Schwarz *et al.*: *Intrinsic Activity*, 2023; **11**(Suppl.1):A2.15
doi:10.25006/IA.11.S1-A2.15
published online: 22 September 2023

INTRINSIC ACTIVITY

27th Scientific Symposium of the Austrian Pharmacological Society

Vienna, 29–30 September 2023

MEETING ABSTRACT

A2.15

Blood–brain barrier – Peptide shuttles for drug delivery into the CNS

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Background: Blood–brain barrier (BBB) permeability is needed for new therapeutics with targets in the central nervous system. Nevertheless, most molecules (proteins in general and over 98% of small molecules) fail to meet this requirement due to the tightly regulated transport processes into and out of the brain [1]. To overcome this limitation, the 'Trojan Horse' approach exploits endogenous receptor-mediated transcytosis, which can shuttle bioactive cargos across the BBB. A recent approach in the field are peptide BBB shuttles but intrinsic proteolytic instability of peptides, resulting in short half-life *in vivo*, remains an important challenge in the development of peptide therapeutics. Especially natural peptides promise solutions for this problem [2], as they can be used as scaffolds for molecular grafting to increase the stability of peptide therapeutics [3].

Methods: A series of peptide probes was designed using the molecular grafting method. As starting point, established peptides BBB shuttle motifs were selected and the sequences were incorporated into the proteolytically stabilized cyclic sunflower trypsin inhibitor 1 (SFTI-1) scaffold. We chose three different linear sequences (COG1410, peptide 22 and MiniAp-4) that are transported via different mechanisms, and designed novel hybrid molecules for bioactivity evaluation. To screen the probes, an endothelial cell monolayer transport assay (human cerebral endothelial cell line hCMEC/D3) was established using liquid chromatography–mass spectrography (LC–MS) for quantification of transport.

Results: The cell monolayer of hCMEC/D3 cells was validated by showing increasing tight junction protein expression until day 6 after seeding cells into the insert and stable expression until day 9. Lucifer-yellow permeability measurements showed that apparent permeability (Papp) values were ~ 2×10^{-6} from day 5 on, which is considered as low permeability in the literature. As control substances, atenolol (negative control), quinidine (positive control) and a reference peptide shuttle were tested in the assay and quantified using LC–MS. Papp values of 1×10^{-6} for atenolol, 8×10^{-6} for quinidine and 1.7×10^{-6} for peptide 22 were obtained.

Discussion: Integrity of the cell monolayer was validated and functionality of the assay set-up was confirmed by testing the reference substances atenolol and quinidine. We were not able to confirm the transport of peptide 22, which was published to be transported across the BBB. Next, we will screen the probes for transport across the BBB in this model and measure serum stability to demonstrate that a nature-derived peptide scaffold increases the stability of an incorporated linear peptide, while still mediating transcytosis. This work provides proof of concept for the design of novel stabilized peptide BBB shuttles.

Acknowledgements: The study was supported by the Austrian Science Fund FWF (grant ZK-81B).

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Keywords: blood–brain barrier – drug transport – peptides

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