MR imaging detects impaired angiogenesis and trabecular bone formation during endochondral bone growth mediated through PKBalpha/Akt1 in gene dosage dependent manner

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Introduction

PKBalpha/Akt1, a protein kinase, is a major mediator of angiogenic signaling, acting downstream of vascular endothelial growth factor (VEGF). PKBalpha/Akt1 deficient mice are smaller (Cho et al., 2001; Chen et al., 2001) and exhibited bone mineralization defects characterized by decreased length and bone mass of long bones (Kawamura et al. 2007; Ulici et al., 2009). Since infiltration of the newly formed blood vessels is required for endochondral bone formation, and PKBalpha/Akt1 mediates intracellular signaling of angiogenesis, we postulated that a vascular deficiency at the site of the long bones could contribute indirectly to impaired bone development in PKBalpha/Akt1 deficient mice. The purpose of this study was to use macromolecular DCE-MRI to examine in vivo vascular changes at the site of long bones in growing PKBalpha/Akt1 deficient mice and to study the impact of PKBalpha/Akt1 gene dosage on trabecular bone formation during endochondral bone growth.

Methods

Male PKBalpha/Akt1 wild type (+/+; n=7), heterozygote (+/-; n=7) or knockout (-/-; n=7) mice were studied by MRI on postnatal day 40 (P40). MRI experiments of 40-day-old male mice were performed at 9.4 T (Bruker BioSpec, Germany) using a linear resonator for excitation and an actively decoupled 2-cm surface coil for detection. 3D gradient echo (3D-GE) images of the left front limb were acquired before and sequentially for 30 minutes after iv injection of biotin-BSA-GdDTPA. At the end of the MRI experiment, 30 minutes after contrast injection, bovine serum albumine (BSA) labeled with rhodamine (BSA-ROX), as an early vascular marker, was iv injected via a tail vein catheter 3-5 minutes prior to animal sacrifice. Left humerus of 4-5 animals in each group were taken for ex vivo μCT for trabecular and cortical bone analysis, whereas the right humerus was taken for histological validation. In addition, the left femur of the same animals was taken for μMRI analysis of trabecular bone.

Results

In the study reported here, vascular defects were demonstrated at long bones, associated with reduced size of PKBalpha/Akt1 null mice, using non invasive DCE-MRI with biotin-BSA-GdDTPA as a macromolecular contrast media (Fig. 1), combined with histological data. Interestingly, reduced vascular density at the growth plate region of the humerus was revealed, not only in PKBalpha/Akt1 null mice, but also in heterozygous PKBalpha/Akt1 mice. Ex vivo μCT and μMRI confirmed impaired trabecular bone formation in 40-day-old null mice and revealed the same impairment in heterozygous mice (Fig. 2).

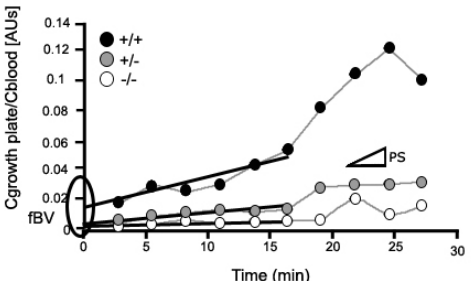

Fig. 1. In vivo dynamic DCE-MRI for 30 minutes post contrast (10 time points) of the role of PKBalpha/Akt1 in growth plate vascularization. For wild type (+/+), heterozygote (+/-), and null mice (-/-), fractional blood volume (fBV) and permeability surface area product (PS) values were calculated from a linear regression of the first 15min, after averaging the concentration of contrast material in the selected ROI (growth plate region) by the concentration in the cephalic vein. The fBV, a parameter for (micro)vascular density, was significantly reduced in +/- and -/- growth plates ($P<0.05$).

Discussion

Reduction of blood vessel invasion, concomitant with impaired trabecular bone formation and shorter long bones, suggests a role for PKBalpha/Akt1 in regulation of overall size and endochondral bone growth indirectly mediated by bone neovascularization in a gene dosage dependent manner. Thus, our study demonstrated vascular and bone developmental defects in PKBalpha/Akt1 null mice, and remarkably also in heterozygous mice, lacking a single copy of the gene. MRI proved to provide high sensitivity for in vivo detection of impaired bone vascularity and for uncovering changes in trabecular bone mineralization.

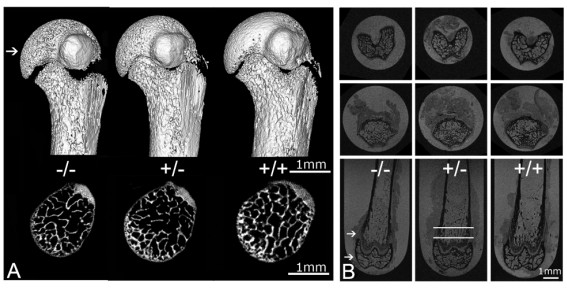

Fig. 2. Ex vivo bone analysis of 40-day-old male mice. From the same animals, humeri were taken for μCT (A), and femora were differently prepared for μMRI analysis (B). (A) Upper panel, surface projection; epiphysis (arrow). Lower panel, trabecular bone of the proximal epiphysis of the humerus. (B) Distal femora of the same animals imaged by μMRI. Arrows on the longitudinal view (lower) show the region of the epiphysis and metaphysis; slices of MR images of the epiphysis (upper) and metaphysis (middle) illustrate the trabecular bone. Tabecular bone analysis of μCT and μMRI suggests impaired trabeclar bone development in the PKBalpha/Akt1 null and heterozygote ($P<0.05$).