

AB011. Live imaging of retinal pericytes: evidence for early calcium uptake, capillary constriction and vascular dysregulation in ocular hypertension glaucoma

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Background: Pericytes are contractile cells that wrap along the walls of capillaries. In the brain, pericytes play a crucial role in the regulation of capillary diameter and vascular blood flow in response to metabolic demand. The contribution of pericytes to microvascular deficits in glaucoma is currently unknown. To address this, we used two-photon excitation microscopy for longitudinal monitoring of retinal pericytes and capillaries in a mouse glaucoma model.

Methods: Ocular hypertension was induced by injection of magnetic microbeads into the anterior chamber of albino mice expressing red fluorescent protein selectively in pericytes (NG2-DsRed). Minimally invasive, multiphoton imaging through the sclera of live NG2-DsRed mice was used to visualize pericytes and capillary diameter at one, two and three weeks after glaucoma induction. *In vivo* fluctuations in pericyte intracellular calcium were monitored with the calcium indicator Fluo-4. *Ex vivo* stereological analysis of retinal tissue prior to and after injection of microbeads was used to confirm our *in vivo* findings.

Results: Live two-photon imaging of NG2-DsRed retinas demonstrated that ocular hypertension induced progressive accumulation of intracellular calcium in pericytes. Calcium uptake correlated directly with the narrowing of capillaries in the superficial, inner, and outer vascular plexuses (capillary diameter: naïve control = $4.7 \pm 0.1 \mu\text{m}$, glaucoma = $4.0 \pm 0.1 \mu\text{m}$, $n=5-6$ mice/group, Student's *t*-test $P < 0.05$). Frequency distribution analysis showed a substantial increase in the number of small-diameter capillaries ($\leq 3 \mu\text{m}$) and a decrease in larger-diameter microvessels ($\geq 5-9 \mu\text{m}$) at three weeks after induction of ocular hypertension ($n=5-6$ mice/group, Student's *t*-test $P < 0.05$).

Conclusions: Our data support two main conclusions. First, two-photon excitation microscopy is an effective strategy to monitor longitudinal changes in retinal pericytes and capillaries in live animals at glaucoma onset and progression. Second, ocular hypertension triggers rapid intracellular calcium increase in retinal pericytes leading to substantial capillary constriction. This study identifies retinal pericytes as important mediators of early microvascular dysfunction in glaucoma.

Keywords: Glaucoma; pericytes; capillaries; *in vivo* two-photon microscopy

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