THE USE OF MAGNETIC RESONANCE ANGIOGRAPHY TO DIRECTLY ASSESS ARTERIOGENESIS IN MICE

Joan M Greve (1,2), Timothy JA Chico (1), Hope Steinmetz (1), Lisa Bernstein (1), Simon-Peter Williams (1), Stuart Bunting (1), Charles A Taylor (2), Nicholas van Bruggen (1)

(1) Physiology Department
Genentech, Inc.
South San Francisco, CA

(2) Dept of Mechanical Engineering
Stanford University
Stanford, CA

INTRODUCTION

Adequate blood supply is provided by a balanced combination of larger caliber arterial pathways feeding into capillary beds that perfuse the tissue or organ. When such a network is damaged or diseased, causing blood flow to be inadequate, function is compromised. Two mechanisms of vascular remodeling, angiogenesis and arteriogenesis, proceed spontaneously in an attempt to restore sufficient blood flow and hence function.

Angiogenesis has been defined as the *de novo* sprouting of capillary beds; it is closely associated with tissue ischemia, may play an important role in acute tissue survival, and can be potentiated by the exogenous application of vascular endothelial growth factor (VEGF). Arteriogenesis is the growth of collateral pathways from pre-existing arterioles; it is associated with inflammation and believed to be driven mainly by mechanical forces [1]. Adequate collateral vessel formation is required for restoration of function [2]. These two mechanisms have been shown to be temporally dissociated in models of peripheral artery disease [3] and the role VEGF might play in promoting arteriogenesis is unclear. To elucidate the role of exogenous VEGF in arteriogenesis, we developed magnetic resonance angiography techniques to directly evaluate arteriogenesis in the hindlimb of C57Bl6 mice.

Data presented here primarily utilized three-dimensional time of flight magnetic resonance angiography (3D TOF-MRA). The ability to detect blood vessels using 3D TOF-MRA is dependent on several factors including the size of the vessel as well as blood flow. Because VEGF increases the expression of nitric oxide (NO) synthase and hence NO, a potent vasodilator, it was necessary to apply an NO synthase inhibitor, L-NAME, in order to discern true vessel enlargement from NO-mediated vasodilation. Similarly, to distinguish changes in vessel size from changes in blood velocity, images acquired using a 2D TOF-MRA technique, which is insensitive to dramatic changes in velocity, were compared to the 3D TOF-MRA data. Results show that VEGF can induce collateral growth.

MATERIALS AND METHODS

All experiments were performed with local IACUC approval in accordance with ethical guidelines. Male 8-10 week old C57Bl6 mice were used for all experiments.

Murine VEGF was formulated at 1mg/ml in a sustained release formulation matrix. Injections consisted of 25µl of 1mg/ml, VEGF or vehicle, at two sites in the left calf.

TOF-MRA was performed at 4.7T (Unity Inova MR system, Varian, Inc., Palo Alto, CA) using a 3cm inner diameter radio frequency volume coil. After a pilot scan to confirm positioning, a 3D TOF-MRA (flip angle = 20°, FOV = $3cm^2$, TR=15ms, TE = 2.6ms, isotropic matrix of 128 zero filled to 256^3) or 2D TOF-MRA (flip angle = 60°, FOV $3cm^2$, TR = 20ms, TE = 1.9ms, isotropic matrix of 256, slice thickness = 0.33mm) sequence was used to acquire data sets. Maximum intensity projection reconstructions were used to visualize data. During data acquisition, animals were maintained on 1.5% isoflurane in 1L medical grade air. Body temperature was monitored and maintained between 37 and 38C using warm air.

To assess the contribution vasodilation might play in the appearance of the MRA's following vehicle or VEGF treatment seven days post injection, baseline 3D TOF-MRA's were acquired followed by acquisition of MRA's while L-NAME was administered via a jugular vein catheter (initial bolus: 3.75mg in 100µl; maintenance infusion throughout image acquisition: 0.32mg per minute).

RESULTS

The 3D TOF-MRA's showed a dramatic increase in the number and extent of angiographically detectable vessels 5 days after injection of exogenous murine VEGF into the calf (Figure 1).

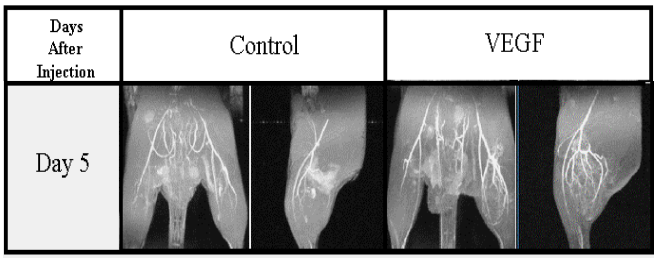

Figure 1. 3D TOF-MRA maximum intensity projections of the lower limbs of mice with and without VEGF treatment.

A small but visible change of vasodilator tone was apparent when the NO synthase inhibitor, L-NAME, was administered intravenously while acquiring 3D TOF-MRA data (Figure 2).

