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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 thirteen 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.

Keywords

Aqueous Humor; Glaucoma; Schlemm's Canal; Trabecular Meshwork; Water Channel

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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; Stamer et al. 1995; Hamann et al. 1998; Stamer et al. 2001). In the ciliary body, two aquaporins, AQP-1 and AQP-4, 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, Vetrivel and Verkman 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, Vetrivel and Verkman 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, Lindsey and Weinreb 2003)). As a result of these limitations, understanding the specific 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 over-expression 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 over-express AQP-1, outflow facility was unaffected. Thus, water movement facilitated by AQP-1 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.

Materials and Methods

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 co-workers (Johnson and Tschumper 1987; Johnson and Tschumper 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, Chan and Ethier 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.

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, Chan and Ethier 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 sec intervals to promote mixing inside of SC as described previously (Stamer, Chan and Ethier 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.

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 Sørensen's buffer) prior 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 minutes in fixation buffer from the Gal-S β-galactosidase reporter gene staining kit (Sigma, St. Louis). Tissue wedges were exposed to X-gal substrate for 7–12 hours at 4°C for complete hydrolysis of the enzyme expressed and full indigo-blue color development. Selected sub-wedges from each quadrant were then embedded in paraffin and sagittally oriented 5µm 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 200x and 400x. 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 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).

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 μ m) 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, Snyder and Regan 1996). Following antibody incubations, tissue sections were washed extensively (4 $\times$ 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 hours incubation and extensive washing before viewing. Background fluorescence was indicated in tissues processed in the absence of primary antibodies. Labeled tissue sections were visualized and captured digitally at identical settings using a Nikon PCM 2000/E800 confocal microscope (Melville, NY USA).

To evaluate expression of recombinant AQP1 in transduced anterior segments, frozen sections from each of four quadrants from control and experimental tissues were stained for AQP1 and evaluated in a binary fashion by two masked observers. Thus, observers first reviewed all confocal images and then went back and scored each as either “endogenous expression” or “elevated above endogenous”. With the forward perfusions data was very clear; every quadrant analyzed for every segment perfused with AQP1 virus was found to be above endogenous (data not shown). For segments retroperfused with AQP1, we found that about half of quadrants were above endogenous; consistent with our previously published study showing preferential expression around collector channels (Stamer, Chan and Ethier 2007). Thus, positive expression is dependent upon the location of radial sections relative to collector channels. If none or only one of quadrants in retroperfused segments was “above endogenous”, then we cut and stained more sections and images were again reviewed. All segments that were retroperfused and included in analysis in the present study were positive for recombinant AQP1 expression in at least two quadrants (data not shown).

Results

Thirteen pairs of perfused human anterior segments in organ culture were used to determine the effects of aquaporin-1 (AQP1) expression on conventional outflow function. Age of donors ranged from 50–89 years (mean: 80 ± 3 years), average time from death to enucleation was 3.7 ± 0.5 hrs, while average time from death to start of perfusion was 27.3 ± 2.7 hours (Table 1). Note that this latter quantity includes the time taken to dissect the eye and mount it in the perfusion dish.

In all anterior segments that received adenovirus encoding AQP1, expression of recombinant AQP1 was clearly above endogenous expression levels in contralateral control segments (figure 1). Interestingly, in segments that received virus via the inlet tubing and subsequent

forward perfusion, we frequently observed elevated expression predominantly in the uveal (inner) meshwork region and little staining in the juxtacanalicular region or in the inner wall of Schlemm's canal, similar to previous studies by our laboratory and others (Ethier et al. 2004; Gonzalez et al. 2004; Liu et al. 2005; Rao et al. 2005; Stamer, Chan and Ethier 2007). Elevated AQP1 expression in the inner meshwork of experimental segments, compared to contralateral segments, caused no significant effect on conventional outflow facility (figure 2), with a mean change in outflow facility of AQP1-perfused segments of $-2.0 \pm 9.2\%$ compared to controls (table 2). All controls were examined for, and found to have, robust β -galactosidase activity (figure 1). Additionally, we observed no significant difference in the morphological condition of control (histology score= 2.2 ± 0.12) and experimental (histology score= 2.2 ± 0.24) anterior segments post-perfusion.

