Polyuria-associated hydronephrosis induced by xenobiotic chemical exposure in mice

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Polyuria-associated hydronephrosis induced by xenobiotic chemical exposure in mice. Am J Physiol Renal Physiol 311: F752–F762, 2016. First published July 20, 2016; doi:10.1152/ajprenal.00001.2016.—Hydronephrosis is a commonly found disease state characterized by the dilation of renal calices and pelvis, resulting in the loss of kidney function in the severest cases. A generally accepted etiology of hydronephrosis involves the obstruction of urine flow along the urinary tract. In the recent years, we have developed a mouse model of hydronephrosis induced by lactational exposure to dioxin and demonstrated a lack of anatomical obstruction in this model. We also showed that prostaglandin E2 synthesis system plays a critical role in the onset of hydronephrosis. In the present study, we found that neonatal hydronephrosis was not likely to be associated with functional obstruction (impaired peristalsis) but was found to be associated with polyuria and low urine osmolality with the downregulation of proteins involved in the urine concentrating process. The administration of an antiuretic hormone analog to the dioxin-exposed pups not only suppressed the increased urine output but also decreased the incidence and severity of hydronephrosis. In contrast to the case in pups, administration of dioxin to adult mice failed to induce polyuria and upregulation of prostaglandin E2 synthesis system, and the adult mice were resistant to develop hydronephrosis. These findings suggest the possibility that polyuria could induce hydronephrosis in the absence of anatomical or functional obstruction of the ureter. It is concluded that the present animal model provides a unique example of polyuria-associated type of hydronephrosis, suggesting a need to redefine this disease state.

HYDRONEPHROSIS IS DEFINED as the dilation of the renal pelvis and calices proximal to the point of obstruction (11). It is found in 1.5–3.3% of all autopsies (11) and in 1–5% of pregnancies on antenatal ultrasonography (22, 50). In the most severe cases of hydronephrosis, kidney function is lost because of destruction of the renal parenchyma. The etiology of hydronephrosis is considered to be obstruction of urine flow at any point along the urinary tract, leading to urine retention and increased backpressure on the renal pelvis and calices (11). The following two types of obstruction are known: anatomical obstruction that occludes the ureter and functional obstruction that impairs urine transport by ureteral peristalsis.

Recent studies of urinary tract development have revealed several genes associated with impaired ureteral peristalsis that leads to hydronephrosis. Discs-large homolog 1 (Dlgh1) is important for the coordinated development of the ureter; genetic ablation of this gene was shown to cause loss of the mesenchymal layer between the smooth muscle and epithelial layers, impair ureteral peristalsis, and cause hydronephrosis (26). Genetic ablation of the angiotensin type 1 receptor (30) or angiotensinogen (27) is known to retard the development of renal pelvis, from which ureteral peristalsis is initiated, and to cause impaired ureteral peristalsis to develop hydronephrosis. Exposure to chemicals, such as mitomycin (31), adriamycin (14), lithium chloride (54), and dioxins (56), also causes hydronephrosis. Dioxin-induced hydronephrosis in mouse pups is pathologically distinct and is characterized by the absence of anatomical obstruction of the ureter (32, 35) and the presence of a critical window during the initial few days after birth (7).

Of the dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most potent congener and exhibits a variety of toxic effects (40) including hydronephrosis during gestation or via lactation (6, 32, 36). The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, is essential for the manifestation of TCDD poisoning as shown in AhR-null mouse studies (15, 29, 35, 43). Endogenous factors downstream of AhR are believed to modulate a variety of toxic effects, but the mechanism of pathogenesis is not clear (56). Among these, genes encoding enzymes for the production of prostaglandins are responsible for the onset of TCDD-induced neonatal hydronephrosis in mice (35, 53, 55). AhR-dependent upregulation of cyclooxygenase-2 (COX-2) (10, 35) was shown to play a critical role in the onset of this disease, and the role this was confirmed by the abrogation of TCDD-induced hydronephrosis by treatment with a COX-2-selective inhibitor (35). COX-2 is an inducible form of cyclooxygenase that converts arachidonic acid to prostaglandin H2 (PGH2). Microsomal prostaglandin E synthase-1 (mPGES-1) transforms PGH2 to prostaglandin E2 (PGE2) and is also required for TCDD-induced hydronephrosis, as was demonstrated by the lack of hydronephrosis in mPGES-1-null mice (53). These studies indicate that PGE2 production, mediated by COX-2 and mPGES-1, plays a critical role in the onset of TCDD-induced hydronephrosis. Despite these findings, the underlying mechanism by which TCDD

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induces hydronephrosis in the absence of anatomical obstruction is not understood.

