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Multi-spectral molecular imaging of capillary endothelium to facilitate preoperative endovascular brain mapping

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Abstract

Object—Brain mapping strategies globally aim to non-invasively localize neurological function to specific regions of the human brain. Preoperative endovascular brain mapping (PEBM) is a novel imaging-based approach designed to visually discriminate functional from silent brain parenchyma and therefore has potential to improve neurosurgical guidance during intracranial resection. In this study, the authors report the first PEBM approach that combines selective molecular targeting of brain endothelium with multispectral (optical) molecular imaging to generate regio-selective endovascular brain maps in preclinical animal models.

Methods—Sprague-Dawley rats and New Zealand white (NZW) rabbits were selectively catheterized and administered a FITC-derivatized tomato lectin-based imaging probe intra-arterially. Following a brief binding/uptake period, saline was infused to remove unbound imaging probe and brains were subsequently harvested for analysis. Gross imaging probe regio-selectivity was documented by multi-spectral molecular imaging of intact brains, with discrete localization and endothelium-directed targeting validated by histology.

Conclusions—We have demonstrated the first endothelium-targeted approach to PEBM that does not require manipulation of the intact blood-brain-barrier or result in vascular occlusion. Furthermore, the presented multi-spectral molecular imaging technique appears to be a suitable methodology for the generation of regio-selective brain maps of vascularized brain parenchyma. Further refinement of the PEBM approach, as well as the development of improved imaging probes, may result in clinical advancement of PEBM where direct visual discrimination of functional and silent brain parenchyma at the time of resection could significantly improve neurosurgical outcomes.

Keywords

multi-spectral molecular imaging; brain mapping; neurosurgical guidance; Wada test; brain endothelium

Introduction

Brain mapping strategies globally aim to localize neurological function to specific regions of the human brain. Today, a diversity of sophisticated brain mapping strategies exists and include electrophysiological methods as well as imaging-based techniques such functional magnetic resonance imaging (fMRI) (1,2,8–10,12). Within the context of clinical neurosurgery, the utility of brain mapping is to determine the presence or absence of neurological function within resection fields. There is growing interest in developing and advancing minimally-invasive strategies suitable for real-time, intraoperative assessment of functional parenchyma within potential resection fields. We recently reported a novel approach termed preoperative endovascular brain mapping (PEBM) which aims to synthesize individualized functional brain maps in patients requiring surgical resection(4). Such maps could facilitate neurosurgical guidance and thus improve clinical outcomes after neurosurgical resections.

The PEBM technique combines superselective intra-arterial catheterization of a distal target artery (e.g. M4 branches, P4 branches) with localized delivery of imaging agents to generate regioselective maps of the corresponding brain tissue. Conceptually, prior to administration of the mapping agent, a super-selective Brevital infusion test is performed to determine the presence or absence of neurological functional activity present in the target brain parenchyma. If it is determined from this provocative testing that resection of the target brain parenchyma will not result in significant loss of neurological function, then the mapping agent can be infused into the same target artery and corresponding parenchyma with the goal of delineating this tissue with sharp, discernable margins. At the time of subsequent craniotomy, if resected tissue is confined to the mapped silent parenchyma, then new neurological deficit may be avoided or minimized.

PEBM-suitable imaging probes can be prepared from a variety of approaches, with each having strengths and limitations (4). In general, PEBM probes should be non-toxic, facilitate clear discrimination between mapped and non-mapped tissues, and must have adequate residency time within the target tissue to serve an entire neurosurgical procedure. In preliminary investigations, we have reported PEBM in rat and rabbit animal models with various dyes and embolic agents (3–7). While these studies demonstrated the conceptual feasibility of PEBM, the imaging reagents used had considerable drawbacks including vascular occlusion in the case of the embolic agents, potential toxicity in the case of nanoparticles, and requirement of blood brain barrier (BBB) manipulation in the case of the non-targeted dyes.

