Selective Decrease in Urinary Aquaporin 2 and Increase in Prostaglandin E2 Excretion Is Associated with Postobstructive Polyuria in Human Congenital Hydronephrosis

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Abstract. This study was undertaken to determine the role of aquaporin 2 (AQP2) in the impaired urinary concentrating capacity observed in patients who underwent pyeloplasty because of congenital unilateral hydronephrosis as a result of pyeloureteral junction disease. Twelve children (mean age, 8 ± 2 mo) were examined in the study. From day 1 to day 5 after surgery, the urine was collected separately from pyelostomy draining only from the postobstructed kidney and from the bladder catheter draining mostly from the contralateral kidney used as internal control. After pyeloplasty, the postobstructed kidney was characterized by a reduced urinary excretion of AQP2 (~54%) associated with polyuria that persisted from day 1 to day 5 (433 ± 58 *versus* 310 ± 74 ml/24 h at day 1; 326 ± 44 *versus* 227 ± 26 ml/24 h at day 5). In parallel, urine osmolality from the postobstructed kidney was significantly reduced compared with the contralateral kidney (111 ± 12 *versus* 206 ± 49 at day 1; 136 ± 24 *versus* 235 ± 65 mOsm/kg at day 5). Creatinine clearance from the postobstructed kidney was not significantly different compared with the contralateral kidney throughout the 4 d after surgery. However, on day 5, creatinine clearance from the postobstructed kidney became significantly lower. Prostaglandin E2 in the urine from postobstructed kidneys was found to be twofold higher than in the contralateral samples (26.0 ± 6.7 *versus* 13.5 ± 2.5 at day 5). It is concluded that (*1*) the selective downregulation of AQP2 in postobstructed kidney may account for the higher excretion of hypotonic urine, and (2) the local increase in prostaglandin E2 synthesis in postobstructed kidney may be involved in AQP2 downregulation and in maintaining a GFR similar to that of the contralateral kidney.

Developmental renal and urinary tract abnormalities are responsible for 54% of chronic renal insufficiency in children, with a prevalence of congenital obstructive nephro-uropathies (1). Among them, ureteral obstruction (UO), with or without concomitant renal failure, is associated with long-term impairment of the ability to concentrate urine. The pathophysiologic mechanisms of postobstructive polyuria and of the defective urinary concentrating capacity, which requires an adequate treatment of the patient, have still to be elucidated in humans. Studies in animal models suggest that the pathophysiology behind the loss of urinary concentrating ability is complex and involves different tubular segments (2,3). These studies have shown impaired water reabsorption at the collecting duct level during bilateral ureteral obstruction (BUO) and massive vasopressin-insensitive polyuria after relief of obstruction, suggesting that BUO can be considered a form of nephrogenic diabetes insipidus (NDI) (4).

The vasopressin-sensitive water channel aquaporin 2 (AQP2) is the chief target for the regulation of collecting duct water permeability (5,6). AQP2 is translocated from intracellular vesicles to the apical membrane of collecting duct cells after vasopressin stimulation and is downregulated in multiple forms of acquired NDI characterized by severe polyuria (7–9).

In a BUO model in rats, it has been observed that postobstructive polyuria and the urinary concentrating defect is associated with reduced trafficking and expression of AQP2 measured after the release of obstruction (10–12). Moreover, in an animal model of unilateral ureteral obstruction (UUO), a reduction in AQP2 expression has been demonstrated not only in the obstructed kidney (23% of control) but also in the contralateral nonobstructed kidney (75% of control), associated with a 150% increase in urine production (13). This suggests that AQP2 regulation depends on both intrarenal and systemic

Received December 16, 2003. Accepted June 28, 2004.
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1046-6673/1510-2705
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000139689.94776.7A

factors, although the specific mechanisms involved still have to be elucidated. In UUO, a balance between vasoconstrictors and vasodilators is important in the regulation of renal blood flow and of filtration rate (14). In a recent study performed in humans, we observed that in the immediate postoperative period, the previously obstructed kidney had an increased urinary excretion of prostaglandin E2 (PGE2) compared with the contralateral kidney. This effect decreased significantly after pharmacologic inhibition of PGE2 synthesis, leading to a significant reduction in glomerular function as measured by inulin clearance (15). If the increased synthesis of PGE2 could maintain the filtration reserve at the glomerular level, then it could also interfere with water excretion and urine concentrating ability at the tubular level. Indeed, it has been shown in the renal inner medulla of rats (16) and in primary collecting duct cells (17) that PGE2 counteracts the action of vasopressin by interfering with AQP2 trafficking (16).

Whereas the decreased expression of AQPs and their role in determining the concentrating inability of obstructed kidney and postobstructive polyuria have been shown in animal models, no information is available in humans. In addition, animal models of ureteral ligation are of limited value for the comprehension of its pathophysiology in humans, as the ligation is produced after birth, when nephrogenesis is already complete. Moreover, it is extrinsic and acts only for a short period of time. In the human fetus, however, UUO begins early, during nephrogenesis, and could be due to a dysregulation of a genetic program involved in the concomitant ureteric and nephron elongation and differentiation (18). For this reason, the distribution and expression of AQPs, in particular AQP2, located in the collecting duct derived from the ureteric bud, could be influenced by congenital obstruction.

The aim of this study was to evaluate urinary excretion of AQP2 during postobstructive polyuria in infants with prenatal diagnosis of unilateral hydronephrosis as a result of unilateral pyeloureteral obstruction. The study in this highly selected group of patients has two fundamental advantages: (*1*) it selects a homogeneous group of patients with unilateral obstruction based on objective criteria, and (*2*) evaluation of urinary excretion of AQP2 and renal function of the postobstructed kidney is compared with the nonobstructed kidney by selective sampling of urine from both kidneys.

