

2-Hydroxypropyl-β-cyclodextrin does not cross the blood-brain barrier (BBB) in +/+ or *Npc1*^{-/-} mice

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Introduction

Cyclodextrins are complex carbohydrates. 2-Hydroxypropyl-¹⁴C-propyl-β-cyclodextrin is composed of seven glycosidic residues complexed into a ring structure. Cyclodextrins have the property of being able to form inclusion complexes with smaller molecules within the hydrophobic cavity formed by the sugar ring (Dodzuik 2006). Cyclodextrins readily complex with the steroid cholesterol and have been demonstrated to deplete intracellular cholesterol from macrophages in tissue culture (Kiladonk et al. 1995). It was shown by Griffin et al. (2004) that allongeneolone complexed with CD delayed the onset of neurodegeneration and reduced the storage of GM2 and GM3 gangliosides in *Npc1*^{-/-} mice. A later publication by Davidson et al. (2009) showed that cyclodextrin treatment alone was effective in reducing neurodegeneration and ganglioside storage and prolonged life in *Npc1*^{-/-} mice. It was naturally assumed that CD was able to enter the brain complex with, and remove, cholesterol from brain cells and be cleared from the CNS by bulk flow and clearance of cerebrospinal fluid to blood whence it could be eliminated by the kidney. However other carbohydrates, for which no transporter exists at the blood-brain barrier (BBB), for example sucrose (342.30 Da) and inulin (~6000 Da) remain almost exclusively in the vascular compartment and do not enter brain (Smith 1988). It was therefore thought imperative to investigate the interaction of CD (MW 1300 Da) with the BBB.

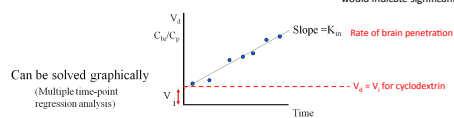
Methods

Brain uptake of 2-Hydroxypropyl-¹⁴C-propyl-β-cyclodextrin (CD - specific activity 6.95mCi/g) was determined in +/+ (BalbC) and *Npc1*^{-/-} mice using two methods. In situ brain perfusion and multi-time-point regression analysis following intraperitoneal administration. For in situ brain perfusion a cannula was inserted into the aorta via the left ventricle, the descending aorta tied off and the upper part of the animal perfused at a rate of 10ml/min with a buffered saline solution containing tracer [¹⁴C]-CD at a radioactive concentration of 0.3µCi/ml. After two or four minutes of perfusion the experiment was terminated by decapitation and the brain removed from the skull. Brain regions were then manually dissected out and their contained radioactivity determined by scintillation counting. Under these conditions a volume of distribution in brain (V_d) is given by the expression $V_d = C_{brain}/C_{plasma}$ where C_{brain} is the CD radioactive concentration in brain, C_{plasma} the radioactive concentration of CD in the perfusate. Units are ml/g⁻¹. For multi-time-point regression analysis animals were injected intraperitoneally with 10µCi of CD and the experiment terminated by decapitation after varied times up to 1 hour. The volume of distribution in brain at different experimental times, was then plotted against experimental time (min). The slope of the resultant line gives a unidirectional influx constant (K_{in}) and the intercept represents the an instantaneous volume of distribution (V_d) or the vascular volume (Begley 1999). All experiments were conducted under general anaesthesia using a mixture of medetomidine HCl 1mg/kg and ketamine HCl 75 mg/kg under the provisions of the Animals Scientific Procedures Act 1986 and Home Office Project Licence 70/6381.

FIGURE 1 Uptake of Solutes by the Brain

$$(1) \text{ Basic equation } \frac{C_b(T)}{C_p(T)} = K_{in} \int_0^T C_p(t) dt + V_d$$

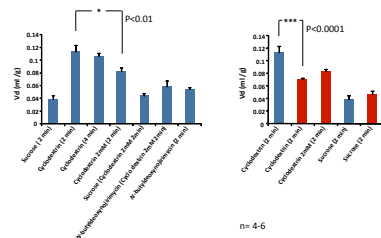
$$(2) \text{ Simplifies } \frac{C_b(T)}{C_p(T)} = K_{in}T + V_d \quad \text{When CNS exposure } C_p \text{ is constant}$$



The slope of a plot of V_d against T gives the unidirectional influx constant, K_{in} . K_{in} represents brain uptake with time. The intercept of the plot on the abscissa gives the value V_d which represents an instantaneous volume of distribution at zero time. This varies from solute to solute. For an inert tracer such as sucrose, with in situ brain perfusion this value approximates to the total vascular volume (0.04-0.05ml/g), with i.p. or i.v. administration of sucrose this value approximates the plasma volume 0.02-0.025 ml/g. If the V_d is substantially greater than these values this would indicate significant binding of tracer at the BBB.

