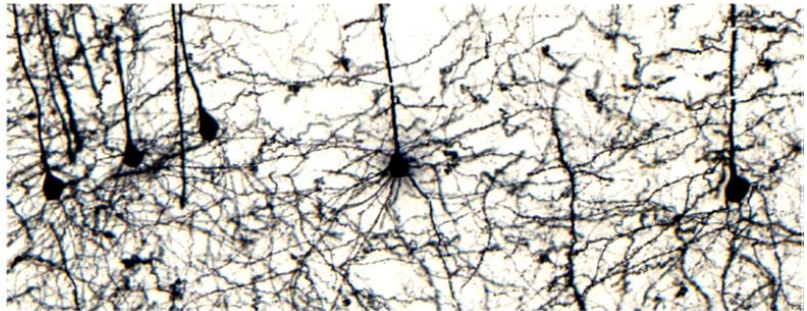


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**5th Hungarian Neuroscience Doctoral Conference for Undergraduate Students,
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26 January 2022, Budapest**

TESTING MODIFIED CYCLODEXTRINS ON CELL CULTURE MODELS OF THE BLOOD-BRAIN BARRIER

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Cyclodextrins (CDs) are cyclic oligosaccharides which can be used in therapy both as active agents or carrier molecules. Some CDs are used as therapeutic drugs, while others are currently tested in clinical studies to treat human neurological diseases, but the exact mode of action of cyclodextrins is unclear. CDs can interact with lipid membranes and selectively remove lipids from cell membranes. However, it is still a question whether CDs are able to cross the blood-brain barrier (BBB). BBB is a protective system that separates the central nervous system from the circulation and is anatomically based on the endothelial cells of the brain capillaries. The main roles of the BBB are creation of ionic homeostasis for neuronal functions, the supply of the brain with nutrients and protection from toxic insults. Most of the therapeutic compounds developed for neurological diseases have limited access to their brain targets because of the BBB. Our research hypothesis is, that coupling CDs with peptides that target BBB receptors may help the binding of these complexes to brain endothelial cells and their penetration across the BBB. In our experiments, we used beta-cyclodextrin (BCD) complexed with adamantane labelled with the fluorescent molecule rhodamine B (BCD/Ad-RhB), and BCD/Ad-RhB complexes modified with a peptide targeting the transferrin receptor of the BBB. These complexes were investigated in toxicity measurements and the entry of the complex into cells was assessed by fluorescence spectrophotometry. We investigated the cellular uptake of BCD-peptide complexes in brain endothelial cells and the permeability of these molecules across a cell culture model of the BBB. We demonstrated that none of the complexes affected the viability of brain endothelial cells in the tested concentration range during two-hour incubation time. The uptake of CDs was temperature dependent suggesting an active, energy demand cellular entry process. The permeability studies showed that the penetration of peptide-modified BCD/Ad-RhB complexes across the BBB was significantly higher as compared to unmodified CDs. Our results may contribute to the future application of different modified cyclodextrins in the therapy of neurological diseases.

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Testing modified cyclodextrins on cell culture models of the blood-brain barrier

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Introduction

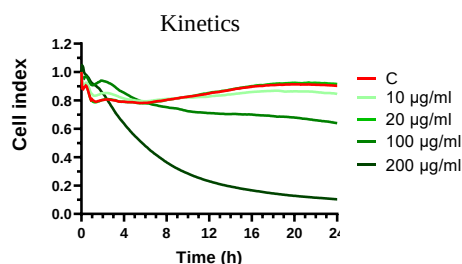
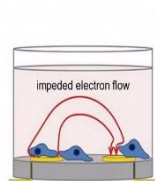
Cyclodextrins (CDs) are cyclic oligosaccharides which can be used in therapy both as active agents or carrier molecules, but the exact mode of action is unclear. CDs can interact with cell membranes and selectively remove lipids. However, it is still a question whether CDs are able to cross the blood-brain barrier (BBB). Our research hypothesis is, that coupling CDs with peptides that target BBB receptors may help the binding of these complexes to brain endothelial cells and their penetration across the BBB.

Methods

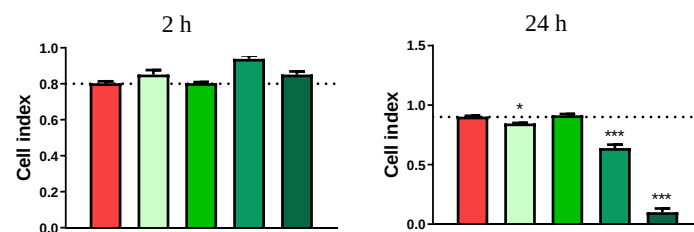
- CD complexes: β CD/Ad-RhB and peptide-modified β CD/Ad-RhB
- BBB model: rat primary brain endothelial cells co-cultured with pericyte and glia cells
- Viability: impedance-based real-time cell monitoring
- Cellular uptake: fluorescent spectrofluorometry from cell lysates after 2 hours incubation, and live-cell confocal microscopy
- Permeability assay: BBB marker molecules (fluorescein (376 Da), Evans blue-albumin (67 kDa) measured by fluorescent spectrofluorometry

Results

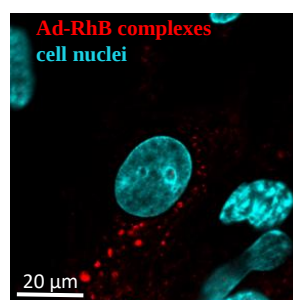
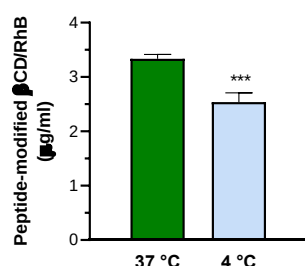
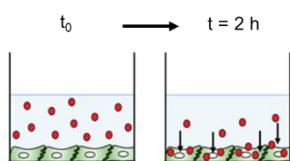
Viability experiments



Peptide-modified β CD/Ad-RhB



Cellular uptake experiments



Conclusions

The uptake of CDs was temperature dependent suggesting an active, energy demand cellular entry. The penetration of peptide-modified β CD/Ad-RhB complexes across the BBB was significantly higher as compared to unmodified CDs. Our results may contribute to the future application of different modified CDs in the therapy of neurological diseases.

Permeability experiments

