Aquaporin Expression in Normal Human Kidney and in Renal Disease

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Abstract. Aquaporins (AQPs), membrane-inserted water channel proteins, play a highly important role in the reabsorption of water from the renal tubular fluid. Experimentally, both in rats and mice, failure to insert functional AQP molecules into renal tubular membranes leads to nephrogenic diabetes insipidus. In humans, most forms of renal disease lead to a reduction in the water handling capacity of the kidney. AQP distribution in various forms of human renal disease has not been documented. Immunohistochemical studies of biopsy samples from a wide range of renal diseases revealed a substantial and striking upregulation of AQP-1 in the glomeruli of most diseased kidneys. AQP-1 expression remained prominent in proximal tubules in all lesions. In contrast, there was judged qualitatively to be a reduction in the amounts of AQP-2 and AQP-3 expression, especially in lesions with substantial interstitial fibrosis and nephron loss, as compared with a healthy region of normal kidneys. The results were quantitatively confirmed by real-time reverse transcriptase–PCR. This is the first documentation of altered AQP expression in human renal disease. The significance of the increased AQP-1 expression requires further studies.

At least nine different aquaporins (AQP) have been described in humans, with AQP-1, AQP-2, AQP-3, and AQP-4 shown to occur in the mammalian kidney (1,2). AQP-1 is found in abundance in the renal cortex, where it occurs on both apical and basolateral membranes of cells of the proximal tubule and descending thin limb. AQP-2, -3, and -4 are restricted to the distal regions of the renal tubules, where they are critically involved in the regulation of water balance of the body. AQP-2 is confined to the apical regions of the principal cells of the distal tubule and collecting tubule and is the principal target for short-term regulation of collecting duct permeability by arginine vasopressin (AVP). Basolateral permeability of these cells is mediated by AQP-3 and AQP-4. The role of AQPs in human pathology is yet to be fully elucidated, although there is abundant experimental evidence from rats that the expression of these proteins is profoundly altered in renal disease (3–5).

Many experimental treatments have a wide influence on AQP expression in the kidney, with consequent manifestation of water balance abnormalities (3). Kwon et al. (1) report that in rats with lithium-induced nephrogenic diabetes insipidus, there is a dramatic reduction not only of AQP-2 but also of AQP-3 levels, whereas there is no reduction in AQP-1 and AQP-4. Fernandez-Llama et al. (6) found that carbon-tetrachloride induced liver cirrhosis in rats was associated with ascites and a water-excretion deficit. AQP-1 levels were elevated, AQP-2 levels were increased with a redistribution to the apical membranes, and AQP-3 levels were elevated. In a rat model of cisplatin-induced nephrotoxicity (2), a reduced expression of AQP-2 and AQP-3 was found in the collecting duct, as well as a significant decrease in AQP-1 in the proximal tubule.

A number of factors are known to alter the ability of the kidney to recover water from the tubular fluid. Broadly speaking, these fall into two classes: those that affect the transtubular osmotic gradient, and those that affect the tubular permeability. In rare cases, mutations in either the AVP receptor protein, which may lead to an inherited failure to respond to AVP, or the AQP-2 gene, which leads to failure to synthesize AQP-2 or, alternatively, to failure to translate to a functional site on the membrane, can occur. The role of renal AQPs in water balance disorders has recently been reviewed (3,4). However, most of the information relating to humans has been extrapolated from animal studies. More commonly, alterations in renal function may lead to an alteration in the ability to retain water. In the clinical setting, most forms of established renal injury are associated with a loss of renal concentrating ability, manifested as nocturia and the inability to concentrate the urine after water deprivation. This is independent of the underlying etiology of the renal disease. It is commonly thought that this is predominantly mediated by an altered response to AVP and binding to its receptor. What has not been demonstrated is the extent to which AQP expression in different groups of renal damage is altered.

