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Aqueous Angiography in Living Nonhuman Primates Shows Segmental, Pulsatile, and Dynamic Angiographic Aqueous Humor Outflow

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Purpose: To evaluate the feasibility of safely performing aqueous angiography in intact eyes of living nonhuman primates (NHPs) for evaluating aqueous humor outflow and segmental patterns.

Design: Cross-sectional, observational study.

Subjects: Six nonhuman primates.

Methods: Aqueous angiography was performed in 6 nonhuman primates. After anesthesia, an anterior chamber (AC) maintainer was placed through a temporal 1-mm side-port wound. Indocyanine green (ICG; 0.4%) or 2.5% fluorescein was introduced (individually or in sequence) into the eye with a gravity-driven constant-pressure system. Aqueous angiography images were obtained with a Spectralis HRA+OCT (Heidelberg Engineering GmbH, Heidelberg, Germany) suspended over the NHP eye using a custom-designed surgical boom arm. Concurrent anterior segment optical coherence tomography (OCT) was performed on distally angiographically positive and negative regions.

Main Outcome Measures: Angiographic patterns described by location, time-course, choice of tracer, and anterior-segment OCT.

Results: Aqueous angiography in the living NHP eye demonstrated mostly stable angiographic patterns. With multimodal imaging, angiographically positive signal co-localized with episcleral veins as identified by infrared imaging and intrascleral lumens, as demonstrated by anterior segment OCT. Sequential aqueous angiography in individual eyes with ICG followed by fluorescein showed similar angiographic patterns. A pulsatile nature of aqueous angiographic outflow was sometimes observed. Aqueous angiographic patterns could also dynamically change. In some instances, positive angiographic flow suddenly arose in regions previously without an angiographic signal. Alternatively, an angiographic signal could suddenly disappear from regions in which an angiographic signal was initially documented.

Conclusions: Aqueous angiography in living NHPs demonstrated segmental and pulsatile patterns with a newly described ability to dynamically shift. These characteristics further the understanding of live aqueous humor outflow biology and may be useful in improving glaucoma surgeries aimed at trabecular meshwork bypass. *Ophthalmology 2017;■:1–11 © 2017 by the American Academy of Ophthalmology*

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A better understanding of the complete conventional aqueous humor outflow (AHO) pathway is necessary. For good reason, traditional AHO investigations have focused on the trabecular meshwork (TM) because it was identified as the primary resistor to AHO in the eye.[1,2] Moreover, glaucomatous eyes showed increased resistance at the level of the TM.[1,2] However, it was apparent that there was also post-TM[3] outflow resistance, which was further increased in glaucomatous compared with normal eyes.[1,4] These basic observations, coupled with the clinical results of variable success in lowering intraocular pressure (IOP) with surgical trabecular bypass in human glaucoma patients, have suggested that the full nature of AHO is more complex and warrants further study.[5–8]

Structural evaluation of AHO pathways has improved with the advent of optical coherence tomography (OCT).[9–11] The TM has been hypothesized to be represented by an interphase shadow,[9] and Schlemm canal and distal outflow lumens show low reflectivity on anterior segment OCT. Three-dimensional reconstructions of distal outflow pathways have been created in whole in postmortem enucleated eyes[12] or in part in live normal human subjects.[10] Automated segmentation algorithms are under development (Dastiridou A et al. IOVS 2016; ARVO Abstract 5119-C0134). In particular, phase-based OCT has shown dynamic motion in TM and post-TM outflow pathways with demonstration of a pulsatile tempo likely related to the cardiac cycle.[13–17]

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Aqueous angiography is a recently developed method to visualize functional AHO as real-time imaging of tracer movement.[18−21] In postmortem pig, cow, and human eyes, similar patterns can be visualized using multiple tracers, such as fluorescein or indocyanine green (ICG).[18−21] Segmental patterns have been observed that may be relevant to trabecular bypass surgeries and their success via concepts of guiding surgical placement toward areas of greater or lesser flow in the eye. Comparisons between ICG and fluorescein aqueous angiography have shown sufficient similarity to conduct interventional studies where ICG is first used to determine a pattern, followed by fluorescein after an intervention to query the effect.[19,20] With this method, regions of initially poor angiographic flow have been shown to be capable of angiographic recruitment for enhanced flow using aqueous angiography−guided trabecular bypass in enucleated human eyes.[20] Limitations translating aqueous angiographic investigations of enucleated eyes to in vivo ones include postmortem cellular necrosis, potential presence of episcleral venous blood clots, and the discontinuation of the episcleral vein from the systemic venous circulation, leading to tracer accumulation on the ocular surface.

Therefore, we conducted real-time aqueous angiography for the first time in intact eyes using living nonhuman primate (NHP) subjects (designated NHP-A through NHP-F). Qualitative observations confirmed a segmental and pulsatile nature of AHO. The observation that AHO patterns could dynamically change in a living eye was a novel discovery.

