

Postmortem Dilation and Imaging Can Aid Research

Pilot study shows that dilation is possible and that fundus photos and OCT imaging can be successfully correlated with histopathology, offering a window into clinical history of donor eyes.

BY TIMOTHY SAUNDERS, MD

Much of the research that we are able to perform with donor eye tissue requires good characterization of the tissue. That is, we want to have a good clinical history of the patient, with any ocular diagnoses and fundus photos or other images to support those diagnoses.

In many cases, however, the clinical history is limited or unavailable. Particularly in the early stages of many ophthalmic diseases, such as glaucoma, macular degeneration, and diabetic eye disease, the patient may not yet have noted any visual symptoms and may not have been under the care of an ophthalmologist. Dissection of the tissue can be very revealing but may also damage the tissue for further study.

I was interested, therefore, in whether we could obtain postmortem images to confirm or add to the clinical history without damaging the tissue. In designing a study to facilitate availability and accessibility of disease-specific tissue for bench researchers, I had the opportunity to compare macular pathology between results of 2 clinical diagnostic methods with an already high degree of structural similarity: histopathology sections and optical coherence tomography (OCT) images. To date, validated corollary studies of retinal OCT images are lacking. Despite this, OCT images are often a main contributor in guiding disease management. If postmortem changes in ocular media clarity and ischemia are kept at a minimum, and there is a small enough window of time between death and tissue delivery, then significant advances in interpretation of these images in donor eyes are possible.

IMAGING METHODS

The first challenge was taking fundus photographs of donor globes. A certain amount of flash has to enter the pupil to obtain the photograph; therefore, a minimum pupillary diameter is necessary to permit adequate light and a good photographic representation. A cursory literature review revealed no prior publications that describe pharmacologic postmortem mydriasis.

My colleagues and I compiled a small cohort of 14 donor eyes to test this. All eyes were obtained from the Lions Eye Institute for Transplant & Research (LEITR), between December 2012 and April 2013 and were evaluated within 24 hours of death.

Dilation was achieved using a clinical dose of 10% phenylephrine and 1% tropicamide dropped onto the external cornea surface in 2 rounds 3 minutes apart. Due to high demand for the tissue and LEITR's strict adherence to quality and service, some of the eyes, which were earmarked for other research projects, could not be dilated until after enucleation had occurred. Pupils were measured by serial external ocular photographs, taken before dilation and then again at 20 and 60 minutes post-dilation.

For the purposes of our research, meaningful dilation was defined as dilation sufficient to permit posterior segment photos. This was reliably achieved in all the eyes in both studies. Although too early to quantify, the research is suggestive that postmortem pharmacologic dilation is possible in fresh donor globes, pre- and postenucleation. (Figure 1).¹ Similar to in vivo dilation,

