Quantitative Dynamic Contrast-Enhanced MRI of Angiogenesis in VEGF-Enhanced Tissue-Engineered Bladder Constructs using Contrast Agents of Different Molecular Weights

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INTRODUCTION

Achieving prompt angiogenesis is a major challenge in engineering functional new tissue [1]. Current strategies such as incorporating vascular endothelial growth factor (VEGF) have shown some success [2], but creating a functional, sustainable blood supply requires a better understanding of the underlying kinetics [3]. Dynamic contrast-enhanced (DCE) MRI is a non-invasive 3D tool for quantitative, serial monitoring of angiogenesis and may aid in this understanding to accelerate the development of effective angiogenic strategies. Its application in tissue engineering has been few [4], and consideration of different contrast agents has not been reported. In this study, we compared two paramagnetic MR contrast agents, Gadomer (MW=17 kDA) and Gd-DTPA (MW=0.57 kDa), for quantifying neovascularization in VEGF-enhanced engineered bladder constructs in a blinded rabbit study. MRI findings were correlated to histological measures of microvessel density (MVD) and permeability.

METHODS

Tissue-engineered bladder constructs enhanced with $VEGF_{121}$ (Sigma, USA) were grafted on the bladder of 12 female New Zealand white rabbits (n=3/VEGF, VEGF=0,10,15,20 ng/g tissue). At 8 days post-implantation, MRI was performed on a 1.5-Tesla scanner (Signa EXCITE TwinSpeed, GE) using an 8-channel transmit/receive knee array coil. A bolus of Gadomer (0.033 mmol/kg) was first administered, followed by Gd-DTPA (0.1 mmol/kg) at least an hour later. DCE-MRI was performed using 3D T1w fast SPGR (TR/TE = 5.18/1.35 ms, FA=15°, BW=31.2 kHz, FOV=12 cm, matrix=256×224×16, SL=3 mm interpolated to 1.5 mm, 1 NEX). T1 maps were acquired pre-injection using a varied flip angle approach [5]. Laparotomy was then performed, and Evans blue (30 mg/kg) was injected into the abdominal aorta to assess permeability. Constructs were harvested and assessed histologically for MVD (CD31-immunostaining) and permeability (595-nm spectrophotometer measurement of extracted Evans blue).

DCE-MRI parameters were calculated from a two-compartment pharmacokinetic model (plasma volume fraction, v_p; transfer constant, K^{trans}) [6] and model-free analysis (area-under-the-concentration-time curve, AUC, normalized to resting dorsal muscle) [7]. The arterial input function (AIF) used in pharmacokinetic analysis was measured in the iliac artery, distal from entry into the slab to minimize in-flow effects. All DCE-MRI parameters were averaged over the construct to yield a mean value for each animal. One-way ANOVA was performed to determine whether DCE-MRI parameters and histology measurements varied significantly with VEGF. Correlation between DCE-MRI and histology was done by Pearson's regression analysis.

RESULTS

MVD was elevated at the highest VEGF (10.76±1.12% vs. 8.86±1.08%, P<0.05) but not amongst lower levels; permeability differences were absent at 8 days post-implantation. Contrast enhancement on MRI increased with VEGF and was better resolved with Gadomer than Gd-DTPA. Gadomer was the better assay for estimating plasma volume, with v_p providing better distinction (P<0.005) than AUC parameters (Fig.1). Correlation of v_p to MVD is significant only for Gadomer (r=0.696, P=0.012) but not Gd-DTPA (r=0.228, P=0.5) (Fig.2). Using Gd-DTPA, AUC_{1min} was the best parameter to distinguish MVD differences (r=0.437, P=0.179). Changes in K^{trans} were insignificant, in keeping with absence of permeability differences.

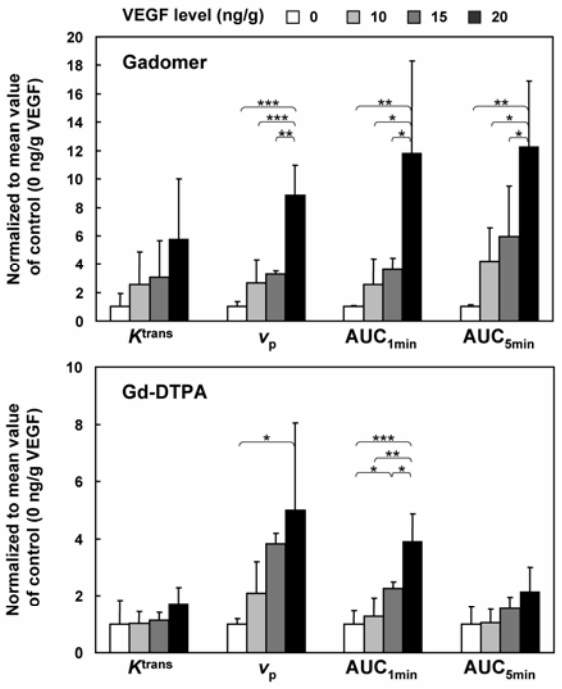

Fig.1 DCE-MRI parameters (K^{trans}, v_p, AUC_{1min}, AUC_{5min}) versus VEGF. With Gadomer, v_p and AUCs are significantly higher at 20 ng/g VEGF, with v_p providing the best distinction. With Gd-DTPA, AUC_{1min} best distinguishes VEGF. Values are mean ± SD (n=3/VEGF). Significance: * (P<0.05), ** (P<0.005), *** (P<0.0005).

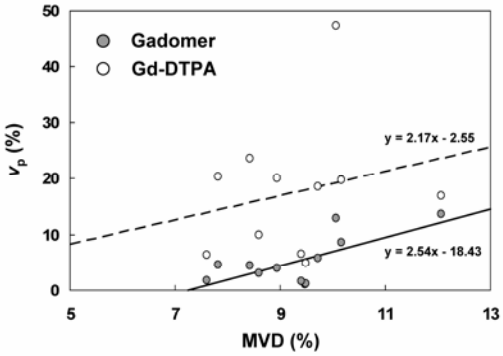

Fig.2 Correlation of DCE-MRI parameter v_p versus microvessel density (MVD). Correlation is significant using Gadomer (r=0.696, P=0.012) but not Gd-DTPA (r=0.228, P=0.50).

CONCLUSIONS

Macromolecular contrast agents are valuable for monitoring angiogenesis in tissue-engineered bladder constructs. Blood volume measurements are possible, and Gadomer provides greater accuracy, precision, and correlation to histological assessment compared to Gd-DTPA. Physiological quantification not possible with model-free approaches can be obtained accurately through pharmacokinetic analysis if accurate T1-mapping and AIF measurements are made. However, if contrast leakage is too rapid to enable accurate pharmacokinetic modeling (as when Gd-DTPA is used), AUC provides a robust model-free alternative to detect vascular differences. Future studies will probe a wider spectrum of blood volumes and permeabilities to refine our DCE-MRI strategy.

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