The aim of the present study was to determine whether TCDD-induced neonatal hydronephrosis is caused by functional obstruction associated with morphological changes in the ureter, papilla, and/or pelvis and to investigate concomitant functional obstruction associated with morphological changes in TCDD-induced neonatal hydronephrosis is caused by functional obstruction associated with morphological changes in TCDD-induced neonatal hydronephrosis is caused by functional obstruction associated with morphological changes in

METHODS

Animals and treatment. The study protocols were approved by the Animal Care and Use Committee of the University of Tokyo. TCDD (purity, >99.1%) purchased from AccuStandard (New Haven, CT) was diluted in corn oil containing 2% n-nonane (Wako Pure Chemicals, Osaka, Japan) as a vehicle. C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan). Mice were housed at 23 ± 1°C and 50 ± 10% humidity on a 12:12-h light-dark cycle. Laboratory rodent chow (Labo MR Stock; Nosan, Yokohama, Japan) and distilled water were provided ad libitum. Parturition was checked twice daily, and the day of birth was designated postnatal day 0 (PND 0). Dams were orally administered either a single dose of TCDD (10, 20, or 80 μg/kg; 20 ml/kg body wt) or an equivalent volume of the vehicle or an equivalent volume of the vehicle. C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan). Mice were housed at 23 ± 1°C and 50 ± 10% humidity on a 12:12-h light-dark cycle. Laboratory rodent chow (Labo MR Stock; Nosan, Yokohama, Japan) and distilled water were provided ad libitum. Parturition was checked twice daily, and the day of birth was designated postnatal day 0 (PND 0). Dams were orally administered either a single dose of TCDD (10, 20, or 80 μg/kg; 20 ml/kg body wt) or an equivalent volume of the vehicle. C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan). Mice were housed at 23 ± 1°C and 50 ± 10% humidity on a 12:12-h light-dark cycle. Laboratory rodent chow (Labo MR Stock; Nosan, Yokohama, Japan) and distilled water were provided ad libitum. Parturition was checked twice daily, and the day of birth was designated postnatal day 0 (PND 0). Dams were orally administered either a single dose of TCDD (10, 20, or 80 μg/kg; 20 ml/kg body wt) or an equivalent volume of the vehicle.

For experiments using adult mice, 12-wk-old C57BL/6J male mice were orally administered either a single dose of TCDD (20 or 80 μg/kg; 20 ml/kg body wt) or an equivalent volume of the vehicle.

Kidney histology. Kidney specimens were fixed in 10% neutralbuffered formalin, cryoprotected in 20% sucrose solution, embedded in OCT compound (Sakura Finetek Japan, Tokyo, Japan), and snap-frozen in liquid nitrogen. Tissues sections (5 μm) were stained with hematoxylin and eosin (HE). Hydronephrosis severity scores were assigned using a previously described scoring system: “0 = no hydronephrosis,” “+1 = slight dilation of the renal pelvis,” “+2 = reduced papilla size and noticeable dilation of the pelvic space,” “+3 = very short papilla and compressed renal tissue,” and “+4 = the most severe hydronephrotic kidney” (4). The representative images for each score are shown in Fig. 1. Two researchers (T. Kawaguchi and N. Fujisawa) who were blinded to the TCDD dose and ID were assigned to judge the scores independently. The incidence of hydronephrosis in the pups was calculated as the percentage of pups with a severity score ≥2, in accordance with previous reports (4, 47).

