert drug carriers because of their cellular effects. These effects, however, depend on which CD (cavity size) and which derivative (type and number of substituents) is used. According to the literature, the widest range of pharmaceutical benefits has been described for Me-β-CDs followed by polysulfated CDs. HP-β-CD demonstrates similar impacts, but lower efficiency compared to Me-β-CD, particularly when the affinity to cholesterol is involved. Application of HP-β-CD is

Table 3. Effects of cyclodextrins in animal models of CNS diseases

Disease model	Cyclodextrin	Active ingredient	Effects	Reference
Niemann-Pick type C disease				
Npc1$^{-/-}$ mutant mice	HP-β-CD	HP-β-CD	Liver cholesterol↓, delayed neurological symptoms	(151)
Npc1$^{-/-}$ mutant mice	HP-β-CD	HP-β-CD	Brain cholesterol & GSL↓, neurodegeneration↓, delayed onset, lifespan↑	(148)
Npc1$^{-/-}$ mutant mice	HP-β-CD	HP-β-CD	Body cholesterol pool↓, neurodegeneration↓, lifespan↑	(153)
Npc1$^{-/-}$ mutant mice	HP-β-CD	HP-β-CD -	Cholesterol pools↓, cerebellar neurodegeneration↓, lifespan↑	(154)
Npc1$^{-/-}$ mutant mice	HP-β-CD	HP-β-CD	Correction of lysosomal defects in CNS, neurodegeneration↓	(152)
APP-overexpessing *Npc1*$^{-/-}$ mutant mice	HP-β-CD	HP-β-CD	Cholesterol pool↓, tau pathology↓, neurodegeneration↓, lifespan↑	(155)
Npc2$^{-/-}$ mutant mice	HP-β-CD	HP-β-CD	Brain cholesterol & GSL↓, neurodegeneration↓, delayed onset, lifespan↑	(148)
Other neurodegenerative diseases				
APP transgenic Tg19959 mice model of AD	HP-β-CD	HP-β-CD	Amyloid-β burden↓, tau pathology↓, cognitive functions↑	(157)
α-synuclein transgenic mice model of PD	Me-β-CD	Me-β-CD	Brain α-synuclein accumulation↓, neuronal integrity↑	(158)
6-OH-dopamine model of PD in rat	HP-β-CD	D-264	Enabled D-264 to exert neuroprotective effect in the CNS	(159)
Reserpine hypolocomotion model of PD in rat	HP-β-CD	D-264	Enabled D-264 to exert neuroprotective effect in the CNS	(159)
Brain Ischemia-Reperfusion				
MCA occlusion-reperfusion in rats	HP-β-CD + PLGA	Puerarin	Brain infarction volume↓, improved EEG	(160)
Hypoxia-ischemia in rats	HP-β-CD	HP-β-CD	Brain infarction size↓, excitotoxicity↓	(161)
Epilepsy				
Pentylenetatrazole-induced convulsions in mice	SBE_7-β-CD	Carbamazepine	Anti-epileptic activity↑	(72)
CNS infections				
Measles encephalitis in mice	α-CD	Ribavirin	Viral load↓	(60,61)
Measles encephalitis in mice	β-CD	Ribavirin	Viral load↓	(62)
Human African trypanosomiasis in mice	HP-β-CD	Melarsoprol	Parasitic load↓, BBB integrity↑	(70)
Human African trypanosomiasis in mice	RAMEB	Melarsoprol	Parasitic load↓, BBB integrity↑	(70)
Brain tumors				
Malignant L9 glioma model in rats	β-CD	Camptothecin	Survival time↑	(162)
Malignant GL261 glioma model in mice	β-CD-based polymer	Rhodamine	Uptake by tumor-associated macrophages	(163)
Malignant C6 glioma model in rats	α-CD	Gadolinium	Cerebral blood volume quantification by MRI	(164)

AD, Alzheimer's disease; α-CD, α-cyclodextrin; APP, amyloid precursor protein; BBB, blood–brain barrier; β-CD, β-cyclodextrin; CD, cyclodextrin; CNS, central nervous system; EEG, electroencephalogram; GSL, glycosphingolipid; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; MCA, middle cerebral artery; Me-β-CD, methylated β-cyclodextrin; MRI, magnetic resonance imaging; PD, Parkinson's disease; PLGA, poly(lactic-co-glycolic acid; RAMEB, randomly methylated-β-cyclodextrin; SBE_7-β-CD, sulfobutyl ether$_7$-β-CD.

still also expected in other diseases in addition to NPC due to the better regulatory status and less drastic effects compared to those of Me-β-CDs. For instance, neurotoxicity induced by hypoxia, glutamate and *N*-methyl-D -aspartic acid can be decreased by HP-β-CD and Me-β-CD via cholesterol depletion both *in vitro* and *in vivo* (161,170,171).