Materials and Methods

Patients and Inclusion Criteria

Retrospectively, the eligibility of all of the children who underwent unilateral pyeloplasty at the Pediatric Nephrology Unit because of a congenital unilateral hydronephrosis was evaluated from January to December 2002. Patients were included when they had histologically documented pyeloureteral junction disease, severe pelvic (anteroposterior pelvic diameter >25 mm) and/or calyceal dilation, split renal function >40%, and no significant histologic alterations of the hydronephrotic kidney.

All patients had a prenatal diagnosis of unilateral hydronephrosis during routine fetal ultrasound and a postnatal diagnosis of pyeloureteral junction (PUJ) pathology. All patients were evaluated after birth by abdominal ultrasound, voiding cystography to exclude vesicoureteral reflux, and mercaptoacetyltriglycine (MAG3) diuretic renography. During the radionuclide scan, split renal function (normal value, 45 ± 5%), parenchymal transit of MAG3, and renal washout were evaluated as described previously (15,19,20).

Surgical intervention consisted of dismembered pyeloplasty in all patients. When an extrinsic compression was noted on the ureteropelvic junction (*i.e.*, vascular ring surrounding ureter), patients were excluded from the study. In addition, a biopsy of the obstructed kidney was performed for clinical purposes during pyeloplasty with the informed consent of parents. The histologic evaluation was carried out using the classical dyes eosin and hematoxylin, Masson's trichromic dye, periodic acid-Schiff dye, and silver periodic acid-Schiff.

On the basis of the results obtained with the histologic evaluation of the biopsy, kidneys that are affected by PUJ may exhibit the following histologic features: (*1*) mature, completely differentiated renal tissue without any histologic lesions as a result of the obstruction or signs of only mild tubular cell damage; (*2*) mature, completely differentiated renal tissue with histologic lesions as a result of the obstruction, such as inflammatory infiltration, and interstitial and periglomerular fibrosis, tubular atrophy, and glomerular sclerosis; and (*3*) immature, incompletely differentiated renal tissue with immature nephrons, persistence of primitive ductal structures, and areas of undifferentiated parenchyma (dysplasia) (Figure 1). For reducing interassay variability, which could depend more on the different degree of renal tissue damage than on the obstruction, only patients who had preoperative split function of obstructed kidney >40% and a biopsy showing mature and well-differentiated renal parenchyma without significant histologic changes (histologic group 1) were considered eligible for the study. Additional exclusion criteria were no prenatal ultrasound record demonstrating hydronephrosis; presence of other concomitant urologic malformations (*e.g.*, megaureter, kidney ectopia); treated or untreated hypertension; a creatinine clearance rate (CrCl) <80 ml/min per 1.73 m^2, calculated using the Schwartz formula; and episodes of urinary tract infections.

Urine Samples

During dismembered pyeloureteral anastomosis, a large multihole tube 6 Cherier was placed inside the pelvis with the tip in the first 2 cm of the ureter as anastomosis tutor. As in routine clinical practice with children who undergo pyeloplasty, the pyelostomy tube as well as the catheter positioned in the bladder during surgery were removed on the fifth postoperative day. From the day of surgery to the fifth postoperative day, urine was collected separately from the pyelostomy tube draining the postobstructed kidney and from the bladder catheter draining mainly the contralateral normal kidney, which was used as control. Therefore, the renal parenchyma was intact, and, for each patient, all of the parameters tested in the obstructed kidney could be compared with the most appropriate control represented by the normal contralateral kidney (Figure 2).

The separate urinary output was collected daily in the morning, and urinary (U) osmolality, Na, creatinine, and AQP2 excretion were tested in each sample. The concentration of creatinine in the urine samples and blood was determined by standard automated techniques. The separate creatinine clearance of both kidneys was calculated using the standard formula CrCl = V*UCr/SCr (ml/min), where UCr is the urinary creatinine (mM), V is the urinary volume (ml/min), and SCr is the plasma concentration of creatinine (mM). An aliquot of each urine sample was stored at −20°C for analysis of AQP2 and PGE2 excretion. Free water clearance was calculated as $Cl_{H2O} = V - (U_{osm} \times V)/S_{osm}$, where V is urine volume, U_{osm} is urine osmolality, and S_{osm} is serum osmolality.

It has to be stressed that for these patients, the calculation of free water clearance and creatinine clearance throughout the 5 d after

