Analysis of Ovarian Vascular Function in PKB[alpha]/Akt1- deficient Mice Using biotin-BSA-GdDTPA Enhanced 3D-MRI

V. Plaks[1], E. Berkovitz[2], B. A. Hemmings[3], N. Dekel[1], M. Neeman[1]

[1]Biological Regulation, Weizmann Institute of Science, Rehovot, Israel, [2]Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel, [3]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Introduction:

Angiogenesis plays a vital role in sustaining reproductive processes such as ovarian function, embryo implantation and placental development. PKB/Akt promotes or inhibits many cellular and physiological processes through phosphorylation of numerous substrates. These proteins are involved in glucose metabolism, transcription, cell cycle regulation, survival, inflammation and angiogenesis. PKB/Akt is known to act downstream of VEGF, a key mediator of angiogenesis via the VEGF receptor/PI3K/PKB signaling cascade that is known to mediate the formation of new blood vessels in pathological conditions such as cancer [1]. In a recent study [2] concerning the role of PKB in fetal development, PKBα null late mouse embryos (E14.5) were smaller with disordered fetal vasculature and increased neonatal mortality and all three major parts of the mutant placentas were smaller compared with WT. These data suggest a novel role for PKBα (PKB isoform, widely expressed in placenta) in the regulation of placental and fetal development. This study focuses on the role of PKBα in maternal angiogenesis, i.e. ovarian function and its implications on embryo implantation and overall fertility. Macromolecular contrast enhanced MRI was applied here to study ovarian vascular function in PKBα - knock out (KO) mice.

Materials and Methods:

Animal model: Sexually matured either non pregnant or pregnant (E5.5) PKBα KO (n=5 total including 2 pregnant and 3 non pregnant) and WT (n=4 total including 2 pregnant and 2 non pregnant) littermates were scanned by MRI [3].

Contrast agents: Biotin-BSA-GdDTPA was prepared as reported [4].

MRI experiments: Animals were placed in a 4.7T horizontal Bruker (Germany) Biospec spectrometer and a whole body excitation coil was used.

3D-GE: a series of images with 15, 5, 30, 50, 70 degrees flip angles were acquired to determine the precontrast R1 (TR 10ms, TE 3.6ms, 2 averages, spectral width 50000Hz, matrix 128x128x64 (zero filled to 128), FOV 5x5x5 cm). Dynamic post contrast images were acquired with a 15 degrees flip angle and animals were followed for 30min after intravenous administration of MR contrast agent (MR-CA) via the tail vein.

MRI data analysis: mean MR-CA concentrations were derived from the precontrast R_1 and using pre- and postcontrast 3D-GE-mean signal intensities at ROIs of ovaries. Changes in CA concentrations were used for derivation of PS (permeability surface area product) and fBV (blood volume fraction i.e. vessel density) [5].

Results:

To test overall maternal fertility, WT and KO females were mated with KO and WT males, respectively (so embryos in both cases were heterozygous for PKBα). No pregnancies were detected in KO females (out of a total of 29 females in 3 separate experiments), except once, when pups died immediately after birth. Implantation sites were detected in KO females upon transferring pre-implantation WT embryos into pseudopregnant KO females, although their numbers was apparently reduced. Subsequently, ovarian function was examined. Upon examination of the post contrast MR images, KO (Fig. 1a) ovaries were almost non- distinguishable from the adjacent tissue exhibiting almost no contrast enhancement, implying less CA accumulation/extravasation in compare to WT that were easily resolved (Fig. 1b). Further analysis of the MRI data revealed that fBV in the KO ovaries was significantly lower than that of the WT ovaries (Fig. 1c, 2 tailed t- test p=0.003). Although reduced, there was no significant difference in the PS measured in the two types of ovaries (Fig. 1d, 2 tailed t- test p=0.068). While significant differences were measured for the ovaries, there was no significant difference between the KO and WT mice in the fBV measured from kidneys or muscle. Weight of non pregnant mice was examined and the KO mice were found to be significantly lighter than the WT mice (20.4±0.4g (n=11) vs. 17.8±0.7g (n=9) average weight respectively, 2 tailed t- test p=0.003). Upon post mortum examination of abdominal fat in older animals (>6 months), the KO mice (10 mice) had less fat than WT mice (15 mice).