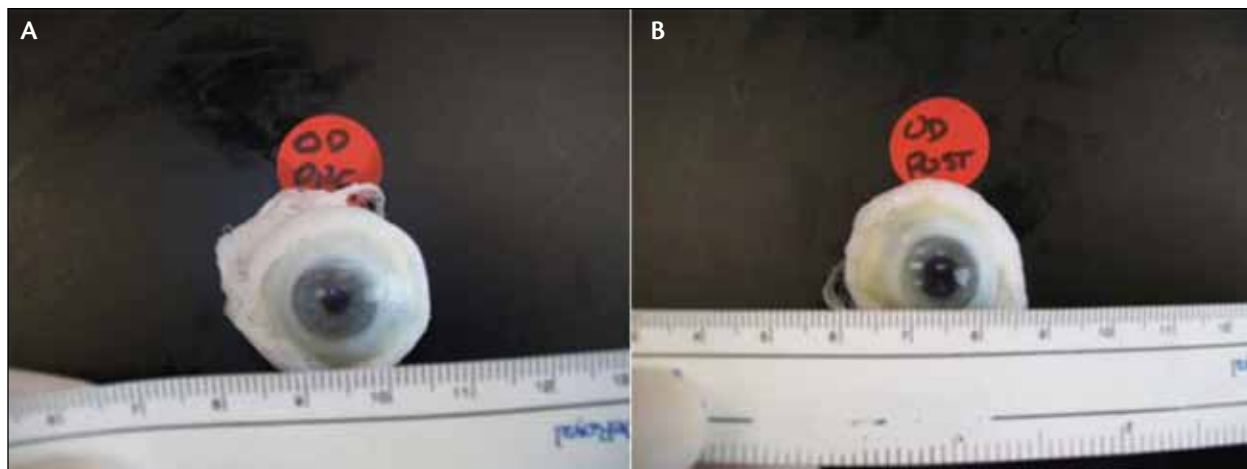


Figure 1. In a photograph taken 24 hours after death and 5 hours after globe procurement, the pupil measures 3.5 mm (A). One hour after instilling dilating drop, the pupil has dilated 2.6 mm to 6.1 mm (B).

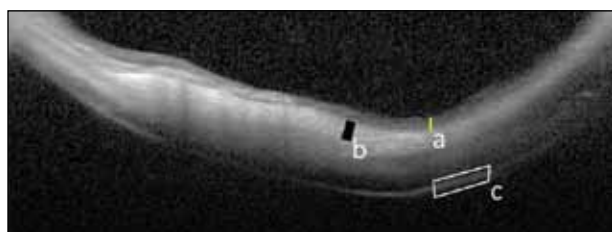


Figure 2. OCT shows thinned retina (A), thinned sclera (B), and retro-orbital reflections, which are possibly Tenon capsule (C).

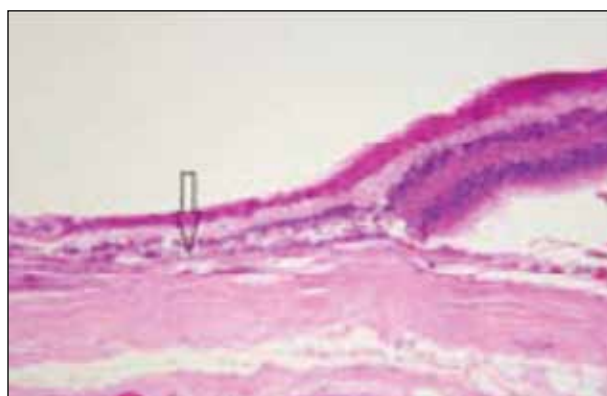


Figure 3. Pathology slide of the same area as seen in Figure 2 shows outer retinal and retinal pigment epithelial obliteration and inner nuclear retinal tissue overlying thin sclera.

the dilation achieved in this small study was varied, from less than 1.0 mm to as high at 2.9 mm.

IMAGING AND HISTOPATHOLOGY

The next step was obtaining multimodality imaging of posthumously dilated eyes and correlating the images with histopathologic findings.

For this study, we obtained 11 whole globes (7 subjects) from LEITR, with the time from death ranging from 11 to 60 hours.² All eyes were dilated as described above. The tissue was stored at 4°C, wrapped in moist gauze. Balanced salt solution was injected through the pars plana to restore physiologic tension. Images were obtained using OCT and a fundus camera. Immediately after the last image was obtained, the eyes were placed in fixative containing 5% glutaraldehyde and 2.5% formalin. Step sectioning of the maculopapular bundle was performed, and a histopathologic exam was conducted.

Correlation of postmortem fundus imaging with histopathology sections was precipitously less meaningful following approximately the 48-hour mark. Media opacities and tissue autolysis were the likely culprits.

In the other eyes, despite variations in artifact, a high degree of structural detail was present and most retinochoroidal structures were easily recognizable. The reflectivity of the retinal pigment epithelium (RPE) line, for example, looked essentially the same as it does in vivo. However, the lumen of primary blood vessels, typically with flow through them, demonstrated a very low reflectivity in the postmortem images. Color photographs of the edematous retina were sufficiently detailed for gross evaluation. Overall, standard OCT imaging was a reliable modality under these conditions, while laser beam modification for enhanced depth imaging or fundus autofluorescence yielded less consistent results.

