Department of Drug Delivery Research[1], Division of Chem-Pharm Drug Evaluation, Janssen Research Foundation, Beerse, Belgium, and Faculty of Pharmacy[2], University of Iceland, Reykjavik, Iceland

The use of chemically modified cyclodextrins in the development of formulations for chemical delivery systems

M. E. Brewster[1] and T. Loftsson[2]

Retrometabolic drug design provides a highly useful and directed approach for identifying new drug candidates with improved therapeutic indices based on predictable/controlled metabolism and/or site-targeted delivery. In the process, formulation becomes an important and integral concern especially for brain-targeting chemical delivery systems (CDS) based on the need for appropriate dosage form stability, solubility and dissolution characteristics. Adjuncts that have been useful in this regard are chemically modified, water soluble cyclodextrin derivatives such a 2-hydroxypropyl-β-cyclodextrin (HPβCD). These starch-derived excipients can interact with drugs via dynamic complex formation resulting in a number of beneficial pharmaceutical effects including increased apparent water solubility and stability as well as improved aesthetic and excipient compatibility properties. This cyclodextrin is approved in a number of product in the US and world-wide. HPβCD has contributed to the development and preclinical/clinical testing of a number of CDS including E2 (estradiol)-CDS, AZT (zidovudine)-CDS, DEX (dexamethasone)-CDS and a neuropeptide CDS based on an enkephalin derivative. In these contexts, HPβCD provided for stable and water-soluble dosage forms intended for parenteral administration.

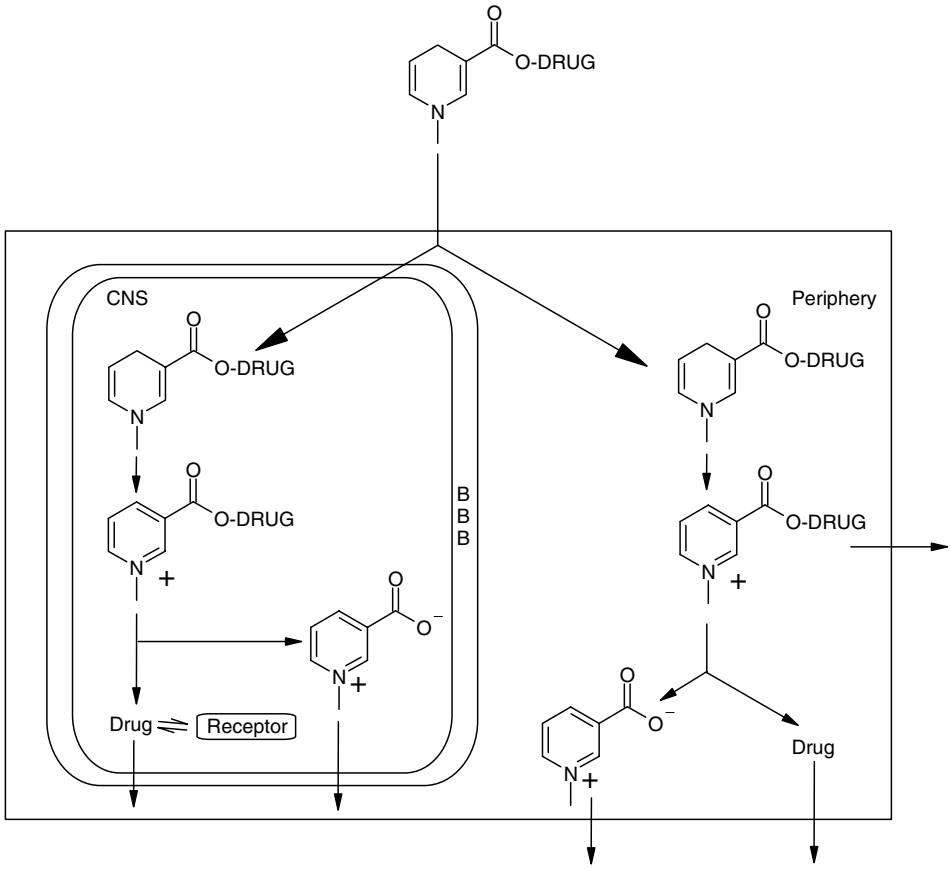
1. Introduction

Historically, drug design has relied on the optimization of compound potency as its primary goal. While this addresses one aspect of the development of new pharmaceutical agents, it ignores several others including toxicity and drug delivery. The result of this single parameter-guided quest for more potent compounds often gives rise to pharmacologically active agents which have greater toxicological liabilities and thus, do not result in any improvement in the therapeutic index. Consequently, no clinical advancements are obtained. The acknowledgment that factors other than potency are important in drug development are embodied in a suite of design guidelines known broadly as the retrometabolic design approach [1–3]. The retrometabolic approach can be divided into several substrategies including the soft drug (SD) methodology [4, 5] wherein drug candidates are generated that have the required biological activity but which are designed to undergo facile and safe deactivation subsequent to receptor activation and chemical delivery systems (CDS) [6–8] which are inactive entities converted through multiple enzymatic/chemical steps to give rise to an active agent. The manipulation of this activation process can also lead to tissue targeting. This short overview will concentrate on the CDS technology although examples of the use of cyclodextrins to improve formulations of SD are available [9–11].

Chemical delivery systems are, in their broadest definition, an active pharmacological agent which is covalently attached to a carrier or targetor in such a way that activity of the agent is lost [1]. CDS differ from prodrugs in that prodrugs generally require a single chemical/enzymatic step to release the active agent while this is a multistep process for CDS derivatives. One of the most successful for the CDS embodiments is a system design to target drug to the brain and central nervous system (CNS) [6, 7, 12, 13]. As outlined in the Scheme, the drug of interest is covalently bound to a functional targetor, in this case, 1-methyl−1,4-dihydronicotinic acid to form an ester [14]. The new conjugate generally has little or no pharmacological or toxicological potential. In addition, the lipophilicity of the ester is higher than that of the starting alcohol or acid and as such can penetrate barriers such as the blood-brain barrier (BBB) in a facile manner. Once the conjugate is in the CNS, it can, in analogy with the NAD(P)H – $NAD(P)^+$ co-enzyme interconversion, undergo transformation to the corresponding quaternary salt which imparts to the conjugate a dramatic change in water solubility with the log P dropping several units. Because of the "tight" nature of intracellular (endothelial) junctions of the BBB [14], this conversion effectively traps the oxidized conjugate within the CNS. In the rest of the body, conversion to the quaternary salt accelerates peripheral excretion by the liver or kidney generating a scenario where CNS levels of the conjugate are high and sustained while peripheral levels fall rapidly. The trapped quaternized conjugate can then hydrolyze giving rise to the active drug which is then free to interact with appropriate receptor to produce the desired effect without stimulation in non-target site areas. This principle has been demonstrated with a number of drugs as is illustrated in Table 1 [7].

