Investigation of the cellular import and export of free and protein-bound cobalamin (vitamin B_{12})

Rasmus Beedholm-Ebsen, MSc

This PhD dissertation was accepted by the Faculty of Health Sciences of the University of Aarhus, and defended on February 29, 2008.

Official opponents: Thomas Ledet, Uffe Holmskov, and Roger Alberto, Switzerland.

Supervisor: Søren K. Moestrup.

Correspondence: Rasmus Beedholm-Ebsen, Ingerslevs Plads 1B, 2., 8000 Aarhus C, Denmark.

E-mail: rasmus@biokemi.au.dk

Dan Med Bull 2008;55:126

ABSTRACT

This PhD dissertation is based on experimental work performed at the Department of Medical Biochemistry, the University of Aarhus. The work was conducted to gain insight into the cellular endocytosis and export of vitamin B_{12}. The main results of this PhD study are presented in three separate manuscripts.

Vitamin B_{12} (cobalamin) is an organic molecule which functions as an essential coenzyme for two metabolic reactions in mammalians. Vitamin B_{12} deficiency is a common condition causing pernicious anemia, which is characterized by megaloblastic anemia and neurological symptoms. Cellular vitamin B_{12} uptake is a complex process involving different binding proteins (intrinsic factor and transcobalamin) and at least three different receptors.

In the first study the receptor-mediated uptake of vitamin B_{12} has been studied in cultured cancer cells. These studies included the use of fluorescently labelled vitamin B_{12} conjugates, which make it possible for the first time to visualize the vitamin by confocal immunofluorescence microscopy. The uptake studies revealed that the endocytosis of transcobalamin bound to fluorescent vitamin B_{12} conjugate correlated with the cell growth in fast-dividing cancer cells and the uptake of free vitamin B_{12} was negligable at low as well as high cell division activity.

The second study describes the vitamin B_{12} export in cultured cells using ^{57}Co-labeled vitamin. In contrast to what has been previously reported, the data evidenced that the vitamin is exported to the cellular environment as a free molecule and the binding to a carrier protein occurs subsequently to export.

In the third study a transporter for cellular export of vitamin B_{12} was searched for by a "bioinformatics" approach, whereby a spectrum of candidate membrane proteins were selected by a subset of functional, structural and cellular location criteria. The role of candidate proteins was investigated by selective RNA silencing with small interfering RNA (siRNA) probes specific for the encoding RNAs. Inhibition of vitamin B_{12} export after inhibition of the expression of one of the candidate proteins by siRNA was followed-up by analysis of the vitamin B_{12} status of mice with targeted disruption of the encoding gene. These data showed low levels of extracellular vitamin B_{12} thus providing further evidence of a role of this particular transporter in cellular export of vitamin B_{12}.

In conclusion, this PhD study provides new information on the cellular vitamin B_{12} import and in particular export by revealing a novel transport mechanism of non-protein bound vitamin B_{12} and the identification of a transporter protein for this process.