In this study, we report a novel molecularly targeted PEBM approach that does not require manipulation of the intact blood-brain-barrier or result in vascular occlusion. Selective molecular targeting of brain capillary endothelium, which is present in sufficient density to provide gross parenchymal contrast if adequately targeted, is achieved through the use of a FITC-conjugated lectin-based molecular imaging probe. We combine the use of this imaging probe with multispectral (optical) imaging to generate selective endovascular brain maps in rats, delineating brain parenchyma of either the left or right hemisphere. In addition, because of the ability to selectively catheterize the P1 segment of the posterior cerebral artery (PCA) in the rabbit brain, we illustrate PEBM of brain parenchyma within the PCA distribution in NZW rabbits. With further refinement of both imaging probes and metrics, and after demonstrating no evidence of systemic toxicity, it is anticipated that these techniques can be eventually attempted in a limited feasibility human trial of patients who require a parenchyma resection.

Materials and Methods

All procedures involving animal use were approved and conducted under the supervision of the Vanderbilt University Institutional Animal Care and Use Committee.

Animal preparation

Sprague-Dawley rats (Harlan, Indianapolis, IN) were used to demonstrate the endothelium-targeted approach to preoperative endovascular brain mapping (PEBM). Rats of mass approximately 350–450g were anesthetized via a single intraperitoneal injection of a ketamine-xylazine mixture (87mg/mL and 13mg/mL respectively, J.A. Webster, Inc., Sterling, MA). Following anesthetization, a carotid cutdown was employed to expose the common carotid (CCA), and the bifurcation of the external carotid (ECA) and the internal carotid (ICA) arteries. The ECA and occipital arteries were occluded with a ligature to enhance selective flow through the ICA. A polyethylene catheter (PE-50, o.d. 0.965mm, i.d. 0.580mm, Intramedic®) was introduced into the lumen proximal to the internal carotid artery (ICA) and was secured within the vessel by suture. Once catheterization was complete, 1 mL of an aqueous 2 mg/mL solution of FITC-derivatized tomato lectin (Vector Laboratories) was directly infused into the right ICA at a rate of 100µL/min using a micro-infusion pump. Following administration of the probe and a static binding period of approximately 10 minutes, 2mL of sterile normal saline was infused through the catheter and into the brain to flush unbound imaging agent from the parenchymal vasculature. Following the final infusion, animals were sacrificed and the brains harvested for ex vivo characterization.

New Zealand white rabbits were used to demonstrate that the PEBM agent could be infused in a selective intracranial artery. Rabbits weighing 3.5–4.8 kg were sedated and anesthetized with acepromazine (Henry Schein, Melville, NY) given subcutaneously, ketamine/xylazine (40/8 mg/kg) given by intramuscular injection (IM), small doses of ketamine, given IM. Maintenance of the anesthesia plane was monitored by blink reflex and changes in heart rate and respiration rate. A femoral cutdown was performed to expose the femoral artery. A 4 French microaccess kit was employed to gain access to the artery. Once the sheath was in place, a Marathon 10 microcatheter with a Mirage 0.008 inch microguidewire (ev3, Neurovascular, Irvine, CA) was inserted through the sheath and maneuvered through the femoral into the basilar artery and then into the P1 segment of the posterior cerebral artery using digital subtraction angiography. Subsequently, 1 mL of an aqueous 0.5 mg/mL solution of FITC-derivatized tomato lectin (Vector Laboratories) diluted in saline directly into the right P1 segment at a rate of 100µL/min using a micro-infusion pump. Following administration of the probe and a static binding period of approximately 10 minutes, 2mL of sterile normal saline was infused through the catheter and into the brain to flush unbound imaging agent from the parenchymal vasculature. Following the final infusion, animals were sacrificed and the brains harvested for ex vivo characterization.

Multi-spectral imaging

Multi-spectral brain images were collected using a CRI Maestro® fluorescence imaging system. Prior to collecting experimental images, spectral libraries were generated for both the imaging agent and endogenous brain tissue autofluorescence. Tissues were illuminated with a xenon lamp through the supplied blue filter set (ex. 445–490nm, em. 515nm long pass). Image cubes were collected from 500nm–720nm at 10nm spectral resolution and processed using the supplied software interface.