We learned in a previous study that adenovirus doses above 6×10^5 PFU that are delivered by forward perfusion effectively deliver genes to cells in the juxtacanalicular region of the meshwork, but unfortunately overload and subsequently kill inner meshwork cells during perfusion. To circumvent such stress on perfused segments in organ culture and to deliver AQP1 specifically to juxtacanalicular and Schlemm's canal cells, we retroperfused virus into a second set of 7 anterior segment pairs. We observed that recombinant protein (β -galactosidase in control and AQP1 in experimental eyes) was expressed in all 7 anterior segment pairs. Similar to β -galactosidase, the expression of AQP1 was found elevated in the endothelial cells of Schlemm's canal and in the juxtacanalicular region of the trabecular meshwork (figure 3). Even though AQP1 was successfully delivered to cells thought responsible for regulating fluid drainage in the conventional pathway, outflow facility was not significantly different than controls. Specifically, the mean change in outflow facility in anterior segments receiving AQP1 adenovirus via retroperfusion relative to contralateral segments was $-3.15 \pm 11.8\%$ (table 2). Analysis of morphological condition of control (histology score= 2.3 ± 0.10) and experimental (histology score= 2.3 ± 0.18) anterior segments post-perfusion revealed no significant differences.

Discussion

The primary finding of the present study was that over expression of AQP1 by cells in the conventional outflow tract does not significantly affect outflow facility of human anterior segments in organ culture. Even when cells in the resistance-generating region of the conventional outflow pathway were specifically targeted, outflow facility of AQP1-overexpressing anterior segments was not different than contralateral segments expressing β -galactosidase. These results indicate that the preferred pathway for water across the JCT-TM and Schlemm's canal inner wall is not by an AQP1-mediated, transcellular route.

Similar to our previous results with adenovirus containing β -galactosidase cDNA, AQP1 adenovirus perfused in the forward direction was predominately intercepted by cells in the uveal meshwork leading to an increased expression of recombinant protein in this region (Stamer, Chan and Ethier 2007). Conversely, when perfused in the retro-direction, AQP1 adenovirus was taken up by cells in the resistance generating region of the conventional tract. Unlike β -galactosidase adenovirus, it was difficult to tell with certainty the degree of penetration of AQP1 into the meshwork due to endogenous AQP1 expression throughout the conventional tract. However, similar to our results with β -galactosidase adenovirus (Stamer, Chan and Ethier 2007), expression of recombinant AQP1 was focal, and likely concentrated near collector channels (not shown). Such a focal distribution of AQP1 in retroperfused segments is not likely responsible for lack of effects on outflow facility. Our previous studies showed that outflow facility can be modulated by retroperfusion, despite focal distribution of retroperfused substances (Ethier et al. 1993; Ethier et al. 1995).

Forward perfusion of virus coding for β -galactosidase caused a facility decrease of $35 \pm 11\%$ in control eye segments over time of perfusion. This decrease was significantly different than the small decrease of $5 \pm 15\%$ seen in segments retroperfused with β -galactosidase virus, but similar to the decrease observed in paired segments perfused with AQP1 virus (32%). The existence of a facility decrease in eyes forward-perfused with β -galactosidase virus (or AQP1 virus) in the present study is consistent with an earlier report by our group (Ethier et al. 2004), although the magnitude of the decrease seen here was about double than the $17 \pm 6\%$ decrease reported earlier. These decreases in facility may be due to cytotoxic effects of the virus in some susceptible inner meshwork cells and subsequent accumulation of cellular debris in the outer meshwork over time of perfusion as observed by others that have perfused adenovirus encoding β -galactosidase (Borras et al. 1999). Also note that, although on average there was no net facility change due to AQP1 over-expression, there was substantial variability in the results, with some eye pairs showing a net facility decrease and others showing a net increase. Some variation is of course expected; however, we could find no obvious explanation for the variation we observed. All eyes in this study passed histology and facility quality control criteria, and there was no correlation between post mortem time, average histology score for a pair, or net facility change. We can only presume that the variation was due to unknown differences in donor tissue.

