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The Stability of Salmon Calcitonin Against Trypsin, Chymotrypsin and Neutrophil Elastase in Human Lung Epithelial Cells

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At least three enzymes (i. e., trypsin, chymotrypsin and neutrophil elastase) are responsible for the degradation of salmon calcitonin (sCT) in the human body [1]. Here, we investigated how these proteinases affect drug disposition of sCT in the lungs.

Stability of sCT was studied in human respiratory epithelial cells. The presence of trypsin, chymotrypsin and neutrophil elastase in cell supernatant and homogenate was studied by Western blot. Enzymes' activities were studied in cell supernatant by measuring UV absorption, caused by interaction with model substrates. *In vitro* stability studies were carried out by adding sCT to cell supernatant, homogenate, as well as on intact cell monolayers, followed by incubation at 37°C for 2 h. Samples were serially withdrawn and analysed by HPLC for sCT content. Degradation of sCT by pure trypsin, chymotrypsin and neutrophil elastase was investigated after incubation for 2 h at 37°C.

We observed that sCT concentrations remained unchanged over the period of 2 h, when incubated in supernatant or on cell monolayers. When cell homogenates were studied, sCT concentrations were reduced to 82.5% (hBEpC), 45.9% (Calu-3), 45.1% (16HBE14o-), 4.3% (A549) and 18.5% (Caco-2), respectively. sCT was also degraded when incubated with pure enzymes. Western blot revealed strong signals for all proteinases in the cell homogenates and weaker, varying expression in supernatants.

Calcitonin is rapidly degraded in homogenate, indicating high intracellular enzymatic activity. Epithelial proteases hence, appear to play a role in interactions of sCT with lung epithelium. Although trypsin, chymotrypsin and neutrophil elastase are secreted *in vitro*, sCT remained intact in supernatant for 2 h. The concentration of the relevant enzymes (indicated by very low measured activities) might be too low to cause degradation.

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