P29 STABILITY OF A DISULFIDE BOND OF CHIMERIC PEPTIDE DURING IN VIVO TRANSCYTOSIS THROUGH THE BRAIN ENDOTHELIAL CELLS

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Drug delivery to the brain is facilitated by the synthesis of chimeric peptides, wherein neuropharmaceuticals are linked to a vector such as an antibody to the transferrin receptor that mediates transcytosis through the blood-brain barrier (BBB). When disulfide linkers are used in the chimeric peptide, it is crucial that the S-S bridge is stable during transit and that cleavage does not occur prematurely within endothelial cells, as the peptide drug moiety would then be sequestered by the BBB instead of passing through it. The present study addressed that problem. As a model drug a metabolically stable opioid peptide, [^{3}H]DALDA (Tyr-dArg-Phe-Lys-NH_2), was used. It was monobiotinylated with NHS-SS-biotin to yield bio-[^{3}H]DALDA. The biotinylated peptide was bound to the vector OX26-SA which is a covalent conjugate of OX26 and streptavidin (molar ratio = 1:1). In vitro treatment of the chimeric peptide, bio-[^{3}H]DALDA/OX26-SA, with a reducing agent, dithiothreitol, released the labeled peptide from the vector by conversion of bio-[^{3}H]DALDA to the desbiotinylated derivative, desbio-[^{3}H]DALDA. After intravenous injection in rats

significant brain uptake of the chimeric peptide was demonstrated compared to negligible uptake of bio-[^{3}H]DALDA without the vector. In vivo microdialysis was applied to sample brain extracellular fluid for 60 min after the intravenous bolus. Chromatographic analysis of the dialysate by reverse phase HPLC did not show the presence of desbio-[^{3}H]DALDA in brain extracellular fluid, thus indicating stability of the chimeric peptide during transport through the BBB.