CDS derivatives are associated with special developmental issues related to their unique mechanisms and this is evident in the preparation and optimization of formulations for the products [15, 16]. The CDS-derived drugs are, by definition, lipophilic to allow BBB penetration and CNS uptake but this property also renders to the compounds poor water solubility. In addition, the oxidative lability of these materials, which is essential for their ability to be trapped within the CNS, and the hydrolytic instability, which allows for drug release from the carrier moiety, combine to limit the pharmaceutical shelf-life of these products. One approach that we have applied to address these dosage form issues is the use of chemically modified cyclodextrins [15, 17]. Cyclodextrins are cyclic sugar oligomers derived from starch containing various numbers of α-1,4-lined glucose residues (α = hexomer (α-CD), β = heptomer (β-CD), γ = octomer (γ-CD), δ = nonamer (δ-CD)) (Table 2) [18, 19]. These oligomaltose systems take the form of a truncated cone wherein the primary hydroxyl functions of the sugar groups are oriented to the narrower end of the torus while the secondary group are oriented to the

Scheme 1: Brain-targeting chemical delivery systems



wider face. This architecture generates a hydrophilic external surface which provides for aqueous solubility and a lipophilic cavity into which appropriately sized molecules can include and form water-soluble complexes. No covalent bonds are formed or broken during guest/host (i.e., drug/cyclodextrin) complex formation and in solution free guest and host molecules are in a dynamic equilibrium with the complex. In addition to increasing the water solubility of the guest substrates, this equilibrium can increase their chemical stability (based on insulating a sensitive portion of the chemical guest from the environment), reduce unaesthetic smells and tastes, convert oils and liquids

Table 1: Application of chemical delivery systems of pharmacologically active agents [7]

Centrally acting amines

Dopamine
γ-Aminobutryic acid
Tryptamine
L-Tryptophan
Adenosine
Desipramine
Tranylcypromine

Steroids

Testosterone
Progesterone and Progestins
Dexamethasone
Estradiol and Other Estrogens

Antiviral Agents

AZT
Acyclovir
Trifluorothymidine
1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-(E)-5-(2-iodovinyl)-uracil
Gancyclovir
(E)-5-(2-iodovinyl)−2'-deoxyuridine
Ribavirin
2′-Fluoro-5-methylarabinosyluracil

Anticancer Agents

Chloroethyl cyclohexyl nitosourea (CCNU)
Chlorambucil

Anticonvulsants

Phenytoin
Valproic acid
Stiripentol
Felodipidine

Antibiotics

Benzyl penicillin
Cephalotin

Others

Naproxen
Tacrine
Propofol
Sulfonamides
LY231617
Nerve Growth Factor

Neuropeptides

DADLE (enkephalin analog)
Kyotorphin analog
Thyrotropin release hormone analog

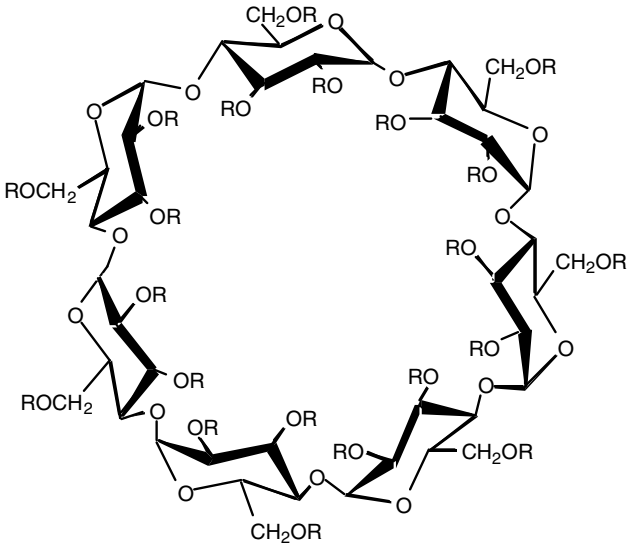
Table 2: Properties of α-, β- and γ-cyclodextrin

Properties	α-CD	β-CD	γ-CD
Glucose units	6	7	8
MW (g/mol)	972	1135	1297
Cavity diameter (Å)	5	6.2	7.9
Cavity volume (Å^3)	176	346	510
Height of torus (Å)	7.9	7.9	7.9
H_2O solubility (%w/v)	14.5	1.85	23.2
Water of crystallization (%)	10.2	13–14	8–18
H_2O per cavity	6	11	17
pKa	12.33	12.20	12.08

to powders and reduce various formulation-based incompatibilities [20]. Unfortunately, the cyclodextrin which is of the best size to form complexes with the majority of drugs, i.e., β-cyclodextrin (β-CD), is itself only poorly water-soluble (Table 2). This results from intramolecular hydrogen bonding and associated high crystal lattice energy. To overcome this limitation, β-CD has been chemically altered to generate derivatives which are more soluble but which also retain the beneficial complexation characteristics of the parent compound. Of the chemical derivatives of β-CD, the most successful to date is 2-hydroxypropyl-β-cyclodextrin (HPβCD) [19, 21–23]. HPβCD is prepared by treating base solubilized β-CD with propylene oxide followed by purification. As with other chemically modified pharmaceutical starches, HPβCD is a statistical mixture of various hydroxypropylated isomers with a defined substitution average and substitution distribution. These materials can be made reproducibly and in large quantities. Importantly, HPβCD is not toxic even at high doses [24, 25] and interacts with lipophiles in a quantitatively similar manner as does β-CD. Since the introduction of HPβCD other chemically modified cyclodextrins have also been prepared and tested including the sulfobutyl ether of β-CD known as SBE-β-CD [26, 27].

