Potassium Citrate/Citric Acid Intake Improves Renal Function in Rats with Polycystic Kidney Disease

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Abstract. Polycystic kidney disease (PKD) has been shown to be exacerbated by acidosis or a low potassium intake, and there is evidence that administration of alkali might have a beneficial effect. This study determined whether ingestion of potassium citrate and citric acid would ameliorate PKD. Healthy normal and heterozygous littermate Han:SPRD rats with autosomal dominant PKD were provided with either tap water or 55 mM \( K_2\text{citrate/67 mM citric acid solution (KCitr)} \) to drink starting at the age of 1 mo. Renal clearance measurements and histologic assessments were performed when the rats were 3 mo old. KCitr intake did not affect body weight or urine flow, but completely prevented the decline in GFR found in untreated rats with PKD. In rats that drank tap water, left kidney GFR averaged \( (\mu l/min per 100 g\ body\ wt) 503 \pm 78 (n = 9) \) in normal animals and \( 242 \pm 56 (n = 6) \) in rats with PKD. In rats that drank KCitr, GFR averaged \( 562 \pm 123 (n = 7) \) in normal animals and \( 534 \pm 103 (n = 7) \) in rats with PKD. Kidneys of rats with PKD were approximately double normal size. KCitr treatment did not affect kidney size, but led to fewer interstitial abnormalities and smaller cysts in cystic kidneys. KCitr ingestion led to a significantly lower \( (P < 0.001) \) plasma \( [K^+] \) in rats with PKD (3.3 \pm 0.2 versus 4.1 \pm 0.2 mEq/L in rats on tap water). Chronic KCitr intake in the young heterozygous Han:SPRD rat with PKD yields a modest improvement of kidney histology and a dramatic improvement in GFR. The mechanism of action of KCitr and the long-term effects of this treatment on renal structure and function in PKD deserve further study.

Autosomal dominant polycystic kidney disease (PKD) is a common genetic disease that often leads to renal failure. The expression of this disease is quite variable, probably because of differences in the primary gene defect, modifier genes, and environmental factors. Practical treatments to halt or slow the progression of PKD in patients are extremely limited (1).

The discovery of the Han:SPRD rat with autosomal dominant PKD has provided a unique opportunity for testing various treatments (2–5). PKD in heterozygous rats of this strain closely mimics the disease in humans. Major advantages of this model are that treatments can be tested in the young animal, before advanced renal failure develops, and that the usual time course (months) of the disease is much shorter than in people (decades). Using these rats, Torres et al. (4) found that acidosis, induced by \( NH_4Cl \) ingestion, resulted in a diminished GFR and greater kidney size in heterozygous rats with PKD. These investigators also found that treatment with alkalinizing salts, e.g., \( KHCO_3 \) and \( NaHCO_3 \), diminished enlargement of cystic kidneys, but they did not find a statistically significant improvement in endogenous creatinine clearance. A potential problem with the alkalinizers was that large doses of bicarbonate led to precipitation of calcium stones in the kidneys. Also, rats ingesting \( KHCO_3 \) solutions (200 or 300 mM) showed impaired growth. Recent studies by Cowley et al. (5) demonstrate the detrimental effects of \( NH_4Cl \) ingestion or a low potassium intake on renal cystic disease in the Han:SPRD rat.

In the present study, the effects of ingestion of a potassium citrate/citric acid solution (KCitr) on renal structure and function in Han:SPRD rats were investigated. KCitr was chosen for several reasons. Citrate, like bicarbonate or carbonate, is an alkalinizer. Citrate forms a soluble complex with calcium in the urine and so prevents intrarenal calculi formation (6). The potassium salt was selected because it would be expected to have less of an effect on BP than the sodium salt. Also, administration of potassium might serve to correct potassium deficiency, a condition known to favor the development of renal cysts (5,7). This study hypothesized that administration of KCitr would ameliorate renal cystic disease. Chronic treatment of cystic Han:SPRD rats with KCitr led to complete normalization of GFR and less severe histologic changes in the kidneys.