Ureter histology. Immunostaining was performed on 4-μm thick, paraffin-embedded urinary tract tissue sections. The primary antibodies used were rabbit anti-mouse galectin-3 (1/100, Santa Cruz Bio-technology (sc-23938)) for staining the epithelium and Cy3-labeled monoclonal anti-smooth muscle actin (SMA) (1/200, Sigma (A 1978)) for staining the smooth muscle layer. Biotin-conjugated anti-rabbit IgG (1/200, Vector Laboratories (BA-1000)) was used as the secondary antibody. Sections were activated in 10 mM citrate using a microwave oven and the endogenous peroxidase activity quenched with 0.3% H2O2 in PBS (pH 7.4). Samples were then incubated with anti-galectin-3 for 1 h and then with anti-IgG for 1 h, followed by incubation in a solution containing anti-SMA, 4',6-diamino-2-phe-

Fig. 1. Representative images of hydronephrotic kidney of male mouse pups at postnatal day (PND) 1. A: normal kidney: severity score 0. B: small gap between the papilla and the renal wall: severity score 1. C: noticeable dilation of the pelvic space: severity score 2. D: short papilla and a larger gap between the papilla and the renal wall: severity score 3. E: virtually no papilla and a thin renal wall: severity score 4. The kidneys for severity scores 2–4 were derived from pups exposed to 20 μg/kg TCDD. Scale bars = 1 mm.
Osmolality was measured using the freezing point depression method. Similar among groups (16.2 ± 0.01 for control, 16.4 ± 0.01 for 20 μg/kg, and 16.0 ± 0.02 for 80 μg/kg groups).

Western blotting. Kidney specimens were homogenized in a buffer containing 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, and 2% sodium dodecyl sulfate (SDS) and were applied to 10% SDS polyacrylamide gels. Proteins separated by electrophoresis were transferred to polyvinylidene difluoride membranes. As primary antibodies, we used rabbit anti-mouse AQP2 (cat. no. 178612; Invitrogen), NKCC2 (cat. no. ARF14388_P050; Aviva Systems Biology), ROMK1 (cat. no. APC-001; Alomone Labs, Jerusalem, Israel), and β-actin (cat. no. 31430, Thermo Scientific) antibodies. The peroxidase-labeled secondary antibody against rabbit IgG (cat. no. SA5-10033; Pierce Biotechnology, Yokohama, Japan) was detected by chemiluminescence reaction.

The abundance of target proteins was quantified through band intensity analysis and normalized to that for β-actin.

Urine analyses. As a first trial, we kept pups with or without dams in a metabolic cage to collect urine, which turned out to be unsuccessful because the pups either did not survive or the dams did not nurse their pups. For neonatal mice, urine was collected from the bladder using a 29-gauge syringe. Any instance of incontinence before the urine collection led to the exclusion of the pup from the urine analyses. Urinary PGE2 concentrations were measured using a prostaglandin E2 EIA kit (Cayman Chemical, Ann Arbor, MI). Urine analyses.

TCDD-induced morphological changes in the urinary tract. We addressed a question whether lactational exposure to TCDD impaired the peristalsis of ureter in neonatal mice. In previous studies, loss of mesenchymal layer of ureter in Dlgh1-null mice was documented to cause impairment of ureteral peristalsis and hydronephrosis (26), and HE-stained ureter was suggested to have possible abnormalities in the mesenchymal layer of TCDD-exposed ureters (35). Then, we histologically examined the ureter of mouse pups nursed by dams administered an oral TCDD dose of 10 μg/kg. Under this experimental condition, the majority of pups lactationally exposed to TCDD developed hydronephrosis by PND 7, which was consistent with previous observations (35, 53). The proximal portion of the ureter was the main target of investigation, as dilation of the ureter and renal pelvis occurs at the ureter proximal to the point of obstruction (11). The layer structure of the ureter was analyzed by staining the smooth muscle and epithelial layers for α-smooth muscle actin (α-SMA) and galectin 3, respectively. Three-layer ureteral structure was preserved in TCDD-exposed pups on PND 3 (Fig. 2, A and D), PND 5 (Fig. 2, B and E), and PND 7 (Fig. 2, C and F). The cross-sectional area of the epithelial layer of TCDD-exposed mice tended to be slightly larger than that of the control group on PNDs 5 and 7, although there was no statistically significant difference (Table 2). The mesenchymal layer (estimated as the cross-sectional area between the smooth muscle and epithelial layers) tended to be slightly smaller in the TCDD-exposed group than in the control group on PND 3, although the difference was not statistically significant. The cross-sectional area of the smooth muscle layer did not differ between these two groups. In addition, no significant difference of α-SMA-positive area in the cross-section was found by image analyses, despite the appearance of a difference in Fig. 2, in the TCDD-exposed group compared with those in the control group.

No hydroureter was observed by gross anatomy or by the analysis of ureter (Fig. 2) in the TCDD-exposed pups. These hydronephrosis by chemical exposure.