Histology and microscopy

Immediately following imaging, fresh brains were sectioned perpendicular to midline and the resulting tissues were either snap frozen for formalin fixed. Fresh frozen tissue was sectioned

(7 μ M slices) and mounted in Vectashield mounting media (Vector Laboratories) containing a DAPI counterstain. Formalin fixed tissues were sectioned similarly and serial slices were stained with either standard haematoxylin and eosin or goat anti-CD34 (Santa Cruz Biotechnology, Santa Cruz, CA). Histological sections were imaged using a Leica DM5000 B inverted fluorescence microscope coupled to a cooled CCD camera and configured with standard DAPI (ex. 350 $\times$ 50nm, em. 460 $\times$ 50nm) and FITC (ex. 495 $\times$ 18nm, em. 535 $\times$ 30nm) filter sets.

Results

Selective PEBM of right hemisphere in rat

Following infusion of the imaging probe and brain resection, selective localization of the imaging probe to the right hemisphere was easily visualized. Spectral un-mixing of the collected fluorescence imaging cubes discriminated tissue autofluorescence, arterial blood, and FITC-lectin fluorescence (Fig. 1). As shown in Fig. 1C/F, the imaging probe was primarily confined to right hemisphere brain parenchyma and did not appear localized in the collection of arteries that comprise the Circle of Willis.

Histological validation of capillary targeting

Specific targeting of brain capillaries by the FITC-lectin imaging probe was validated by histology (Figs. 2A–D). As shown, brain capillary endothelial cells on the right hemisphere, as visualized by anti-CD34 staining (Fig. 2B), appeared brightly fluorescent when serial unstained tissues were examined by fluorescence microscopy (Fig. 2C). Importantly, cross-sections of stained capillaries demonstrated that the imaging probe did not cause vascular occlusion, as shown in Fig. 2C, inset. Brain tissues examined from the left hemisphere (un-mapped) exhibited minimal accumulation of the imaging probe, indicating the desired regio-selectivity inherent to the PEBM approach.

Selective PEBM of right PCA distribution in rabbit

Selective mapping of the right posterior cerebral artery (PCA) vascular distribution was achieved in NZW rabbits. Spectral un-mixing of collected fluorescence imaging cubes in the rabbit discriminated tissue autofluorescence, arterial blood, and FITC-lectin fluorescence (Fig. 3A–L). Dorsal (Fig. 3A–D) and ventral (Fig. 3E–H) views of rabbit brain exhibited clear delineation of the right PCA distribution with well defined tissue margins, which were further documented following cut-down of the left and right PCA distributions (Fig. 3I–L, dorsal view). Regio-selective localization of the imaging probe to brain capillaries was validated by histology (Fig. 4).

Discussion

Lectins are unique class of carbohydrate-binding proteins found ubiquitously in nature, where their unique specificity for discrete carbohydrate and glycoprotein residues gives them a role in cellular and molecular recognition (11). Capitalizing upon this specificity, lectin-based imaging reagents have been prepared and are commercially available for use in histological applications where it may be desirable to differentiate various types of vascular endothelium. Such probes may have considerable utility within the *in vivo* setting as tissue specific molecular imaging reagents as we illustrate here. For example, we found that FITC-derivatized tomato lectin appeared to predominately localize to venous and capillary endothelium, as opposed to arteries, and could be visualized within intact brain specimens. Because of this fact, and because capillaries are densely distributed throughout the brain parenchyma, targeting the capillary bed appears to be an effective surrogate for brain parenchymal mapping. Furthermore, capillary targeting is an attractive feature of our approach because it circumvents the need for brain

mapping agents to cross the blood brain barrier and it facilitates the use of a wide range of imaging probes ranging in size from small molecules to antibodies, without the undesirable side-effect of vascular occlusion. Additionally, the preclinical PEBM approach shown here utilizes optical imaging which is highly sensitive, yet rapid and inexpensive to use.

