

Layer-Specific Retinal and Choroidal Blood-Flow MRI of Retinitis Pigmentosa in Mice

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INTRODUCTION: Retinitis pigmentosa (RP), the most common inherited retinal degeneration, causes photoreceptor death and blindness and affects 1.5 million people worldwide (1). In addition to degeneration of the anatomical structure, the vasculature of the retina is attenuated in RP (1,2). The retina is nourished by two separate vasculatures: the retinal and choroidal vessels. The retinal vessels are located in the inner retina next to the vitreous, and the choroidal vessels are behind the retinal pigment epithelium. The photoreceptor cells (outer nuclear layer and inner/outer segments) between these two vascular layers are avascular (4). Optical imaging techniques, widely used to study the retina, have difficulty resolving choroidal BF (ChBF) and retinal BF (RBF). In contrast, MRI has recently been used to measure quantitative RBF and ChBF in mice at 42x42x400 μm (5). In this study, BF MRI was used to study layer-specific RBF and ChBF at different stages of disease in a mouse model of RP.

METHODS: The rd10 mouse model of RP on a C57BL/6J background was used, with wild type (WT) C57BL/6J mice used for controls. MRI was done on mice at post natal days 25, 35, and 60 (P25, P35, and P60) (n=32 total). Mice were imaged under ~1.1% isoflurane in 30% oxygen and spontaneous breathing. Respiration rate, heart rate, oxygen saturation and temperature were maintained within normal ranges. MRI was performed on a 7T/30cm Bruker scanner with a 150 G/cm gradient and a small surface eye coil with active decoupling (diameter=6mm) and a circular coil (diameter=8mm) for arterial spin labeling placed at the heart (6). Images were acquired in coronal orientation with gradient-echo EPI with FOV=6x6 mm, matrix=144x144 zero-filled to 256x256, 2 segments, a single 0.4 mm slice, TR=3.0s per segment, TE=9.7ms, labeling duration=2.6s, and post labeling delay=350ms. BF images in ml/g/min were calculated as in (7). Profile analysis was used to average BF along the retinal length (4). Histology was acquired at P25 and P60 to assess anatomical degeneration. Eyes were enucleated, fixed in 10% paraformaldehyde, embedded in paraffin, sectioned at 8 μm , and stained with hematoxylin and eosin. Statistical analysis used ANOVA with Bonferroni correction used for post-hoc tests.

RESULTS: BF images from WT mice had two BF layers corresponding to RBF and ChBF, but the RBF layer became difficult to detect in rd10 mice (Figure 1). Figure 2 shows group averaged BF profiles along the depth of the retina from WT and rd10 mice at P25, P35, and P60. In WT mice, RBF and ChBF layers were separated. In P25 rd10 mice, the RBF and ChBF layers were clearly present, but were not well separated. In rd10 mice at P35 and P60, the ChBF layer was still clearly present, but RBF was difficult to detect. A small hump that was not separated from the ChBF layer could generally be found at P35 but not P60. Due to the loss of most of the avascular layer (outer nuclear layer and inner/outer segments), RBF and ChBF are expected to no longer be separated by a region of minimal BF. Figure 3 summarizes RBF and ChBF from WT and rd10 mice at all age groups. From histology, the outer nuclear layer and inner/outer segments of rd10 mice were substantially thinned at P25. Entire retinal thickness at P25 was 229 \pm 8 μm in WT and 150 \pm 5 μm in rd10 mice (mean \pm SD, p<0.01). At P60 these layers were further degenerated, and other layers of the retina were also thinned. Entire retinal thickness at P60 was 231 \pm 8 μm in WT and 98 \pm 7 μm in rd10 mice (mean \pm SD, p<0.01).

DISCUSSION: In rd10 mice, RBF tended to decrease overtime, and was significantly reduced at P60. ChBF was unaffected up to P60. In humans, RBF (2) and ChBF (8) are reduced. In rat (9) and cat (3) models of RP, RBF was compromised while ChBF was not significantly affected. The findings herein are in agreement that RBF is reduced and with the findings of unchanged ChBF in animal models. Although the retina has essentially completed degeneration by P60, this is still a young age in mice, so it remains possible that ChBF is eventually reduced at later time points.

