Dichloroacetate treatment accelerates the development of pathology in rodent autosomal recessive polycystic kidney disease

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Gattone VH II, Bacallao RL. Dichloroacetate treatment accelerates the development of pathology in rodent autosomal recessive polycystic kidney disease. Am J Physiol Renal Physiol 307: F1144–F1148, 2014. First published September 18, 2014; doi:10.1152/ajprenal.00009.2014.—Dichloroacetate (DCA) is a toxicant by-product from the chlorination disinfection process for municipal water. The levels would not affect people with normal renal and liver function. However, people with impaired renal or liver function may have an increased susceptibility to DCA toxicity as those are the organs affected by DCA. People (and rodents) with polycystic kidney disease (PKD) are polyuric, drink more fluids, and have both renal and liver pathology. In PKD, renal tubules and biliary epithelial cells proliferate to form cysts, which can eventually cause renal and/or liver dysfunction. Therefore, PKD may be a predisposing condition with an increased sensitivity to DCA toxicity. PKC rats are an orthologous model of human autosomal recessive PKD and were treated with 75 mg/l DCA in their drinking water. Male and female PKC and male Sprague-Dawley rats were treated from 4 to 8 wk of age, after which the severity of the renal and liver pathology induced by DCA were assessed. Only male PKC rats were adversely affected by DCA treatment, with an increase in the severity of renal cystic disease evinced by an increase in cystic enlargement and proteinuria. In conclusion, the chlorination byproduct DCA may adversely affect those with a preexisting renal disease, especially those who are polydipsic, like those with PKD.

Polycystic kidney disease; chlorination byproducts; dichloroacetate; rat; water

Polycystic Kidney Disease (PKD) is a relatively common inherited disease with a distinctive pathology consisting of epithelial cell-lined cysts in the kidney and fibrocytic pathology in the liver. The two prominent forms of PKD include one that is inherited as an autosomal dominant trait [autosomal dominant PKD (ADPKD)], which typically affects adults, whereas the other is transmitted as an autosomal recessive trait [autosomal dominant PKD (ARPKD)], which usually affects the young, infants through young adults. However, there are several other inherited forms of renal cystic disease, including nephronophthisis, Meckel syndrome, Bardet Biedl syndrome, Oro-facial-digital syndrome, and medullary cystic disease (13). One of the common features of all of these conditions is their lack of a urine concentration capability, leading to polyuria and polydipsia. The PKC rat model has been particularly useful in the evaluation of several factors involved in cystic pathology progression (10). The PKC rat has a mutation in the same gene that causes human ARPKD and develops both renal and hepatic pathology similar to the human condition (20, 34). Like in other renal cystic diseases, these rats are polyuric and polydipsic. This abnormality is due to alterations within the kidney caused by the disease, which ultimately leads to an increase in vasopressin-induced renal cAMP. To reduce vasopressin, this PKC model was induced to drink even more water, which reduced the severity of the cystic disease (22). When the renal vasopressin V2 receptor was pharmacologically antagonized to lessen the effect of vasopressin, disease severity was reduced in rodents (11). Therefore, humans with PKD are encouraged to drink increased amounts of water daily, and those who would be treated with V2 receptor antagonists would necessarily increase water intake. However, drinking an increased amount of water could amplify potentially toxic effects of any carcinogens or toxicants present in drinking water.

Dichloroacetate (DCA) is a potential metabolic-targeting therapy for cancer (21). Considering the proliferative nature of PKD, it may be considered a form of neoplasia. Some PKD clinicians advocate treating PKD as a form of neoplasia (12), in which case DCA may function as a treatment for PKD. DCA functions as an antiproliferative agent by inhibiting mitochondrial pyruvate dehydrogenase kinase, switching the mitochondria from glycolysis to glucose oxidation. The increase in glucose oxidation increases mitochondrial H2O2 production and stimulates cancer cell apoptosis. Cystic kidneys already have an increase in apoptosis (36), and if DCA increases the apoptosis of mutant cystic cells, then the PKD may be reduced. However, inhibition of apoptosis is also seen as a potential treatment for PKD (29). Cystic kidney disease is also associated with increased renal oxidative stress (19), and DCA may add to that oxidative stress. If increased oxidative stress contributes to the progression of the cystic disease, then DCA treatment could exacerbate the renal cystic disease. It has been shown that further increasing oxidative phosphorylation in the cystic kidney can exacerbate the disease, as was found in the rat model of Meckel syndrome (18). Furthermore, reducing antioxidants can aggravate rodent PKD (30). Those with PKD drink more water, and most drink municipal water that has been treated, thus containing chlorination byproducts like DCA. Therefore, it will be necessary to determine what affect these byproducts may play in the disease progression in those with underlying renal and/or liver disease, like PKD.