Because the PEBM technique could potentially enable neurosurgeons to resect portions of brain parenchyma without any new neurological deficit, this approach could become useful in clinical neurosurgery. One possible application in clinical neurosurgery includes using PEBM to map and resect all neurologically nonfunctional/silent brain tissue surrounding a partially resected high-grade infiltrating glioblastoma multiforme (GBM). Other possible applications include resections performed for refractory epilepsy or other possible functional neurosurgery resections. An additional role for our described PEBM approach could be in the synthesis of vascular brain atlases, both in preclinical animal models as well as in humans. Such research tools, constructed from anatomical and functional mapping of selective brain vascular territories, could play a crucial role in the assessment and outcome prediction for numerous cerebrovascular or brain parenchymal diseases. For example, clinically accurate and precise knowledge of specific cerebrovascular perfusion territories at a cellular or molecular level could be useful in the study of the mechanism of stroke, as well as treatment alternatives, neuroprotection strategies, and response to therapy.

Conclusions

We have demonstrated the first endothelium-targeted approach to PEBM that does not require manipulation of the intact blood-brain-barrier or result in vascular occlusion. We have also demonstrated for the first time that PEBM can be successfully administered to a selective intracranial artery in an animal model. The presented multi-spectral molecular imaging technique appears to be a suitable methodology for the generation of regio-selective brain maps of vascularized brain parenchyma. Further development and refinement of the molecular imaging techniques and brain mapping probes may enable future advancement of the technique to the clinical setting.

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Figure 1. Selective mapping of right hemisphere brain parenchyma via molecularly targeted multi-spectral fluorescence imaging in normal rat

Tissue autofluorescence (A/D), arterial blood (B/E), and specific imaging probe fluorescence (C/F) emissions were spectrally resolved (Figs. 1A–C dorsal view, and Figs. 1D–F ventral view) following administration and binding of capillary endothelium-targeted imaging probe. Selective accumulation of the imaging probe within the capillaries, in preference to the arteries, was observed in the right hemisphere (note the clear visualization of the Circle of Willis that is spectrally resolved from both autofluorescence and imaging probe fluorescence). Pseudo-color overlay of arterial blood (red) and imaging probe fluorescence (green) in brain, dorsal and ventral views, respectively (G/H).

Figure 2. Histological validation of endothelial-targeted PEBM

(A) Hematoxylin and eosin (H&E) staining of normal brain taken from the mapped (right) hemisphere, magnification 50X. (B) anti-CD-34 staining of a serial section of adjacent brain tissue shown in (A) was used to visualize capillary endothelium, magnification 200x. (C/D) Fresh frozen, unstained sections of tissue collected from mapped (right, C) and un-mapped contralateral (left, D) hemispheres revealed primary imaging probe localization to capillary endothelium in the mapped hemisphere, magnification, 200x. (C, inset) Representative cross-section of mapped capillary demonstrating no discernable vessel occlusion following imaging probe administration.

Figure 3. Selective PEBM of the right posterior cerebral artery (PCA) vascular distribution in rabbit

Tissue autofluorescence (A, E, I), arterial blood (B, F, J), and specific imaging probe fluorescence (C, G, K) emissions were spectrally resolved following administration and binding of capillary endothelium-targeted imaging probe. Dorsal (Fig. 3A–D) and ventral (Fig. 3E–H) views of rabbit brain exhibited clear delineation of the right PCA distribution with reasonably well defined tissue margins, which were further documented following cut-down of the right and left PCA distributions (Fig. 3I–L, dorsal view).

Figure 4. Histological validation of endothelial-targeted PEBM in right rabbit PCA

(A) anti-CD-34 staining of section of mapped right PCA in rabbit brain tissue shown illustrating the density of capillary endothelium, magnification 200x. (B) Fresh frozen, unstained serial section of tissue collected from mapped (right) hemisphere revealed significant imaging probe localization to capillary endothelium in the mapped(right) hemisphere, magnification, 200x. (C) anti-CD-34 staining of section of unmapped (left) PCA in rabbit brain tissue illustrating similar density of capillary endothelium to the mapped hemisphere. (D) Fresh frozen, unstained serial section of tissue collected from non-mapped (left) hemisphere, magnification, 200x.