Based on the successful example of NPC1 therapy, the efficacy of CDs has been tested in animal models of other lysosomal storage disorders with neurodegeneration. CD treatment is essentially of equal benefit to *NPC2*$^{-/-}$ mice, which have a gene defect responsible for 5% of cases in human NPC disease, suggesting that CD can replace the function of NPC1 protein, NPC2 protein, or an entire cholesterol shuttling mechanism controlled by NPC proteins (148) (Table 3). However, CD administration in mouse models of GM1 gangliosidosis and mucopolysaccharidosis type IIIa, two severe inherited human metabolic disorders characterized by accumulation of cholesterol and glycosphingolipids, had no detectable benefit (148). Similarly, HP-β-CD treatment could not delay motor impairment and Purkinje cell loss in a knock-out mouse model of mucolipidosis II, a lysosomal storage disorder caused by lack of *N*-acetylglucosamine-1-phosphotransferase resulting in

loss of the Npc2 protein involved in the lysosomal export of cholesterol and sphingolipids in the brain (172).

Other Neurodegenerative Diseases

The CD-mediated cholesterol modulation changes the action of various proteins located in the lipid rafts such as receptors, transporters, and ion channels having significant role in the pathogenesis of stroke, cerebral hypoxia/ischemia, traumatic brain injury, Alzheimer's disease, and Parkinson's disease (171). HP-β-CD and also the unmodified β-CD and α-CD can inhibit the aggregation of proteins such as amyloid-β and α-synuclein (173) that is a hallmark in the brain pathology of Alzheimer's and Parkinson's diseases and other neurodegenerative disorders. The effect is concentration dependent and proved *in vivo* by treating Tg19959 transgenic mice overexpressing amyloid precursor protein (APP), a mouse model of Alzheimer's disease (157) (Table 3). Treated animals showed reduced levels of membrane cholesterol and upregulated the genes involved in cholesterol trafficking including ABCA1 and NPC1 (157). ABCA1 is a key regulator of amyloid-β aggregation and deposition (174), and ABCA1-mediated amyloid-β clearance is an important factor in the removal of amyloid-β from the brain in decreased amyloid-β deposition and reduced amyloidogenic processing of APP (157). Subcutaneous HP-β-CD administration for 4 months starting at postnatal day 7 could prevent tau pathology in hippocampus and cortex and improve cognitive functions, spatial learning and memory in APP transgenic mice (157).

Alterations in brain cholesterol and lipid homeostasis and increased expression of caveolin-1 can be seen in Huntington's disease. Mutation of huntingtin gene leads to neurodegenerative disease characterized by motor, behavioral and cognitive dysfunctions (175). Total cholesterol levels were increased in human caudate nucleus from Huntington's disease patients and in primary striatal neurons from knock-in mice expressing full-length mutant huntingtin (175). *In vitro* treatment of cells expressing huntingtin with β-CD or simvastatin, a cholesterol-lowering drug, reduced cholesterol accumulation and high levels of cholesterol-enriched domains caveolin-1 and glycosphingolipid GM1 and protected the cells against *N*-methyl-D-aspartate mediated excitotoxicity (175) (Table 3). As an interesting new therapeutic approach, modified amphiphilic β-CD is used as efficient and safe vector during repeated intracerebral injections of short interfering RNAs (siRNAs) in mice, an experimental treatment resulting in selective alleviation of motor deficits in a model of Huntington's disease (176).

Decreasing the cholesterol levels in transgenic α-synucleinopathy mice using Me-β-CD resulted in a decrease in oligomeric α-synuclein accumulation *in vivo*, suggesting the its therapeutic use in Parkinson's disease (158). HP-β-CD excipient increased the *in vivo* efficacy of D-264, a D3 preferring dopamine D2-D3 receptor agonist drug, in reserpinized and 6-OH-dopamine induced unilateral lesioned rats, animal models of Parkinson's disease (159).