Materials and Methods

Immunohistochemistry

Archival human renal biopsy samples of differing pathologies and normal human kidney tissue (nephrectomy specimens) were processed for immunohistochemistry by means of standard techniques.
Tissue was fixed in 4% buffered formaldehyde and embedded in wax. Sections were cut at 4 μm, blocked in 1% BSA, and reacted with the primary antibody. They were then washed in PBS and reacted with the secondary antibody, anti-IgG horseradish peroxidase (PO488, DAKO). After washing again, the sections were reacted with diamobenzidine (DAKO) and counterstained with hematoxylin, dehydrated, and mounted in DPX. Antigen retrieval techniques did not improve the signal obtained. The primary antibodies used were (1) anti-rat AQP-1 from Alpha Diagnostics; (2) anti-human AQP-2 made by Chiron Mimetopes, Australia (antiserum in rabbits was raised against a synthetic peptide from the 15 C terminal end of human AQP-2 [amino acids 257 to 271: VELH-SPQSLPRGTKA]); and (3) anti-AQP-3 and anti-AQP-4, obtained from Santa Cruz Biotechnolog. Negative controls were carried out by either omitting the primary antibody or blocking with the appropriate peptide. Immunostaining in the renal biopsy specimens was compared semiquantitatively to the immunostaining evident in the normal human kidney specimens.

Confirmation of the endothelial location of AQP-1 within the glomerulus was achieved by labeling β-laminin in basement membranes with a mouse monoclonal anti-human β-laminin antibody (Santa Cruz Biotechnology), visualized with rabbit anti-mouse IgG coupled to Texas red (Molecular Probes). AQP-1 was identified in the same sections with rabbit anti-rat AQP-1 (Alpha Diagnostics) and a secondary antibody (goat anti-rabbit IgG) coupled to Alexa 633 (Molecular Probes). Fluorescence was detected with a Zeiss confocal microscope (LSM 510).

The specificity of AQP-1 (Alpha Diagnostics), AQP-2 (Chiron Mimetopes, Australia) and AQP-3 (Santa Cruz Biotechnology) was verified by Western blot test of human cortical kidney samples. Protein samples (approximately 25 μg per lane) were separated by 12% SDS-PAGE (BioRad Mini Protein II) and electroblotted onto Immobilon PDVF membranes (Millipore). After blocking with 5% milk in Tris buffer, pH 7.0, containing Tween 20 (TBST), the membranes were incubated in either preimmune serum, antisemum, or affinity-purified serum overnight at 4°C. They were washed in TBST, then incubated with the secondary antibody, rabbit or goat Ig-–horseradish peroxidase (P488, P489, DAKO) (1:3000 dilution) for an hour at room temperature. The product was detected with the PicoWest Supersignal chemiluminescent kit (Pierce Chemicals) and visualized at room temperature. The product was detected with the PicoWest Supersignal chemiluminescent kit (Pierce Chemicals) and visualized at room temperature. The product was detected with the PicoWest Supersignal chemiluminescent kit (Pierce Chemicals) and visualized at room temperature.

**Real-Time Reverse Transcriptase–PCR**

A limited number of renal biopsy specimens (2 to 3 mg each) that had been stored at −80°C were available for RNA extraction. The samples, together with a number of similarly sized samples from cortices of normal kidneys, were used for real-time reverse transcriptase–PCR (RT-PCR) and matched with those in which immunohistochemistry was carried out. Sufficient sample had been taken at the time the biopsy was taken to ensure that some was frozen at −70°C in Tissue-Tek O.C.T. (Sakura Finetechnical Co., Japan). Of all the renal biopsy samples investigated, only one included some medulla, and this could clearly be identified from the histology. The number of glomeruli in each of these biopsy samples was estimated to be between 10 to 20. These biopsy samples were individually extracted for total RNA with Trizol (Life Technologies). Chloroform was added, and the sample was mixed and spun at 10,000 g for 10 min at 4°C. The supernatant was carefully removed and the RNA precipitated by the addition of 0.1 vol 3 M sodium acetate and 2.5 vol cold ethanol and 0.5 μl glycogen (20 mg ml−1) at −20°C overnight. The RNA was spun again, air dried, and resuspended in 20 μl Tris/EDTA buffer. The RNA was reverse transcribed to cDNA with Superscript II (Invitrogen). The number of steps was kept to a minimum to prevent loss of RNA at each stage.