In conclusion, high-resolution BF MRI could detect BF changes at different stages of disease in the retina of the rd10 mouse model of RP. Future studies could measure functional BF changes to physiological stimulations. Since MRI provides a non-invasive method to pinpoint anatomical and vascular changes *in vivo*, it could be used for longitudinal studies of retinal degeneration and monitoring of potential treatments.

Reference: 1) Berson, IOVS 1993, 34:1659. 2) Grunwald et al, Am J Ophthalmol 1996, 122:502. 3) Nilsson et al, IOVS 2001, 42:1038. 4) Cheng et al, PNAS 2006, 103:17525. 5) Muir et al, NMR Biomed 2010, in press. 6) Muir et al, MRM 2008, 60:744. 7) Shen et al, JCBFM 2005, 25:1265. 8) Falsini et al, IOVS 2010, in press. 9) Li et al, ISMRM 2010, Stockholm. Supported in part by R01 EY014211, R01 EY018855, VA MERIT.

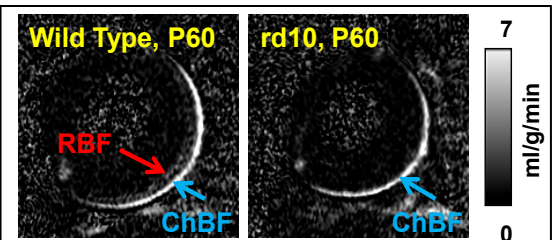


Figure 1. Quantitative BF maps of wild type and rd10 mouse eyes at P60 at 42x42x400 μm .

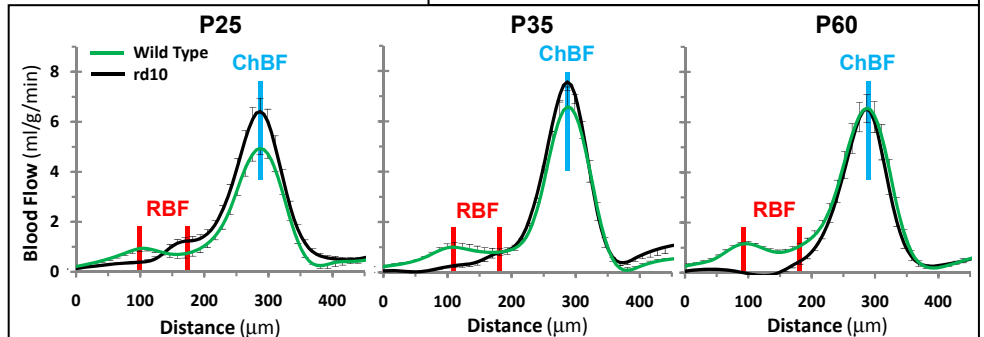


Figure 2. Group-averaged BF profiles from wild type and rd10 mice at P25, P35, and P60 (mean \pm SEM, n are the same as in Figure 3). The blue lines indicate the ChBF layer, and the red lines indicate the RBF layer. The ChBF layer of wild type and rd10 mice were aligned. Two red lines are shown in each plot to indicate the RBF layer of both wild type and rd10 mice, which had different locations.

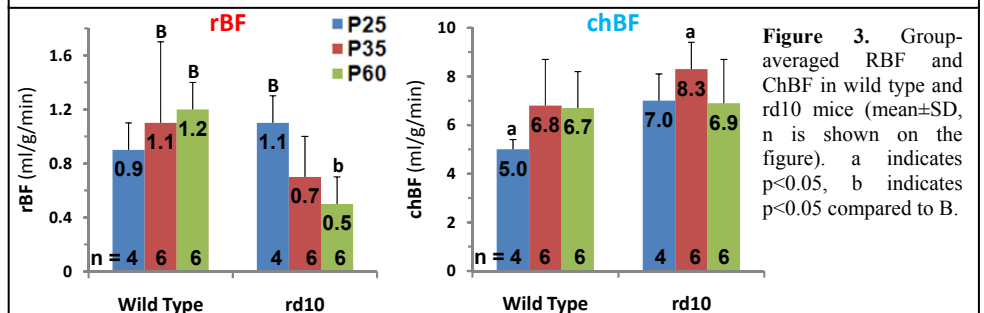


Figure 3. Group-averaged RBF and ChBF in wild type and rd10 mice (mean \pm SD, n is shown on the figure). a indicates p<0.05, b indicates p<0.05 compared to B.