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How cyclodextrins can mask their toxic effect on the blood–brain barrier

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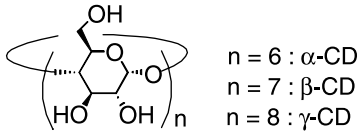
Abstract—The toxicity of monosubstituted *n*-alkyldimethylammonium-β-cyclodextrins (DMA-C_n-CD with $n = 2$, 4 and 12) towards endothelial cells of an in vitro model of the blood–brain barrier (BBB) was evaluated and compared to that of the native β-CD. DMA-C_{12}-CD was found to be non-toxic below 10 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 the endothelial cells has been measured.

The understanding of the mechanisms of transport through the blood–brain barrier (BBB) constitutes one of the most exciting goals of many researchers throughout the world. Indeed, a better comprehension of the functioning of these barriers may lead to improved treatments of neurodegenerative diseases such as Parkinson's or Alzheimer 'syndromes.' The special nature of the BBB lies in the presence of tight junctions between the endothelial cells of the BBB which prevent any possibilities of paracellular transport.

During the last three years, we have focused our attention on the use of cyclodextrins (CDs—cyclic oligosaccharides composed of 6, 7 or 8 glucose units named α-, β- or γ-cyclodextrin, respectively—Scheme 1) to evaluate their potential to interfere with the cellular membranes and thus make possible the transfer of drugs from the blood to the brain.

CDs and their derivatives are widely used in the pharmaceutical field to improve the dissolution rate, chemical stability and bioavailability of drugs.[1] Nonetheless, only a few studies have been reported so far concerning the blood–brain barrier (BBB), and the results are rather contradictory.[2]



Scheme 1. Chemical structure of native cyclodextrin.

To clarify the role played by cyclodextrins on the BBB, we recently carried out a systematic study on native and modified CDs which clearly demonstrated that their ability to cross the BBB not only depended on their size but also on the presence of substituents such as methyls or hydroxypropylated groups.[3] Thus, the native CDs appeared to be the most toxic CDs. The toxicity of the native α-CD was higher than that of the β-CD whose toxicity was superior to that of the γ-CD. Whereas the chemical modification of β-CD (hydroxypropylated or methylated CDs) did not affect the toxicity of this CD, differences were observed for the α- and γ-CD. It was found that α-CD removed phospholipids and that β-CD extracted phospholipids and cholesterol. γ-CD was less lipid-selective than the other CDs. No structure/permeability relationship has been observed according to the nature and chemical modifications of CDs.

Keywords: Cyclodextrins; Blood–brain barrier; Toxicity; Permeability; Endothelial cells; In vitro model.
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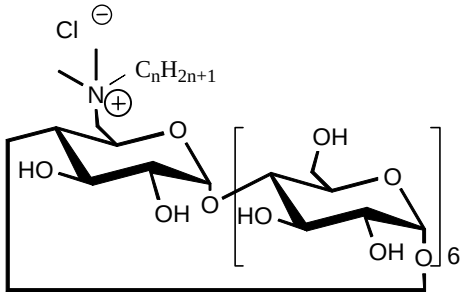
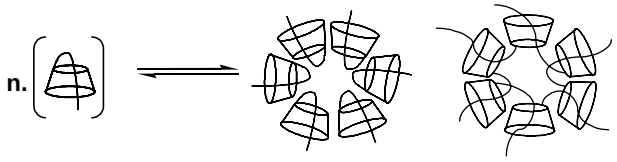


Scheme 2. Structure of dimethylalkylammonium-β-cyclodextrin (DMA-C_n-CD) $2 \leqslant n \leqslant 16$.

In this context, it was of great interest to evaluate the behaviour of a series of monosubstituted *n*-alkyldimethylammonium-β-CD derivatives (DMA-C_n-CDs with $2 < n < 16$) which we have recently synthesized (Scheme 2).[4]

Actually, these ammonium CD derivatives might be able to interact with the phosphates at the surface of the BBB endothelial cells and the alkyl chain might facilitate an interaction with the lipidic bilayer of the membranes.[5] Moreover, when the length of the alkyl chain was sufficient ($n > 4$), a self-inclusion process was demonstrated by surface tension measurements and NMR spectroscopy. Depending on the hydrophobicity of the medium, the alkyl chain could be spread outside of the CD cavity or included inside it.