Table 1. Primer sequence

<table>
<thead>
<tr>
<th>Target Name</th>
<th>Primer Sequences (5’ to 3’)</th>
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<tbody>
<tr>
<td>AQP2</td>
<td>GCT GTC AAT GCT CTC CAC AA</td>
</tr>
<tr>
<td></td>
<td>AGG CAA AGA TGC ACA GCA C</td>
</tr>
<tr>
<td>COX-2</td>
<td>TGT GAA CAA TCA AAC ATG CTG</td>
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<tr>
<td></td>
<td>GCG TAA ATT CCA AGC TAA GT</td>
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<tr>
<td>Cyclophilin B</td>
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</tr>
<tr>
<td></td>
<td>TGT GAG CCA TGG TGT TGG</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>GGC ACC TCT CTT CAC CCT A</td>
</tr>
<tr>
<td></td>
<td>GAA TCT CTC CTT CTT TGG</td>
</tr>
<tr>
<td>mPGES-1</td>
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<td></td>
<td>GGC CTC AGA AGA GAG ACC AT</td>
</tr>
<tr>
<td>NKCC2</td>
<td>GCT TTA GAC AGG CTC TGG A</td>
</tr>
<tr>
<td>ROMK</td>
<td>GCC TGG TTA AGA CAA TGC AG</td>
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AQP2, aquaporin 2; COX-2, cyclooxygenase-2; mPGES-1, microsomal prostaglandin E synthase-1; NKCC2, NaK2Cl cotransporter; ROMK, renal outer medullary potassium channel.
observation was consistent with our previous study (35) and is in contrast to the reported observations in Dlgh-1 null mice (18, 26).

We then analyzed whether TCDD exposure induces morphological changes in the kidneys of neonatal mice, since Agtr1-null and agt-null mice were reported to have an abnormally small pelvis that is considered to hamper peristalsis (27, 30). The degree of pelvic development was determined as the relative papilla length normalized to the minor axis length of the kidney or the kidney width. The relative papilla length in TCDD-exposed pups tended to be smaller than that in the control pups on PNDs 3 and 5, with a significant difference on PND 7 (Table 2).

To examine whether the morphological changes observed in the present study were associated with functional impairment of ureteral peristalsis, we measured the frequency of peristaltic movement. We did not find any change in gross appearance (Supplementary Movie files: Control-pelvis.avi for a control pup; Supplementary Material for this article is available online at the Journal website) or significant differences in the frequency between the TCDD-exposed and control mice on PNDs 3, 5, and 7 (Table 2). These pups were too small to be analyzed for the contractility (magnitude and its rate) by determining intraureteral pressure.

**TCDD increases urine output in mouse pups.** Since we observed that TCDD-exposed pups often had dilated bladders full of urine, we hypothesized that lactational exposure to TCDD increases urine production in mouse pups. We collected urine directly from the bladder and determined its weight as a measure of urine output. The day of urine collection was set at PND 14 because younger pups were prone to incontinence. The weight of bladder urine collected at 1500 during the light period was significantly larger in the TCDD-exposed pups than that in the control pups (Fig. 3B), although dose dependency was less obvious than that observed during the light period. These results demonstrate that TCDD induces excessive urine output in neonatal mice.

To address whether the TCDD-dependent increase in urine output is a cause of TCDD-induced neonatal hydronephrosis, the overproduction of urine in the TCDD-exposed pups was suppressed by daily administration of DDAVP, an antidiuretic agent, from PND 1 to PND 14. A suppressive effect of DDAVP on urine overproduction in TCDD-exposed pups was conspicuous at 6 h after the last administration of DDAVP; urine output of DDAVP-treated pups exposed to TCDD was significantly smaller than that of DDAVP-untreated pups exposed to TCDD and was not different from that of pups not exposed to TCDD (Fig. 3C). The suppressive effect of DDAVP in TCDD-exposed pups was less clear at 23 h post-DDAVP administration than at 6 h; urine output of DDAVP-treated pups exposed to TCDD was not significantly changed compared with that of DDAVP-untreated pups exposed to TCDD or that of pups not exposed to TCDD (Fig. 3D). These results are consistent with this drug’s elimination half-life of several hours (1). Histological analysis revealed that administration of DDAVP significantly decreased the TCDD-induced hydronephrosis (Table 3). Analysis of the relationship between the severity of hydronephrosis and urine output revealed a significant positive correlation (Fig. 3E) \( r = 0.61; P = 0.00019 \).