DCA is a toxicant byproduct of water chlorination and is present at some level in essentially all public drinking water. The Environmental Protection Agency established Stage 2 Disinfection and Disinfection Byproduct Rules that suggest a level of 60 μg/l DCA in drinking water, yet the Environmental
Protection Agency’s Information Collection Rule evaluation of major water processing centers described significant levels of five halogenated acetic acid compounds [which include trichloroacetic acid (TCA) and DCA] present in most water supplies. DCA is also a metabolite of trichloroethylene (TCE), which is a common environmental toxin (15), particularly in manufacturing areas and around military bases. DCA at higher concentrations causes liver and kidney tumors. DCA induces DNA hypomethylation, which may be part of its mode of action in tumorigenesis (4, 28). TCE liver tumorigenesis appears to be largely due to its metabolites, DCA and TCA (4). Therefore, people with chronic renal pathology who are polydipsic may be more sensitive to the toxic effects of TCE, DCA, and/or TCA because of increased water intake and the long-term nature of their disease. Those with ADPKD as well as ARPKD are polydipsic and have kidney and liver pathology, the principal target organs of DCA toxicity. Therefore, PKD patients may be particularly affected over an extended period of time by an increased daily exposure to drinking water with DCA, TCA, and/or TCE. The present study found that providing drinking water with DCA to PCK rats exacerbated the renal disease, but only in male rats. Therefore, understanding the potential effect of toxicants found in treated drinking water may be especially important for those renal conditions associated with polydipsia, like PKD.

MATERIALS AND METHODS

All experiments were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine. Four-week-old male and female PCK rats used in these experiments were bred at Indiana University from breeders provided by Charles Rivers Laboratories (20). All rats were fed ad libitum with 7002 6% Teklad mouse-rat diet (Harlan, Indianapolis, IN). As a normal non-cystic control, male Sprague-Dawley rats were similarly treated. At 4 wk of age, male rats were provided with drinking water ad libitum with either 75 mg/l DCA or regular tap water until 8 wk of age, the period of active cystic development and enlargement. The dose of DCA in the water was based on the assumption that a human consumes 2 liters of water per day with a maximum allowable concentration of DCA in water of 60 μg/l. At an average dose of 120 μg/day and 30 yr of exposure, this results in a 18.8 mg/kg lifetime dose for a 70-kg human. Rats drink ~1.2 ml water/day, with an exposure of 30 days. This gives a daily dose of 0.09 mg/day for a

Table 1. Effect of DCA in drinking water on the progression of polycystic kidney disease

<table>
<thead>
<tr>
<th></th>
<th>Male PCK Rats</th>
<th>Female PCK Rats</th>
<th>Male Sprague-Dawley Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DCA</td>
<td>Control</td>
</tr>
<tr>
<td>Number of rats/group</td>
<td>15</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>3.26 ± 0.4</td>
<td>3.55 ± 0.3*</td>
<td>2.33 ± 0.3†</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>4.81 ± 0.14</td>
<td>5.69 ± 0.09*</td>
<td>1.56 ± 0.03</td>
</tr>
<tr>
<td>Kidney weight/body weight</td>
<td>1.47 ± 0.03</td>
<td>1.60 ± 0.04*</td>
<td>15.3 ± 1.4</td>
</tr>
<tr>
<td>Cyst volume density, %</td>
<td>11.4 ± 1.1</td>
<td>17.0 ± 2.4*</td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td>Cyst volume, ml</td>
<td>0.54 ± 0.06</td>
<td>0.99 ± 0.15*</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>Cyst volume/body weight</td>
<td>0.16 ± 0.02</td>
<td>0.28 ± 0.04*</td>
<td>14.4 ± 2.1</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dl</td>
<td>16.6 ± 0.8</td>
<td>18.5 ± 0.7</td>
<td>1.14 ± 0.21</td>
</tr>
<tr>
<td>Proteinuria (dipstick)</td>
<td>1.7 ± 0.2</td>
<td>2.4 ± 0.2*</td>
<td>1.46 ± 0.6†</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>19.7 ± 0.5</td>
<td>21.7 ± 0.5*</td>
<td>6.24 ± 0.18</td>
</tr>
<tr>
<td>Liver weight/body weight</td>
<td>6.01 ± 0.12</td>
<td>6.12 ± 0.10</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>119 ± 4</td>
<td>151 ± 8*</td>
<td>2.4 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Fibrosis score was based on a scale of 1+ to 4+. Dipstick protein was evaluated on a scale from 0 to 4+. DCA, dichloroacetate. *P ≤ 0.05 with DCA treatment; †P ≤ 0.05 compared with male PCK rats.