Although the *in vitro* antiprion effect is also explained partly by cholesterol depletion from the lipid rafts, the stabilization of the prion protein structure by both β-CD and Me-β-CD, but not by α- or γ-CD, might also play a role (177). However, daily oral administration of 0.16% HP-β-CD alone, or in combination with p53 inhibitor pifitrin α, was inefficient in Syrian hamsters inoculated with 1% scrapie brain homogenates; it did not change PrP^{Sc} expression or the manifestation of clinical symptoms (178).

Brain Ischemia-Reperfusion

Puerarin, a poorly water-soluble isoflavonoid, was used in HP-β-CD inclusion complex and added to poly(lactic-co-glycolic acid) (PLGA) nanoparticles to increase entrapment efficiency (160). The effect of these puerarin nanoparticles was compared to that of control and puerarin groups in middle cerebral artery occlusion-reperfusion model in rats (160). Puerarin nanoparticles significantly decreased brain infarct volume measured by CT scan, improved cortical EEG parameters, and reduced neuropathological changes (160). Intraperitonal injection of HP-β-CD within 30 min of hypoxia-ischemia decreased the infarction size and reduced neuronal excitotoxicity in hippocampus of rats (161).

Epilepsy

Oral administration of carbamazepine in a complex with SBE_7-β-CD resulted in higher antiepileptic activity than carbamazepine alone in mice with pentylenetetrazole-induced convulsion model of epilepsy (72).

CNS Infections

CDs can be used to increase the efficacy of antiviral treatments in CNS infections. Intraperitoneal injection of ribavirin complexed with α-CD (in a molar ratio 1:3) could significantly increase ribavirin concentration in brain tissue and decreased cerebral viral load in mouse model of measles encephalitis (60,61). Similarly, ribavirin-β-CD complexes (in a molar ratio 1:1) also reduced viral load in the brain in the same model compared to the changes induced by ribavirin only (62).

In a mouse model of human African trypanosomiasis, oral administration of melarsoprol-CD inclusion complexes formed by HP-β-CD or RAMEB was more effective than melarsoprol alone (70). The complexes rapidly cleared *Trypanosoma* parasites from the CNS, restored BBB integrity and reduced the severity of infection-induced neurological symptoms (70). Pharmacokinetic

and tissue distribution studies in mice had previously indicated that brain tissue accumulation of HP-β-CD inclusion complex of melarsoprol was ten times higher than that of melarsoprol nanosuspension (179). HP-β-CD complex was suggested to be used for the treatment of cerebral trypanosomiasis or brain tumors, and the nanosuspension for treatment of refractory leukemia where limitation of cerebral toxicity is an important consideration (179).

Although no comparison is available for antimycotic treatment with or without CD complexes, it is known that CD-containing pharmaceutical formulations of intravenous itraconazole or voriconazole were effective in the treatment of CNS infections in human patients suffering from aspergillosis (180–182), human histoplasmosis presenting with stroke and meningitis (183), and murine coccidioidal meningitis (184).

Brain Tumors

Alhough blood-tumor barrier in cerebral malignancies is usually more permeable than the intact BBB, effective treatment of brain tumors is still a difficult issue. 6-O-capro-β-CD nanoparticles containing camptothecin, a topoisomerase I inhibiting plant alkaloid, decreased tumor growth and significantly increased median survival by 27% on an intracranial rat xenograft model using L9 gliosarcoma cells, whereas the anticancer drug in PLGA or poly(citric acid) polymeric nanoparticles did not change the survival time (162). It was confirmed that β-CD-based polymer-rhodamine nanoparticles can enter the tumor in GL 261 glioma model in mice because tumor-associated microglia cells and macrophages phagocytosed the nanoparticles and migrated into the tumor (163). Due to the impermeability of α-CD across the BBB and blood-tumor barrier, a newly developed magnetic resonance imaging (MRI) preclinical contrast agent—gadolinium per (3,6-anhydro)-α-CD—was used for the quantification of cerebral blood volume in tumor regions, in healthy brain tissue, and in the contralateral hemisphere of C6 glioma tumor-bearing rats (164).

Cyclodextrins as Excipients for CNS-Acting Drugs

Although direct beneficial effects were not published about specific BBB or brain-related effects, CDs are the pharmaceutical excipients of choice in several formulations for treating CNS diseases such as epilepsy (72,185–189) or multiple sclerosis (190,191). Formulations of antiepileptics could include β-CD for semicarbazone (185), HP-β-CD for semicarbazone (186) and carbamazepine (187), and SBE-β-CD for carbamazepine (72) and topiramate (188,189). HP-β-CD was applied in the oral formulation of cladribine (190,191), a drug developed for treatment of multiple sclerosis, that ultimately did not receive FDA approval.