Methods

Test Subjects

This study was carried out in accordance with the Declaration of Helsinki and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and with approval by the Institutional Animal Care and Use Committee of Beijing Institute of Xieerxin Biology Resource. Rhesus macaque monkeys (NHPs; *Macaca mulatta*) involved in this study were all purchased from Beijing Institute of Xieerxin Biology Resource, which is one of the largest NHP centers in the northern part of China. They specialize in breeding laboratory NHPs for studies routinely done through Beijing Tongren Hospital and Capital Medical University. Six adult (5 male and 1 female) NHPs were used (Table 1). They had a mean weight of 9.65 kg (range, 7.5−12.3 kg) and mean age of 15.5 years (range, 10−25 years). Each NHP was chosen by pre-examination of the eye at cageside to identify animals with minimal conjunctival pigmentation. Imaging of the animals was done at the Beijing Institute of Xieerxin Biology Resource in a dedicated procedure room. The NPHs were individually housed at a temperature of 16°C to 28°C. Feeding regimens were standard animal feeds containing various kinds of vegetables and fruits. No NHP that participated in this study was sacrificed.

The animals were anesthetized with Zoletil 50 (tilteamine/zolazepam; Laboratorios Virbac, Bogota, Columbia, S.A.). The initial dose was 5 mg/kg intramuscular for each NHP.[22] Inhalational general anesthesia was not used, as the mask size precluded the imaging device coming close enough to obtain images. The NHPs were then secured to the procedure table with soft restraints similar to those used in human surgeries. Topical 0.5% proparacaine hydrochloride (Alcaine; Alcon Laboratories, Fort Worth, TX) was applied, and the eye was sterile prepped with entoiodine swabs (Likang Disinfectant H-Tech. Co., Shanghai, China). Eyelashes were covered with Steri-Strips (R1457; 3M Company, Pasadena, CA), the face draped (D1022; Cardinal Health, Los Angeles, CA), and a sterile wired lid speculum placed. Investigators themselves were sterile masked, capped, and gloved. All instruments were both disposable and suitable for human surgery or were autoclaved and cooled prior to use. Throughout the procedure, the veterinary technician or head veterinarian at Beijing Institute of Xieerxin Biology Resource monitored the NHP in the room. Prior to regaining consciousness or at any sign of discomfort, NHPs were dosed with additional topical anesthetic and/or given additional intramuscular Zoletil 50 (one third to one half of the initial dose). After imaging, the anterior chambers were flushed with Ringer's solution (RS), wounds hydrated, a 10-0 nylon suture placed to secure the wound, and topical 0.3% tobramycin/0.1% dexamethasone ointment (TobraDex; Alcon, Fort Worth, TX) applied. All NHPs were monitored postoperatively daily and treated appropriately, with no complications noted.

Table1. Details of Nonhuman Primate Subjects

Identification	Sex	Weight (kg)	Age (yrs)
NHP-A	Male	12.3	15
NHP-B	Female	7.5	13
NHP-C	Male	10.5	25
NHP-D	Male	9	11
NHP-E	Male	8	10
NHP-F	Male	10.6	19

NHP = nonhuman primate.

Aqueous Angiography

Aqueous angiography was performed similar to the method previously described[18−21] but modified for intact eyes of living subjects. The NHP palpebral fissure precluded an inferior approach and covered much of the ocular surface posterior to the limbus. Therefore, the first step was to place superior and inferior peripheral, mid-depth corneal 8-0 prolene traction sutures (Ethicon, Somerville, NJ) that were used to rotate the eye. This also meant that unlike prior publications[18−20] with postmortem eyes, simultaneous 360-degree appreciation of aqueous angiographic patterns around the limbus was not possible. Since the primary purpose of this study was to demonstrate aqueous angiography in intact eyes of living NHPs and to document potential segmental outflow patterns, this suture was necessary to move the eye for imaging different locations. Then, a Lewicky anterior chamber maintainer (BVI Visitec, Alcester, UK) was inserted through a 1-mm side port (Alcon) into the anterior chamber from a temporal approach. All eyes studied were right eyes, given the orientation of the room and ethical guidelines to leave 1 nonmanipulated eye in each NHP. Pharmaceutical-grade RS (CR Double-Crane, Beijing, China), identical to what is used for human surgery, was introduced into the anterior chamber from a reservoir height set ∼10 inches above the eye to provide a gravity-delivered constant pressure of ∼18.7 mmHg.

Given the use of live animals, supine imaging was necessary. Heidelberg Engineering provided a prototype surgical FLEX module (mobile stand equipped with an arm) that allowed the standard clinical table-mounted Spectralis HRA+OCT (Heidelberg Engineering GmbH, Heidelberg, Germany) camera head to be suspended for supine imaging (Fig 1). The FLEX module allowed for stable 3-dimensional manipulations and positioning of the