RESULTS

This short-term DCA treatment (75 mg/l in drinking water) was well tolerated for the 4- to 8-wk treatment period. An acceleration of the PKD renal cystic pathology was documented in male PCK rats but not in either female PCK rats or male Sprague-Dawley rats (Table 1). In male PCK rats, DCA treatment led to an increase in total kidney weight and liver weight; however, when expressed as a percentage of total body weight, only the kidney was statistically increased. Histopathological analysis revealed an increase in renal cystic pathology (cyst volume density, total cyst volume (in ml), and as volume expressed as a percentage of body weight; Table 1 and Fig. 1). As expected, kidney weights and liver weights of normal male Sprague-Dawley rats were smaller than their comparable male PCK values. Interestingly, both serum cholesterol and albumin were increased by DCA in both male and female PCK rats, whereas urinary dipstick proteinuria was increased only in DCA-treated male PCK rats. The body weight increase asso-
ARPKD primarily affects children with both a rapidly progressive infantile phenotype as well as an adolescent to young adult phenotype, whereas liver pathology can be more problematic in the adolescent/young adult phenotype of ARPKD. Both kidney pathology tends to predominant in the earlier form. Kidney pathology tends to dominate in the earlier form. Kidney pathology tends to dominate in the earlier form.

DISCUSSION

PKD is a relatively common inherited disease that primarily causes cyst formation and fibrosis in both the kidney and liver. In ADPKD, the development of end-stage renal disease requiring renal replacement therapy (kidney transplant or dialysis) occurs in later midlife (50–60 yr of age). Men tend to develop renal failure earlier than affected women with ADPKD (6). ARPKD primarily affects children with both a rapidly progressive infantile phenotype as well as an adolescent to young adult form. Kidney pathology tends to predominate in the earlier phenotype, whereas liver pathology can be more problematic in the adolescent/young adult phenotype of ARPKD. Both conditions have significant morbidity and mortality, and there is no currently approved treatment to slow these diseases.

PKDs generally affect the liver and kidney, the two organs principally involved by toxicants like DCA, a chlorination byproduct in drinking water. Since those with PKD are generally polyuric and polydipsic, they would be affected disproportionately by toxicants commonly found in drinking water. The slowly progressive nature of ADPKD and adolescent-type presentation of ARPKD would provide a long period where such toxicant injury could take place. At toxic levels to rodents with normal liver and kidney function, DCA can cause liver or kidney tumors. Renal cystic epithelial cells have an abnormal/immature phenotype, a phenotype comparable in many ways to slow-growing neoplasms (12). However, the relentless progression of PKD ultimately leads to organ dysfunction. Epithelial proliferation is a central feature in all forms of renal cystic disease. Therefore, some interventions have approached PKD as a neoplastic condition and used cancer chemotherapies [taxol for testicular cancer (24) and PKD (37), EGF tyrosine kinase inhibitors for lung cancer (16) and PKD (32), rapamycin for renal cell carcinoma (7) and PKD (33, 38), and roscovitine for cancer (17) and PKD (3)]. While DCA has been proposed as a relatively novel cancer treatment (21), DCA was found to exacerbate the disease in male PCK rats. DCA induced oxidative stress, and the cystic kidney is already under increased oxidative stress, possibly because of reduced renal blood flow in PKD (31). Therefore, further oxidative stress from DCA could further exacerbate the renal environmental factors, cystic disease, and renal dysfunction, as seen in Meckel syndrome 3 rats (18).