Initial information about the role of AQP1 in the conventional outflow tract were provided using primary cells that were isolated from human donor eyes (Stamer et al. 2001). In these experiments, AQP1 expression was manipulated using adenovirus vectors that carried AQP1 cDNA oriented in the sense or antisense direction. Antisense AQP1 knocked down AQP1 protein expression by $\sim 70\%$ and was associated with a decrease in TM resting cell volume by $\sim 8\%$. Overexpression of AQP1 by TM cells did the opposite, increasing resting intracellular volume by $\sim 9\%$, and consequently decreasing paracellular permeability of trabecular cell monolayers. These results suggest that altering AQP1 levels could significantly alter outflow dynamics. However, in the present study, increasing AQP1 expression in the JCT region did not significantly impact bulk facility. Thus, AQP1 overexpression in outflow cells did not appear to either restrict the paracellular movement of water through the JCT or across the inner wall of SC (and decrease facility) or increase transcellular movement of water across cells (and increase facility). Thus, the role of AQP1 in the resistance-generating region of the conventional tract in situ appears to involve cell volume regulation in a range that does not affect the regulation of bulk water movement; similar to AQP1's apparent role in other tissues (Krane et al. 2001; Chan, Butterworth and Hazell 2004; Kuang et al. 2004; Liu et al. 2006). Alternatively, the effects of AQP1 overexpression on bulk outflow are small, and within the span of variability (9–12%) of the model system used in the present set of experiments.

Given that AQP1 is ubiquitously expressed in the conventional outflow tract yet does not appear to regulate bulk fluid flow, this study raises the question: *what is the function of aquaporin-1 channels in the conventional tract, particularly in the inner meshwork?* We propose that endogenous AQP1 channels may accommodate rapid volume changes that could occur in the conventional outflow tract when the outflow tissues are subjected to mechanical deformation. Such an idea is particularly relevant for TM cells that reside on the lamellar beams, where they presumably provide no appreciable resistance to flow due to the wide opening between beams. The unique environment of the conventional drainage tract subjects resident cells to continuous mechanical deformation, both repetitive and intermittent (Ethier 2002). For example, during ocular accommodation, the TM is stretched between the scleral spur and Schwalbe's line, with strain transmitted throughout conventional outflow tissues via tendons that originate in the ciliary body and attach to the basement membrane below the SC inner wall. Moreover, conventional drainage tissues are continually perturbed due to everyday activities such as ocular pulse, blinking, squinting, or eye rubbing (Coleman and Trokel 1969; Ramos and Stamer 2008). Such events can rapidly and transiently elevate intraocular

pressure up to 100 mm Hg. When pressure is just raised from 8 to 30 mmHg in monkey eyes, the calculated magnitude of stretch of inner wall cells is about 50% [based on a calculation in (Ethier 2002) using Grierson and Lee's data (Grierson and Lee 1977)]. As tissues deform, the resident cells are forced to change volume, and AQP could be playing a key role in accommodating the rapid volume change of TM cells. Interestingly, in skeletal muscle a similar role for AQP4 has been hypothesized, where aquaporins are thought to facilitate the rapid transfer of water from blood to muscle during periods of intense activity, such as exercise (Frigeri et al. 2004). If this hypothesis is true in the trabecular meshwork, the presence of AQP1 on uveal and corneoscleral meshwork cells emphasizes the dynamic biomechanical environment of the conventional outflow pathway. To test this hypothesis, future studies might examine the effect of AQP1 knockdown or over-expression on the viability and volume regulation capability of trabecular cells exposed to mechanical stress.

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Figure 1. AQP1 expression in conventional outflow tissues of human anterior segments in organ culture that were transduced with adenovirus via injection into inlet tubing during forward perfusion

Panels A and B are images of radially-oriented sections through outflow tissues that have been probed with antibodies specific for AQP1, taken with the same confocal microscope settings. Panel A shows expression levels of endogenous AQP1 in conventional outflow tissues of a segment transduced with β -galactosidase (β -gal) adenovirus by injection into the inlet tubing. Inset box in panel A shows background fluorescence of secondary antibody staining in the absence of AQP1 IgGs. Panel B shows AQP1 expression levels (endogenous plus recombinant) in the contralateral segment after transduction with AQP1 adenovirus. Notice elevated AQP1 IgG labeling in uveal meshwork of panel B (arrows). Panels C and D are histological sections of same anterior segments shown in panels A and B (perfused with β gal and AQP1, respectively) that were developed for β gal activity and counterstained with Hematoxylin and Eosin. Note β -gal labeling of inner TM in panel C. Box in panel D shows approximate region of TM displayed in panels A and B. Bar=50 μ m.

Figure 2. Outflow facility traces for human anterior segment pairs in organ culture that were transduced with AQP1 adenovirus

Panel A shows results from a representative experiment in which, following approximately 140 hours of baseline perfusion, one anterior segment was injected with AQP1 adenovirus (red line) or β -galactosidase adenovirus (control, black line) via the inlet tubing while perfused in the forward direction. Panel B shows results from a representative experiment in which, following approximately 170 hours of baseline perfusion, one segment was exposed to AQP1 adenovirus (red line) or β -galactosidase adenovirus (black line) using the retroperfusion technique. Spikes in facility traces are due to perturbations when media supply syringes are reloaded.

