Successful knockdown of aquaporin 1 (AQP1) protein expression in American eel (*Anguilla rostrata*) gastrointestinal tract using direct-infusions of vivo-morpholinos

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Vivo-morpholinos are artificial nucleic acids that can block the protein production from a particular gene. Direct intestinal infusions of vivo-morpholinos were used to 'knockdown' (reduce) the amount of aquaporin 1 water channel protein to be able to see differences in function with or without the protein.

Previous experiments with vivo-morpholinos were performed in attempt to knockdown the expression of aquaporin 1 (AQP1) water channel protein in the gastrointestinal tract of the American eel (*Anguilla rostrata*)[1]. These experiments used peritoneal injections of the vivo-morpholinos entry route in order to elicit the knockdown of AQP1 protein. However the level of knockdown achieved was minimal with a marginally significant reduction in AQP1 protein in the intestine and no change in rectal AQP1 protein levels[1]. In this further study a more invasive route of vivo-morpholino ingress into the animal was employed, where they were infused directly into the intestine via a surgically implanted cannula. This was performed in order to improve upon the level knockdown achieved in the previous study.

In American eels, AQP1 protein is expressed in the apical membrane of surface epithelia of the intestine and rectum. Additionally it is also found at even higher levels in underlying vascular endothelia (data not shown) and this is common to European[2] and Japanese[3] eels. A variety of correlative data suggests that AQP1 likely plays an important role in rectal/intestinal water absorption in eels when they inhabit the marine environment[2,3]. However due to a lack of specific inhibitors of the AQP1 protein, it has so far proven impossible to directly demonstrate a role for AQP1 in intestinal/rectal trans-cellular water absorption. To determine the role of AQP1, eels were infused with AQP1-specific vivo-morpholinos in order to 'knockdown' AQP1 protein expression as a precursor to further experiments that will measure the effect of AQP1 protein knockdown on actual intestinal/rectal water absorption.

Freshwater eels were anesthetized in 1000 ppm MS-222 until unresponsive. A small incision (1-2cm) was made with a scalpel blade on the ventral side of the animal adjacent to the anterior intestine. A cannula was inserted into the anterior intestinal lumen through a hypodermic needle. The cannula was held in place with multiple knots using Ethilon nylon 4-0 monofilament suture material. Each knot was anchored to into the body wall with sutures. The incision was then closed with knotted sutures. The end of the cannula was sealed with paraffin wax. The fish was placed in a piece of PVC pipe with ends sealed with mesh to restrict movement of the fish that might result in cannula dislodgement. After a recovery period of one day fish were anesthetized in 500 ppm MS-222 and AQP1-specific vivo-morpholinos (Gene Tools LLC; 1.25 μmol/kg fish weight) dissolved in phosphate buffered saline (PBS) was infused into the anterior intestinal lumen through the cannula. This regimen was performed one day before eels were transferred to seawater and was then carried out for a further three days. The following day, fish were anesthetized and sacrificed, and the posterior intestine and rectum dissected out. The surface epithelium (and underlying non-muscle tissue) of each tissue was scraped off with a microscope slide and processed essentially as previously described[1]. The above procedures were performed in accordance with IACUC approved protocols. The level of AQP1 protein was determined using Western blotting using an eel AQP1-specific antibody as previously described[1].

As shown in Figure 1, the vivo-morpholinos appeared to have a significant effect on AQP1 protein expression levels in comparison to control fish, especially in the rectum, with a slightly lesser effect seen in the posterior intestine. The blots were further quantified, with the level of AQP1 protein significantly lower in posterior intestine (-40%) and rectum (-44%). The resulting method for vivo-morpholino knockdown of AQP1 protein expression will consequently be utilized further in future studies where vivo-morpholino-treated and control PBS-treated eels will have their intestines removed post-mortem and these will be used to measure any differences in net trans-epithelial water influxes that occur between these two groups.

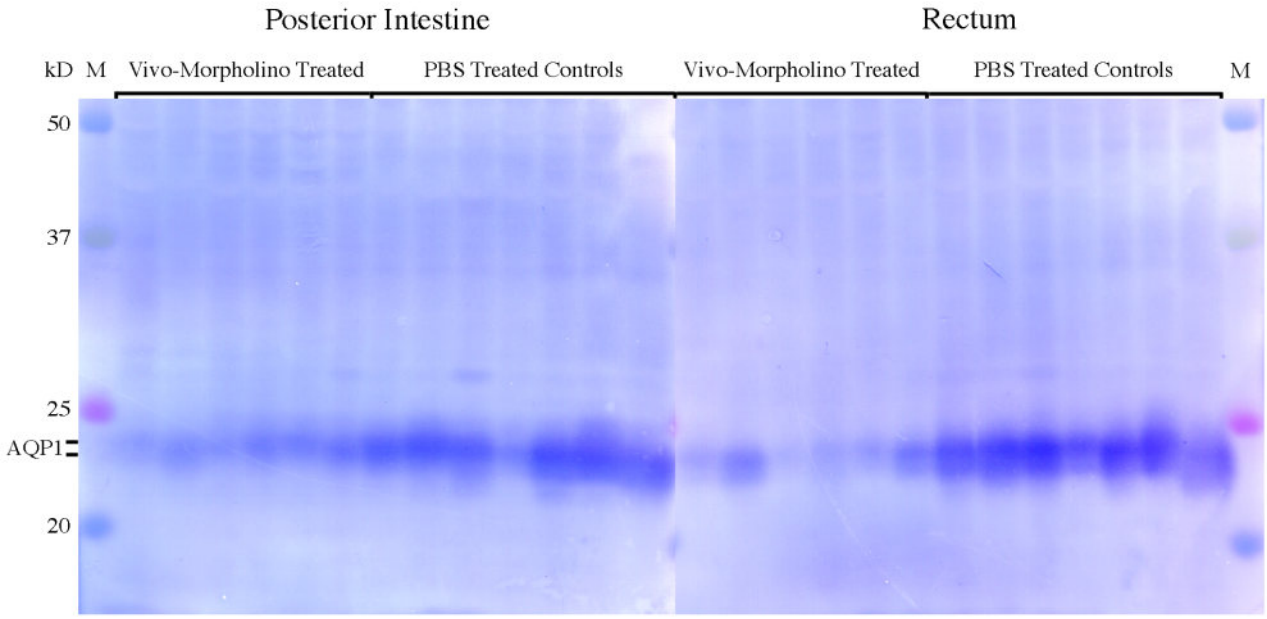

Figure 1. Western blots of crude membrane extracts from either the posterior intestinal or rectal epithelial scrapes of American eels (*Anguilla rostrata*) infused with either vivo-morpholinos dissolved in PBS (n=6) or PBS alone (n=7). Fish were infused for 4 successive days for sacrifice. The Western blots were incubated with an rabbit anti-eel aquaporin 1-specific polyclonal antibody and a goat-anti-rabbit alkaline phosphatase linked secondary antibody and developed using NBT/BCIP enzyme substrate. M = Kaleidoscope Rainbow Molecular Weight Markers (Biorad) with sizes shown in kilo daltons (kD). Two AQP1 protein bands are also indicated.

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