Real-time PCR was carried out by the TaqMan Applied Biosystems Sequence Detection Systems 7700. Primers were as follows: TGG CTG TGG GAT TAA CCC TG (forward) and GTG TGC TGA AGT TGT GTG TGA TC (reverse) for AQP-1; CCA CCT CCT TGG GAT CCA TT (forward) and GTG AC GAC ACA GCT GGA GCC A (reverse) for AQP-2; CCC ATC GTG TCC CCA CTC (forward) and GCC GAT CAT CAG CTG GTA CA (reverse) and for AQP-3. Amplicons were 6FAM-CGG TCC TTT GCC TCC GCG G for AQP-1; 6FAM-CAC CGG CTG CTC TAT GAA TTC TGC C for AQP-2; and 6FAM-TGG CCT CCA TTG CGG GTG TCT TC for AQP-3. Each amplicon and primer set was checked through BLAST search (NCBI, NLM) to ascertain that the sequences were unique for each human AQP. Amplicons (VIC-AAC CAT GTG ACT TTG TCA CAG CCC AAG AT) and primers (AGA TGA GTA TGC CTG CCG TGT forward; GCT TAC ATG TCT CCA TTC AAC TTA reverse) for β2-microglobulin in the human kidney, which would act as an endogenous control, was also used. AQP-4 was not investigated by real-time RT-PCR because most of the renal biopsy samples were from the cortical region of the kidney. Real-time PCR was carried out according to the manufacturer’s instructions, and relative Ct values (ΔΔCt; Applied Biosystems Sequence Detection Systems Instruction Manual) (9) calculated. Amplification efficiency (control) is referred to as 1.00 because each analysis was carried out on one run, including the control dilution series and the endogenous control.

The amplification of the cDNA showed clear and distinct sigmoid curves (data not shown), indicating that the RNA was clean and amplification of the reverse-transcribed cDNA was uncontaminated.

**Results**

**Immunohistochemistry**

The archival renal biopsy samples analyzed were (numbers of each shown in parentheses); mesangial proliferative glomer-
ulonephritis (immunofluorescent negative) (4); IgA nephropathy with mesangial proliferative changes (5); lupus nephritis (4); membrane-proliferative glomerulonephritis (1); minimal change glomerulonephritis (4); membranous nephropathy (1); acute interstitial nephritis (3); interstitial nephritis in association with vasculitis (3); crescentic glomerulonephritis associated with vasculitis (2), chronic glomerulonephritis (2), arteriolar nephrosclerosis (6), and chronic lithium-induced nephropathy (2).

Figure 2 shows distribution of AQPs 1 to 4 in the normal human kidney. AQP-1 is located on the basolateral and apical membranes of the proximal tubules and descending thin limb of the loop of Henle (Figure 2, a through c). It is absent from other parts of the nephron and the collecting ducts. It is also found in the descending vasa recta (Figure 2d), and a weak positive stain is evident in the glomerular capillaries.

AQP-2 is localized to the apical membranes of the collecting duct principal cells. It is absent from the intercalated cells of the collecting duct (Figure 2e). AQP-3 (Figure 2f) and AQP-4 are present in the basolateral membrane of the principal cells of the collecting duct. AQP-4 was only present in the collecting duct cells.