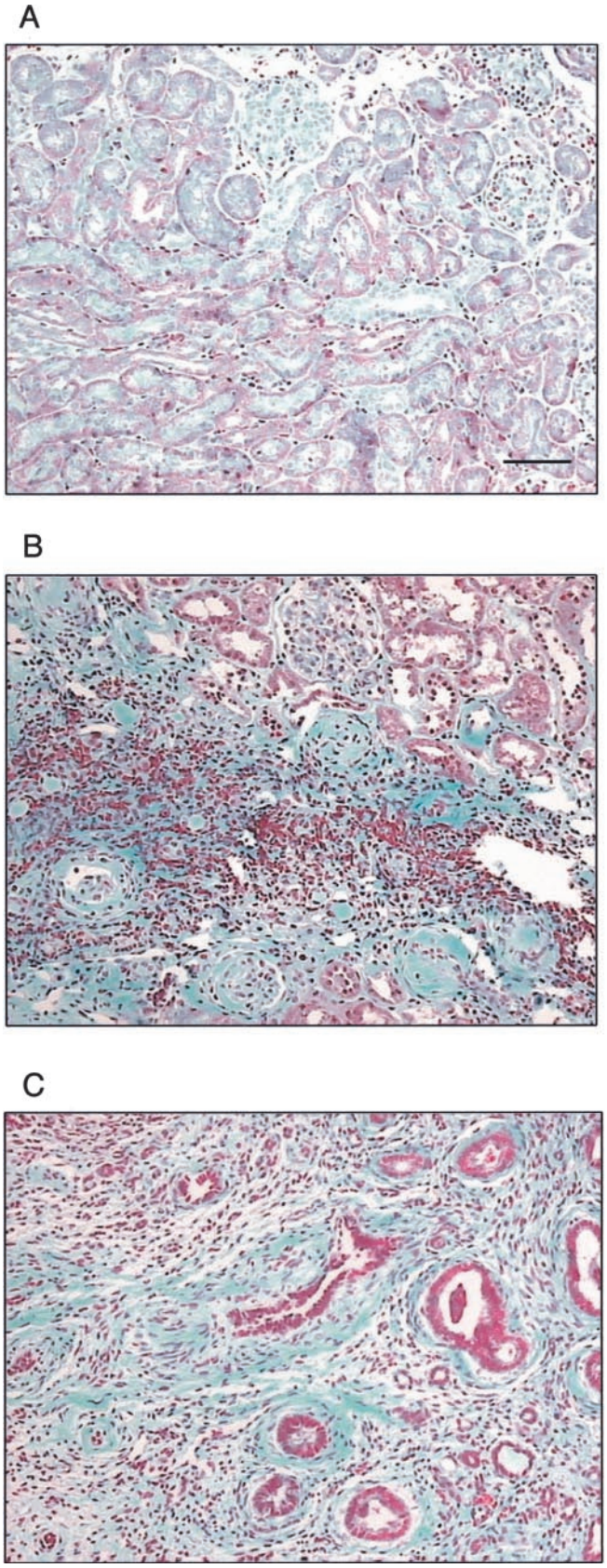

Figure 1. Renal histology of congenital hydronephrotic kidney with pyeloureteral junction (PUJ) disease submitted to pyeloplasty. (A) Normal histology. (B) Chronic interstitial nephropathy. (C) Renal dysplasia. Bar = 50 μm.

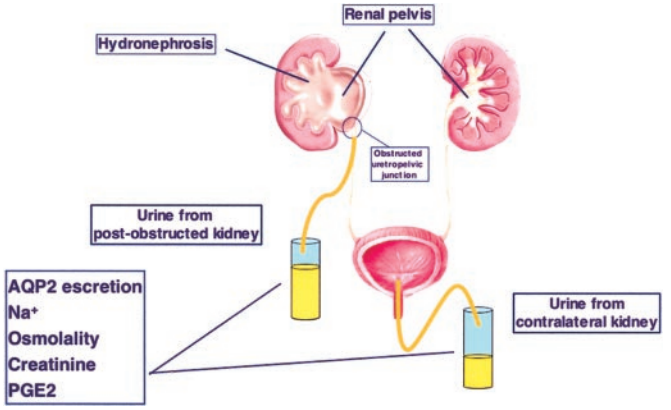

Figure 2. Urinary concentrating defects in unilateral PUJ obstruction. From the first day of surgery to the fifth day after intervention, urine was collected separately from the pyelostomy tube draining the postobstructed kidney and from the bladder catheter draining mainly the contralateral kidney used as control. Aquaporin 2 (AQP2) excretion, urine output, osmolality, sodium, creatinine, and creatinine clearance were determined throughout the 5 d after surgery in each sample.

surgery was based on the S_{osm} measured on the first day. In fact, because of the patients' ages, it was considered not ethical to obtain a blood sample for each day of follow-up.

Evaluation of AQP2 Excretion

AQP2 excretion was evaluated by semiquantitative Western blotting in both urine samples, *i.e.*, those collected from the postobstructed kidney and the contralateral kidney as described previously (21,22). Briefly, urine samples from each patient were spun down at 3000 rpm for 10 min at 4°C to remove cellular debris in the presence of the following protease inhibitors: 2 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml pepstatin; 150 μg of creatinine equivalent of each sample was then concentrated by ultrafiltration using Centricon tubes (Millipore, Bedford, MA) with 10,000 D cutoff according to the protocol provided by the manufacturer. Concentrated proteins were subjected to immunoblot analysis to semiquantify the amount of AQP2 in the sample. Immunoblotting of urine samples using anti-human AQP2 antibody was performed as described previously (21). Antigen-antibody reactions were visualized using the substrates 0.56 mM 5-bromo-4-chloro-3-indolyl phosphate and 0.48 mM nitro blue tetrazolium in 10 mM Tris-HCl (pH 9.5). The density of the 29-kD band of AQP2 that showed up was measured by densitometry and semiquantified using the NIH software.

PGE2 Measurements

PGE2 excretion in urine samples was evaluated by a standard ELISA kit (Pantec, srl, Torino, Italy).

Statistical Analyses

Values are presented as mean ± SEM. Comparisons between groups were made by paired *t* test. $P < 0.05$ was considered significant.

Patient Risks

All of the procedures that were performed to monitor the patients and to obtain the biologic materials analyzed in the present study are performed routinely in our unit for clinical purposes. Written informed parental consent was requested for this study.

Results

Patients and Renal Function

Clinical and instrumental details of the patients are reported in Table 1. Hydronephrosis was diagnosed during gestation by fetal ultrasound scan at a mean age of 31.5 ± 1.4 gestational wk. The mean anteroposterior diameters of the pelvis prenatally, measured at the first month after birth and just before surgery, were 15.4 ± 2, 20 ± 1.7, and 29.8 ± 1.8 mm, respectively. The mean presurgery split renal function (MAG3) of the hydronephrotic kidney was 46 ± 1.8%; the age at surgery was 8 ± 2 mo, and the body weight was 7214 ± 614 g.