In this study, we first focused our attention on the supramolecular arrangement of DMA-C_n-CDs in water. Light scattering measurements have evidenced the existence of nanoparticles of various diameters (from 50 to >1000 nm), proving that free DMA-C_n-CDs were involved in an equilibrium process with their aggregated forms (micelles...) (Scheme 3) as already observed previously.[6]



Scheme 3. Possible structures of aggregates of DMA-C_n-CD.

To assess the benefit that might be obtained on the transport through the BBB using these modified CDs, studies of toxicity and endothelial permeability have been carried out on an in vitro model of the BBB.[7]

The toxicity of DMA-C_n-CDs was evaluated as follows: the integrity of the brain endothelial cell monolayer during exposure to CDs was checked by determination of the endothelial permeability coefficient (Pe) of [^{14}C]sucrose across the BBB.[8] Sucrose diffused very slowly across the BBB in physiological conditions both in vitro and in vivo.[9] It was used as an indicator of the functional integrity of the tight junctions sealing the cells together, and a Pe(sucrose) higher than 1×10^{-3} cm min^{-1} was indicative of a leaky BBB. In our in vitro BBB model, the Pe(sucrose) across the monolayer was less than 1×10^{-3} cm min^{-1} (mean value of 0.60–0.05 $\times$ 10^{-3} cm min^{-1}) in the control conditions. The thresholds of toxicity of three DMA-C_n-CDs ($n = 2$, 4 and 12) have been determined and compared to that measured with the native β-CD. Figure 1 shows the variation of Pe(sucrose) as a function of the CD concentration for each CD (Fig. 1).

Once CDs were deposited on the luminal chamber of the coculture system, the evolution of Pe(sucrose) was very different according to the presence and the length of the alkyl chain. Actually, though the native β-CD topped the threshold of toxicity when its concentration was over 1 mM (Pe(sucrose) = 2.81×10^{-3} cm min^{-1} at a 2.5 mM concentration), DMA-C_2-CD was slightly toxic at 2.5 mM (Pe(sucrose) = 1.07×10^{-3} cm min^{-1}) and DMA-C_4-CD or DMA-C_{12}-CD remained non-toxic (Pe(sucrose) = 0.85×10^{-3} and 0.40×10^{-3} cm min^{-1}, respectively). At 5 mM, DMA-C_2-CD appeared to be as toxic as β-CD (Pe(sucrose) = 4.00×10^{-3} cm min^{-1}) and DMA-C_4-CD became toxic (Pe(sucrose) = 1.88 $\times$ 10^{-3} cm min^{-1}). At this concentration, only DMA-C_{12}-CD did not alter the integrity of the cell monolayer (Pe(sucrose) = 0.49×10^{-3} cm min^{-1}). The effect was more marked when increasing the CD concentration to 10 mM. Indeed, the toxicity of β-CD, DMA-C_2-CD and DMA-C_4-CD was greatly enhanced

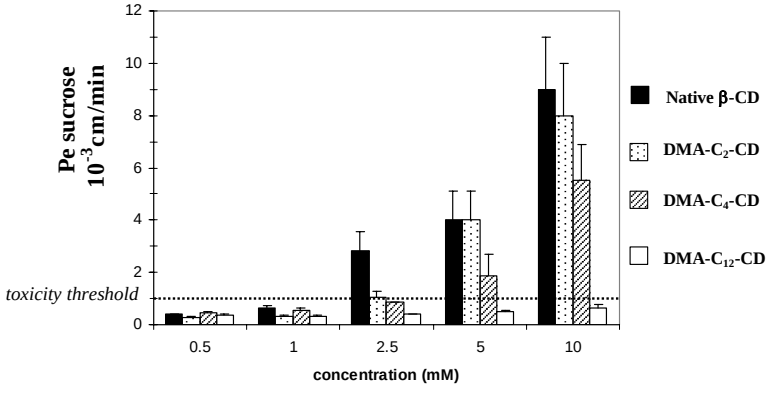


Figure 1. Effect of DMA-C_n-CD on the endothelial permeability coefficient for sucrose after 4 h incubation at 37 °C.

(Pe(sucrose) = 9.00×10^{-3}, 8.00×10^{-3} and 5.50×10^{-3} cm min^{-1}, respectively). By contrast, DMA-C_{12}-CD always remained non-toxic ((Pe(sucrose) = 0.64×10^{-3} cm min^{-1}). Consequently, obstructing the CD cavity by a long alkyl chain resulted in a non-toxic effect of β-CDs on the endothelial cells.