**Mechanism of urine dilution by TCDD in mouse pups.** The osmolarity of urine was significantly lower in the TCDD-exposed mouse pups than in the control pups (Fig. 4A). Since channels and transporters in the kidney, such as aquaporin 2 (AQP2), NaK2Cl cotransporter (NKCC2), and renal outer medullary potassium channel (ROMK), are known to be associated with urine concentration mechanisms (25, 41, 46), we...
assessed the expression of these molecules by quantitative RT-PCR and Western blotting. The expression of AQP2, NKCC2, and ROMK mRNAs (Fig. 4, C–E) and proteins (Fig. 4, F–H) were significantly decreased by TCDD.

Urinary concentration is regulated by the antidiuretic hormone arginine vasopressin (AVP), which is secreted from the posterior pituitary gland into the blood. In the kidney, AVP binds to V2 receptors on the principal cells of the collecting duct to promote AQP2-dependent water reabsorption. In this urine concentrating system, insufficient AVP secretion results in overproduction of dilute urine (48). Therefore, we analyzed whether the TCDD-induced defect in the urine concentrating system is related to insufficient AVP secretion. The urine AVP level was not altered by TCDD administration at a dose of 20 μg/kg but tended to increase at a dose of 80 μg TCDD/kg (Fig. 4B), which indicates that AVP secretion was not suppressed by TCDD. This result suggests that TCDD disrupts the urine concentrating system at the site located in the kidney but not in the brain.

**TCDD-induced upregulation of PGE2 production.** Urine PGE2 levels increased 8.6- and 9.6-fold from that in controls after TCDD exposure at 20 and 80 μg/kg, respectively (Fig. 5A). Concomitantly, the mRNA expression of genes encoding COX-2 and mPGES-1, enzymes for the production of PGE2, was significantly increased by TCDD administration (Fig. 5, B and C). In addition, mRNA of CYP1A1, an indicator gene of TCDD exposure (28), showed a dose-dependent increase (Fig. 5D).

**Suppressed response to TCDD-exposure in the kidney of adult mice.** The incidence and severity of hydronephrosis was negligible in adult male mice 14 days postadministration of TCDD at 20 or 80 μg/kg; only one kidney of a mouse exposed to 80 μg/kg of TCDD was affected by a mild degree of hydronephrosis (score 2). These findings strikingly differ from those of mouse pups (Table 3) and are consistent with the previous observation that TCDD-induced hydronephrosis is developmental-stage specific and occurs only during a critical window during the fetal and neonatal periods (7, 8). Thus we hypothesized that factors required for the development of TCDD-induced neonatal hydronephrosis are absent in adulthood. Oral administration of TCDD at a dose of 0, 20, or 80 μg/kg in adult male C57BL/6J mice significantly increased the renal expression of CYP1A1, an indicator of TCDD exposure (28), in a dose-dependent manner as assessed 7 days postadministration (Fig. 6A). This finding confirmed the retention of TCDD in the kidneys. In contrast to the findings in TCDD-exposed pups (Fig. 5, B and C), almost no upregulation of COX-2 and mPGES-1 was observed in TCDD-exposed adult mice (Fig. 6, B and C). Furthermore, TCDD exposure induced a prominent increase in urine output in neonatal mice (Fig. 3, A–D) but not in adults (Fig. 6G). Moreover, TCDD did not decrease expression of AQP2, NKCC2, or ROMK in adults (Fig. 6, D–F).

**DISCUSSION**

The present study addresses questions on the pathogenesis of neonatal hydronephrosis in a mouse model, in which no anatomical obstruction is present (35). Our initial hypothesis was that loss of mesenchymal layer of the ureter could be a cause of TCDD-induced neonatal hydronephrosis because such a ureteral abnormality is thought to result in abnormal peristalsis and hydronephrosis as well as hydroureter in the absence of any anatomical obstruction of the ureter (18, 26). Genetic ablation of sonic hedgehog also results in a similar phenotype in terms of presence of hydronephrosis and hydroureter, and absence of the mesenchymal layer, although no data on peristalsis is available (57). Our investigation on the ureter, however, revealed that TCDD-induced neonatal hydronephrosis has a distinct phenotype in terms of a preserved three layer structure, absence of hydroureter, and normal gross appearance and frequency of ureteral peristalsis. The absence of hydroureter implies that no obstruction of the ureter or lower urinary tract exists as hydroureter develops in the ureter proximal to the point of obstruction (11). Taken together, we conclude that alterations in the ureteral structure and/or in the ureteral peristalsis, if any, probably do not significantly contribute to the development of TCDD-induced neonatal hydronephrosis.

