LABORATORY INVESTIGATION

Microvascular Perfusion Changes following Transarterial Hepatic Tumor Embolization

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ABSTRACT

Purpose: To quantify changes in tumor microvascular (< 1 mm) perfusion relative to commonly used angiographic endpoints.

Materials and Methods: Rabbit Vx2 liver tumors were embolized with 100–300-μm LC Bead particles to endpoints of substasis or complete stasis (controls were not embolized). Microvascular perfusion was evaluated by delivering two different fluorophore-conjugated perfusion markers (ie, lectins) through the catheter before embolization and 5 min after reaching the desired angiographic endpoint. Tumor microvasculature was labeled with an anti-CD31 antibody and analyzed with fluorescence microscopy for perfusion marker overlap/mismatch. Data were analyzed by analysis of variance and post hoc test (n = 3–5 per group; 18 total).

Results: Mean microvascular density was 70 vessels/mm^2 ± 17 (standard error of the mean), and 81% ± 1 of microvasculature (ie, CD31$^+$ structures) was functionally perfused within viable Vx2 tumor regions. Embolization to the extent of substasis eliminated perfusion in 37% ± 9 of perfused microvessels ($P > .05$ vs baseline), whereas embolization to the extent of angiographic stasis eliminated perfusion in 56% ± 8 of perfused microvessels. Persistent microvascular perfusion following embolization was predominantly found in the tumor periphery, adjacent to normal tissue. Newly perfused microvasculature was evident following embolization to substasis but not when embolization was performed to complete angiographic stasis.

Conclusions: Nearly half of tumor microvasculature remained patent despite embolization to complete angiographic stasis. The observed preservation of tumor microvasculature perfusion with angiographic endpoints of substasis and stasis may have implications for tumor response to embolotherapy.

ABBREVIATIONS

FITC = fluorescein isothiocyanate, SACE = subjective angiographic chemoembolization endpoint, TRIP = transcatheter intraarterial perfusion

Although transarterial embolization and chemoembolization have been performed for decades, there are relatively few quantitative data describing the effects of embolotherapy on the tumor microenvironment. Currently, angiography is used to visualize tumor-supplying arteries and monitor reduction in blood flow in these arteries to gauge residual tumor perfusion and treatment endpoints. However, only arteries > 1 mm in diameter can be sufficiently resolved with angiography, and smaller vessels remain difficult to evaluate despite magnification and other efforts to optimize imaging. This is a significant limitation, as tumor microvasculature, the small blood vessels (10–30 μm) responsible for nutrient delivery and oxygen exchange to individual tumor cells, are typically approximately 100 times smaller than arteries resolved with angiography. This highlights the importance of understanding changes in tumor microvascular

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An Appendix and Figures E1 and E2 are available online at *www.jvir.org*

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perfusion in the optimization of embolotherapy. Although the relationship between angiographic embolization endpoints and global tumor perfusion has been quantified (1), the effect of embolization on the extent of tumor microvascular perfusion has not been well defined.

There are established tools and techniques used to assess microvascular density and perfusion within tumors in preclinical models and clinical pathology. For example, the CD31 antibody (which binds a glycoprotein expressed by endothelial cells) can provide an indicator of the microvascular density in tumors, and CD31 can be complemented by intravascular delivery of perfusion markers such as Hoechst (which binds nucleic acids) or lectins (which bind glycoproteins in the endothelial wall) to identify the fraction of functionally perfused microvasculature in histologic sections (2,3). Fluorescently conjugated lectins allow simultaneous polychromatic visualization of perfused vasculature (3). In addition, advanced microscopes with integrated mechanical slide scanning capability can now provide high-resolution (< 1 μm) histologic images over large tissue regions (> 1 cm) that permit regional/geographic microvasculature mapping of entire tumor cross sections. When perfusion markers are administered appropriately and analyzed with quantitative techniques, the influence of transarterial embolization on tumor microvascular perfusion may be determined.

