Does estrogen deficiency promote the development of glaucoma?

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INTRODUCTION

Glaucoma is the second leading cause of blindness in the world and is typically a slowly progressing neurodegenerative disease. It is characterized by a gradual loss of retinal ganglion cells (RGCs), which leads to vision loss. The most common form of glaucoma, occurring in 70 to 90% of patients, is primary open angle glaucoma (POAG) **.**

Acompelling epidemiological features of POAG is that its incidence shows a striking sex-related difference. Women have a significantly lower incidence of POAG, as compared to men, until the age of 80 years. This sex-related difference has been linked to the extent of lifetime estrogen exposure. Indeed, there is a strong association between increased estrogen exposure and a reduced POAG risk. Conversely, studies have shown that a decreased exposure (i.e. early loss of estrogens), due to late onset of menstrual cycles, oral contraceptive use, early menopause, early surgical removal of the ovaries, and a shorter duration between menarche to menopause, confers an increased risk of POAG.

We hypothesize that an early estrogen deficiency accelerates the aging of the optic nerve and predisposes to glaucomatous damage. We further hypothesize that estrogen administration will remove these risks and serve as a novel preventive treatment for glaucoma, and in particular, POAG. To begin to test this hypothesis, we examined whether estrogen deprivation is associated with heightened IOP, RGC loss and glaucoma in an animal model.

DESIGN & METHODS

We obtained breeding pairs of C57BL/6J - aromatase knockout (ArKO) heterozygous mice (Dr. Nabil J. Alkayed; Oregon Health & Science University, Portland, OR) to generate ArKO mice and their wildtype (WT) controls. The ArKO mice harbor a targeted disruption of exon IX in the cyp19 gene and possess no aromatase activity. Aromatase catalyzes the conversion of androstenedione to estrone and the conversion of testosterone to estradiol. In the absence of aromatase, the synthesis of estrogens is completely eliminated. All mice were genotyped at least twice to confirm their genetic background. At 12 and 24 weeks of age, we measured in a masked fashion the IOP (n = 6 consecutive IOP measurements/value, 3 values/eye/day, 2 consecutive days) in the left and right eyes of conscious mice (n = 8/group/sex). Animals were then sacrificed and retinas were processed for the analysis and quantitation of RGCs. Unpaired t-tests were used for statistical analyses.

DIAGRAM

IOP was measured with a TonoLab tonometer at the central cornea of conscious mice that were secured in DecapiCone bags.

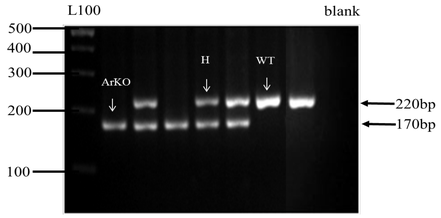


Figure 1. Genotyping of ArKO and WT mouse tissue samples by PCR and agarose gel electrophoresis. Products with a single 170 base pair (bp) or 220 bp band identified ArKO or WT mice, respectively. PCR products with both bands identified heterozygous (H) mice. The L100 lane contained a 100 bp ladder, and the blank lane was for a negative control of PCR reactions.

Table 1.

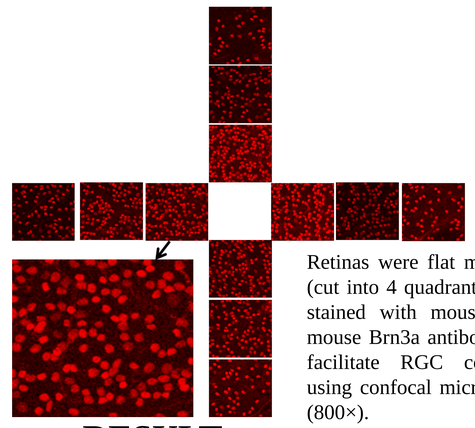
IOP (mean±SE,mmHg) in different groups

Time Point	Genotype	Female (n=8)	Male (n=8)
12-week	WT	13.48 ± 0.15	13.83 ± 0.20
	ArKO	14.54 ± 0.17	13.52 ± 0.17
24-week	WT	12.32 ± 0.19	14.35 ± 0.17
	ArKO	14.89 ± 0.17	13.46 ± 0.17

Table 2.

RGC numbers (mean±SE) in ArKO and WT mice

Time Point	Genotype	Female (n=8)	Male (n=8)
12-week	WT	1249 ± 25.31	1351 ± 23.6
	ArKO	1136 ± 29.25	1288 ± 16.48
24-week	WT	1213 ± 32.73	1214 ± 24.44
	ArKO	1129 ± 34	1208 ± 42.73



Retinas were flat mounted (cut into 4 quadrants) and stained with mouse anti-mouse Brn3a antibodies to facilitate RGC counting using confocal microscopy (800×).

