USPIO-enhanced ΔR2* MR-Relaxometry for in-vivo Monitoring of Fracture Healing

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Introduction

Angiogenesis is an essential progress in embryogenesis, but it also occurs in cancer, arthritis, wound and fracture healing. The fracture healing process could be divided into four temporal phases: inflammation, soft callus (fibrocartilage) formation, hard callus (osteogenic) formation and bone remodeling. Angiogenic invasion of the soft callus by vascular endothelial cells and capillary in-growth (as stimulated by pro-angiogenic factors) seems to be essential for a proper fracture healing, and impairment of vascular recruitment will result in delayed bone union or non-union[1,2].
Recently, USPIO-enhanced ΔR2* MR-relaxometry has been successfully explored for non-invasive characterization of the tumor mircovasculature and anti-angiogenic cancer therapy monitoring[3,4]. Susceptibility-corrected multi-gradient-echo MR-relaxometry allows for accurate and robust determination of the vascular volume fraction (VVF), as a surrogate marker of the histological microvessel density (MVD)[5]. The purpose of this study was to evaluate susceptibly-corrected ΔR2* MR-relaxometry for *in-vivo* monitoring of angiogensis during bone fracture healing in a rat model.

Material and Methods

A standard closed fracture healing model was used and approved by the institutional animal care committee. The right femur shaft of female Sprague-Dawley rats (totally n=16) was fractured and stabilized with an intramedullar PLLA (poly-L-lactideacrylate) nail under deep anesthesia conditions. After surgery at day 0, 7, 14, 21, and 28 (n=8), MR imaging was performed on a clinical 3.0 Tesla MR system (Achieva, Philips) by using a solenoid receive only coil with diameter of 70 mm. USPIO-induced (80 µmol/kg SHU 555 C, Bayer-Schering Pharma) changes of R2* (ΔR2*) were measured with a multi-gradient-echo MR-relaxometry sequence (TR/ TE: 600/ 4.6–56.7 ms; number of echoes: 25; ΔTE: 2.2 ms; slice thickness: 2 mm; matrix size, 256 x 256; field of view, 200 x 200 mm; and flip angle: 30°). Sagittal parametric ΔR2* maps were computed and the mean ΔR2* value and vascular volume fraction (VVF) were measured in ROI, selected in the perifractural bone area. MR results were compared with the microvessel density (MVD) by immunohistochemistry in successively sacrificed control rats on day 7, 14, 21, and 28 (n=2 each) (anti- smooth muscle actin (SMA) antibody fraction quantified by digital imaging analysis, Olympus Microscope BX41 and Image-pro plus 5.0 software, Media cybernetics Inc., USA). For statistical analysis a Mann-Whitney U test with significance $p < 0.05$ was performed. Values are given as mean ± SD of the mean.

Results

ΔR2*-maps of the fractured femur were obtained without relevant susceptibility artifacts of the intramedullar PLLA nail (Fig. 1). MR-Relaxometry revealed a significant initial increase of ΔR2* values from day 0 to 14 after surgery followed by a decrease at day 21 that was stable until day 28 (ΔR2* day 0: 5.4±3.8s^{-1}, day 7: 31.4±3.5s^{-1}, day 14: 35.9±4.7s^{-1}, day 21: 23.9±3.3s^{-1}, and day 28: 5.4±3.8s^{-1}; $p<0.05$) (Fig. 2). The calculated VVF showed similar time course with a maximum peak at day 7 and 14 (VVF day 0: 1.6±1.2%, day 7: 11.8±3.8%, day 14: 10.9±3.7%, day 21: 6.4±2.6%, and day 28: 8.8±3.8%; $p<0.05$). Immunohistochemistry corroborated the MR results showing the highest MVD at day 14 (MVD day 7: 0.48±0.2%, day 14: 1.64±0.4%, day 21: 1.2±0.6%, and day 28: 1.22±0.3%).

Discussion

USPIO-enhanced ΔR2* MR-relaxometry allows an *in-vivo* monitoring of angiogenic changes in fracture healing. Therefore, this technique may facilitate experimental studies in bone repair, investigation of its underlying mechanisms, and possible novel molecular therapies. Moreover, the amount of rodents scarified for histological analysis of callus formation could be significantly reduced in future research settings.

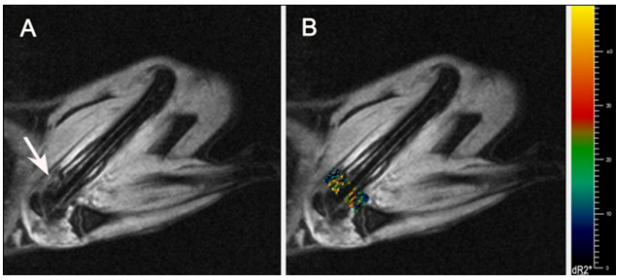

Figure 1: Exemplary MRI 14 days after surgery. (A) Morphologic T2-w image with soft callus formation at the distal femur fracture (arrow). (B) Parametric ΔR2* overlay; notice the higher ΔR2* valus at the perifractural callus formation adjacent to the intramedullar PLLA nail.

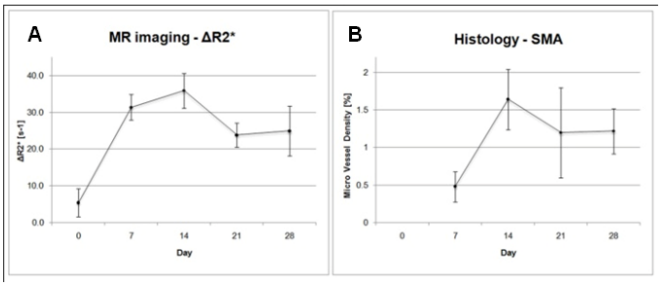

Figure 2: Correlative MRI and histological microvessel density (MVD). (A) ΔR2* and (B) MVD time curves from day 0 to day 28 after closed fracture of the distal femur.

References: [1] Schindeler et al. Sem Cell Dev Biol (2008; p 459-466). [2] Hausman et al. Bone (2001; p 560-564). [3] Bremer at al. Radiology (2006; p 214-220). [4] Reichardt et al. Neoplasia (2005; p 847-853). [5] Persigehl et al. Radiology (2010; p 781-789).