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Aquaporin-1 expression and conventional aqueous outflow in human eyes

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ABSTRACT

Aquaporin channels facilitate the enhanced permeability of secretory and absorptive tissues to water. In the conventional drainage tract, aquaporin-1 is expressed but its contribution to outflow facility is unknown. The purpose of the present study was to determine the effect of elevated aquaporin-1 expression by cells of the human conventional drainage pathway on outflow facility. Using 13 pairs of human anterior segments in organ culture, we modified aquaporin-1 protein expression in outflow cells using adenovirus encoding human aquaporin-1. Contralateral anterior segments served as controls and were transduced with adenovirus encoding beta-galactosidase. By confocal immunofluorescence microscopy, we observed that inner trabecular meshwork cells from anterior segments exposed to adenovirus (via injection into the inlet tubing during perfusion) had increased aquaporin-1 protein expression compared to endogenous levels. In contrast, elevation of aquaporin-1 protein in outer meshwork cells (juxtacanalicular region) and Schlemm's canal required transduction of adenovirus into anterior segments using retroperfusion via episcleral veins. Regardless of exposure route, outflow facility of experimental segments was not different than control. Specifically, overexpression of aquaporin-1 in the inner meshwork resulted in an average facility change of $-2.0 \pm 9.2\%$, while overexpression of aquaporin-1 in the resistance-generating region changed outflow facility by $-3.2 \pm 11.2\%$. Taken together, these results indicate that a transcellular pathway, mediated by aquaporin-1, does not contribute significantly to bulk outflow through the conventional aqueous outflow tract of human eyes.

1. Introduction

Aquaporins are expressed by ocular tissues responsible for the formation and removal of aqueous humor. In the conventional drainage tract, aquaporin-1 (AQP1) is expressed abundantly in all regions including the inner (uveal and corneoscleral) and outer (juxtacanalicular, JCT) trabecular meshwork, plus the inner wall of Schlemm's canal (Stamer et al., 1994, 1995, 2001; Hamann et al., 1998). In the ciliary body, two aquaporins, AQP1 and AQP4, are expressed and localize to the non-pigmented epithelial cells. The contribution of these two aquaporins to the production of aqueous humor has been clearly demonstrated in AQP null mice (Zhang et al., 2002). Thus, AQP deletion reduced intraocular pressure by 1–2 mmHg, depending upon the mouse strain and type/number of AQPs deleted. Interestingly, outflow facility measurements in AQP1 null mice were not significantly different than littermate controls (Zhang et al., 2002).

While results with AQP1 null mice suggest that AQP1 does not contribute to bulk outflow, interpretation of those results requires caution for several reasons. First, there are likely multiple compensatory mechanisms in the conventional drainage pathway (site of intraocular pressure regulation) to offset the loss of a single protein such as AQP1. Second, the effects of AQP1 deletion on outflow facility (hydrostatically driven) are expected to be more difficult to detect because hydrostatically driven water permeability has been shown to be less affected by the absence of AQP1 than is osmotic-driven water permeability (Bai et al., 1999). Third, it is technically more difficult to measure intraocular pressure and outflow facility in rodents than in humans. Fourth, appreciable differences in the anatomy and physiology of aqueous humor drainage exist between mice and humans. For example, the rodent trabecular meshwork is architecturally less complex than in the human eye; moreover, conventional outflow in mice accounts for significantly less of the total outflow (roughly a quarter of total, compared to about three-quarters for humans (Aihara et al., 2003)). As a result of these limitations, understanding the specific

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contribution of AQP1 to conventional outflow in humans requires the use of a more relevant model system, such as human anterior segments in organ culture (Johnson and Tschumper, 1987).

The purpose of the present study was to determine whether alteration in AQP1 expression by cells of the conventional drainage tract influences outflow facility in perfused human anterior segments in organ culture. We hypothesized that overexpression of AQP1 by cells in the resistance-generating region (including JCT-TM and SC) of the conventional drainage pathway would increase bulk outflow facility by increasing transcellular water movement. Using forward and retroperfusion gene delivery, we differentially transduced cells of the conventional drainage tract with adenoviruses that encode AQP1. We observed that when cells of either the inner or outer meshwork (including Schlemm's canal) were successfully transduced to overexpress AQP1, outflow facility was unaffected. Thus, water movement facilitated by AQP1 in the human conventional outflow pathway cannot be appreciably increased over steady state levels, suggesting a preferred non-AQP1-dependent route for water through the JCT-TM and inner wall of Schlemm's canal.

2. Materials and methods

2.1. Forward perfusions

Paired, human eyes free of any known ocular disease were obtained post mortem from two sources: the Eye Bank of Canada (Ontario Division; Toronto) and National Disease Research Interchange (Philadelphia). Eyes were stored in moistened chambers at 4 °C until time of dissection. The method of anterior segment forward perfusion was similar to that described by Johnson and coworkers (Johnson and Tschumper, 1987, 1989; Johnson, 1996), with modifications described in detail previously by our group (Ethier et al., 2004; Gottanka et al., 2004b). After dissection and mounting into culture chambers, the anterior segments were perfused with Dulbecco's modified eagle medium (DMEM) containing antibiotics (0.17 mg/ml gentamycin, 0.25 μg/ml amphotericin-B, 100 units/ml penicillin and 100 μg/ml streptomycin; all from Sigma, St. Louis, USA), 1% fetal bovine serum (FBS, Sigma, St. Louis, USA) and 250 μg/ml bovine serum albumin (Sigma, St. Louis, USA). Segments were perfused at a constant flow rate of 2.5 μl/min and intraocular pressure was measured continuously. Typically after 3–5 days of perfusion, a stable baseline facility was reached in both segments and adenovirus (6.6×10^5 PFU) was injected into inlet tubing in some eyes as previously described (Ethier et al., 2004) or adenovirus was administered by retroperfusion as described previously (Stamer et al., 2007). After introduction of adenovirus by either method, eyes were further perfused in the forward direction for an additional 5 days while continuously measuring pressure.

