Dynamic Contrast-Enhanced MRI for Quantifying VEGF-Enhanced Neovascularization in Tissue-Engineered Bladder Constructs

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INTRODUCTION

One major challenge in tissue engineering of large organs is achieving immediate perfusion and monitoring the engineered construct over time to ensure successful integration with surrounding tissue. Dynamic contrast-enhanced MRI (DCE-MRI) offers a non-invasive alternative to conventional methods of graft harvest and histology for on-going assessment of angiogenesis. However, despite its value in oncology (1,2), its application in tissue engineering and its appropriateness to the vascular characteristics of engineered tissue have remained largely unexplored (3).

In this study, we investigated the ability of DCE-MRI for quantitative assessment of angiogenesis in engineered bladder constructs and compared commonly used pharmacokinetic analysis techniques for the best distinction of microvessel density (MVD) in a blinded animal study.

METHODS

Constructs of rabbit bladder acellular matrix-hyaluronic acid were fortified with vascular endothelial growth factor (VEGF) at three concentrations (0,10,20 ng/g of tissue) to enhance angiogenesis. These hybrids were implanted onto the anterior bladder wall in nine rabbits (3 at each VEGF level). At successive times post-implantation (1, 2, and 3 weeks), one rabbit from each VEGF group underwent MRI on a clinical 1.5-Tesla system (Signa LX, GE). DCE-MRI was performed by monitoring a bolus injection of Gd-DTPA (0.1 mmol/kg, Magnevist) using 3D T1w fast SPGR (TR/TE = 9.3/2.1 ms, θ=15°, BW=15.6 kHz, FOV=12 cm, matrix=256×192×12, SL=3 mm, 1 average). A pre-injection T1 map was acquired using a modified Look-Locker approach (4). Grafts were immediately harvested and whole-mount immunostained with CD31. The MVD, measured as the microvascular area (μm^2), was determined using Simple PCI software in the central region of the graft by serial confocal laser scanning microscopy.

Two pharmacokinetic approaches recommended for tumor studies (5) were considered. The first was Tofts model (6), modified to estimate the plasma volume (v_p) in addition to the transfer constant (K^{trans}) and the extravascular extracellular space (EES) volume fraction (v_e). The second was the uptake integral approach (7), where an estimate of the area-under-the-concentration-time curve (AUC) normalized to resting dorsal muscle was calculated for the first 1, 2, and 8 minutes post-injection. DCE-MRI analysis was performed in uniformly enhancing ROIs on 4 to 5 contiguous 3-mm slices to cover the central portion of the construct where immunohistochemistry was performed to determine MVD. A two-tailed Student's t-test was performed to determine whether DCE-MRI parameters (K^{trans}, v_p, v_e, AUC_{1min}, AUC_{2min}, AUC_{8min}) and MVD were significantly different for varying concentrations of VEGF, and whether MRI could correctly distinguish different VEGF levels at various times post-implantation. Finally, DCE-MRI was compared to MVD by Pearson's correlation.

RESULTS

The ability of DCE-MRI to distinguish different VEGF preparations at each post-implantation time point was tested on the assumption of increased DCE-MRI values with higher VEGF. Distinction was poorest using v_p (4 incorrect classifications). Only K^{trans}, AUC_{2min}, and AUC_{8min} correctly classified all cases. Furthermore, AUC_{8min} had the most consistent precision ($P<0.05$ except in one case) and, hence, the best discrimination power.

Immunohistochemistry revealed higher MVD with increased levels of VEGF at all timepoints. MVD was significantly higher in grafts prepared with a high dose of VEGF compared to a low dose ($P=0.014$ versus 0.21). In Fig.1, comparison to DCE-MRI shows that accompanying minimal MVD changes at low VEGF were much larger increases in AUC_{8min} (1.5×) and K^{trans} (3×). In contrast, for the two-fold increase in MVD observed at high VEGF, changes in K^{trans} were insignificant and resulted in overall poor correlation with MVD (Fig.2a. $r=0.572$, $P=0.11$). However, significant correlation to MVD was observed with AUC_{8min} (Fig.2b. $r=0.705$, $P=0.034$). In fact, AUC_{8min} was the only DCE-MRI parameter to demonstrate a significant increase ($P=0.038$) consistent with MVD changes at high VEGF.

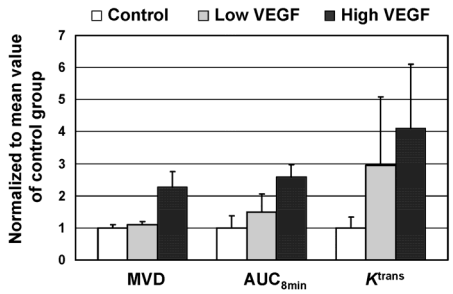

Fig.1 Microvessel density (MVD) and DCE-MRI in different VEGF groups. Shown are mean ± SD normalized to the mean of the control group.

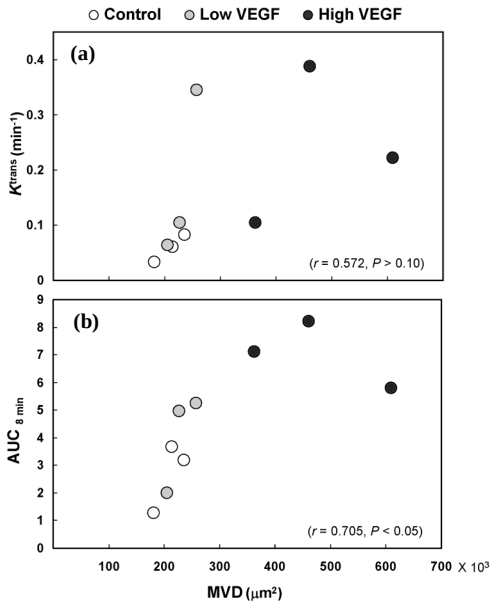

Fig.2 Correlation between (a) K^{trans} and (b) AUC_{8min} versus MVD.

CONCLUSIONS

Our study demonstrates that DCE-MRI with Gd-DTPA can quantitatively assess neovascularization in tissue-engineered bladder constructs using accepted analysis methods. This ability will aid in the development and investigation of strategies for enhancing angiogenesis in engineered human organs.

Comparison of DCE-MRI approaches shows that AUC is the most robust. AUC_{8min} provides the most precise discrimination of different VEGF preparations and is significantly correlated to MVD. Greater variability is seen with Tofts' K^{trans} and v_p, which are more sensitive to noise and inaccurate estimates of the input function. Discrepancies between MVD and DCE-MRI may indicate vessel changes other than density. For example, at low VEGF, enhanced vessel permeability may explain substantial increases in DCE-MRI parameters. Beyond a maximum VEGF level, rapid growth of dense but poorly perfused vessels may explain the modest increases in DCE-MRI. Future studies should incorporate larger contrast agents and permeability assessment to devise an optimal DCE-MRI strategy.

REFERENCES

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