In the renal biopsy specimens, the striking finding was the greatly increased expression of AQP-1 in the glomeruli of all specimens, including the “minimal change” lesions (Figures 3, 4, and 5). AQP-1 is clearly localized to the glomerular endothelium (Figure 5, a and b, arrows), as is evidenced by its presence interior to β-laminin, which demarcates the basement membranes. AQP-1 staining was also evident in the sclerosed glomeruli. AQP-1 staining was widespread and prominent in all viable proximal tubules on the specimens. In immunofluorescence-negative mesangial proliferative glomerulonephritis specimens, there was an apparent loss of AQP-1 (Figure 3, a and b) staining on the basolateral membranes of the tubules, but AQP-2 (Figure 3c) still showed moderate amounts of expression even although the cells were atretic. Similar patterns were apparent in IgA nephropathy (Figure 3, d through f), interstitial nephritis (Figure 4, a and b), and lupus nephritis (Figure 3, h through j) biopsy specimens. In minimal change specimens, where no detectable changes are seen on routine light microscopy, there was a clearly visible increase in AQP-1 expression in the glomerulus and a loss of basolateral staining of AQP-1 in the proximal tubules. AQP-2 appeared normal (Figure 4, e through h).

AQP-2 immunostaining was present in the cortical and distal collecting duct tubules but was reduced in the lesions of chronic interstitial nephritis, nephrosclerosis, and chronic glomerulonephritis due to a greater degree of interstitial fibrosis. A similar pattern in AQP-3 staining was seen. AQP-4 staining was absent, presumably the result of the lack of inner medullary tissue on the biopsy specimens that contain tissue predominantly from the cortex and juxtamedullary regions of the kidney. A semiquantitative analysis has been made for the four AQPs and is presented in Table 1.

**Real-Time RT-PCR**

Real-time RT-PCR is a recently developed technique (9) that gives an estimate of the changes in the abundance of the mRNAs. The distribution in terms of relative amounts (ΔΔCₜₜ) of AQPs are shown in Figure 6. In renal disease samples, AQP-2 is decreased compared with the normal kidney. AQP-1 mRNA was increased in biopsy specimens of crescentic glomerulonephritis with vasculitis and immunofluorescence-negative mesangial proliferative glomerulonephritis, but in other cases, it remained unchanged compared with the normal kidney. Increases in the AQP-3 mRNA expression were seen in crescentic glomerulonephritis with vasculitis, IgA nephropathy, and nephrosclerosis. There was a decrease in AQP-3 mRNA expression in lupus nephritis and with kidneys that had been exposed to chronic lithium treatment. Real-time RT-PCR gives an estimation of how much the AQPs have changed in disease states relative to that expressed in the normal kidney.

**Discussion**

The role of AQPs and the regulation of water permeability has been the subject of intense investigation. They are now regarded as the main route for transmembrane water movement (3). The majority of studies have involved experimental animal models, and only limited human studies on normal kidney tissue (3,10,11) have been reported. Alterations in renal AQP expression have not been studied in human renal diseases. We have examined the expression (by means of immunohistochemistry and real-time RT-PCR) of AQPs 1 to 4 in various
forms of renal disease. The most striking observation was the increase in AQP-1 immunostaining especially in the glomerular endothelium, but also in the tubules of diseased kidneys. In the normal human renal tissue, we were able to document only minute amounts of AQP-1 immunostaining in glomeruli as opposed to the prominent immunostaining in the proximal tubules. In all forms of renal disease examined, AQP-1 immunostaining in the glomerulus was clearly increased compared with normal tissue. Even in the minimal change nephropathy biopsy specimens, glomerular AQP-1 immunostaining was clearly increased. This upregulation in immunostaining was supported by an increase in AQP-1 mRNA expression as measured by real-time RT-PCR in certain renal diseases, such as crescentic glomerulonephritis due to vasculitis and immunofluorescence-negative mesangial proliferative glomerulonephritis. Devuyst et al. (12) have reported that AQP-1 is very...
specifically located in capillary and venule endothelium but not in small-sized arteries in human peritoneum. Interestingly, these authors found that AQP-1 expression was remarkably stable in clinical conditions characterized by peritoneal inflammation, although eNOS was upregulated in three patients with ascites and/or peritonitis.