The urine output, osmolality, sodium, AQP2, creatinine, and CrCl of both kidneys were determined throughout the 5 d of postsurgery. The urine output from the postobstructed kidney was higher than that from the contralateral kidney during the 5 d of follow-up (Figure 3). The difference between the two kidneys was statistically significant starting from day 1 (433 ± 58 *versus* 310 ± 74 ml/24 h) up to day 4 (360 ± 44 *versus* 221 ± 34 ml/24h). On day 5, the difference between the two kidneys was reduced and became not statistically significant (326 ± 44 *versus* 227 ± 26 ml/24 h; Figure 3).

Similarly, the osmolality of the urine from the postobstructed kidney was lower than that from the contralateral kidney during the 5 d of follow-up, indicating impaired urinary concentrating ability (Figure 4). This difference remained significant until day 4 of follow up (157 ± 29 *versus* 256 ± 64 mOsm/kg). On day 5, the difference no longer achieved statistical significance (136 ± 24 *versus* 235 ± 65 mosm/kg; Figure 4).

Urinary sodium (normalized for creatinine) from the postobstructed kidney was significantly higher than from the contralateral kidney on day 1 and day 4 (18.98 ± 3.2 *versus* 10.7 ± 1.8 and 21.78 ± 2.9 *versus* 17.2 ± 2.2, respectively). On day 5, the difference diminished and was no longer statistically significant (21.95 ± 4.9 *versus* 14.9 ± 2.3l; Figure 5). Moreover, sodium excretion from both kidneys increased significantly from day 1 to day 2 (Figure 5). Urine creatinine was found to be significantly lower in the postobstructed kidney throughout the 5 d after surgery (Figure 6). CrCl from the postobstructed kidney was not significantly different compared with the contralateral kidney throughout the first 4 d after surgery, becoming significantly lower than from the contralateral kidney on day 5 (4.83 ± 0.65 *versus* 7.06 ± 1.06 ml/min; values not normalized for the body surface; Table 2).

Table 1. Clinical details of patients[a]

Male/female	10/2
Age of fetal diagnosis (wk)	31.5 ± 1.4
Age at pyeloplasty (mo)	8 ± 2
Weight at pyeloplasty (g)	7214 ± 614
AP ∅ at fetal ultrasound (mm)	15.4 ± 2.0
AP ∅ at prepyeloplasty (mm)	29.8 ± 1.8
Renal function at renography MAG3 (%)	46 ± 1.8
Serum creatinine (μmol/L)	41.4 ± 1.8

[a] AP ∅, anteroposterior diameter of renal pelvis; MAG3, mercaptoacetyltriglycine.

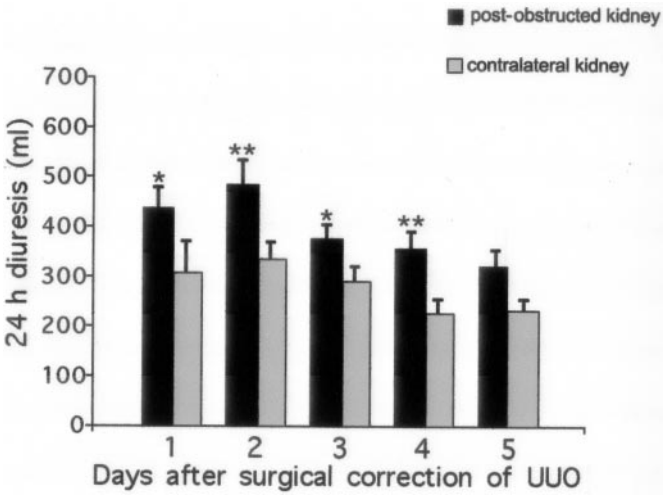

Figure 3. Urine output. Urine was collected at 24-h intervals throughout the 5 d after surgical correction of unilateral PUJ obstruction. Urine output from the postobstructed kidney was significantly higher throughout the first 4 d after relief from obstruction. The difference between the two kidneys was NS on day 5. All values are expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$, $n = 12$, t test for paired data.

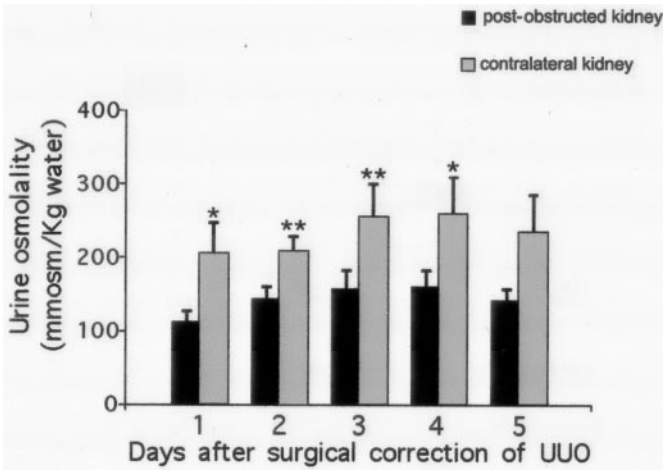

Figure 4. Urine osmolality. Comparison of urine osmolality in the postobstructed kidney and the contralateral kidney for 5 d after surgical correction of unilateral PUJ obstruction. On day 1, the osmolality of the urine from the postobstructed kidney was significantly lower than from the contralateral kidney; the significant difference persisted for 4 d. All values are expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$, $n = 12$, t test for paired data.