To get information about the ability of DMA-C_n-CD to cross the BBB, a study on the luminal-to-abluminal transport was performed on our model.[8] In particular, it was of great interest to compare β-CD and DMA-C_{12}-CD to evaluate their respective role on the BBB knowing that their thresholds of toxicity were very different. In non-toxic conditions (<1 mM), the percentages of passage of these CDs were very close (20% for β-CD and 18% for DMA-C_{12}-CD (determined by LC–MS analysis)). On the other hand, at 5 mM, β-CD was toxic and its percentage of passage could not be determined. By contrast, though DMA-C_{12}-CD was not toxic at 5 mM, 30% of DMA-C_{12}-CD was recovered in the abluminal compartment.

The masked amphiphilic structure of DMA-C_{12}-CD was believed to be responsible for both the non-toxic character of this CD and its permeability through the BBB. Actually, the above observations were perfectly consistent with the hypothesis that the CD cavity was hindered by the alkyl moiety and so became unable to extract the phospholipids or cholesterol of the membrane. Therefore, the membrane integrity was preserved. This also gives indirect proof of the extracting role of the native β-CD towards the constitutive molecules of the BBB as previously established.[3] Interestingly, despite its non-toxic character, the percentage of passage of DMA-C_{12}-CD was 30% in toxic conditions for β-CD (5 mM). Experiments are currently under progress to determine whether the CD could cross the BBB in its single form or if the aggregated forms were necessary.

As a conclusion, DMA-C_{12}-CD was not toxic towards the BBB endothelial cells below 10 mM and could thus be used in larger amounts than β-CD. Thanks to the remarkable properties of DMA-C_{12}-CD, we envisage to use this CD to improve drug delivery through the BBB.

Acknowledgment

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8. *Transport studies*. The transport studies were conducted at 37 °C in buffered Ringer's solution at pH 7.4. Prior to the transport experiment, cell monolayers were washed with Ringer's solution. At the start of the experiment, 2.5 ml of buffered Ringer's solution was added to wells of a six-well plate. One insert containing a confluent BCEC monolayer was placed in the first well of the six-well plate. Then 1.5 ml of buffered Ringer's solution containing CDs or not (control) at the required concentrations was placed at time 0 in the apical compartment. The plates were then placed on a rocking platform. At selected times (30, 60 and 120 min after the addition of the solution containing CDs), the insert was moved to other wells of the plate to minimize backdiffusion of the compound to the upper compartment. Three inserts with the brain capillary endothelial cell (BCEC) monolayer and three without cells were assayed for each solution. Amounts of CD in the lower compartment were analyzed by high-performance liquid chromatography (mobile phase: acetonitrile–water gradient). The conditions were as follows: a Finnigan P4000 pump and LCQ-DUO mass spectrometer detector (Thermo Finnigan, Courtaboeuf, France) and a column Hypersil C18 BDS (150 × 3.2 mm). Using the same procedure, the integrity of the BCEC monolayer was checked by adding [^{14}C]sucrose (58 mCi/mmol) (Amersham Biosciences Inc. (Piscataway, NJ)) in the upper compartment containing the tested solutions. Amounts of radiotracers in the lower compartment were measured in a liquid scintillation counter (Tri-Carb 2100TR; PerkinElmer Life and Analytical Sciences, Boston, MA). *Data analysis and calculation.* The amount of sucrose crossing the BBB was expressed in endothelial permeability (Pe, centimetres per minute). The cleared volume was calculated as described by Siflinger-Birnboim et al.[10] by dividing the amount of compound in the receiver compartment by the drug concentration in the donor compartment at each time point. The average cumulative volume cleared was plotted versus time, and the slope was estimated by linear regression analysis to give the mean and standard deviation of the estimate. The slope of the clearance curve with inserts alone and inserts with BCEC monolayer is equal to PSf and PSt, respectively, where PS = the permeability surface area product. The units of PS and S are microlitres per minute and square centimetres, respectively. The PS value for endothelial monolayer (PSe) was obtained as follows: 1/PSe = 1/PSt − 1/PSf. To generate the endothelial permeability coefficient Pe (centimetres per minute), the PSe value was divided by the surface area of the insert. For the CDs, results were

expressed as a percentage of transport across the BCEC monolayer alone and were obtained from the transport across the inserts coated with collagen and seeded with BCECs and the transport across the inserts coated only with collagen.

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