Once included in the cyclodextrin cavity, the guest molecule may be released through complex dilution, by replacement of the included guest by some other suitably sized molecule such as dietary lipids or, if the complex is located in close approximation to a lipophilic biological membrane such as the oral mucosa, the guest may be transferred to the matrix for which it has the highest affinity.

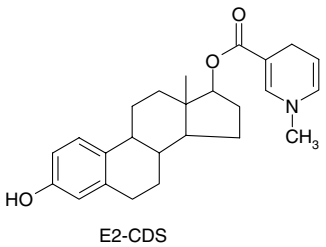
The regulatory status of the cyclodextrins continues to evolve. β-Cyclodextrin is used in a number of formulations in numerous countries throughout the world. The excipient is also compendial in the US (USP24/NF19) and Europe (Ph. Eur. 3rd Ed.). In addition, an initiative has begun to add β-CD to the Generally Regarded as Safe (GRAS) list maintained by the US Food and Drug Administration (FDA) [28]. HPβCD is available in approved products in the US, Europe and the rest of the world including Sporanox® (itraconazole) oral and i.v. solution, a hydrocortisone-based mouth wash (Iceland) and an indomethacin-based eye drop (France). The i.v. dose of HPβCD can be as high as 16 g/day in the Sporonox i.v. product and as high as 8 g/day in the oral formulation [29–33]. Recently, an i.m. formulation of Zeldox (ziprasidone) which contains SBE-β-CD has been approved in Sweden while an HPγCD eye drop solution for Voltaren (diclofenac) has been approved in France. These properties of cyclodextrins including their proven safety in man make them potentially useful excipients for CDS. This has been borne out in the literature where numerous examples have been published [7, 17, 34, 35]. Several of these applications will be presented to illustrate the applicability of HPβCD to these important drug candidates.



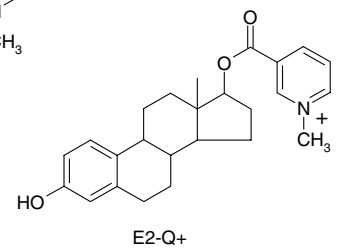
R = −H, β-cyclodextrin (β-CD)
R = $-CH_2CHOHCH_3$ or −H, 2-hydroxypropyl-β-cyclodextrin (HPβCD)
R = $-(CH_2)_4SO_3Na$ or −H, sulfobutylether-β-cyclodextrin (SBEβCD)

2. Estradiol-CDS

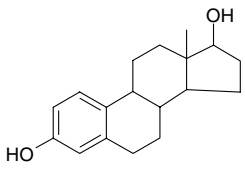
Estradiol (E2) exerts potent actions in the CNS of both males and females. These lipophilic steroids have been implicated in the treatment of Alzheimer's disease, stroke, obesity, decreased libido as well as more traditional uses such as in the treatment of post-menopausal hot flushes and prevention of bone loss [17]. Unfortunately, peripheral exposure to estrogens has also been associated with cancer and metabolic disease. Targeting and trapping of estrogens to the brain may be useful in their safe exploitation as therapeutic modalities. A CDS for estradiol was prepared to test this hypothesis [36–40]. The CDS was constructed so that the 1-methyl-1,4-dihydronicotinate was linked to the 17-alcohol of estradiol (E2). Consistent with the operating principles of the CDS, the conjugated estradiol is more lipophilic than the parent steroid (log P of 4.5 (E2-CDS) versus 3.8 (E2)) while the quaternary nicoti-



E2-CDS



E2-Q+



E2

nate salt is significantly less lipophilic (log P of −0.14 (E2-Q+)) providing for the CNS trapping [39]. Animal studies bear out the CDS hypothesis. A single i.v. administration at doses as low as 500 μg/kg to ovariectomized rats elicited prolonged CNS-mediated pharmacological effects (3 to 6 weeks) using various endpoints including luteinizing hormone (LH) suppression and reduced weight gain [17, 37]. In castrate male rats, copulatory behavior was re-established over similarly prolonged periods [17]. Pharmacokinetically, the E2-CDS rapidly distributed subsequently to parenteral injection with concomitant appearance of the corresponding quaternary salt (E2-Q+). The salt was readily lost from the peripheral circulation but its elimination from the CNS was very slow. The half-life of the E2-Q+ in plasma was about 1 hour while brain levels disappeared with a half-life of 10 days [41]. The trapped E2-Q+ then hydrolysed to give rise to elevated levels of E2 in the CNS even though plasma levels were within the normal range [42].

In preliminary studies, E2-CDS was administered in the water miscible organic solvent, dimethyl sulfoxide (DMSO). The use of DMSO provided for adequate solubilization of the water insoluble E2-CDS and it is known to be a relatively safe substance [43]. However the solvent was clearly not useful in the context of human dosing prompting a search for more appropriate formulations. HPβCD was selected for assessment based on the arguments made in the introductory part of the article. HPβCD formed a useful complex with E2-CDS increasing the apparent aqueous solubility of the compound from 62 ng/ml to 21 mg/ml in an aqueous 43.5% (w/v) HPβCD solution (that is an increase in solubility of >300,000-fold) [16]. The phase solubility diagram (Fig. 1), suggested an A_p-type solubility isotherm based on the definitions of Higuchi and Connors [44]. This indicates 1 : 1 (drug to cyclodextrin) complexation at low HPβCD concentration and higher order complexation (i.e., 1 : 2) at higher HPβCD concentrations. A freeze-dried complex of the drug and excipient contained approximately 20 mg of E2-CDS per gram of complex. Stability studies of the dry powder indicated that cyclodextrin complexation also stabilized the dihydronicotinate as illustrated in Table 3 [16]. The data suggest a greater than four-fold increase in solid-state stability. In solution, the oxidative stability of E2-CDS in the absence and presence of HPβCD was assessed using a redox buffer containing potassium ferricyanide. As illustrated in Fig. 2, increasing concentrations of HPβCD reduces the second-order rate constant for oxidation such that at concentrations >5% w/v, the oxidation rate is suppressed 90% compared to solutions of the redox buffer