AQP2 Excretion in Postobstructed Kidney and Relationship to Increases in PGE2 Synthesis

Figure 7 reports the evaluation of AQP2 excretion from the two kidneys. AQP2 excretion from postobstructed kidneys on day 1 from surgery was significantly lower (~54%) than the value measured in the urine from the contralateral kidney.

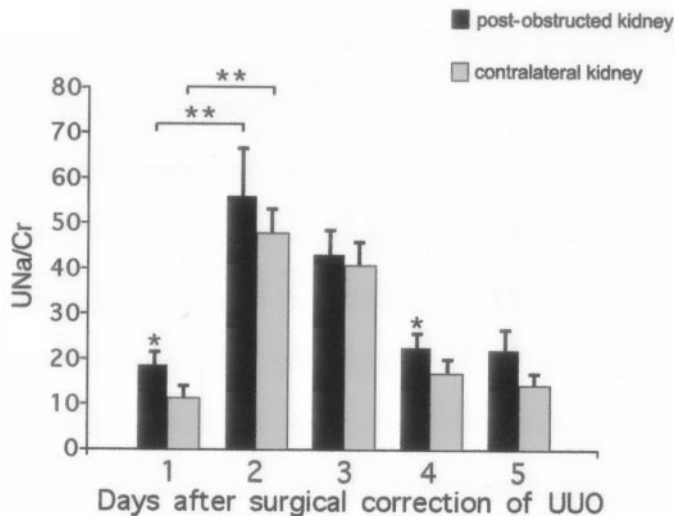

Figure 5. Sodium excretion. Urine sodium concentration in the postobstructed kidney and the contralateral kidney was measured for 5 d after surgical correction of unilateral PUJ obstruction. Sodium excretion from the postobstructed kidney was significantly higher than from the contralateral kidney from days 1 to 4. All values are expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$, $n = 12$, *t* test for paired data.

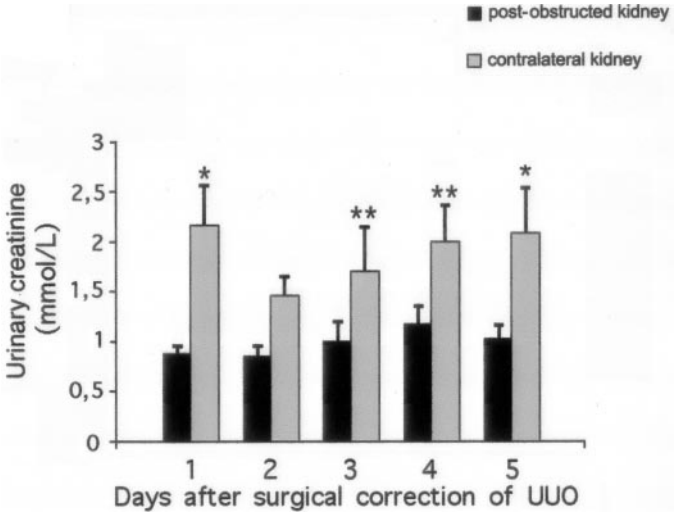

Figure 6. Urinary creatinine. Urine creatinine was significantly lower in the postobstructed kidney throughout the 5 d. All values are expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$, $n = 12$, *t* test for paired data.

Reduced AQP2 excretion persisted unaltered for 4 d after release. However, on day 5, the difference in AQP2 excretion between the two kidneys diminished by ~22% and became NS. The free water clearance was higher in the postobstructed kidney throughout the 5 d after surgery (in ml/min: day 1, 0.183 ± 0.032 *versus* 0.110 ± 0.049, $P = 0.05$; day 2, 0.168 ± 0.034 *versus* 0.062 ± 0.022, $P = 0.001$; day 3, 0.131 ± 0.024 *versus* 0.060 ± 0.025, $P = 0.004$; day 4, 0.104 ± 0.022 *versus* 0.024 ± 0.035, $P = 0.005$; day 5, 0.115 ± 0.022 *versus* 0.040 ± 0.030, $P = 0.02$ in the postobstructed kidney *versus* the contralateral kidney, respectively). These findings are consistent with an impairment of collecting duct water reabsorption and suggest that there is an inverse correlation between AQP2 excretion and free water clearance, which is consistent with a reciprocal functional association between the two parameters.

Urinary PGE2 excretion was also determined in the urine samples from both kidneys. On day 1 after surgery, PGE2 excretion (normalized for creatinine) from the postobstructed kidney was nearly twice as high as that from the contralateral kidney and remained so throughout the study (26.0 ± 6.7 *versus* 13.5 ± 2.5, $n = 13$, $P < 0.05$; Figure 7). On day 5, the difference between the two kidneys was not statistically significant, mainly because of the high sample variability (31.3 ± 15.9 *versus* 14.88 ± 2.6; Figure 8).

Discussion

Congenital obstruction of the urinary tract is a common condition with an incidence amounting to 3 to 4 of every 1000 deliveries (1). The condition is characterized by long-term impairment of the kidney's ability to concentrate urine. Unilateral obstruction is associated with disruption of tubule function, resulting in impaired concentrating capability of the obstructed kidney associated with the risk of progressive ischemia (3,11,23).

In animal models, ureteral obstruction is associated with downregulation of renal AQPs (10–13,24) as well as of major renal Na transporters (25). Although a decreased expression of AQPs and their role in determining the concentrating inability of the obstructed kidney and the postobstructive polyuria are well demonstrated in animal models, no direct evidence of AQP downregulation in human unilateral obstruction has been reported so far.

In this study, we demonstrated that in patients who underwent pyeloplasty because of unilateral PUJ obstruction, AQP2 excretion from postobstructed kidneys 24 h after surgery was significantly lower (~54%) than from the contralateral kidney. Reduced AQP2 excretion persisted for 4 d after release. On day 5, the difference in AQP2 excretion between the two kidneys diminished (~22%) and was no longer statistically significant.

