Lipoaminoacid Analogues of Leu-Enkephalin: Permeability and Stability Experiments

Cecile Cros[1], Istvan Toth [1, 2] and Joanne T. Blanchfield[1]

[1]School of Molecular and Microbial Sciences, The University of Queensland, Australia.

[2]School of Pharmacy, The University of Queensland, Australia

THE UNIVERSITY OF QUEENSLAND

Introduction

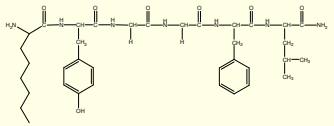
A technique to study the delivery of neuroactive drugs to the brain via nasal delivery involves the addition of the radioactive label ^3H_3C-CO to the N-terminus of peptides. Liquid scintillation counting is then used to analyse the level of radioactivity in the brain and various other organs. However, this method measures the level of radioactivity in the organs without certainty that the radiolabelled marker is still attached to the peptide.

This study presents Caco-2 cell permeability assays on acetylated analogues of Leu-enkephalin modified by the addition of a lipoamino acid (Laa) at the N- or at the C-terminus of the peptide. Lipoamino acid are synthetic amino acids with a hydrophobic side chain. They have been shown to increase stability to degradation and permeability through biological membranes. They are synthesised as a racemic mixture of isomer D and L.

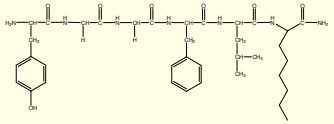
The second part of this study presents a human plasma stability assay on a racemic lipoamino acid Leu-enkephalin derivative.

Leu-Enkephalin analogues

- The peptides were synthesised by solid phase peptide synthesis with MBHA rink amide resin following Fmoc based procedures and HBTU/DIEPA activation protocol. The efficiency of the couplings were evaluated by ninhydrin assay.



C8-Enk

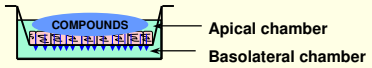


Enk-C8

* both D and L stereoisomers of Laas used in synthesis to afford two diastereomers of both peptides.

Methods

- Caco-2 Permeability assay: Caco-2 cells form tight monolayer structures when seeded on to polycarbonate filters. To assess permeability, a 200 µM solution is placed on the apical side of the monolayer and samples are taken from the basolateral side at predetermined time points. Concentrations found in the basolateral chamber are measured by LCMSMS analysis.



The apparent permeability of the compound is then calculated applying the formula:

$$P_{app} = dC/dt \times V_r/(A \times C_0)$$

Where dC/dt is the ready state rate of the change in the chemical concentration (M/s);

V_r is the volume in the receiver chamber;

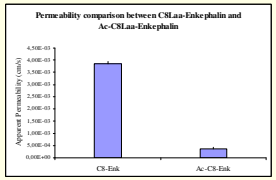
A is the surface area of the cell monolayer;

C_0 is the initial concentration in the donor chamber.

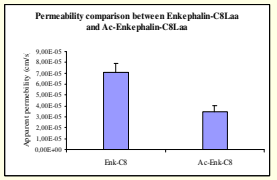
- Plasma stability assay: This assay was performed by mixing 300µL of a solution of C8Laa-Enkephalin (1mg/mL) with 300µL of plasma prewarmed at 37.5°C. At chosen time points, 50µL of the plasma mixture was sampled, placed on ice, and 75µL acetonitrile added to precipitate the plasma proteins. Samples were analysed by HPLC.

Caco-2 permeability results

N-terminal lipoamino acid derivatives

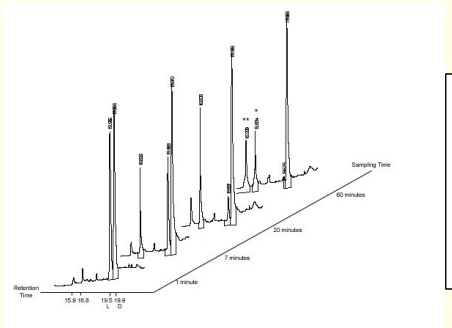


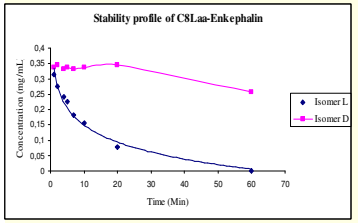
C-terminal lipoamino acid derivatives



- N-terminal analogues showed greater permeability than the C-terminal analogues. However, in both cases, the permeability decreases with acetylation of the N-terminus of the compound.

Plasma Stability assay result





- Plasma Stability of C8Laa-enkephalin showed that the diasteriomer bearing the L-Laa (R_t = 19.5 minutes) was degraded quickly. A degradation product* at R_t = 16.8 min increased in intensity as the L-diastereomer disappeared. After 60 minutes incubation, the L-diastereomer had nearly completely disappeared, and the intensity of the diastereomer bearing the D-Laa (R_t = 19.9 minutes) started to decrease. The intensity of the first degradation product at 16.8 min also begins to decrease, at the same time as a second degradation product** at 15.9 min starts to increase.

Conclusions

- Acetylation decreased the Papp of leu-enkephalin analogues showing that acetylation of the analogues created compounds with different properties. This can be considered with the observation that the acetylated compounds were significantly less water soluble than the parent analogues causing precipitation of the compounds in the apical chamber, which could explain the decrease in permeability.

- The plasma stability experiment showed that the L-Laa-Enk was degraded faster than the D-Laa-Enk peptide and both diastereomers appear to degrade to different products suggesting different degradation pathways. Enzymes usually cleave L-amino acid peptide bonds, consequently, using exclusively the D isomer of lipoamino acid could be the best way of stabilising a compound. The permeability of those analogues and the identity of the degradation products is still under investigation.