Table 3: Stability (as indicated by half-life in days) of E2-CDS and the E2-CDS/HPβCD complex in the solid state at various temperatures

Temperature (°C)	E2-CDS	E2-CDS/HPβCD
23	295	1447
60	20.4	76.2
70	8.7	31.5
80	4.6	11.0

that do not contain the cyclodextrin derivative [16]. Complexation with HPβCD shielded the chemically labile portions of the E2-CDS molecule against attack by water molecules, hydroxy ions and ferricyanide ions in the aqueous media. The stable, water-soluble complex was then tested in animals to compare its performance to the original DMSO systems. The two dosage forms were equivalents with regard to pharmacokinetic and biological activity parameters [17]. After i.v. administration, the E2-CDS was rapidly released from the complex through dilution and binding to plasma proteins. No precipitation of E2-CDS occurred when the aqueous HPβCD formulation was diluted with blood. Route of administration studies found that the E2-CDS/HPβCD complex could be successfully administered bucally in addition to parenteral routes. Through HPβCD complexation, E2-CDS was solubilized in the aqueous saliva and carried to the surface of the buccal mucosa. At the surface, the lipophilic E2-CDS partitions from the cyclodextrin cavity into the lipophilic mucosa. The large hydrophilic HPβCD molecule as well as the E2-CDS/HPβCD complex are unable to permeate lipophilic membranes, such as the buccal or intestinal mucosa. Safety of the E2-CDS/HPβCD complex was then determined in a number of toxicity assessments in preparation for human testing. The studies found that neither E2-CDS nor its complex with HPβCD was mutagenic (Ames test). In subacute (14 day) and subchronic (90 day) dosing in rats and monkeys, the no observable adverse effect dose was the maximum used in the protocol i.e., 5 mg/kg in monkeys and 2.5 mg/kg in rats [17].

Two sets of clinical studies have been carried out using E2-CDS complexed with HPβCD. The first was designed as an open label, i.v. rising dose tolerance study in healthy, post-menopausal volunteers [17, 45]. Each subject received E2-CDS in a 20% w/v solution of HPβCD with drug doses ranging from 10 μg to 1.28 mg. There was no adverse effect associated with the treatment and potent pharmacological action was observed including a dose-re-

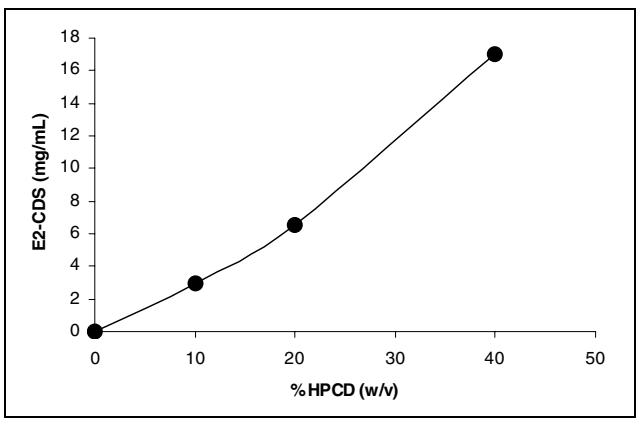


Fig. 1: Phase solubility relationship for E2-CDS and HPβCD

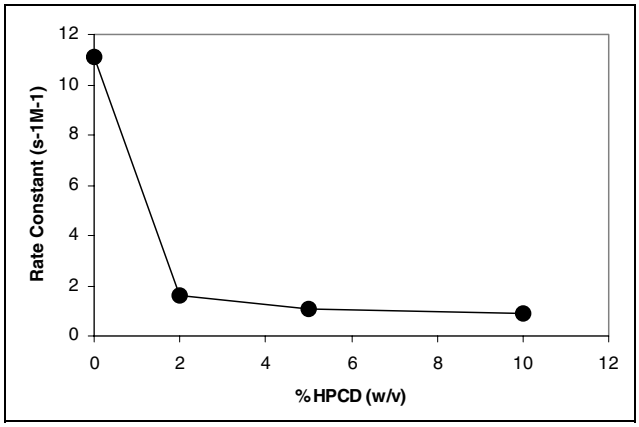


Fig. 2: Effect of HPβCD on the second-order rate constant for ferricyanide-mediated oxidation

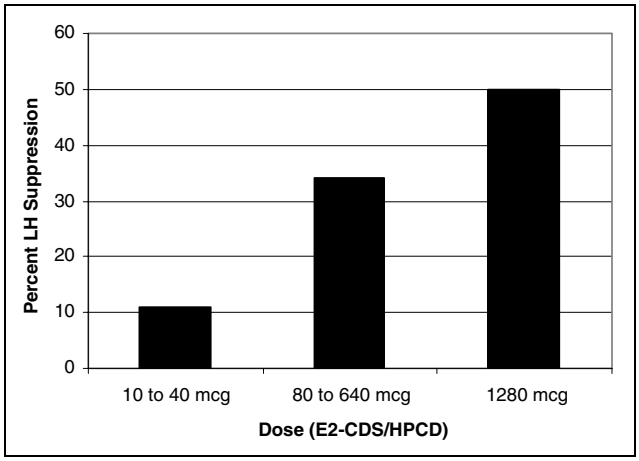
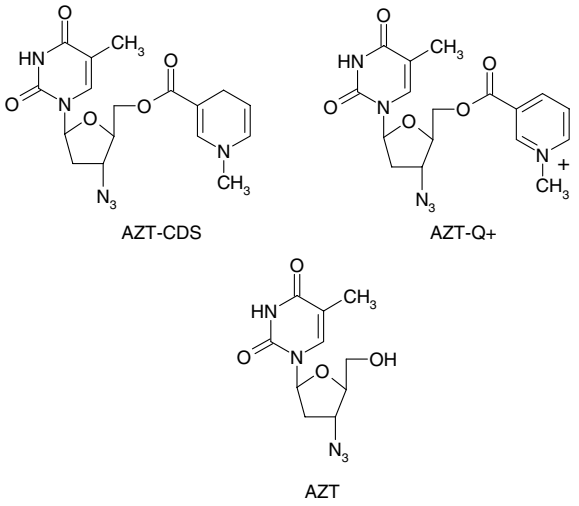


