Activation of the hypoxia-inducible factor (HIF) pathway by roxadustat improves glucose metabolism in human primary myotubes

Selina Mäkinen[1,2]*, Sreesha Sree[1,2]*, Tuulia Ala-Nisula[3], Henric Kultalahti[1,2], Peppi Koivunen[3], Heikki A. Koistinen[1,2]†
*equal contribution

tuulia.ala-nisula@oulu.fi

[1]Minerva Foundation Institute for Medical Research, Tukholmankatu 8, 00290 Helsinki, Finland

[2]Department of Medicine, University of Helsinki and Helsinki University Hospital, PO 340, Haartmaninkatu 4, 00029 HUS, Helsinki, Finland

[3]Biocenter Oulu, Faculty of Biochemistry and Molecular Medicine, Oulu Center for Cell-Matrix Research, University of Oulu, PO 90014, Aapistie 7A, Oulu, Finland

Hypoxia-inducible factor prolyl 4-hydroxylase (HIF-P4H) enzymes regulate cellular adaptation to hypoxic conditions. Inhibiting HIF-P4Hs stabilizes hypoxia-inducible factors (HIFs) and therefore activates the HIF pathway. Animal model data suggest that HIF-P4H inhibitors could act as a treatment for metabolic disorders with insulin resistance.

In this study, roxadustat, a pan-HIF-P4H inhibitor approved for the treatment of renal anemia, was used on primary human myotubes. From a cohort of men with normal glucose tolerance and type 2 diabetes (T2D), *M. vastus lateralis* biopsies were taken and primary skeletal muscle cell cultures were established. Stabilization of HIF and its target gene expression was examined with Western blotting and qPCR. Glucose uptake and glycogen synthesis were studied with radioactive tracers. Glycolysis and mitochondrial respiration rates were measured with a Seahorse analyzer.

Treatment with roxadustat stabilized HIF1α and induced expression of HIF target gene mRNAs for *GLUT1*, *HK2*, *MCT4*, and *HIF-P4H-2* in myotubes from donors with normal glucose tolerance, with a blunted response in myotubes from donors with T2D. mRNAs for *LDHA*, *PDK1* and *GBE1* were induced similarly in myotubes from donors with both groups.

Roxadustat treatment enhanced insulin stimulated glucose uptake in normoglycemic donors myotubes. Roxadustat led to increased glycolytic rate in myotubes of both groups. Exposure to roxadustat also reduced basal mitochondrial respiration in both groups. Roxadustat enhanced insulin-stimulated glycogen synthesis in T2D myotubes.

To conclude, perturbations that activate the HIF response, such as roxadustat, may have beneficial effects in the treatment of insulin resistance and T2D.