We found out that TCDD has an action to increase urine output in the mouse pups. The standard method for the analysis of urine output utilizes 24-h urine collected in a metabolic cage. However, it was impossible to apply this method to mice during a lactational period. Thus we collected urine directly from the bladder and used its weight as a surrogate measure of...
urine output. This alternative method of urine collection has inevitable drawbacks; the urine volume retained in the bladder is affected by the timing of urination and is distinctly regulated by the circadian rhythm (51). To minimize these unavoidable confounding effects, we prepared a sufficiently large number of pups (n = 13–15/group) and assessed possible changes in urine output in diurnal and nocturnal phases by setting up experimental conditions at two different clock time points. We observed an increase in urine output in the TCDD-exposed pups from that in the control pups in four independent experiments (Fig. 3, A–D). These results demonstrate for the first time that TCDD increases urine volume in mouse pups.

TCDD-induced polyuria (Fig. 3) and low urine osmolality (Fig. 4A) raised the possibility that TCDD exposure perturbs secretion of the antidiuretic hormone AVP from the pituitary gland, thereby decreasing the AVP concentration in the blood and reabsorption of water at the collecting duct, leading to polyuria. However, this possibility was negated by the obser-

Table 3. Incidence and severity of hydronephrosis in male mouse pups

<table>
<thead>
<tr>
<th>PND</th>
<th>TCDD, μg/kg</th>
<th>DDAVP, 1 μg/kg</th>
<th>n (pup/dam)</th>
<th>Severity</th>
<th>Incidence, %</th>
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<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
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<tr>
<td>14</td>
<td>0</td>
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<td>13/4</td>
<td>6</td>
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<td>5</td>
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Column-titles represent as follows. “PND”: the postnatal day when pups were analyzed; “n (pup/dam)”: the number of pups and dams in each group; “Severity”: the number of pups of each severity score; “Incidence”: percentage of affected pups on a litter basis. *P < 0.05 vs. TCDD 0 μg/kg group, †P < 0.05 vs. TCDD 20 μg/kg group, by Fisher’s exact test with Bonferroni’s correction.
vation that the urine AVP level, considered to reflect the blood AVP level, did not decrease but rather tended to increase in response to TCDD exposure (Fig. 4B). A plausible underlying mechanism could be the interference by PGE2 in the renal AVP system (17, 34, 59) in which an increase in PGE2 concentration (Fig. 5A) reduces AVP-activated membrane AQP2 (59) and/or total AQP2 (Fig. 4, C and F) (2), resulting in a decrease in water permeability at the collecting duct (17, 34) to produce dilute urine (Fig. 4A) (2). Hydronephrosis accompanied with polyuria and low urine osmolality has been reported in another mouse model of a disrupted renal AVP system (58), in which renal V2 vasopressin receptor (V2R) is genetically ablated by a nonsense mutation. The role of AQP2 in the onset of hydronephrosis is not clear because there are some inconsistent observations on the abundance of AQP2 and its localization in the different experiments, such as pharmacological inhibition of V2R (5), nonsense mutation of V2R (58), and our present (Fig. 4) and previous studies (2, 35, 55). Studies on short-term regulation and long-term adaptation of apical-trafficking, endocytosis, and expression of AQP2 may elucidate the underlying mechanism. It is also not clear whether urine dilution has a relationship with hydronephrosis severity; no TCDD dose dependency for the decrease in urine osmolality was observed (Fig. 4A), which was in contrast to the TCDD dose dependent increase in the hydronephrosis severity (Fig. 3E). An additional potential mechanism could be the PGE2-mediated inhibition of Na⁺/H⁺ exchanger 1 (31), which increases urine output. However, it is unclear whether NKCC2 or ROMK is a direct target of PGE2.