Fig. 3: Effect of various doses of E2-CDS/HPβCD on mean LH suppression relative to baseline in post-menopausal volunteers



lated suppression of serum LH concentrations (Fig. 3). When compared with E2 (also solubilized with HPβCD), the effect of E2-CDS on the reduction of LH was greater while peripheral estrogen levels were much lower after treatment with the CDS. In a second study, a similar population was assessed and a broaden protocol applied [46]. The clinical trial included an i.v. treatment arm, a buccal treatment portion to assess feasibility of that route of administration and a comparitor arm (oral treatment with 1.0 mg Progynova® (estradiol valerate)). For both buccal and i.v. dosing E2-CDS was administered in 20% aqueous HPβCD w/v. All treatments were well tolerated. The i.v. dosing provided similar results to the initial study with dose-dependent LH and FSH suppression. Based on the i.v. data the bioavailability after buccal treatment was approximately 25%. A comparison of either the i.v. and buccal administration with oral estrogen therapy indicated that the E2-CDS was superior with regard to activity and/or reduction of peripheral estradiol concentrations. In the case of E2-CDS, HPβCD was found to dramatically increase water solubility, increase solution and solid-state stability and to generate a formulation that was useful for animal and human testing.

3. Azidothymidine-CDS

AIDS encephalopathy is a significant and frequent complication of infection by the human immunodeficiency virus (HIV) [47]. Unfortunately, this component of AIDS is difficult to treat because of the effective exclusion of several potentially useful antiviral agents by the BBB. A pertinent example is azidothymidine (AZT or zidovudine). This was the first FDA-approved drug for the treatment of AIDS and while effective against the peripheral components of the disease, it poorly penetrates the CNS and has only marginal effects against AIDS encephalopathy [48]. Several groups have applied the CDS technology to AZT and similar nucleoside reverse transcriptase inhibitors [49–52]. The generated CDS (in which the dihydronicotinate is attached at the 5′ sugar hydroxyl function) was shown to readily penetrate the brain and CNS to deliver therapeutic levels of the antiviral agent. In several test animals (mice, rat, rabbit and dog) the AZT-CDS was several times more effective that AZT itself in delivering AZT to the brain [49–52]. As with E2-CDS, AZT-CDS was designed to be lipophilic to allow for BBB transit and enzymatically labile to provide for CNS trapping and like E2-CDS, DMSO served as an early vehicle. The optimization of a parenteral form for AZT-CDS was assessed using the following design criteria relative to the DMSO solution: a 10 to 50 mg/ml dosage form with a low potential for venous irritation and extravasation injury, a similar or improved pharmacokinetic profile and low systemic toxicity [53].
Solubility assessments demonstrated good solubility in DMSO, dimethyl acetamide and benzyl alcohol but poor solubility in ethanol, water, detergents and oils which eliminated surfactants and emulsion-based formulations. Various cyclodextrins were also examined but simple equilibration of a cyclodextrin solution and the AZT-CDS, or treatment of an aqueous cyclodextrin solution with a concentrated solution of the AZT-CDS in a water miscible solvent, followed by equilibration gave poor results [53]. The highest loading that could be prepared using these methods was approximately 4 mg AZT-CDS/g complex (in this case HPβCD). An alternate approach was suggested by Pitha and Hoshino in which the drug and HPβCD was dissolved in an alcohol, ideally ethanol, followed by solvent evaporation [54]. This approach was impractical due to the poorly solubility of AZT-CDS in ethanol and the resulting large volumes needed. In any case, best results were obtained using methanol as a solvent which generated a lyophilized complex containing 26 mg AZT-CDS/g complex. Another line of research was to include pH adjustment. AZT-CDS, like the parent thymidine, has an ionizable proton at the N3 imine position. AZT-CDS is soluble in dilute NaOH or KOH and a solution could be made using 0.05 M Na_3PO_4 (pH 12) but these systems were not physically stable and precipitated soon after they were prepared. The possibility of combining the pH solubilization effects with those of HPβCD were then considered [55, 56]. Screening studies with an aqueous 43.5% (w/v) HPβCD solution and various basic buffers, including TRIS, K_2CO_3 and Na_3PO_4 were performed. The data obtained suggested significant increases in solubility could be reached with complex loadings of between 30 and 70 mg/g complex. These promising data directed our reseach efforts to the formation of AZT-CDS salts which were then solubilized in aqueous HPβCD solutions. The sodium, potassium and ammonium salts of AZT-CDS were screened and the potassium salt selected based on its stability and ease of preparation. Simple addition of the K^+ AZT-CDS to water resulted in a solution with a pH between 11 and 12 and which was not physically stable. A matrix was then developed to optimize the solution. Best results were obtained with a 30 mg/ml solu-

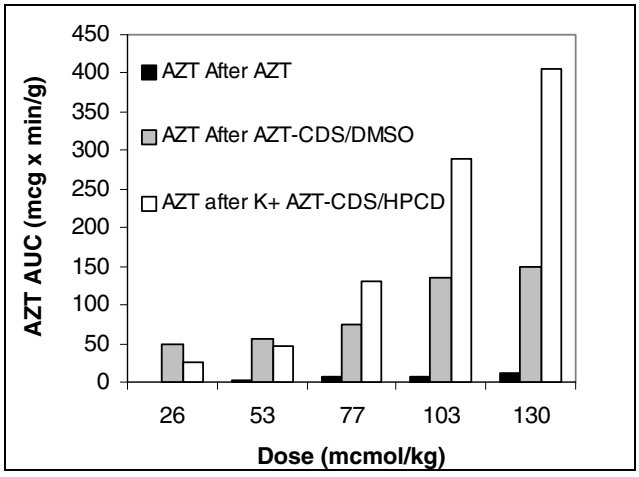


Fig. 4: Effect of CDS and formulation on brain exposure of AZT after treatment wit AZT, AZT-CDS in DMSO or K+ AZT-CDS in HPβCD

