NASAL DELIVERY OF PEPTIDES NANOFIBERS FOR ISCHAEMIC STROKE

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Background: Stroke is the second highest cause of death and the third leading cause of disability worldwide, with ischaemic stroke subtype accounting for 85% of cases. Recombinant tissue plasminogen activator (r-tPA) is the only FDA-approved thrombolytic agent, effective when administered intravenously within 4.5 hours after stroke onset, thus benefiting only 1-5% of patients. Angiotensin-(1-7) [Ang-(1-7), H_2N-DRVTIHP-COOH] has been shown to be neuroprotective by binding the Mas receptor and exerting anti-inflammatory, angiogenic, vasodilatory and anti-oxidative activities. Ang-(1-7) do not cross the blood-brain barrier in significant amounts and is cleared from the blood in less than 30 minutes. Our working hypothesis lies in engineering novel lipidized Ang-(1-7) analogs by the palmitoylation of the free hydroxyl group of Tyrosyl[4] residue to trigger the self-assembly of the peptide amphiphile (PA) in stable nanofibers that will be both enzymatically stable and brain permeable, while conserving binding to the Mas receptor. Formulating nano-in-microparticles based on mucoadhesive polymers of intermediate molecular weight, such as quaternised glycol chitosan (QGC) and quaternised pullulan (QPUL), we will direct and maximise the uptake of the new PAs via a direct nose-to-brain non-invasive delivery strategy for the early treatment of stroke patients.

Methods: Peptides were synthesized using solid phase peptide synthesis and characterized (ESI-MS, HPLC, FTIR, NMR). Self-assembly studies were undertaken using pyrene and thioflavin T (ThT) assays and complemented with transmission electron microscopy studies (TEM), while conformational studies using circular dichroism (CD) under different physiological pH, different temperatures and upon dilution were also carried out. The enzymatic stability in mouse plasma (50%v/v) and liver and brain homogenates (50%w/v) was assessed. An oxidative stress model was developed using organotypic brain slices treated with hydrogen peroxide and dihydroethidium (DHE) to measure superoxide in live cells. Novel QGC and QPUL polymers were synthesized and characterized (NMR, FTIR, GPC-LALLS), while nano-in-microparticles were prepared using spray-drying.

Results: ESI-MS, FTIR and NMR confirmed the synthesis and lipidisation of Ang-(1-7) lipopeptides as well as their parent analogues. Conformational studies revealed a stable β-sheet secondary structure of the lipopeptides upon different physiological pH, temperatures up to 37°C and upon dilution. Critical aggregation concentrations (CAC) studies indicated the formation of micelles (46.93 ± 6.81 nm) at 5.77 ± 1.62 μM (pyrene assay) and nanofibers (length: 0.2-1μm, width: 29.99 ± 5.65 nm) at concentrations above 54.16 ± 9.06 μM (ThT assay) in PBS (pH 7.4, 10 mM). Stability studies of lipidised analogues showed less than 20% degradation in 24h compared to Ang-(1-7) that was more than 50% degraded within 46, 5, and 11 minutes in plasma, brain and liver homogenates respectively, and was completely degraded after 4h. Coronal 300-μm thick slices treated with 10 mM H_2O_2 had ≥50% of cells producing superoxide compared to control, both in hippocampus and cortex. QGC (MW 60 KDa, DA 6.57%, DQ 11.46%) and QPUL (MW 60 KDa, DQ 32. 22%) polymers were synthesised and employed to prepare nano-in-microparticles with 1% peptide loading. Permeability studies across nasal lamp epithelia mucosa and across RPMI2650 cell layers and an all human blood-brain barrier model are pending.

Conclusions: Novel enzymatic stable Ang-(1-7) analogues were synthesized, able to form stable nanofibers stabilized by β-sheets. Their efficacy is being tested in *ex vivo* models of ischaemic stroke (oxidative stress, oxygen-glucose deprivation). Nano-in-microparticles have been prepared to enable nose-to-brain delivery, while their permeability will be tested using *ex vivo* nasal epithelial mucosa and cell monolayers, as well as an all human blood-brain barrier model.