

Dose-dependent effects of Bevacizumab in human HCC xenografts

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Introduction: Bevacizumab (Avastin), an anti-VEGF humanized monoclonal antibody, has demonstrated positive clinical treatment effect and is currently approved for treatment of colorectal carcinoma and NSCLC in combination with standard chemotherapy. Preclinical studies have shown decrease in microvascular density. We aim to determine if DCE MRI is a sensitive enough technique to detect changes in fractional intravascular volume and blood flow after administration of a single dose of bevacizumab.

Materials and Methods:

Mice: Twenty-seven male BALB/c mice (6 weeks old, 30 ± 2g) formed the study population & were under 1-2 % isoflurane during the scanning. They were maintained according to the Guide for the Care & Use of Laboratory Animals (NIH). They were implanted with human-derived hepatocellular carcinoma xenograft line, sensitive to bevacizumab therapy. Two dose levels were investigated: low dose (n = 4) at 1 mg/kg and high dose (n=8) at 10 mg/kg. Mice were scanned at baseline, day 1 and 5 after therapy. Fifteen control mice were similarly imaged after receiving Xolair (Omalizumab, a human anti-IgE antibody) at 10 mg/kg. Tumors were sectioned and stained immunohistochemically to identify CD34 for quantification of microvessel density.

DCE-MRI: MRI was performed on a 7T scanner (Bruker ClinScan, Bruker BioSpin MRI GmbH, Germany). A 3D VIBE sequence was used with following parameters: TR = 3.04 ms, TE = 1.23 ms, FOV = 36 × 36 mm, 128 × 128 matrix, 8 slices with thickness of 1 mm, & temporal resolution 2 s. Five sets of baseline images were acquired with $\alpha = 6^\circ$ & 14° . It was followed by a dynamic sequence of 130 sets of images ($\alpha = 14^\circ$). A dose of 100 μ L of Gd-DOTA (Dotarem, Guerbet SA, France) at 1 mmol/kg was injected through the tail vein after the first set of dynamic images.

Data Processing: Region of interests corresponding to the xenograft and major artery were manually outlined. Microcirculatory parameters such as blood volume were derived from the two-compartment model as described by Brix.

Results:

Treatment	F (ml/100ml/min)			v ₁ (%)		
	BL	D1	D5	BL	D1	D5
Xolair 10	79.08 ± 28.28	101.14 ± 54.11	105.59 ± 33.39	7.32 ± 3.47	8.36 ± 3.21	8.68 ± 3.59
Bevacizumab 1	133.73 ± 27.91	70.22 ± 19.35	77.85 ± 23.77	9.67 ± 1.72	9.29 ± 4.15	4.51 ± 1.62
Bevacizumab 10	95.26 ± 26.69	51.82 ± 16.15	39.45 ± 14.14	10.02 ± 3.85	4.35 ± 1.77	2.15 ± 0.64

Baseline blood flow and volume for the control group was 79.08 ± 28.28 ml/100ml/min and 7.32 ± 3.47 %, respectively. The values were 133.73 ± 27.9 ml/100ml/min and 9.67 ± 1.72 % for the group treated with bevacizumab 1mg/kg and 95.26 ± 26.69 ml/100ml/min and 10.02 ± 3.85 % for the group dosed with bevacizumab 10mg/kg.

A similar reduction of 46.18 ± 15.39 % and 37.88 ± 37.31 % in blood flow at Day 1 was observed in the group treated with bevacizumab 1mg/kg and 10mg/kg, respectively. However, a further reduction of 31.55 ± 38.03 % at Day 5 was found in bevacizumab 10mg/kg, but not in 1mg/kg, which actually saw an increase of 11.90 ± 33.06 %. An increase of 28.18 ± 58.16 % in blood flow at Day 1 was observed in the control group.

A reduction of 52.69 ± 24.77 % in blood volume at Day 1 was observed in the group treated with bevacizumab 10mg/kg, while a reduction of 6.10 ± 31.86 % was observed in those treated with bevacizumab 1mg/kg. An increase of 29.31 ± 66.84 % at Day 1 was observed in the control group. There is a good correlation between percentage of vessel area derived by CD34 staining with percentage of blood volume derived by the two-compartment model ($r = 0.806$, $p = 0.0002$).

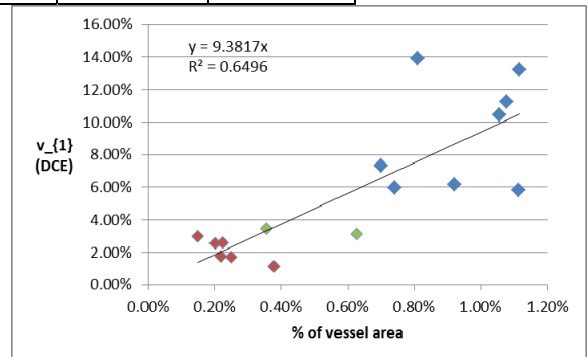


Fig 1. Correlation between percentage of vessel area derived by CD34 staining with blood volume derived by the two-compartment model

Conclusion: The results showed that the effects of bevacizumab are dose-related. A more gradual drop of blood volume was observed in group treated with bevacizumab 1mg/kg. On the other hand, a steep drop of blood volume was observed in group treated with bevacizumab 10mg/kg. The results suggested potential of DCE MRI as a sensitive biomarker of microvascular density. This facilitates translation and clinical application to early time points after anti-angiogenic therapy. This study validates DCE MRI as a reliable non-destructive testing method for time course observation of MVD changes in animals and facilitates future experiments to study drug resistance.

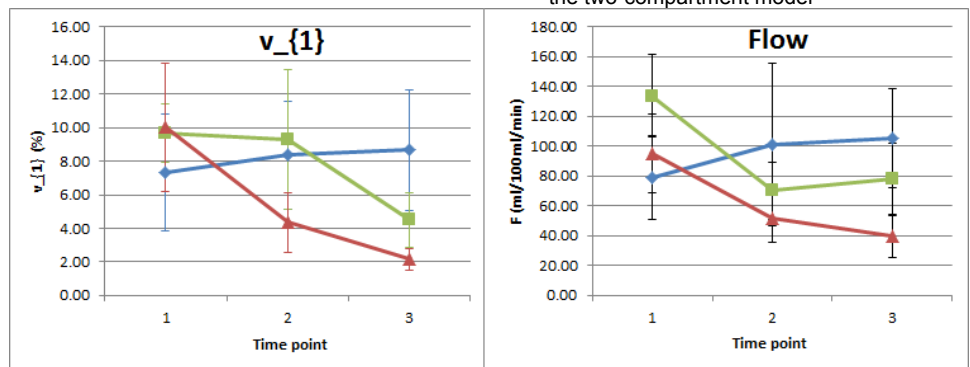


Fig 2. Plot of blood volume and blood flow for all time points in control (blue) and treated groups (1mg/kg: green, 10mg/kg: red)