Figure 3. AQP1 expression in conventional outflow tissues of human anterior segments in organ culture that were transduced with adenovirus using the retroperfusion technique

Panels A and B are images of radially-oriented sections through outflow tissues that have been probed with antibodies specific for AQP1, taken with the same confocal microscope settings. Panel A shows expression levels of endogenous AQP1 in conventional outflow tissues of an anterior segment transduced with β -galactosidase (β -gal) adenovirus via retroperfusion. Inset box in panel A shows background fluorescence of secondary antibody staining in the absence of AQP1 IgGs. Panel B shows AQP1 expression levels (endogenous plus recombinant) in the contralateral segment after transduction with AQP1 adenovirus. Notice elevated AQP1 IgG labeling in inner wall/JCT region of panel B (arrows). Panels C and D are histological sections of anterior segments shown in panels A and B (perfused with β gal and AQP1, respectively) that were developed for β gal activity and counterstained with Hematoxylin and Eosin. Note β -gal labeling in SC/JCT region of panel C. Bar=50 μ m.

Table 1

Summary of characteristics of eye donor tissue

Donor	ID	Age (yr)	Gender	Death to Enucleation (hr)	Death to Perfusion (hr)	Histology Score
1	675	74	M	4.5	38.5	2.4
	676					1.6
2	707	50	M	5.0	26.5	2.4
	708					2.8
3	719	89	M	5.0	11.8	1.9
	720					1.8
4	755	85	M	6.6	45.0	2.4
	756					2.9
5	775	78	M	2.2	35.6	2.1
	776					2.3
6	777	75	F	3.5	34.3	1.9
	778					1.9
7	931	78	F	2.8	22.8	2.8
	932					2.3
8	939	72	F	6.3	22.5	2.1
	940					2.3
9	949	88	M	2.8	23.5	2.9
	950					2.5
10	969	81	M	3.0	32.0	2.0
	970					1.6
11	985	83	M	3.0	24.0	2.3
	986					2.2
12	987	82	F	3.0	22.3	2.2
	988					2.8
13	989	88	M	0.0	16.6	2.3
	990					2.2
Mean	(±SEM)	80±2.48	9M 4F	3.67±0.52	27.34±2.67	AQPI= 2.2±0.13 βgal= 2.3±0.09

M=male, F=female

Table 2 Summary of facility data for anterior segments exposed to AQP1 adenovirus.

Donor	ID	Method	Adenovirus	Co	Cd	Net Change (%)
1	676	FP	AQP1	0.27	0.27	18.92
	675		βgal	0.37	0.20	
2	708	FP	AQP1	0.16	0.10	16.35
	707		βgal	0.13	0.06	
3	719	FP	AQP1	0.06	0.05	-16.67
	720		βgal	0.07	0.07	
4	756	FP	AQP1	0.17	0.17	0.00
	755		βgal	0.13	0.13	
5	776	FP	AQP1	0.40	0.13	-34.89
	775		βgal	0.46	0.31	
6	777	FP	AQP1	0.50	0.20	4.29
	778		βgal	0.42	0.15	
		Exp Mean	(±SEM)	0.28±0.08	0.15±0.03	-2.0±9.2
		Con Mean	(±SEM)	0.26±0.08	0.15±0.04	
7	931	RP	AQP1	0.30	0.31	29.65
	932		βgal	0.38	0.28	
8	939	RP	AQP1	0.43	0.39	-58.14
	940		βgal	0.43	0.64	
9	949	RP	AQP1	0.42	0.29	10.87
	950		βgal	0.55	0.32	
10	970	RP	AQP1	0.20	0.28	-17.14
	969		βgal	0.14	0.22	
11	986	RP	AQP1	0.10	0.10	20.00
	985		βgal	0.10	0.08	
12	987	RP	AQP1	0.24	0.21	-1.39
	988		βgal	0.36	0.32	
13	989	RP	AQP1	0.17	0.16	-5.88
	990		βgal	0.18	0.18	
		Exp Mean	(±SEM)	0.27±0.05	0.25±0.04	-3.15±11.8
		Control Mean	(±SEM)	0.31±0.07	0.29±0.07	

ID, identification; FP, forward perfusion; RP, retroperfusion; Exp, experimental; Co, starting facility (μl/min/mmHg); Cd, ending facility 5 days after application of virus (μl/min/mmHg)