A previous study on normal human renal tissue demonstrated AQP-1 to be localized predominately in proximal tubule cells, and found a small amount of AQP-1 expression in the capillary endothelial cells of the glomeruli (11). However, they did not examine changes in AQPs in human renal disease.

It is not clear why AQP-1 should be increased in the different forms of renal disease. AQP-1 plays a critical role in the preservation of proximal tubule water handling and urinary concentration. In a few cell culture studies, changes in AQP-1 and AQP-3 expression have been demonstrated (13,14).

Figure 4. Distribution of aquaporin (AQP) 1 to 3 in human renal pathology as detected by immunostaining with horseradish peroxidase. (a through d) Acute interstitial nephritis. (a) AQP-1 in localization in the glomerulus; original magnification, ×60. (b) AQP-1 localization in the proximal tubules; original magnification, ×60. (c) AQP-2 localization in the apical membranes of the collecting duct cells; original magnification, ×60. (d) AQP-3 distribution in collecting duct cells; original magnification, ×60. (e through h) Minimal change nephropathy. (e) AQP-1 localization in the glomerulus; original magnification, ×60. (f) AQP-1 localization in the proximal tubules; original magnification, ×60. (g) AQP-2 localization on the apical membranes of the collecting duct cells; original magnification, ×60. (h) AQP-3 distribution in collecting duct cells; original magnification, ×60. (i through l) Chronic crescentic glomerulonephritis. (i) AQP-1 localization in the glomerulus; original magnification, ×60. (j) AQP-1 localization in the proximal tubules; original magnification, ×60. (k) AQP-2 localization on the apical membranes of the collecting duct cells; original magnification, ×60. (l) AQP-3 distribution in the collecting duct cells; original magnification, ×60.
AQP-1 is upregulated after exposure to contrast material or osmotic stress in proximal tubule epithelial cells in culture (13), and similarly, AQP-3 in MDCK cells increases in response to a hyperosmolar challenge (14). Similarly, a protein challenge produced a marked increase in AQP-1 and AQP-3 message in proximal tubule epithelial cells (15). In one study that looked at the expression of AQPs in human peritoneal membrane samples, AQP-1 was shown to be localized to the capillary and venule endothelium and was postulated to be the water channel in the human peritoneum for water transport (12). Increased expression of AQP-1 in glomeruli after injury as demonstrated in this study would suggest a role for AQP-1 in maintaining osmotic equilibrium. One can postulate that renal injury, regardless of etiology, produces increased stress on cell integrity, and that the increased expression of AQP-1 is an adaptive response to this. Further studies are required to elucidate the significance of these immunohistological observations.

Changes in AQP-2 and AQP-3 immunostaining and mRNA seen in this collection of human renal biopsy specimens are similar to those documented in diseased rat kidneys (3). Reduction in nephron numbers and the presence of interstitial fibrosis reduces the amount of AQP-2 and AQP-3. Clinically, this would be associated with the inability of the kidney to concentrate urine and the consequent manifestation of nocturia, as is seen with this condition.

In summary, AQP expression in various forms of renal disease demonstrates a reduction in AQP-2 and AQP-3 expression associated with a loss of nephrons and the presence of chronic interstitial fibrosis. A more striking finding was the

### Table 1. Semiquantitative assessment of localization of aquaporins (AQP) 1 to 4 in normal and kidneys sampled by biopsy

