THE HEMODYNAMICS OF ANGIOGENESIS: ANGIOGRAPHIC STUDY USING THE RABBIT HIND LIMB ISCHEMIA MODEL

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INTRODUCTION

Therapeutic angiogenesis is the induction of new vessel growth to treat occlusive vascular disease. Gene therapy employed to express various proangiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), is the primary means of therapeutic angiogenesis. Clinical trials of therapeutic angiogenesis to treat peripheral and coronary arterial occlusive diseases have produced mixed results. Small phase 1 clinical trials of angiogenesis induced by plasmids encoded to express proangiogenic factors provided promising outcomes. However, a large, double-blinded, placebo-controlled randomized trial of intracoronary and intravenous injections of recombinant VEGF protein did not sustain significant clinical benefit at one-year follow-up [1]. Thus, much *in vitro* and *in vivo* research is currently ongoing to refine techniques of therapeutic angiogenesis.

Angiographic assessment of angiogenesis utilized in both the clinical arena and in the research laboratory has been limited to the analysis of singular angiographic images. The most common imaging technique used to quantify outcomes of proangiogenic therapy is the calculation of an angiographic score. This score represents the total number of contrast-opacified vessels crossing the 2.0 mm squares of a grid overlay divided by the total number of squares and then multiplied by 100. We developed a novel approach to quantify the level of angiogenesis by modeling the temporal variation of contrast intensity throughout an angiographic sequence. The dynamic information extracted from the images of angiographic sequences may then be mathematically modeled, and the model parameters provide information about the transport of contrast through the specified region of interest. We hypothesize that these model parameters serve as quantifiable indices of induced angiogenesis.

MATERIALS AND METHODS

Rabbit Hind Limb Ischemia Model

The rabbit hind limb model of ischemia is a well accepted *in vivo* model used to study proangiogenic therapy. In this investigation, the left external iliac artery was ligated just distal to the internal iliac artery. Subsequently, a segment of the femoral artery was resected extending from the lateral femoral circumflex artery to the descending genicular-popliteal bifurcation. The surgical procedure was performed in accordance with the guidelines of the Animal Care and Use Committee of the University of Miami. Figure 1 depicts the hind limb arterial tree prior to (A) and after surgery (B).

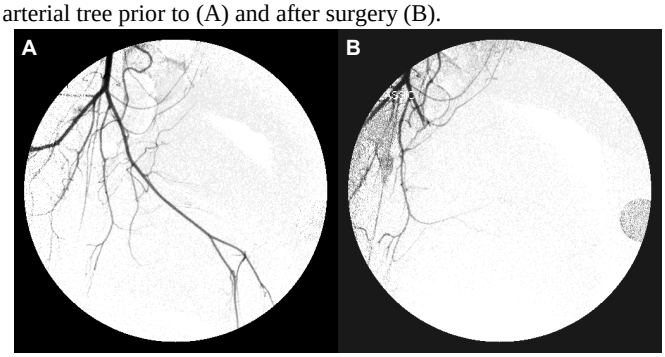

Figure 1. Digitally subtracted angiographic (DSA) images of the left lower limb of the rabbit before (A) and after (B) surgery.

Angiography and Contrast Injection

Angiographic sequences were acquired at a rate of 30 frames per second using an Angiostar Plus (Siemens, Forchheim Germany). The acquired images have a resolution of 512x512 pixels. Angiography was performed prior to and after the surgical intervention and then at three follow-up time points, namely one, three, and six weeks. In all cases, an arterial port was created in the right common carotid artery and at the follow-up in the left carotid artery. A 4 Fr straight catheter (Tempo, Cordis, Miami Lakes FL) was navigated retrograde into the descending aorta. The tip of the catheter was positioned at the level of the intervertebral disc between L3 and L4. Imaging chain parameters, such as the peak voltage and the pulse width, were all kept constant for each angiographic session. Angiographic contrast (Visipaque-320, Amersham Health, Buckinghamshire UK) was injected using a

computer controlled micro-injection pump, designed and built within our laboratory. A total of 5 ml of contrast was injected at a rate of 3 ml/s. Injection pressure measurements were recorded to ensure repeatability.

Gene Therapy

In this series of experiments, one half of the rabbits received 600 μl of an adenoviral vector with a gene sequence inserted to lead to the expression of VEGF. The viral vectors were delivered intramuscularly at randomly selected sites throughout the thigh muscle. The second half of the animals received no treatment, and served as controls.

