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Transport and metabolism of methionine enkephalin in human nasal epithelial primary cell culture system

H. Vu Dang, R. U. Agu, R. Kinget and N. Verbeke

Laboratorium voor Farmacotechnologie en Biofarmacie, K.U. Leuven, Campus Gasthuisberg O&N, Herestraat 49, B-3000, Leuven, Belgium

Methionine enkephalin, which is known to act primarily as neurotransmitter or neuromodulator in pain transmission, has recently received a considerable attention due to its potential therapeutic uses. However, methionine-enkephalin-related treatment has been restricted by its rapid degradation in biological systems. To investigate the potential of using methionine enkephalin following nasal administration, in-vitro experiments were carried out to determine the nasal transport and metabolism of methionine enkephalin in cell monolayers of human nasal epithelium based on air-liquid interface culture method.

In this study, the transport and metabolism of methionine enkephalin (Met-Enk) were kinetically investigated in human nasal epithelial cell culture by HPLC analysing the parent peptide and its major metabolite, des-tyrosyl methionine enkephalin (Des-Tyr-Met-Enk). This pentapeptide was found to undergo extensive degradation by aminopeptidases with first-order kinetic rate constant $k = 2.58 \times 10^{-2} \text{ min}^{-1}$. The inhibitory effects of protease inhibitors, bestatin and puromycin, were also concentration-dependently studied. At equimolar concentration, bestatin had a greater inhibitory effect than puromycin. In the absence of protease inhibition and absorption enhancement, only 0.1% of Met-Enk (3000 μM) transported across the cell monolayers after 2 h incubation period. Except for 1 mM puromycin, the combinations with 2% dimethyl- β -cyclodextrin (DM β CD), 0.5% sodium glycocholate (GC-Na) and 1 mM bestatin did not result in a significant increase in Met-Enk transport, respectively. Co-administration with both protease inhibitors and absorption enhancers achieved a pronounced increase in the amount of Met-Enk transport. Especially, combination with 1 mM puromycin and 0.5% GC-Na resulted in 7% Met-Enk transport (Table 1).

Table 1 % Met-Enk transport across the cell monolayers

Experimental conditions	% Met-Enk transport
MET-ENK alone	0.1 \pm 0.01
MET-ENK + puromycin 1 mM	1.5 \pm 0.12*
Met-Enk + bestatin 1 mM	0.2 \pm 0.12
Met-Enk + GC-Na 0.5%	0.2 \pm 0.21
Met-Enk + GC-Na 0.5% + puromycin 1 mM	7.0 \pm 0.32*
Met-Enk + GC-Na 0.5% + bestatin 1 mM	2.6 \pm 0.45*
Met-Enk + DM β CD 2%	0.1 \pm 0.12
Met-Enk + DM β CD 2% + puromycin 1 mM	2.0 \pm 0.32*
Met-Enk + DM β CD 2% + bestatin 1 mM	0.3 \pm 0.41

*Significantly different from the control (Met-Enk alone)

Unfortunately, the increases in Met-Enk transport as a result of co-administration with a combination of protease inhibitors or absorption enhancers were directly proportional to trans-epithelial electrical resistance reduction (97%) and sodium fluorescein transport across cell monolayers (32.5%). Based on the data of this study, bestatin and puromycin alone or in combination with DM β CD or GC-Na resulted in increased nasal Met-Enk permeation but deleterious to the nasal epithelium. Therefore, the use of these protease inhibitors to formulate the peptide should be done with caution, especially for Met-Enk related sub-acute and chronic treatment.