Urinary excretion of AQP2 is a good marker for the diagnosis of water metabolism disorders related to alteration of AQP2 trafficking/expression in humans (21,22,26–29). In this study, AQP2 excretion was evaluated in comparison with its internal control (nonobstructed contralateral kidney), thus overcoming the high interindividual variability of the parameter. The urinary output measurements revealed a significant increase in postobstructed kidney diuresis during the first 24 h, which persisted until the end of the follow-up. This observation in humans is in agreement with the data reported in animal models, showing a higher urinary output from the postobstructed kidney (24). We also found that the osmolality of the urine from the postobstructed kidney was significantly lower than from the contralateral kidney. Moreover, urinary sodium normalized for creatinine from the postobstructed kidney was significantly higher than in the contralateral kidney on day 1

Table 2. Changes in creatinine clearance (ml/min) in patients after the relief of obstruction[a]

	Days after Surgical Correction of UUO				
	1	2	3	4	5
Postobstructed kidney	6.30 ± 0.81	7.64 ± 0.80	6.25 ± 0.90	5.88 ± 0.92	4.82 ± 0.65
Contralateral kidney	6.99 ± 1.05	6.93 ± 0.80	6.52 ± 0.81	5.62 ± 0.90	7.05 ± 1.06[b]

[a] UUO, unilateral ureteral obstruction. Values are mean ± SEM.
[b] $P < 0.05$ *versus* postobstructed kidney, *t* test for paired data.

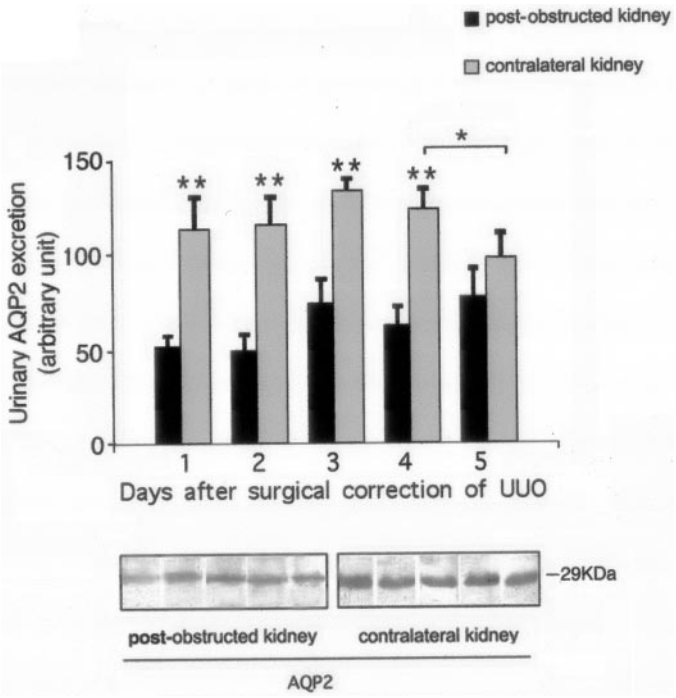

Figure 7. AQP2 excretion. AQP2 excretion was semiquantified by immunoblotting in creatinine equivalent of urine samples collected for 5 d after surgical correction of unilateral PUJ obstruction. Urinary AQP2 excretion was significantly lower from the postobstructed kidney than from the contralateral kidney for the first 4 d of follow-up. On day 5, the difference in AQP2 excretion between the two kidneys diminished. All values are expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$, $n = 12$, *t* test for paired data.

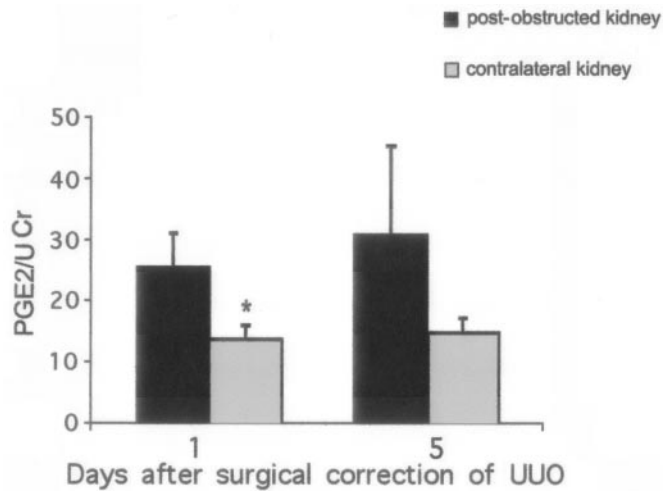

Figure 8. Prostaglandin E2 (PGE2) excretion. On day 1 after surgery, PGE2 excretion in the postobstructed kidney was nearly twice as high as from the contralateral kidney. The values were normalized for urinary creatinine. All values are expressed as means ± SEM. *$P < 0.05$, $n = 12$, *t* test for paired data.

and day 4. Taken together, these findings suggest that the capacity of the postobstructed kidney to concentrate urine is reduced. Urine creatinine was found to be significantly lower in the postobstructed kidney throughout the 5 d. In contrast, CrCl from the postobstructed kidney was not significantly different from that from the contralateral kidney throughout the first 4 d after surgery, whereas on day 5, CrCl from the postobstructed kidney had become significantly lower than from the contralateral kidney.