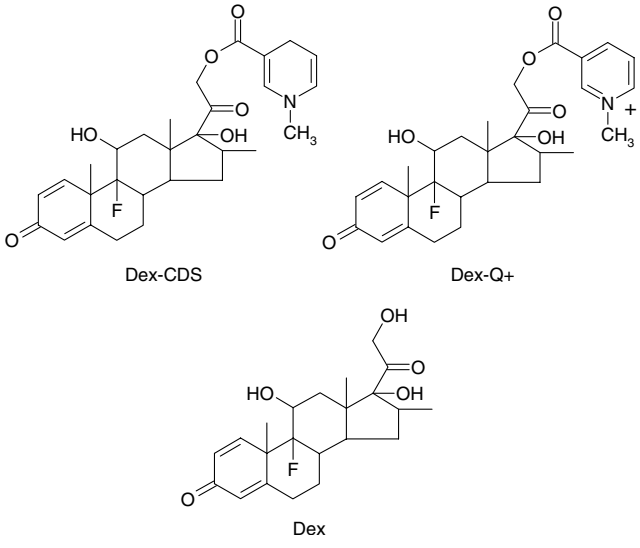
tion of the K^+ AZT-CDS in an aqueous solvent containing 15% (w/v) HPβCD and 0.005 M Na_3PO_4. This solution had a pH of 10.7 and an osmolality of 282 mOsm/kg. Testing of the formulation in a rat-tail vein model of venous irritation found that the formulation was similar to saline or the formulation in which the active principle was absent [53]. Organic solvent containing vehicles were notably more toxic. Pharmacokinetic studies were then performed in the rat comparing AZT, AZT-CDS in DMSO or K^+ AZT-CDS in the HPβCD vehicle at five doses equimolar to 26, 53, 77, 103 and 130 μmol/kg AZT. AZT-CDS in DMSO, provided for trapped AZT-Q^+ and increased levels of AZT (between 10 and 100-fold) in the brain relative to AZT dosing which was consistent with other studies. Blood levels were lower after AZT-CDS compared to AZT treatment providing a favorable brain to blood ratio. Administration of K^+ AZT-CDS in aqueous HPβCD solutions delivered similar peripheral levels of AZT levels as those generated by AZT-CDS in DMSO. In brain, however, the K^+ AZT-CDS systems were more efficient, generating AZT levels that were as high as 3-times those found after AZT-CDS/DMSO treatment (Fig. 4) [53]. The results show that AZT-CDS was effectively released from the complex after i.v. administration. The formulation fulfils the design requirements based on concentration, safety, tissue distribution and pharmacokinetics. The dosage form was useful in various mechanistic and more advance pharmacokinetic evaluations. A rising dose study in rats, for example, using the K^+ AZT-CDS/HPβCD system revealed that increasing doses of the CDS generated disproportionably larger AUC in brain, meaning that the brain to blood ratios increased from 20% at low doses to 80% at high doses [57]. The higher AZT levels may be related to auto-inhibition of efflux mechanisms which are known to eliminate AZT from the brain. In the dog, AZT-CDS generated higher AZT brain tissue levels (2 to 3-fold) and lower blood levels (−46%) than did AZT itself resulting in an improved brain to blood ratio [58].

4. Dexamethasone-CDS

Glucocorticoids, such as dexamethasone (DEX), are useful anti-inflammatory agents and are widely used in the treatment of the tumor-related edema [59]. Vasogenic edema is associated with increases in intracranial pressure (ICP) which can seriously complicate the disease prognosis since ICP is coupled with headaches, seizures and if not controlled cerebral herniation and brain stem compression. While high dose steroid treatment can be beneficial, it is complicated by numerous side effects and by poor permeability of certain compounds in the series through the BBB. These concerns were address by development of a CDS for dexamethasone using tools described herein. In this case, the dihydronicotinate was attached to the 21-alcohol position [60]. The poor water solubility of the DEX-CDS required a formulation optimization process. A cyclodextrin complex was considered and based on a formulation matrix a systems prepared in which the DEX-CDS was suspended in an aqueous 0.01 M borate buffer containing 5% (w/v) glucose and 45% (w/v) HPβCD [61]. Lyophilization generated a powder containing 26 mg of DEX-CDS/g complex. The availability of a water soluble DEX-CDS formulation allowed its testing in a brain rat tumor model. In these studies, the DEX-CDS/HPβCD or dexamethasone (also solubilized in HPβCD) were injected into rats containing a malignant fibrous histiocytoma implanted in sub-cortical white matter. The BBB integrity was measured by an Evans Blue extravasation method. Administration of the dexamethasone or DEX-CDS/HPβCD at 0, 8 and 24 h (i.e., the protocol that was found to be optimal for dexamethasone in the model) demonstrated significantly reduced BBB disruption compared with vehicle. In addition, the DEX-CDS was more potent than both dexamethasone and vehicle. When a single dose of the dexamethasone, Dex-CDS or vehicle were administered, only the DEX-CDS was active (Fig. 5) [61]. These data point to both a pharmacodynamic and pharmacokinetic advantage of the CDS, an estimation that was possible only because of the availability of a suitable formulation.

5. Enkephalin-CDS

As is the case with several of the examples already discussed, the delivery of centrally acting peptides to the CNS can be difficult due to the BBB. In the case of pep-



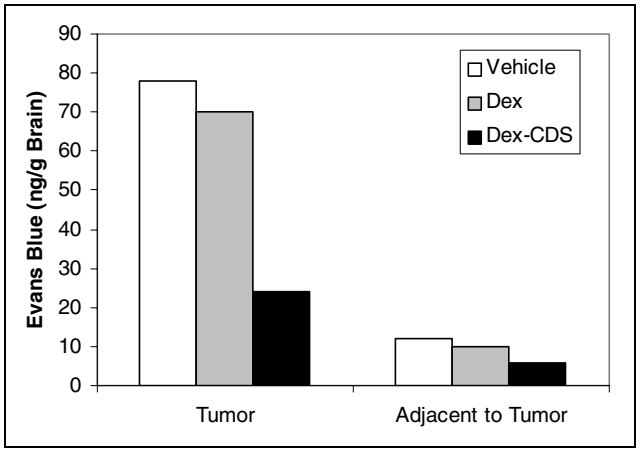
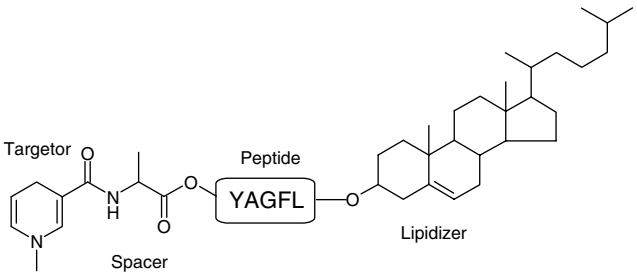