<table>
<thead>
<tr>
<th>Disease</th>
<th>AQP1 PT</th>
<th>AQP1 VR&lt;sub&gt;desc&lt;/sub&gt;</th>
<th>AQP1 glom</th>
<th>AQP2 DCT</th>
<th>AQP3 DCT</th>
<th>AQP4 DCT</th>
</tr>
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<tr>
<td>Normal</td>
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<td>3+</td>
<td></td>
<td>3+</td>
<td>3+</td>
<td>1</td>
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<tr>
<td>Mesangial proliferative glomerulonephritis</td>
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<td>3+</td>
<td>3+</td>
<td>2</td>
<td>3</td>
<td>2+</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>2+</td>
<td>2+</td>
<td>2</td>
<td>2</td>
<td>2+</td>
<td></td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2+</td>
<td></td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>2+</td>
<td>2+</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crescentic glomerulonephritis</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1+</td>
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<td></td>
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<tr>
<td>Chronic lithium nephropathy</td>
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<td>Trace</td>
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<tr>
<td>Chronic glomerulonephritis</td>
<td>2+</td>
<td>Trace</td>
<td></td>
<td>1+</td>
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<tr>
<td>Minimal change</td>
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<td>3</td>
<td></td>
<td>1+</td>
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</table>

*Kidneys were sampled when sufficient material was available for analysis. A blank space indicates that the appropriate antigen was not detected, not necessarily that it was not present. Range is 0 to 3+. A indicates normal kidney; B, crescentic glomerulonephritis with vasculitis; C, IgA mesangial proliferative glomerulonephritis; D, immunofluorescence negative mesangial proliferative glomerulonephritis; E, lupus nephritis; F, nephrosclerosis; G, chronic lithium nephropathy; and H, minimal change.
uniformly increased expression of AQP-1, especially in glo-
meruli, in association with all forms of renal disease investi-
gated. Further studies are required to determine the signifi-
cance of these observations.

Acknowledgments
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References
1. Kwon TH, Laursen UH, Marples D, Maunsbach AB, Knepper
MA, Frokiaer J, Nielsen S: Altered expression of renal AQPs and
Na\(^+\)/H\(^+\) transporters in rats with lithium-induced NDI. Am J Physiol
279: F552–F564, 2000
2. Kishore BK, Krane CM, Di Julio D, Menon AG, Cacini W:
Expression of renal aquaporins 1, 2 and 3 in a rat model of
MA: Aquaporins in the kidney: From molecules to medicine.
Physiol Rev 82: 205–244, 2002
4. Knepper MA: Molecular physiology of urinary concentrating
mechanism: Regulation of aquaporin water channels by vaso-
5. Preston GM, Agre P: Isolation of the cDNA for erythrocyte
integral membrane of 28 kilodaltons: Member of an ancient
water channel family. Proc Natl Acad Sci U S A 88: 11110–
11114, 1991
Nielsen S, Knepper MA: Dysregulation of renal aquaporins and
Na-Cl cotransporter in CCl\(_4\)-induced cirrhosis. Kidney Int 58:
216–228, 2000
7. Marples D, Christensen S, Christensen EI, Ottosen PD, Nielsen
S: Lithium-induced downregulation of aquaporin-2 water chan-
1845, 1995
8. DiGiovanni SR, Nielsen S, Christensen EI, Knepper MA: Regula-
tion of collecting duct water channel expression by vasopressin in
9. Medhurst AD, Harrison DC, Read SJ, Campbell CA, Robbins
MJ, Pangalos MN: The use of TaqMan RT-PCR assays for semiquan-
titative analysis of gene expression in CNS tissues and
Hebert SC, Kaissling B: Human cortical distal nephron: Distri-
bution of electrolyte and water transport pathways. J Am Soc
11. Maunsbach AB, Marples D, Chin E, Ning G, Bondy C, Agre P,
Nielsen S: Aquaporin-1 water channel expression in human
JP, Pouthier D, Goffin E: Aquaporin 1 and endothelial nitric
oxide synthase expression in capillary endothelia of human peri-
inducible water channel in cultured mIMCD-3 cells. J Am Soc
14. Matsuzaki T, Suzuki T, Takata K: Hypertonicity-induced expres-
sion of aquaporin 3 in MDCK cells. J Am Soc Nephrol 13: C55–C63,
2001
studies of aquaporins 1 and 3 expression in cultured human
proximal tubular cells: Upregulation by transferrin but not albu-