MRI detection of Rapamycin and AP-Cav therapeutic rescue from endothelial over expression of Akt in transgenic mice

K. Ziv[1], T. L. Phung[2], O. Brenner[3], K. Walsh[4], L. E. Benjamin[2], M. Neeman[1]

[1]Biological regulation, Weizmann Institute of Science, Rehovot, Israel, [2]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, United States, [3]Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel, [4]Boston University, Boston, Massachusetts, United States

Introduction Akt/PKB is a serine/threonine protein kinase that is activated by various growth factors and cytokines, including many angiogenic factors such as VEGF (1, 2). Akt signaling in endothelial cells regulates multiple critical steps in angiogenesis through its downstream effectors, mTOR and eNOS among others. We applied MRI to investigate vascular function in a binary transgenic mouse model that expresses constitutively active Akt (myrAkt) in endothelial cells in an inducible manner. Induction of myrAkt expression in endothelial cells in transgenic mice leads to progressive fatal edema that can be quantified by MRI. The aim of this work was to investigate the effects of inhibition of mTOR and eNOS on vasodilation and edema induced by sustained endothelial Akt activation.

Materials and Methods tTA:VE-cadherin x TET:myrAkt double transgenic mice were generated by crossing TET:myrAkt mice with tTA:VE-cadherin mice. In these mice, myrAkt was expressed only in endothelial cells, and expression was suppressed by addition of tetracycline in the drinking water. Expression of myrAkt was induced for 7 days prior to MRI scan by withdrawal of tetracycline. Therapy experiments included daily intraperitoneal injections of rapamycin (4 mg/kg/day), or antennapedia-caveolin-1 peptide (AP-Cav, 2.5 mg/kg/day) for 7 days from the time of tetracycline withdrawal. Dynamic contrast enhanced MRI using biotin-BSA-GdDTPA was done as reported previously (3) on a 4.7T Bruker Biospec spectrometer. Precontrast R1 was determined by non-linear fit using 3D-GE variable flip angle data (3). R1 weighted 3D-GE data (TE=3.561ms; TR=10ms; 128x128x64 with FOV of 12x12x6cm; acquisition time per image163sec; total followup after administration of the contrast material 30 min) was analyzed for derivation of the blood volume fraction (fBV) as reported previously (4). The blood volume fraction (fBV) in the tissue was determined as the ratio of biotin-BSA-GdDTPA concentration extrapolated to the time of contrast material administration to the concentration of the contrast material in blood (as measured in the vena cava). Following MRI measurements the mice were euthanized by anesthesia overdose and tissues were retrieved for histological staining.

Results Previous MRI results showed that expression of myrAkt resulted in a systemic increase in blood volume in myrAkt double transgenic mice as compared to single transgenic control mice. This increase was also manifested by the dilution of the contrast material in the circulation as measured in the vena cava at time zero (5). Although the same amount of contrast material was administered intravenously, the concentration of biotin-BSA-GdDTPA in the vena cava in myrAkt double transgenic mice was significantly lower than in single transgenic control mice ($p<0.05$; 2-tail unpaired t-test; control single transgenic: n=6, double transgenic myrAkt untreated mice: n=6). However, the significant increase in blood volume was prevented in double transgenic myrAkt mice that were treated with rapamycin ($p<0.05$; 1-tail unpaired t-test; double transgenic myrAkt rapamycin treated mice: n=4, double transgenic myrAkt untreated mice: n=6). A smaller un-significant effect was observed in double transgenic myrAkt mice treated with the eNOS inhibitor AP-Cav ($p>0.05$; 1-tail unpaired t-test; double transgenic myrAkt AP-Cav treated mice: n=3, double transgenic myrAkt untreated mice: n=6) (Figure 1). Histological sections revealed edema in many organs of the double transgenic untreated mice, and these pathological changes were suppressed by rapamycin or AP-Cav (e.g. skeletal muscles; Figure 2). Blood volume fraction (fBV) of the brain was higher in myrAkt untreated mice compared to myrAkt mice that were treated with Rapamycin or AP-Cav, this increase was statistically significant (untreated mice vs rapamycin treated mice $p<0.05$, untreated mice vs AP-Cav treated mice $p<0.05$; untreated mice n=6, rapamycin treated mice n=4, AP-Cav treated mice n=3, one way ANOVA).

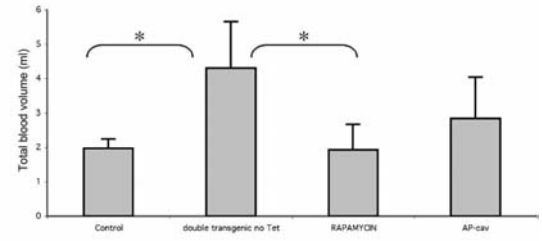

Figure 1. MRI derived changes in blood volume following treatment of double transgenic myrAkt mice with rapamycin or AP-Cav. Mice were given drinking water without tetracycline for 7 days. A significant rise in blood volume was observed by tetracycline withdrawal ($p=0.0077$). Treatment with rapamycin significantly blocked the increase in blood volume in myrAkt mice ($p=0.0066$), while treatment with AP-Cav caused a smaller un-significant change ($p=0.079$).

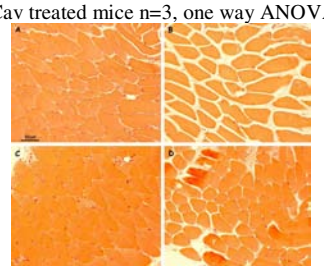

Figure 2. Akt induced edema in skeletal muscle. H&E staining is presented for striated muscle of the hind limb. Mice were given drinking water without tetracycline for 7 days. Histological sections were taken from: control mice (A), double transgenic untreated mice (B), double transgenic rapamycin treated mice (C) and double transgenic AP-Cav treated mice. Magnification x20.

Conclusions Sustained activation of myrAkt in endothelial cells reproduces the phenotype of constitutive activation by VEGF, resulting in systemic vasodilation that is detectable by MRI and led to progressive edema. Inhibition of the downstream signaling of Akt by rapamycin rescued the mice and the effects were detectable by MRI. Thus, functional analysis of the vasculature by MRI can be used for in vivo kinetic analysis of the VEGF signaling cascade activated endothelial cells in transgenic mouse models.

Acknowledgements

This work was supported by the Israel Science Foundation (MN) and by NIH R01 HL7109-01 (LEB).

References

1.Shiojima, I. and Walsh, K. Role of Akt signaling in vascular homeostasis and angiogenesis. Circ Res., *90:* 1243-50., 2002. 2.Franke, T. F., Kaplan, D. R., Cantley, L. C., and Toker, A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science., *275:* 665-8., 1997. 3. Ziv, K., Nevo, N., Dafni, H., Israely, T., Granot, D., Brenner, O., and Neeman, M. Longitudinal MRI tracking of the angiogenic response to hind limb ischemic injury in the mouse. Magn Reson Med., *51:* 304-11., 2004. 4. Dafni H, C. B., Ziv K, Israely T, Goldshmidt O, Nevo N, Harmelin A, Vlodavsky I and Neeman M The Role of Heparanase in Lymph Node Metastatic Dissemination:Dynamic Contrast-Enhanced MRI of EB Lymphoma in Mice. Neoplasia., *in press:*, 2004.5. Ziv K, Phung TL., Brenner O, Walsh K, Benjamin LE and Neeman M. Abstract 2705 of ISMRM 2005. 2005.