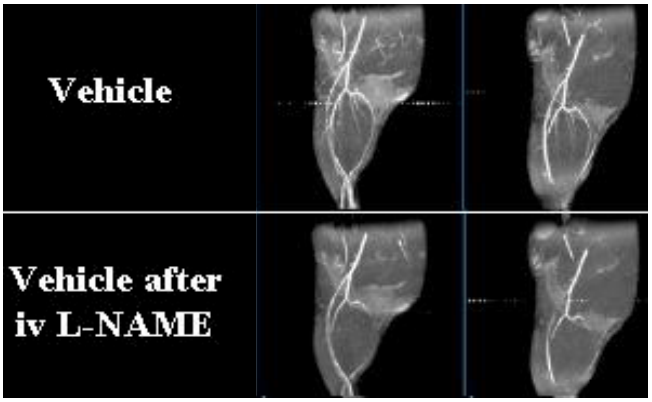

Figure 2. 3D TOF-MRA data of the lower limbs of mice, acquired prior to and during intravenous administration of L-NAME.

Application of L-NAME intravenously did not abolish the remarkable increase in detectable vessels due to exogenous VEGF (Figure 3).

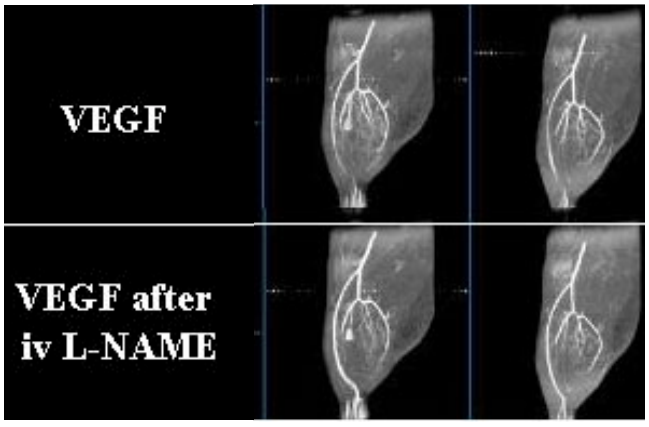

Figure 3. 3D TOF-MRA data of the lower limbs of mice acquired prior to and during intravenous administration of L-NAME, 7 days after injection of murine VEGF in the calf.

The increase in vessel density was comparable using a 2D or 3D TOF-MRA to evaluate the same animals (Figure 4).

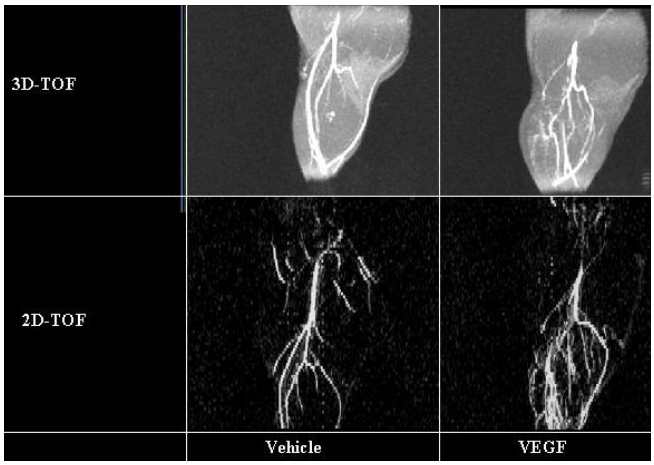

Figure 4. 3D TOF-MRA and 2DTOF-MRA maximum intensity projections of the lower limbs of mice with and without VEGF treatment.

DISCUSSION

Using 3D TOF-MRA we were able to longitudinally monitor the progression of arteriogenesis, directly and in vivo, and qualitatively assess the effects of the application of exogenous VEGF. The 2D TOF-MRA images demonstrate that the increase in trackable vessels is not due to marked alterations in blood flow in the vessels of interest. Furthermore, the ineffectual application of L-NAME substantiates that the increase in angiographically detectable vessels is not simply due to vasodilation. It was concluded that the results presented here are due to actual arterial expansion and not due to changes in blood velocity or vasodilation.

Although these data clearly show that VEGF can promote arteriogenesis, it has been challenging to quantify, *in vivo*, the functional benefits that are believed to accompany the anatomical changes illustrated here. Therefore, it is highly desirable to implement a magnetic resonance technique that is inherently quantifiable or lends itself more readily to quantification through post acquisition image analysis so that characteristic properties of the vascular network, e.g. resistance, might be calculated and utilized to elucidate the relationship between angiogenesis and arteriogenesis, as they are inextricably linked.

REFERENCES

1. Helisch, A., Schaper, W., 2000, "Angiogenesis and Arteriogenesis – Not Yet for Prescription," Zeitschrift fur Kardiologie, Vol. 89, pp. 239-44.
2. Schaper, W, 2000, "Quo Vadis Collateral Blood Flow? A Commentary on a Highly Cited Paper," Cardiovascular Research, Vol. 45, pp. 220-3.
3. Hershey, J. C., Baskin, E. P., Glass, J. D., et al., 2001, "Revascularization in the Rabbit Hindlimb: Dissociation Between Capillary Sprouting and Arteriogenesis," Cardiovascular Research, Vol. 49, pp. 618-25.