To investigate whether the increase in urine output is responsible for the onset of hydronephrosis, we used DDAVP to suppress the increased urine production in TCDD-exposed pups; the level of urine output was comparable to the control pups (Fig. 3C) or an intermediate level compared with those of

Fig. 4. TCDD-induced decrease in urine osmolality. Dams were administered TCDD (0, 20, or 80 μg/kg) on PND 1. Urine and kidney of pups was collected on PND 14. A: Urine osmolality (mosmol/kg H2O) of control or TCDD-exposed mouse pups. B: concentration of AVP in urine normalized to those of creatinine. Renal mRNA levels of aquaporin 2 (AQP2; C), NaK2Cl cotransporter (NKCC2; D), and renal outer medullary potassium channel (ROMK; E) were normalized to that of cyclophilin B. Renal protein expression analyzed by Western blotting for AQP2 (F), NKCC2 (G), and ROMK (H). Insets are bands in Western blot analysis, and the graphs indicate the relative abundance of target proteins normalized to that of β-actin. *P < 0.05 vs. control (TCDD 0 μg/kg) by Tukey-Kramer’s post hoc test.
the control and TCDD-exposed pups (Fig. 3D) depending on the time after the administration of DDAVP. Suppression of polyuria with DDAVP was shown to be sufficient to attenuate or even prevent hydronephrosis (Table 3), which indicates the possibility that the increased urine output is essential for TCDD-induced neonatal hydronephrosis. This notion is further supported by two other findings that 1) urine output is positively associated with the severity of hydronephrosis (Fig. 3E), and 2) polyuria is not exhibited in the TCDD-exposed adults (Fig. 6G) that are resistant to developing hydronephrosis compared with neonates. Other possibilities could be that 1) it is DDAVP itself and not its antidiuretic action that suppresses the

Fig. 5. TCDD-induced increase in renal PGE$_2$ synthesis. Dams were administered TCDD (0, 20, or 80 $\mu$g/kg) on PND 1. Urine and kidney of pups was collected on PND 7. A: urine PGE$_2$ concentration, and renal expression of mRNAs for cyclooxygenase-2 (COX-2; B), microsomal prostaglandin E synthase-1 (mPGES-1; C), and CYP1A1 (D) were normalized to that for cyclophilin B. *P < 0.05 vs. control (TCDD 0 $\mu$g/kg), †P < 0.05 vs. TCDD 20 $\mu$g/kg group, by Tukey-Kramer’s post hoc test.

Fig. 6. Suppressed response to TCDD-exposure in the kidneys of adult mice. Adult male mice were orally administered a single dose of TCDD (0, 20, or 80 $\mu$g/kg). Renal expression of mRNAs for CYP1A1 (A), COX-2 (B), mPGES-1 (C), AQP2 (D), NKCC2 (E), and ROMK (F) in adult mice, 7 days postadministration of TCDD, were normalized to that for cyclophilin B. G: weight of urine collected from mice 14 days postadministration. *P < 0.05 vs. control (TCDD 0 $\mu$g/kg), †P < 0.05 vs. TCDD 20 $\mu$g/kg group, by Tukey-Kramer’s post hoc test.
onset and/or progression of hydronephrosis; 2) TCDD modulates the tubuloglomerular feedback system and increases the glomerular filtration rate to augment tubular flow and urine production; 3) the deterioration of the kidney parenchyma can be induced by the augmented tubular flow and by other yet-unclarified actions of TCDD; and 4) the primary cause could be medullary developmental delay, which could result in decreases in medullary transporter mRNAs and insufficient urine concentrating capacity. In addition, one or a combination of these potential mechanisms might contribute to the development of hydronephrosis.