RESULT

The IOP levels in both 12- and 24-week old female ArKO mice were significantly ($p < 0.0001$) higher than those of age- and sex-matched WT controls. The mean increase in IOP ranged from 1.1 mmHg (7.9%) in the 12-week-, to 2.6 mmHg (19.7%) in the 24-week-old mice, respectively. These changes were accompanied by significant ($p < 0.05$, 12-week; $p < 0.05$, 1 tail, 24-week) decreases in RGC numbers in the ArKO female mice, relative to controls. In contrast, estrogen deficiency did not lead to an increased IOP in male mice. There was, however, a significant reduction in RGC counts in the 12- ($p < 0.05$), but not 24-, week-old male ArKO mice, as compared to their age- and sex-matched WT controls.

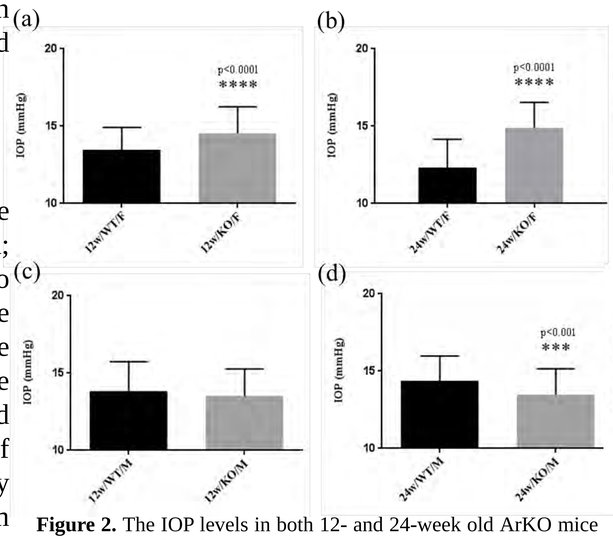


Figure 2. The IOP levels in both 12- and 24-week old ArKO mice were compared with age- and sex-matched WT controls. (a) The IOP levels in 12-week old female ArKO mice were significantly ($p < 0.0001$) higher than WT controls; (b) The IOP levels in 24-week old female ArKO mice were significantly ($p < 0.0001$) higher than WT mice; (c) Estrogen deficiency did not alter the IOP in 12-week old male ArKO mice compared with WT control; and (d) The IOP levels in 24-week old male ArKO mice were significantly ($p < 0.0005$) less than those of WT mice .

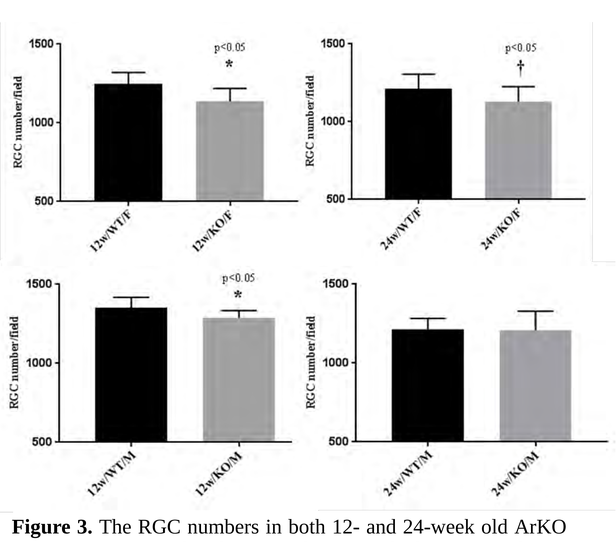


Figure 3. The RGC numbers in both 12- and 24-week old ArKO mice were compared with age- and sex-matched WT controls. (a) RGC numbers significantly ($p < 0.05$, 12-week) decreased in the ArKO female mice, relative to controls; (b) RGC numbers significantly ($p < 0.05$, 24-week, † one-tailed T-test) decreased in the ArKO female mice compared with controls; (c) RGC counts were significantly ($p < 0.05$) less in 12-week, but not 24-week (d), ArKO male mice compared with WT controls. (12w - 12 week; 24w - 24 week; WT - wild type; KO - knockout; F - female; M - male)

CONCLUSION

Our results support our hypothesis that estrogen deprivation promotes the development of glaucoma.

NEXT STEPS

Our immediate plans are to continue to test our hypotheses. More specifically, we seek to: [a] examine whether estrogen deprivation in 4 week-old ArKO mice is associated with heightened IOP and RGC loss, as compared to WT controls; and [b] determine whether estradiol (E2) treatment can reverse this condition. Female mice will be treated with placebo or E2 for 15, 30 and 60 days. The age at the initiation of therapy will be determined by the results in our '4-week-old' series of experiments. We will monitor RGC cell counts and IOP. We anticipate that E2 therapy will correct the glaucomatous changes induced by estrogen deprivation.

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