2.2. Retroperfusions

After reaching a stable facility within physiological limits, adenovirus was introduced into some anterior segments in a retrograde fashion via a technique we have called "retroperfusion" (Stamer et al., 2007). Briefly, a small plastic strip sealed with grease on its lower edge was placed into the clamping ring of the perfusion dish to make a fluid-tight fence encircling the limbus. Media containing an adenoviral construct expressing the lacZ reporter gene (a gift from Dr Karsten Peppel at Duke University) or aquaporin-1 (6×10^6 PFU/ml) under control of the cytomegalovirus promoter was pipetted behind this fence to submerge the limbus, and intrachamber pressure was lowered to −1.0 mmHg for 30–60 min, and then maintained at 0 mmHg for an additional 60 min. During the zero pressure time period, intrachamber pressure was occasionally varied ±1.0 mmHg for 15-s intervals to promote mixing inside of SC as described previously (Stamer et al., 2007). After retroperfusion and the 0 mmHg maintenance period, conventional (forward) perfusion was restarted and continued for 5 days. Net facility change was computed as the percentage facility change of the experimental eye minus the percentage facility change of the contralateral control eye.

2.2.1. β-Galactosidase activity and morphological analyses

To terminate experiments, perfusion was stopped; anterior segments were removed from the culture chambers, and washed with phosphate-buffered saline, pH. 7.5. Anterior segments were rapidly cut into four quadrants and some wedges from each quadrant of both segments were fixed in Universal Fixative (2.5% paraformaldehyde, 2.5% glutaraldehyde in Sörensen's buffer) prior to embedding in Spurr's plastic according to standard methods for thin sections, and stained with Toluidine Blue (Gottanka et al., 2004a) for morphological studies. Some wedges were embedded in OCT compound (Sakura Finetek USA, Inc, Torrance, CA) and rapidly frozen for immunofluorescence microscopy studies. In addition, representative wedges from segments perfused with adenovirus encoding beta-galactosidase were incubated for 10 min in fixation buffer from the Gal-S β-galactosidase reporter gene staining kit (Sigma, St. Louis, MO). Tissue wedges were exposed to X-gal substrate for 7–12 h at 4 °C for complete hydrolysis of the enzyme expressed and full indigo-blue color development. Selected subwedges from each quadrant were then embedded in paraffin and sagittally oriented 5-μm sections of the chamber angle region were cut, counterstained with hematoxylin and eosin and visualized by light microscopy.

Toluidine blue stained sections (0.5 μm) were radially oriented and viewed by light microscopy using an Olympus BH-2 upright microscope at magnifications of 200× and 400×. All sections were scored in a masked fashion by two observers according to a 0–4 grading scheme that was described previously (Wan et al., 2006). Briefly, the worst score is 0 and means that no cells are present in the TM or only a few swollen cells and/or inner wall disruption is present. A score of 1 means that there are only a few cells in the TM, typically in the juxtacanalicular tissue (JCT), but existing cells show little or no swelling and the inner wall is intact. A score of 2 indicates that the JCT is well populated with cells, while the corneoscleral and uveal meshwork contain few or no cells and the inner wall is intact. A score of 3 means that the JCT and most of the corneoscleral meshwork is filled with cells; most cells look good (no swelling) and the inner wall is intact. The best score is 4, corresponding to a TM with cells present everywhere in the JCT and corneoscleral meshwork (uveal TM not considered); inner wall intact. The final reported grade for each anterior segment was calculated by averaging the scores of all four quadrants in an anterior segment from two observers (Table 1).

2.2.2. Confocal immunofluorescence microscopy

Indirect immunofluorescence microscopy was performed on human anterior segment tissues following perfusion. Tissue wedges that included angle structures in OCT compound from four quadrants of each anterior segment were cryosectioned (8 μm) in a radial direction. Sections on slides were fixed in 50% methanol/50% acetone and air-dried. Fixed sections of tissue were rehydrated in PBS, blocked with 10% goat serum in PBS containing 0.1% Triton X-100 and incubated overnight with affinity-purified AQP1 polyclonal immunoglobulin-Gs (IgGs, 1:1000 dilution) (Stamer et al., 1996). Following antibody incubations, tissue sections were washed extensively (4×4 ml $\times$ 15 min) in phosphate-buffered saline containing 0.1% Triton X-100. Specific binding of antibodies was detected using CY3-conjugated goat anti-rabbit IgG at a 1:1000 dilution (Jackson Immunoresearch Laboratories, West Grove, PA) with 2 h incubation and extensive washing before viewing.