Image Processing

Images acquired during the experiments were archived on digital optical discs in DICOM XA multiframe file format. These images were subsequently converted to the tagged image file (tif) format for processing in Matlab (The Mathworks, Natick MA). An elliptical region of interest (ROI) was created with dimensions and orientation defined based on the femur. Using an image with the vessels clearly opacified, a digital mask isolating the arteries within the ROI was created (white is 1 and black is 0). This mask was then multiplied by all of the images of the respective angiographic sequence (Figure 2). The spatially averaged temporal variation of the grayscale intensity was then calculated. A second mask was created by inverting the previous mask in order to isolate angiographic blush, which is the region of the image with increased intensity caused by the transport of contrast into small vessels. The procedure for measuring the grayscale intensity was then repeated using this mask

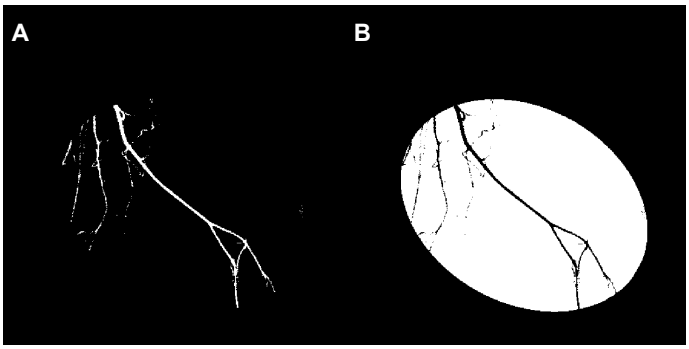

Figure 2. Masks employed to quantify the grayscale intensity within the large arteries (A) and the angiographic blush (B) for the pre-surgery case.

MATHEMATICAL MODELING

The following model was applied to the time history of the spatially average grayscale intensity for each case. This model was applied to data obtained using both types of masks (Figure 2). The first component of the model is a lagged-normal density function which represents the convective transport of contrast through the ROI [2]. The second term of the model is related to the error function and signifies the stagnation of contrast in the flow separated regions within the arteries in addition to the venous return within the ROI. The observation period is insufficient to permit the return of the grayscale intensity to zero, thus requiring this second term. Model parameters were obtained using a constraint optimization algorithm that minimizes the difference between the experimental data and the model in a least squares sense.

$$f(t)=\rho_1\int_0^t \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{(\eta-\mu)^2}{2\sigma^2}} \bullet \frac{1}{\tau} e^{\frac{(t-\eta)}{\tau}} d\eta + \rho_2\int_0^t \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{(\eta-\mu)^2}{2\sigma^2}} d\eta$$

RESULTS & DISCUSSION

Preliminary results are demonstrated in Figures 3 and 4. This data was acquired at the one week follow-up time point. The data obtained using the mask of the large arteries (Figure 2a) delineates noticeable differences between the control and the treated cases, which are quantified by the model parameters (Table 1). These differences are more pronounced when comparing the results from the mask that isolates the angiographic blush. At this juncture, statistical assessment has not been performed since the experiments are ongoing. However, the preliminary data indicates that the model describes the observed physical transport of contrast through the ROI. Moreover, we anticipate that the model parameters will serve as indices to quantify the level of angiogenesis.

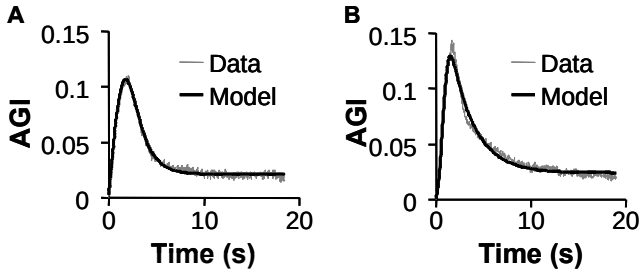

Figure 3. The average grayscale intensity (AGI) within the large arteries at the one week follow-up point for the control (A) and the treated (B) cases.

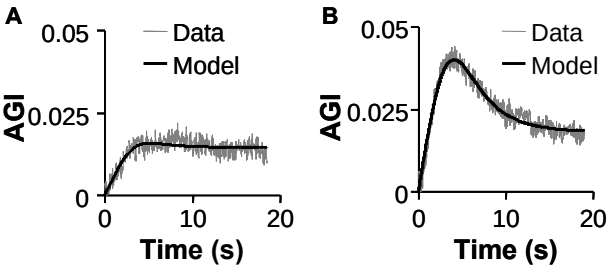

Figure 4. The average grayscale intensity (AGI) outside the large arteries (angiographic blush) at the one-week follow-up point for the control (A) and the treated (B) cases.

		ρ_1	ρ_2	τ	μ	σ
Large Artery Mask	*Control*	0.09	0.02	1.36	0.71	1.02
	Treated	0.11	0.02	2.44	0.76	0.48
Angiographic Blush	*Control*	0.002	0.014	3.0	0.9	2.0
	Treated	0.025	0.019	3.12	1.1	1.2

Table 1. Model parameters for the cases depicted in Figures 3 & 4.

REFERENCES

1. Henry, T.D., *et al.*, 1999 "Double blind, placebo controlled, trial of recombinant human vascular endothelial growth factor: the VIVA trial," J. Am. Coll. Cardiol., 33, p. 384A.
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