Results

FIGURE 2 Cyclodextrin in-situ brain perfusion in +/+ and *Npc1*^{-/-} mice at 6-8 weeks of age



*The volume of distribution V_d of sucrose at 2m is 0.039 ml/g (3.9%) in both +/+ and *Npc1*^{-/-} mice and approximates to the vascular space

*In +/+ mice the V_d for CD is 0.113 ml/g at 2m and does not increase when the perfusion time is increased to 4m and indicates no further movement of CD into brain across the BBB.

*In +/+ mice addition of 2mM excess CD to the perfusate displaces a significant amount of CD from the BBB

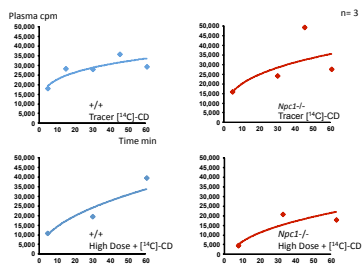
*In +/+ mice the addition of 2mM CD has no effect on the V_d for sucrose.

*These data suggest a significant binding of the CD to the BBB with no further penetration into brain.

*In *Npc1*^{-/-} mice the V_d for CD is significantly smaller than in +/+ mice.

*In *Npc1*^{-/-} mice the presence of 2mM excess cyclodextrin the V_d for is slightly but not significantly increased. The V_d for CD. This value now approximates the value obtained under the same conditions in +/+ mice.

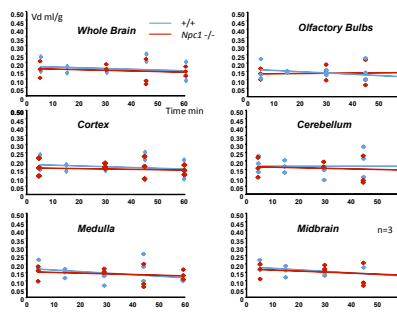
FIGURE 3 Plasma radioactivity of CD after tracer and high dose intraperitoneal administration in 6-8 week mice



*When tracer [¹⁴C]-CD is administered i.p. it is quickly absorbed and plasma levels rapidly plateau.

*When high dose CD (4000mg/kg) containing [¹⁴C]-CD is administered i.p. absorption is slower as the mixture is more viscous but by 30m plasma levels are similar to tracer CD.

FIGURE 4 Multi-time-point regression plot of tracer [¹⁴C]-cyclodextrin in 6-8 week old mice

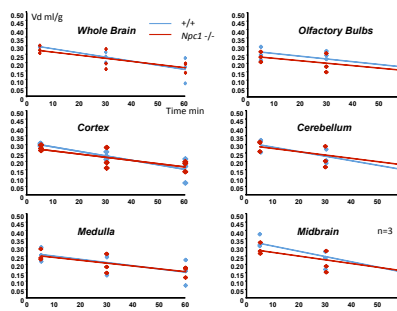


*In whole brain and the regions investigated there is no slope to the plot indicating no penetration with time of CD into the brain.

*The volume of distribution (V_d) for CD is very large 0.075-0.300 ml/g indicating a considerable binding of CD to the BBB.

*There is no difference in the data between +/+ and *Npc1*^{-/-} mice.

FIGURE 5 Multi-time-point regression plot of high dose [¹⁴C]-cyclodextrin in 6-8 week old mice



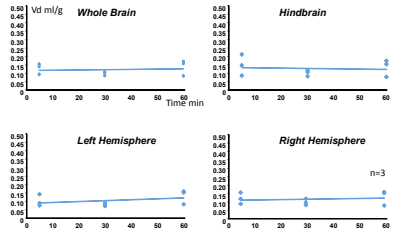
*In all brain regions there is an apparent negative slope to the plot. This is an pharmacokinetic artifact produced by the lower plasma values for CD at the shorter time points.

*The data still indicate no penetration of CD into the brain.

*The data still indicate a high level of binding of CD to the BBB.

*There is no difference in the data between +/+ and *Npc1*^{-/-} mice.

FIGURE 6 Multi-time-point regression plot of tracer [¹⁴C]-CD in P7 +/+ mice



*In 7 day old mice there is no evidence for brain penetration of CD.

*There is a similarly high binding of CD to the BBB.

*There is no difference in the BBB to CD between the 7day and 6-8 week old +/+ animals.

Conclusions

*None of these data collected so far, indicate that CD enters the brain.

*CD does however interact with the BBB in a number of unexpected ways.

*There appears to be a high degree of binding to the BBB possibly an interaction with the plasma membrane glycoplayc.

*This binding appears to be lower in the *Npc1*^{-/-} mice.

*With in situ perfusion 2mM CD displaces tracer CD from the BBB in +/+ mice but increases tracer binding in *Npc1*^{-/-} mice.

*This may suggest two binding sites, one high affinity low capacity and one low affinity high capacity and that the ratio of binding sites may change in the mutant mice with more low affinity high capacity sites exposed.

*The high binding of CD to the BBB may be associated with its ability to remove cholesterol from the brain.

*The interaction of CD with the BBB needs to be further investigated and the mechanism of how CD can remove cholesterol from the brain without entering the CNS needs elucidation.

*There is good evidence that CD administration in NPC disease reduces neuropathology but the mechanism of action is still unclear.

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