The objective of the present study was to quantify changes in tumor microvascular (< 1 mm) perfusion relative to commonly used angiographic embolization endpoints in intrahepatic Vx2 tumors in a rabbit model. Transcatheter arterial delivery of fluorescently conjugated lectins and high-resolution microscopy were used to identify changes in microvascular perfusion caused by embolization to angiographic substasis and stasis.

MATERIALS AND METHODS

The materials and methods presented here are abbreviated. Additional details can be found in the **Appendix** (available online at *www.jvir.org*) (4).

Delivery of Embolic Material to Intrahepatic Vx2 Tumors

Femoral access and catheter placement in the proper hepatic artery was obtained with a 3-F sheath (Cook, Bloomington, Indiana) and 2.4-F microcatheter (Progreat, Terumo, Somerset, New Jersey). Routine fluoroscopy with a model 9600 or 9900 Elite Digital Mobile Super C-arm (GE Healthcare/OEC Medical Systems, Waukesha, Wisconsin) guided catheter placement and confirmed catheter tip location. Intrahepatic Vx2 tumors were identified with angiography as shown in **Figure 1**.

LC Bead particles (100–300 μm; Biocompatibles BTG UK, Farnham, United Kingdom) were mixed at a 1:20 ratio in saline solution/Isovue 300 contrast agent (1:1 ratio; Bracco Diagnostics Inc., Monroe Township, New Jersey) and delivered under fluoroscopic monitoring to the desired angiographic endpoints of substasis or stasis. Embolization to substasis was defined as delivery of a fixed bead volume (0.05 mL total bead volume) that resulted in occlusion of tumoral and peritumoral vessels while maintaining patency of the feeding hepatic artery. Embolization to stasis was defined by complete occlusion of tumoral and peritumoral vessels as well as the more proximal feeding hepatic artery branches, with apparent reflux on further injection. The total bead volume required to reach complete stasis ranged from 0.15 mL to 0.3 mL. Intermittent angiography was performed throughout the embolization to monitor antegrade flow and reflux, and angiographic endpoints were evaluated by one of two interventional radiologists (each with more than 6 y of clinical experience). Following embolization, the animals were euthanized, and tumor tissue was harvested, flash-frozen, and stored at −80 °C before ex vivo analysis.

Microvascular Perfusion Assessment

Pre- and posttreatment microvascular perfusion was evaluated by administering different fluorophore-conjugated perfusion markers through the catheter before embolization to assess native tumor perfusion (lectin/fluorescein isothiocyanate [FITC]) and 5 minutes after reaching the desired angiographic endpoint to assess the embolization effect (lectin/Texas red). The perfusion markers were delivered at a rate of 1 mL/min (0.5 mg of lectin in 5 mL of heparinized saline solution; tomato lectins; Vector Laboratories, Burlingame, California). Animals treated with coadministration ($n = 3$) and sequential administration ($n = 5$) of perfusion markers served as control groups, and animals embolized to the extent of substasis ($n = 5$) and stasis ($n = 5$) served as treatment groups (18 subjects evaluated in total).

Euthanasia was performed 5 minutes after the final perfusion marker was delivered, and livers were harvested and flash-frozen. Frozen tissue was cryosectioned and then stained for cell nuclei (4′,6-diamidino-2-phenylindole; Invitrogen, Grand Island, New York) and blood vessels (CD31; Abcam, Cambridge, Massachusetts; Cy5 conjugated with Zenon labeling; Invitrogen). Microvascular perfusion markers were delivered through the catheter from the same catheter tip location used for embolization. Epifluorescence imaging was conducted with a 10× objective on a Scanscope FL system (Aperio, Vista, California) equipped with a monochrome CCD camera. Image processing software (ImageScope; Aperio) was used to export grayscale images as full-resolution TIFF files for each fluorescent channel.

Image Analysis

Image acquisition and display parameters were held constant to allow for quantitative comparison between