The present study evaluated the relatively early treatment of PCK rats with DCA-treated drinking water. While the concentration of DCA was greater than that associated with publicly treated water, it was designed to mimic the long-term effects of accumulated DCA intake. The DCA treatment primarily affected the male PCK renal pathology, accelerating the cystic change and increasing severity of the already present proteinuria. These data support a worsening of the renal disease despite a continued normal blood urea nitrogen. A similar increase in renal cystic enlargement was seen in the Pcy mouse model of human nephronophthisis type 3 similarly treated from 4 to 8 wk of age with DCA (unpublished findings). However, significant renal function needs to be lost before azotemia becomes evident. Male PCK rats also exhibited increased total liver weight; however, because DCA-treated rats were slightly larger than their controls, liver weight as a percentage of body weight was not statistically different. There were no data to suggest that this short period of DCA treatment caused any further pathology to the liver. DCA treatment was also associated with an increase in serum cholesterol and albumin in both male and female PCK rats. PCK rats normally develop increased serum cholesterol and triglyceride levels later in disease (20), and in this study, DCA treatment was associated with an early increase in serum cholesterol. There is no understanding for the basis for higher cholesterol that is normally observed in PKC rats. Therefore, the increase associated with DCA is further underscored by the observation that DCA lowers serum cholesterol in cases of hypercholesterolemia (27). A sex dimorphism was present in DCA-treated PCK rats, which is relatively unique for any intervention tried on rodent models of PKD.

The progression of hepatorenal fibrocystic diseases in rodents and people can be influenced by numerous genetic and epigenetic factors. The progression of PKD appears to be influenced by a number of genetic factors, including the nature
of the gene mutation [ARPKD (2)], PKD1 mutations [ADPKD (14)], and age of onset (26). However, there are a number of epigenetic and environmental factors that can also influence the rate of cystic disease progression, including water intake (22), acute renal injury (25), modulation of cellular Ca2+ (inhibition with Ca2+ channel blockers (23)), or augmentation of cellular Ca2+ effects with calcimimetics (5). Cellular Ca2+ can become a critical issue in PKD, as the PKD2 gene product, polycystin 2, is a Ca2+ channel and PKD cells are known to already have a lower concentration of cellular Ca2+. Interestingly, in endometrial carcinoma cells that were sensitive to DCA-induced apoptosis, DCA reduced cellular Ca2+ compared with insensitive carcinoma cells (35). These data may suggest a possible Ca2+-associated pathway that DCA could use to influence renal disease progression.

To date, interventions used to modify the rate of PKD progression have had a similar effect on both males and females when evaluated other than those using sex-associated hormones. It is unclear why males with ADPKD progress to renal failure earlier than do females (6); however, in the Han:SPRD rat model, male hormones clearly appeared to exacerbate the disease (9), whereas female hormone attenuated the renal cystic disease in orchietomized male rats (1). However, in the present study, male PCK rats were affected, whereas female PCK rats were not. There is sexual dimorphism in the severity of the disease in humans (6) as well as in Han:SPRD Cy/+ rats (8) and PCK rats (20). The present study is unique in that the treatment selectively enhanced the disease progression in the kidney of a specific sex. This finding suggests that the preclinical testing of interventions probably should be evaluated in both males and females.

Those renal patients that have a urine concentration defect (i.e., PKD) and/or are encouraged to drink increased amounts of water (i.e., nephrolithiasis patients) have be at an increased risk of further kidney damage from water-associated toxicants or organic pollutants. Water toxicants and environmental pollutants that require liver and renal metabolism and elimination may generally be a problem for renal patients. Most of these agents can be eliminated by filtering systems designed for home use. The data presented here may initiate a discussion on the potential benefit to renal patients of using relatively inexpensive home water filtration systems that eliminate these types of agents.

DISCLOSURES

R. L. Bacallaoro has received $10,000 in consultation fees from Otsuka Pharmaceuticals of America for advice on therapy for patients with autosomal dominant polycystic kidney disease.

AUTHOR CONTRIBUTIONS

Author contributions: V.H.G. and R.L.B. conception and design of research; V.H.G. and R.L.B. performed experiments; V.H.G. and R.L.B. analyzed data; V.H.G. and R.L.B. interpreted results of experiments; V.H.G. prepared figures; V.H.G. and R.L.B. drafted manuscript; V.H.G. and R.L.B. edited and revised manuscript; V.H.G. and R.L.B. approved final version of manuscript.

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