Fig. 5: Effect of 2 mg/kg DEX or equimolar DEX-CDS on BBB integrity as measured by Evans blue extravasation at 30 h after a single drug administration. The Dex-CDS treatment was significantly more effective ($p < 0.05$) that either vehicle or DEX.



tides, the BBB acts not only as a physicochemical barrier but also as a histochemical screen. That is, many peptides are destroyed by the numerous enzymes present at the BBB interface such as aminopeptidases, arylaminidases and enkephalinases. One method for the non-invasive delivery of small peptides to the CNS is the CDS but special modifications have to be made for this specific purpose [8]. An example is the delivery of the enkephalinic pentapeptide, Tyr-D-Ala-Gly-Phe-D-Leu (YAGFL) [62]. To generate a CDS of this compound, both the amino and carboxylic acid termini are derivatized. At the amino terminus, the dihydronicotinamide is attached via a spacer (i.e., alanine) to aim hydrolysis towards endopeptidase cleavage and especially towards hydrolysis by alanyl aminopeptidase. At the C-terminal end, a large bulky lipophilic group (cholesterol) is attached. This serves the dual function of protecting the Gly-Phe bond from hydrolysis by endopeptidase as well as of lipidizing (increasing the log P) of the conjugate [62]. The cholesterol ester can be cleaved by esterases or lipases. These changes dramatically alter the overall appearance of the peptide in compliance with CDS requirements but it also increases the formulation challenge. The "molecularly packaged" peptide, which is at this point poorly water-soluble, was formulated in a mixture of aqueous 50% (w/v) HPβCD solution and ethanol [62, 63]. The use of this formulation in rats allowed for the elucidation of the metabolic profile of the CDS including the eventual release of the active peptide as well as the demonstration of its biological activity.

6. Conclusions

Water-soluble cyclodextrins in general, and HPβCD in particular, have been identified as useful non-toxic excipients for poorly water-soluble drugs. They have been successfully applied to various CDS as well as to other components of the retrometabolic design loop making it possible to test these drug delivery systems in animals and humans. HPβCD provides for favorable pharmacokinetic and tissue binding profiles relative to their administration in organic-solvent based vehicles. As the regulatory status of cyclodextrins advance, their use will most likely expand.

This research paper was presented during the 3rd Conference on Retrometabolism-Based Drug Design and Targeting, May 13–16, 2001, Amelia Island, Florida, USA.

References

1 Bodor, N: Adv. Drug. Deliv. Rev. **14**, 157 (1994)
2 Bodor, N.: Pharmazie **55**, 163 (2000)
3 Bodor, N.: J. Controlled Rel. **62**, 209 (1999)
4 Bodor, N.; Buchwald, P.: Pharmacol. Ther. **76**, 1 (1997)
5 Bodor, N.; Buchwald, P.: Med. Res. Rev. **20**, 58 (2000)
6 Bodor, N.; Farag, H.; Brewster, M.: Science **214**, 1370 (1981)
7 Prokai, L.; Prokai-Tatrai, K.; Bodor, N.: Med. Res. Rev. **20**, 367 (2000)
8 Bodor, N.; Buchwald, P.: Adv. Drug Deliv. Rev. **36**, 229 (1999)
9 Bodor, N.: Drustrup, J.; Wu, W.: Pharmazie **55**, 206 (2000)
10 Reddy, I.; Khan, M.; Wu, W.; Bodor, N.: J. Ocul. Pharmacol. Ther. **12**, 159 (1996)
11 Alberth, M.; Wu, W.; Winwood, D.; Bodor, N.: J. Biopharm. Sci. **2**, 115 (1991)
12 Bodor, N,: S. T. P. Pharma Sci. **7**, 43 (1997)
13 Bodor, N.; Buchwald, P.; Huang, M.: SAR QSAR Environ. Res. **8**, 41 (1998)
14 Bodor, N.; Brewster, M.: Pharmacol. Ther. **19**, 337 (1983)
15 Brewster, M.; Loftsson, T.; Estes, K.; Mullersman, G.; Derendorf, H.; Bodor, N.; in: Huber, O.; Szejtli, J. (Eds.): Proceedings of the Fourth International Symposium on Cyclodextrins. p. 399, Kluwer Academic Publishers Dordrecht 1988
16 Brewster, M.; Estes, K.; Loftsson, T.; Perchalski, R.; Derendorf, H.; Mullersman, G.; Bodor, N.: J. Pharm. Sci. **77**, 981 (1988)
17 Brewster, M.; Simpkins, J.; Bodor, N.: Rev. Neurosci. **2**, 241 (1990)
18 Szejtli, J.: Cyclodextrins and Their Inclusion Complexes, Akademiai Kiado Budapest 1982
19 Loftsson, T.; Brewster, M.: J. Pharm. Sci. **85**, 1017 (1996)
20 Brewster, M.; Simpkins, J.; Hora, M.; Stern, W.; Bodor, N.: J. Parenteral Sci. Tech. **43**, 231 (1989)
21 Pitha, J.; Milecki, J.; Fales, H.; Pannell, L.; Uekama, K.: Int. J. Pharm. **29**, 73 (1986)
22 Pitha, J.; Pitha, J.: J. Pharm. Sci. **74**, 987 (1985)
23 Yoshida, A.; Arima, H.; Uekama, K.; Pitha, J.: Int. J. Pharm. **46**, 217 (1988)
24 Brewster, M.; in: Duchêne, D. (Ed.): New Trends in Cyclodextrins and Derivatives. p. 313, Editions de Santé Paris 1991
25 Brewster, N.; Estes, K.; Bodor, N.: Int. J. Pharm. **59**, 231 (1990)
26 Rajewski, R.; Traiger, G.; Bresnahan, P.; Jaberaboansari, J.; Stella, V.; Thompson, D.: J. Pharm. Sci. **85**, 1142 (1996)
27 Zia, V.; Rajewski, P.; Stella, V.: Pharm. Res. **18**, 667 (2001)
28 Szejtli, J.: Cyclodextrin News **10**, 1 (1996) (see Fed. Reg. **61** (164) Docket No. 96G–0324.)
29 Boogaerts, M.; Maertens, J.; Van Der Geest, R.; Bosly, A.; Michaux, J.; Van Hoof, A.; Clleren, M.; Woestenborghs, R.; De Beule, K.: Antimicrob. Agents Chemother. **45**, 981 (2001)
30 Cross, L.; Bagg, J.; Oliver, D.; Warnock, D.: J. Antimicrob. Chemother. **45**, 95 (2000)
31 Glasmacher, A.; Hahn, C.; Molitor, E.; Marklein, G.; Sauerbruch, T.; Schmidt-Wolf, I.: Mycoses **42**, 591 (1999)
32 Cross, L.; Bagg, J.; Aitchison, T.: Antimicrob. Agents Chemother. **44**, 425 (2000)
33 Stevens, D.: Pharmacotherapy **19**, 603 (1999)
34 Raghavan, K.; Loftsson, T.; Brewster, M.; Bodor, N.: Pharm. Res. **9**, 743 (1992)
35 Anderson, W.; Simpkins, J.; Brewster, M.; Bodor, N.: Drug Des. Del. **2**, 287 (1988)
36 Brewster, M.; Estes, K.; Bodor, N.: J. Med. Chem. **31**, 244 (1988)
37 Brewster, M.; Bartruff, M.; Anderson, W.; Druzgala, P.; Bodor, N.; Pop, E.: J. Med. Chem. **37**, 4237 (1994)
38 Brewster, M.; Druzgala, P.; Anderson, W.; Huang, M,; Bodor, N.; Pop, E.: J. Pharm. Sci. **84**, 38 (1995)
39 Bodor, N.; McCornack, J.; Brewster, M.: Int. J. Pharm. **35**, 47 (1987)
40 Simpkins, J.; McCornack, J.; Estes, K.; Brewster, M.; Shek, E.; Bodor, N.: J. Med. Chem. **29**, 1809 (1986)
41 Mullersman, G.; Derendorf, H.; Brewster, M.; Estes, K.; Bodor, N.: Pharm. Res. **5**, 172 (1988)