INTERESTING FINDINGS

In a highly myopic eye, OCT showed retinal atrophy and high subretinal reflectivity, resembling a disciform

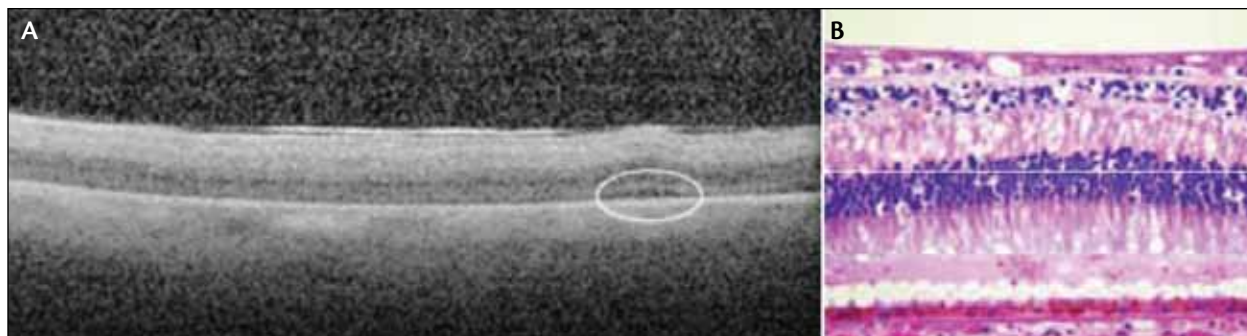


Figure 4. Both the OCT (A) and pathology images (B) from this eye show signs of serous retinal fluid, indicating a small retinal detachment.

scar, and reflective signals in the retro-orbital space (Figure 2). Comparison to the pathology slide (Figure 3) revealed an advanced posterior staphyloma with inner nuclear layer resting on very thinned, bare sclera.

In another eye, for which we had minimal ophthalmic clinical history, OCT convincingly detected, and histopathology reproduced, a small serous neurosensory retinal detachment, the size of which I consider typical during the course of treating wet age-related macular degeneration with anti-VEGF agents (Figure 4).

DISCUSSION

These cases illustrate the value of not only being able to obtain postmortem imaging, but to also correlate the imaging with histopathologic findings. Pre-death clinical records may also be extremely important in confirming diagnoses and, along with localizing tissue plane techniques, they will be the future direction of this study.

To the best of our knowledge, this is the first study to evaluate the utility of standard clinical imaging techniques on unfixed postmortem eyes.

The uniqueness and reliability of LEITR resources enables us to now redirect our investigations to the earliest time frame possible, the window of time between 0 and 8 hours postmortem. In generating images, clarity and best results are heavily time-reliant (the fresher the tissue, the better the images), which raises additional questions about the ideal protocol for future research on donor eyes.

SUMMARY

LEITR is currently assessing whether other researchers who use its tissue may be able to enhance their studies with postmortem dilation and imaging, according to Director of Business Development Patrick Gore, RN, CEBT. "We anticipate this may be useful to a number of researchers who would like to better characterize the

tissue they use for cutting-edge research in glaucoma and retina," he said. "It would be helpful, for example, to obtain optic nerve head images prior to dissection or cell culturing."

With further refinements, it is entirely plausible that postmortem imaging can help researchers diagnose posterior segment disease with a high degree of accuracy. Our goal in conducting these studies is translational. The hope is that such techniques could not only improve utilization of donor tissue for disease-specific research but also provide greater insight into the pathogenesis and progression of conditions of the retina, macula, and choroid through the identification after death of undiagnosed, early-stage disease. ■

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1. Golen JR, Saunders T, Sprehe N, et al. Can meaningful pharmacological dilation of pupils occur after death? Paper presented at: the Association for Research in Vision and Ophthalmology Annual Meeting; May 2013; Seattle.
2. Saunders T, Gore P, Sprehe N, et al. Postmortem imaging of the posterior segment of eyes with histopathologic correlation. Paper presented at: the Association for Research in Vision and Ophthalmology Annual Meeting; May 2013; Seattle.

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