Potential Therapeutic Applications of Cyclodextrins

Antimicrobial Effect

The antimicrobial effect of cholesterol-interacting CDs (Me-β-CD in most experiments) against various viruses like HIV or influenza and bacteria including *E. coli, cholera, Salmonella* was also explained by the cholesterol depletion inhibiting the adhesion of pathogenic cells to host cells (99). Inhibition of cell adhesion via disruption of lipid rafts is utilized when Me-β-CD or HP-β-CD are considered as contraceptives (192). Another theory explains the effect of various tailored β- and γ-CD derivatives against pore-forming bacteria such as anthrax, *Streptoccoccus aureus* and *Clostridium perfringens* (193). CDs having seven- or eight-fold symmetry similar to that of the pores formed by pathogens can perfectly block the material flux through the pores, thus inhibiting the infection as proven *in vivo* in mice with pneumonia caused by *Streptoccoccus aureus*.

Vascular and Immune Systems

Depletion of membrane cholesterol with β-CDs inhibits platelet aggregation indicating therapeutic potential in the treatment of atherosclerosis (194). Intravenous administration of HP-β-CD resulted in reduced atherosclerotic regions in thoracic aorta of hereditary hyperlipidemic Watanabe rabbits (195).

Cholesterol depletion from cells has an influence on the immune system as well, among the effects it enhances the expression of mediators of inflammation, activates T-cells, regulates signaling pathways (196–198). Therapeutic benefits of cholesterol depletion with β-CDs against immunosenescence due to aging have also been studied (199).

The α-CD derivatives, especially DIMEA and trimethyl-α-CD, interact with phospholipids in the cell membrane and disrupt lipid rafts causing similar effects to those of Me-β-CDs; therefore, the pharmaceutical effects might be similar although less studied (200). The therapeutic effect of DIMEA against endotoxin shock caused by lipopolysaccharides was proven in mice (201). Because γ-CD derivatives do not interact remarkably with cell membrane constituents their intrinsic therapeutic effect has not been studied thoroughly, except that of sugammadex. Some γ-CD derivatives were found active in plugging the pores caused by bacterial exotoxins. Other derivatives planned for the capture of specific molecules have special therapeutic applications like β-CD dimers in age-related macular degeneration (202).

Anticancer Effects

Disrupting lipid rafts by cholesterol depletion using Me-β-CD results in reduced expression of proteins responsible for signaling, cell proliferation and angiogenesis, inhibiting tumor development in mice (203–206). A

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further improvement of the anticancer effect was observed with folate-appended Me-β-CD (207). This targeting to tumor cells overexpressing folate receptors resulted in remarkable impacts *in vivo*, reducing tumor size and enhancing survival of mice compared to treatment with Me-β-CD or doxorubicin, a well-known antitumor agent (208).

The CD polysulfates, β-CD tetradecasulfate and γ-CD docosadisulfate, were found to mimic biological carbohydrate polysulfates such as heparin and chondroitin sulfate (209). They are characterized by a high number of sulfate groups in a molecule providing steric hindrance of the entries of the cavities and thus inhibition of the inclusion complex formation. In these molecules, CDs are not hosts, only backbones for the sulfate moieties. Their antiangiogenic, anticancer, and antirheumatic effects were demonstrated, and the inhibition of restenosis after surgery was also proven (210–212).

Conclusions

CDs are important as both excipients and active pharmaceutical ingredients in the treatment of neurological diseases. They are present as solubilizers in many centrally acting marketed drugs like antiepileptics. HP-β-CD received orphan drug designation for the treatment of Niemann-Pick type C disease, which prompted further research to reveal the potential therapeutic use of CDs in lysosomal storage diseases, neurodegenerative diseases, stroke, neuroinfections, and brain tumors. At the same time, new promising CD derivatives and CD nanoparticles are being developed for drug delivery to the CNS. The BBB is a key player in both drug delivery to the CNS and pathomechanism of many neurological diseases. Although several biological effects of CDs were studied on models of the BBB, we are far from understanding the complex interactions between CDs and the brain endothelium. In this context, further research should focus on revealing the effects of CDs on brain endothelial cells at the molecular level including lipid changes at the BBB.

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