Discussion:

Analysis of MRI data revealed reduced vessel density in ovaries of PKBα KO as compared to WT females. We speculate that reduced vessel density in the KO ovaries along with the absence of spontaneous pregnancies in this model and the apparent reduction in the number of implantation sites after embryo transfer imply that normal ovarian function is perturbed. The observed effects of PKBα/Akt1 deficiency which were seen in the ovaries but not in other organs such as the kidney and muscle, are consistent with an effect mediated through angiogenesis, as the ovary is a unique example of physiological angiogenesis occurring in adult animals. In addition, PKBα KO females exhibited reduced body weight and reduced fat mass. This observation might be associated with the role of PKBα in glucose metabolism (PKB is known to inhibit GSK3β that inhibits glucose metabolism; alternatively, PKB activates mTOR which may also regulate growth). Indirectly, the reduced body fat may influence the hormonal balance of the PKBα KO females subsequently contributing to perturbed ovarian function and impaired fertility.

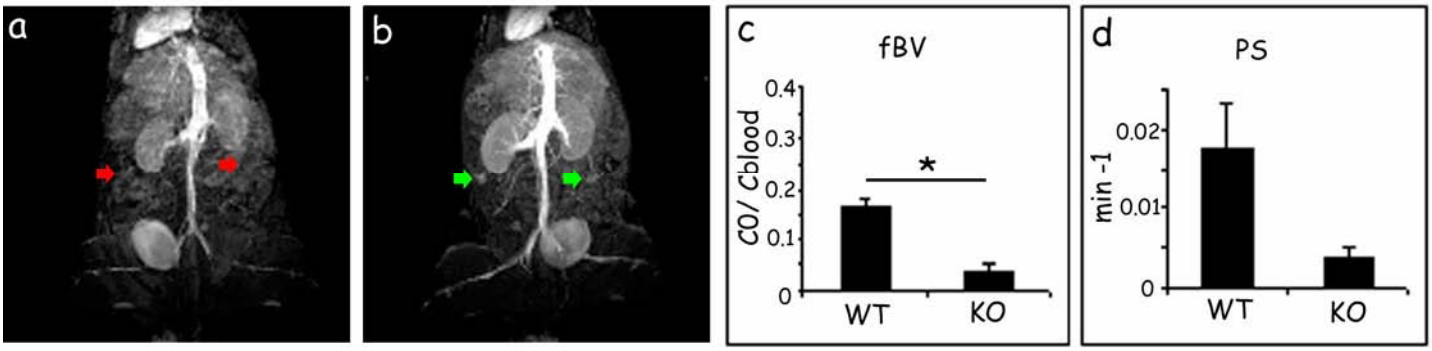

Fig 1. Biotin-BSA-GdDTPA enhanced PKBα KO ovaries (a, apparent ovary localization marked with a red arrow) versus well resolved WT ovaries (b, ovary localization marked with a green arrow) 15 min post contrast. Maximal intensity projections are presented. fBV in the KO ovaries was significantly lower than that of the WT ovaries (c, * p=0.003) but although reduced, the difference in PS was not significant (d; p=0.068).

References:

[1] Riesterer, O., et al (2004). Oncogene. 23(26): 4624- 4635.
[2] Yang, Z.Z., et al (2003). J Biol Chem. 278(34):32124-32131.
[3] Plaks, V., et al (2005). Magn Reson Med. Submitted.
[4] Dafni, H., et al. (2003). Magn Reson Med. 50(5):904-14.
[5] Israely, T., et al (2003). Biol Reprod. 68(6):2055-64