42 Sarkar, D.; Friedman, S.; Yen, S.; Frautschy, S.: Neuroendocrinol. **50**, 204 (1989)
43 Brayton, C.: Cornell Vet. **76**, 61 (1986)
44 Higuchi, T.; Connors, K.: Adv. Anal. Chem. Instrum. **4**, 117 (1965)
45 Howes, J.; Bodor, N.; Brewster, M.; Estes, K.; Eve, M.: J. Clin. Pharmacol. **28**, 951 (1988)
46 Brewster, M.; Howes, J.; Griffith, W.; Garty, N.; Bodor, N.; Anderson, W.; Pop, E.; in: Szejtli, J.; Szente, L. (Eds.): Proceedings of the Eight International Symposium on Cyclodextrins. p. 507, Kluwer Academic Publishers Dordrecht 1996
47 Pajeau, A.; Roman, G.: Psychiatr. Clin. North Am. **15**, 455 (1992)
48 Schmitt, F.; Bigley, J.; McKinnis, R.; Logue. P.; Evans, R.; Drucker, J.: N. Engl. J. Med. **319**, 1573 (1988)
49 Little, R.; Bailey, D.; Brewster, M.; Estes, K.; Clemmons, R.; Saab, A.; Bodor, N.: J. Biopharm. Sci. **1**, 1 (1990)
50 Brewster, M.; Anderson, W.; Bodor, N.: J. Pharm. Sci. **80**, 843 (1991)
51 Chu, C.; Bhadti, V.; Doshi, K.; Etse, J.; Gallo, J.; Boudinot, F.; Schinazi, R.: J. Med. Chem. **33**, 2188 (1990)
52 Lupia, R.; Ferencz, N.; Lertora, J.; Aggarwal, S.; George, W.; Agrawal, K.: Antimicrob. Agents Chemother. **37**, 818 (1993)
53 Brewster, M.; Loftsson, T.; Amselem, S.; Friedman, D.; Yogev, A.; Anderson, W.; Helton, D.; Dinculescu, A.; Bodor, N.; Pop, E.: Int. J. Pharm. **125**, 17 (1995)
54 Pitha, J.; Hoshino, T.: Int. J. Pharm. **80**, 243 (1992)
55 Tinwalla, A.; Hoesterey, B.; Xiang, T.; Lim, K.; Anderson, D.: Pharm. Res. **10**, 1136 (1993)
56 Liu, F.; Kildsig, D.; Mitra, A.: Pharm. Res. **9**, 1671 (1992)
57 Brewster, M.; Anderson, W.; Helton, D.; Bodor, N.; Pop, E.: Pharm. Res. **12**, 796 (1995)
58 Brewster, M.; Anderson, W.; Webb, A.; Pablo, L.; Meinsma, D.; Moreno, D.; Derendorf, H.; Bodor, N.; Pop, E.: Antimicrob. Agents Chemother. **41**, 122 (1997).
59 Criscuolo, G.: Yale J. Biol. Med. 66, 277 (1993)
60 Anderson, W.; Simpkins, J.; Brewster, M.; Bodor, N.; Neuroendocrinol. **50**, 9 (1989)
61 Siegal, T.; Soti, F.; Biegon, A.; Pop, E.; Brewster, M.: Pharm. Res. **14**, 672 (1997)
62 Bodor, N.; Prokai, L.; Wu, W.; Farag, H.; Jonnalagadda, S.; Kawamura, M.; Simpkins, J.: Science **257**, 1698 (1992)
63 Bodor, N.; Wu, W.; in: Labandeira, J.; Vila-Jato, J. (Eds.): Proceedings of the Ninth International Symposium on Cyclodextrin. p 251, Kluwer Academic Publishers Dordrecht 1999

Marcus E. Brewster. Ph.D.
Department of Drug Delivery Research
Janssen Research Foundation
Turnhoutseweg 30
2340 Beerse BELGIUM
mbrewste@janbe.jnj.com