This may reflect a physiologic adaptation of the obstructed kidney, which persisted after relief from the obstruction, as a result of local as opposed to systemic signals, which may be responsible for maintaining renal function in the previously obstructed kidney. Several local factors may contribute to the regulation of renal function in obstructed kidneys. For instance, it was shown recently that endothelin, a potent vasoconstricting peptide, may play a crucial role in some types of renal disease, such as UUO (30).

In the present study, we suggest that PGE2 may represent one of these local signals. In fact, in the patients examined in this study, we found that PGE2 normalized to creatinine measured in urine from the postobstructed kidney was approximately twofold higher than in samples from the contralateral kidney 24 h after the release of the obstruction and remained higher on day 5, although the differences became not statistically significant.

In a previous study, we showed that in patients who were affected by unilateral PUJ obstruction, in the immediate postoperative period, urinary excretion of PGE2 from the previously obstructed kidney (but not from the normal contralateral kidney) was higher than from the contralateral normal renal unit (15). The pharmacologic inhibition of PGE2 synthesis using aspirin led to a significant reduction in glomerular function (15).

PGE2 has been predicted to play an important role in the development of polyuria in many acquired forms of NDI (31). It is interestingly that, using primary rat inner medullary col-

lecting duct cells, we have recently shown that the signaling pathway underlying the diuretic effects of PGE2 includes Rho activation and subsequent formation of F-actin stress fibers. In turn, stabilization of the actin network hinders AQP2-bearing vesicle fusion to the apical membrane under vasopressin activation (17).

Besides AQP2, previous studies of UUO in rats have highlighted the importance of AQP3 and AQP4 located in the principal cells as well as of AQP1 located in the proximal tubule for the formation of concentrated urine, and the expression of all of these AQPs was found to be reduced in rats with UUO (11,24). Because of the impossibility of gaining access to the kidney in humans, we have tested urinary excretion only of AQP2.

The observed AQP2 downregulation may partially explain the postobstructive polyuria and defective urinary concentrating ability in children with congenital urinary tract obstruction. However, it has to be pointed out that a reduced AQP2 excretion has been observed in several water-losing disorders. At present, it is unclear whether this is involved in the pathogenesis of the disorder or is a concomitant event.

On the basis of these findings, we can speculate that locally increased PGE2 synthesis in the postobstructed kidney may be involved in impairment of AQP2 trafficking, which in turn could have a diuretic effect associated with a reduction in AQP2 excretion. This may contribute to postobstructive polyuria and defective urinary concentrating ability in children with congenital urinary tract obstruction. Conversely, an increase in PGE2 synthesis made the GFR (CrCl) similar to that of the contralateral kidney. Our data also suggest that some changes occur starting from day 5 after the relief of obstruction, when the GFR of the postobstructed kidney became significantly lower, whereas the differences in urinary output, osmolality, sodium excretion, AQP2 excretion, and PGE2 excretion between the two kidneys became not statistically different. This may support the conclusion that in patients who are affected by congenital unilateral PUJ obstruction, the obstructed kidney mimics normal renal function as a result of local increases in PGE2, which, conversely, may be involved in determining the postobstructive polyuria possibly through downregulation of AQP2. Finally, from a clinical point of view, the polyuria and the change in the urinary excretion of AQP2 and PGE2 recorded suggest that the obstruction was really present and then resolved after pyeloplasty.

Acknowledgments

This work was supported by funds from the Italian Ministero della Ricerca Scientifica e Tecnologica, from the Laboratorio Analisi del Gene, and from a grant of Regione Veneto.

We thank Anthony Green for providing linguistic advice.

References

1. Ardissino G, Dacco V, Testa S, Bonaudo R, Claris-Appiani A, Taioli E, Marra G, Edefonti A, Sereni F: Epidemiology of chronic renal failure in children: Data from the ItalKid project. *Pediatrics* 111: e382–e387, 2003
2. Curhan GC, Zeidel ML: Urinary tract obstruction. In: *The Kidney*, edited by Brenner B, Rector FC, Philadelphia, W.B. Saunders, 1996, pp 1936–1958
3. Klahr S, Morrissey J: Obstructive nephropathy and renal fibrosis. *Am J Physiol Renal Physiol* 283: F861–F875, 2002
4. Yarger WE, Aynedjian HS, Bank N: A micropuncture study of postobstructive diuresis in the rat. *J Clin Invest* 51: 625–637, 1972
5. Knepper MA, Inoue T: Regulation of aquaporin-2 water channel trafficking by vasopressin. *Curr Opin Cell Biol* 9: 560–564, 1997
6. Brown D: The ins and outs of aquaporin-2 trafficking. *Am J Physiol Renal Physiol* 284: F893–F901, 2003
7. Marples D, Christensen S, Christensen EI, Ottosen PD, Nielsen S: Lithium-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla. *J Clin Invest* 95: 1838–1845, 1995
8. Marples D, Frokiaer J, Dorup J, Knepper MA, Nielsen S: Hypokalemia-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla and cortex. *J Clin Invest* 97: 1960–1968, 1996
9. Bichet DG: Nephrogenic diabetes insipidus. *Am J Med* 105: 431–442, 1998
10. Frokiaer J, Marples D, Knepper MA, Nielsen S: Bilateral ureteral obstruction downregulates expression of vasopressin- sensitive AQP-2 water channel in rat kidney. *Am J Physiol* 270: F657–F668, 1996
11. Li C, Wang W, Kwon TH, Isikay L, Wen JG, Marples D, Djurhuus JC, Stockwell A, Knepper MA, Nielsen S, Frokiaer J: Downregulation of AQP1, -2, and -3 after ureteral obstruction is associated with a long-term urine-concentrating defect. *Am J Physiol Renal Physiol* 281: F163–F171, 2001
12. Kim SW, Cho SH, Oh BS, Yeum CH, Choi KC, Ahn KY, Lee J: Diminished renal expression of aquaporin water channels in rats with experimental bilateral ureteral obstruction. *J Am Soc Nephrol* 12: 2019–2028, 2001
13. Frokiaer J, Christensen BM, Marples D, Djurhuus JC, Jensen UB, Knepper MA, Nielsen S: Downregulation of aquaporin-2 parallels changes in renal water excretion in unilateral ureteral obstruction. *Am J Physiol* 273: F213–F223, 1997
14. Yarger WE, Schocken DD, Harris RH: Obstructive nephropathy in the rat: Possible roles for the renin-angiotensin system, prostaglandins, and thromboxanes in postobstructive renal function. *J Clin Invest* 65: 400–412, 1980
15. Montini G, Sacchetto E, Murer L, Dall'Amico R, Masiero M, Passerini-Glazel G, Zacchello G: Renal glomerular response to the inhibition of prostaglandin E2 synthesis and protein loading after the relief of unilateral ureteropelvic junction obstruction. *J Urol* 163: 556–560, 2000
16. Zelenina M, Christensen BM, Palmer J, Nairn AC, Nielsen S, Aperia A: Prostaglandin E(2) interaction with AVP: Effects on AQP2 phosphorylation and distribution. *Am J Physiol Renal Physiol* 278: F388–F394, 2000
17. Tamma G, Wiesner B, Furkert J, Hahm D, Oksche A, Schaefer M, Valenti G, Rosenthal W, Klussmann E: The prostaglandin E2 analogue sulprostone antagonizes vasopressin-induced antidiuresis through activation of Rho. *J Cell Sci* 116: 3285–3294, 2003
18. Pope JCT, Brock JW 3rd, Adams MC, Stephens FD, Ichikawa I: How they begin and how they end: Classic and new theories for the development and deterioration of congenital anomalies of the kidney and urinary tract, CAKUT. *J Am Soc Nephrol* 10: 2018–2028, 1999