There exist several mouse lines that develop hydronephrosis accompanied with polyuria and low urine osmolality. These include BsndNeo(R8L) line, which was created in a process to produce a model mouse line (BsndR8L) of Barter syndrome type IV (37); Aqp2Δ230 line (45); Slc12a1tm1Tkh line (46); Slc12a1Δ129F line, which was created by N-ethyl-N-nitrosourea (ENU)-driven mutagenesis (20); and Hoxb7-CreMib1fr mice lacking Mib1 in the ureteral bud-derived progeny cells in the kidney (19). The hydronephrosis in these mice and in V2R deficient (58) mice exhibits heterogeneity in the timing of onset, severity, and additional characteristics such as nephrocalcinosis, lethality, and electrolyte balance, and with respect to the cell types affected by the mutations. Since an increase in urine volume and decrease in urine osmolality are the commonly shared characteristics of these hydronephrotic mice in spite of the heterogeneity, the polycystic and low urine osmolality are thought to be associated with hydronephrosis in these mice. The level of polycystic in these mice shows a three- to eightfold increase in urine output in the affected groups from that in the respective control groups. This is comparable to or slightly larger than that observed in TCDD-exposed pups (3.0 ± 0.3-fold increase in the TCDD groups from that in the controls; Fig. 3, A–D). Such a difference in the fold increase could be attributed, at least in part, to the difference in the stage of kidney development in model animals; our study used 14-day-old pups, whereas the other studies used adult mice. In neonates, the urine concentrating mechanism is still under development (24, 52). In fact, the urine osmolality in case of 14-day-old control pups in the present study (0.79–0.89 mosmol/kgH2O) (Fig. 4A) was much lower than that reported for adult mice (1.1–3.2 mosmol/ kgH2O) (19, 20, 37, 45, 46, 58).

The onset of TCDD-induced neonatal hydronephrosis in mice is confined to the initial few days after birth (7), while adult mice are resistant to TCDD-induced hydronephrosis. These observations suggest the following three possible mechanisms for the observed temporal differences in the pathogenesis: the first possibility is that pups ingest TCDD from milk into the body and accumulate it in the kidneys (35, 36), which results in a higher concentration in the target organ than that in adults. The second possibility is that AhR, the receptor for TCDD (13), is more efficiently activated by TCDD in the kidneys of pups than in those of adults. The third possibility is that the prostaglandin synthesis pathway, upregulation of which is critical to the development of hydronephrosis in pups (35, 53), responds to TCDD-induced activation of AhR more readily in the developing kidney than in the adult kidney. The first and second possibilities can be rejected because an indicator gene for TCDD exposure, CYP1A1 (28), was found to be increased in pups and adults to a similar extent (Figs. 5D and 6A), which indicates that TCDD was deposited in the kidney and caused activation of AhR to a similar extent, irrespective of the age of the mice. The third possibility is supported by the observation that TCDD does not upregulate the PGE2-synthesis pathway genes COX-2 and mPGES-1 in adult mice (Fig. 6, B and C).

COX-2 expression in the kidney is regulated in a developmental stage-specific manner and peaks around PND 7 in mice (12, 21, 35). Genetic or pharmacological blockade of COX-2 has been shown to result in immature nephrons and the consequent nephropathy (9, 21, 33, 39). Perinatal cyclooxygenase inhibition by COX-2 inhibitors in humans is, therefore, a clinically relevant concern (23, 38). On the other hand, excessive COX-2 activity also induces adverse effects, defects in urine concentrating mechanisms, and hydronephrosis in the developing kidney, as demonstrated in the present study. In the light of the above discussion, optimal COX-2 activity appears to be indispensable to normal kidney development.

A generally accepted definition is that hydronephrosis is the disease state having a dilation of the renal calices and pelvis proximal to the point of obstruction (11). However, neonatal hydronephrosis induced by chemical exposure (such as TCDD exposure) is linked to polyuria, rather than ureteral obstruction. Hydronephrosis occurring in mutant mice is associated with overproduction of diluted urine (19, 20, 37, 45, 46, 58), a finding that is common in TCDD-induced neonatal hydronephrosis. As for the etiological explanation, it is entirely conceivable that an excessive increase in urine output overwhelms the renal capacity to expel urine, which leads to destruction of the renal parenchyma by the urine backpressure. Thus, hydronephrosis should be redefined to include the nonobstructive type, as follows: “hydronephrosis is a disease state involving dilation of the renal calices and pelvis due to insufficiency in urine-expelling capacity relative to the urine production.” The presence of anatomical or functional obstruction serves to limit the urine-expelling capacity. The most common type of hydronephrosis, which involves anatomical or functional obstruction of the ureter, occurs even with normal urinary output and is considered to represent an extreme end of this disease spectrum. The other end of the spectrum is the nonobstructive type of hydronephrosis manifesting as antenatal hydronephrosis in humans (49, 50). This type of hydronephrosis may be induced by the overproduction of urine, which overwhelms the urine-handling capacity of the kidney.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

W.Y., N.N., F.M., and C.T. conceived and designed the research; W.Y., T.K., T.A., N.N., and N.F. performed experiments; W.Y., T.K., T.A., N.N.,

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