19. Zucchetta P, Carasi C, Marzola MC, Murer L, Passerini-Glazel G, Rigamonti W, Zacchello G, Bui F, Montini G: Angiotensin converting enzyme inhibition worsens the excretory phase of diuretic renography for obstructive hydronephrosis. *J Urol* 165: 2296–2299, 2001
20. Gordon I, Colarinha P, Fettich J, Fischer S, Frokier J, Hahn K, Kabasakal L, Mitjavila M, Olivier P, Piepsz A, Porn U, Sixt R, van Velzen J; Paediatric Committee of the European Association of Nuclear Medicine: Guidelines for standard and diuretic renography in children. *Eur J Nucl Med* 28: BP21–BP30, 2001
21. Valenti G, Laera A, Pace G, Aceto G, Lospalluti ML, Penza R, Selvaggi FP, Chiozza ML, Svelto M: Urinary aquaporin 2 and calciuria correlate with the severity of enuresis in children. *J Am Soc Nephrol* 11: 1873–1881, 2000
22. Valenti G, Laera A, Gouraud S, Pace G, Aceto G, Penza R, Selvaggi FP, Svelto M: Low-calcium diet in hypercalciuric enuretic children restores AQP2 excretion and improves clinical symptoms. *Am J Physiol Renal Physiol* 283: F895–F903, 2002
23. Moody TE, Vaughn ED Jr, Gillenwater JY: Relationship between renal blood flow and ureteral pressure during 18 hours of total unilateral ureteral occlusion. Implications for changing sites of increased renal resistance. *Invest Urol* 13: 246–251, 1975
24. Li C, Wang W, Knepper MA, Nielsen S, Frokiaer J: Downregulation of renal aquaporins in response to unilateral ureteral obstruction. *Am J Physiol Renal Physiol* 284: F1066–F1079, 2003
25. Li C, Wang W, Kwon TH, Knepper MA, Nielsen S, Frokiaer J: Altered expression of major renal Na transporters in rats with unilateral ureteral obstruction. *Am J Physiol Renal Physiol* 284: F155–F166, 2003
26. Saito T, Ishikawa SE, Sasaki S, Nakamura T, Rokkaku K, Kawakami A, Honda K, Marumo F: Urinary excretion of aquaporin-2 in the diagnosis of central diabetes insipidus. *J Clin Endocrinol Metab* 82: 1823–1827, 1997
27. Nakamura T, Saito T, Kusaka I, Higashiyama M, Nagasaka S, Ishibashi S, Ishikawa SE: Decrease in urinary excretion of aquaporin-2 associated with impaired urinary concentrating ability in diabetic nephropathy. *Nephron* 92: 445–448, 2002
28. Ivarsen P, Frokiaer J, Aagaard NK, Hansen EF, Bendtsen F, Nielsen S, Vilstrup H: Increased urinary excretion of aquaporin 2 in patients with liver cirrhosis. *Gut* 52: 1194–1199, 2003
29. Pedersen RS, Bentzen H, Bech JN, Nyvad O, Pedersen EB: Urinary aquaporin-2 in healthy humans and patients with liver cirrhosis and chronic heart failure during baseline conditions and after acute water load. *Kidney Int* 63: 1417–1425, 2003
30. Moridaira K, Morrissey J, Fitzgerald M, Guo G, McCracken R, Tolley T, Klahr S: ACE inhibition increases expression of the ETB receptor in kidneys of mice with unilateral obstruction. *Am J Physiol Renal Physiol* 284: F209–F217, 2003
31. Howard RL, Bichet DG Schier: Hypernatremia and polyuric states. In: *The Kidney: Physiology and Pathophysiology*, edited by Seldin DW, Giebisch G, New York, Raven, 1992, pp 1753–1778