Materials and Methods

Experiments were performed on 13 male heterozygous Han:SPRD rats with PKD and 16 normal littermates. The breeding stock was obtained from the Polycystic Kidney Program at the University of Kansas, courtesy of Dr. Benjamin D. Cowley Jr. All animals were allowed free access to a diet containing 24% protein and 6% fat (Teklad 6% mouse/rat diet 7002, Harlan, Madison, WI). Beginning at 1 mo of age and until 3 mo of age, animals were provided with a solution of 55 mM tripotassium citrate/67 mM citric acid (KCitr) or tap water to drink. The KCitr had an osmolality of 240 ± 3
mosmol/kg H$_2$O ($n=10$) and a pH of 4.2 ± 0.04 ($n=9$). In 12 3-mo-old rats, two animals per cage, daily consumption of KCitr averaged 16.7 ± 2.5 ml/100 g body wt ($n=6$), a normal fluid intake. The experimental design included four groups, because both normal rats and rats with PKD drank tap water or the KCitr. Whether the animals were normal or had PKD was determined by gross inspection of the kidneys after completion of renal clearance measurements. All experiments were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Before experiments, the rats were deprived overnight of food but had free access to tap water or KCitr. They were anesthetized with the thiobarbiturate Inactin (Byk Gulden, Konstanz, Germany). 130 mg/kg body wt, intraperitoneally. Each animal was placed on a heated table, and rectal temperature (monitored with a probe) was kept at 37°C. The trachea was cannulated, and a slow flow of moistened 35% O$_2$/65% N$_2$ was passed over the opening of the cannula. A femoral vein was cannulated for infusions. One milliliter of 6 g/dl fraction V bovine serum albumin (Sigma, St. Louis, MO) in 0.9% NaCl was administered intravenously during the surgical preparation. To measure GFR, a prime dose of a solution of polyfructosan (PFS), a synthetic inulin (Laevosan Co., Linz, Austria), was injected intravenously, followed by a sustaining infusion of 3% PFS in 0.9% NaCl at 3 ml/h. A femoral artery was cannulated for periodic blood sampling (0.25 ml) and for measuring BP with a pressure transducer (Gould-Statham, Hato Rey, Puerto Rico). The abdomen was opened by a midline incision, a cannula was inserted into the bladder to drain urine from the right kidney, and the left ureter was cannulated for urine collection. After 2 h for equilibration, urine was collected for three 20-min periods, with mid-period blood sampling. Urine and plasma samples were analyzed for PFS with an anthrone method (8) and for potassium with an atomic absorption spectrophotometer (Instrumentation Laboratory, Wilmington, MA). GFR was calculated from the rate of excretion of PFS divided by the plasma PFS concentration. All data for a single animal (three clearance periods) were averaged.

After completion of the clearance measurements, in a few experiments an arterial blood sample was collected for anaerobic measurement of blood pH. Subsequently, a solution of 5 mg of bromodeoxyuridine (BrdU)/ml in 0.9% NaCl was infused intravenously over several minutes at a dose of 1 ml/100 g body wt, to assess DNA synthesis (cell proliferation rate). After 1 h, the kidneys were fixed with 1% glutaraldehyde in Tyrode’s solution by retrograde aortic perfusion at an applied pressure of 150 to 170 mmHg for 20 min. The kidneys were removed and placed in fixative solution for several days in the refrigerator and were then weighed, sliced with a razor blade, immersed in 0.1 M cacodylate solution, pH 7.25, and embedded in paraffin for routine light microscopy. Some sections were stained with hematoxylin and eosin. Others were stained for BrdU (BrdU staining kit, Zymed Laboratories, South San Francisco, CA); standard procedures were followed, except that kidney sections were exposed to 0.17% trypsin for 60 min at 37°C to improve BrdU detection. Slides were coded, so that all evaluations were done in a blinded manner.

Histologic evaluation of kidney sections included the following. First, all cystic kidneys were examined for interstitial changes (widening of intermephron spaces, fibrosis, presence of inflammatory cells), using an arbitrary scale of 0 to 3, where 0 represents the normal condition and 3 represents severe changes. On two separate occasions, scoring of cystic kidneys was in agreement 92% of the time. Second, individual tubule/cyst lumen areas were measured in five to eight random regions of outer cortex using an Image 1 measuring program (Universal Imaging, West Chester, PA). Each region had an area of $1.1 \text{ mm}^2$, and up to 50 individual tubule/cyst lumen areas were measured per region, for a total of 250 measurements per kidney. Mean and median values were calculated. Third, at a low magnification ($\times 12.5$), four random regions of the cortex (each region had an area of $6.6 \text{ mm}^2$) of 13 cystic kidneys were videotaped, and the number of cysts with lumen areas greater than $30,000 \mu\text{m}^2$ was measured. Fourth, the number of BrdU-positive nuclei in 60 nonoverlapping areas of cortex was determined at a magnification of $\times 400$ in sections from all kidneys. The total area evaluated per kidney was 2.54 mm$^2$. BrdU-positive nuclei in cysts or tubule epithelial cells and in the interstitium were counted separately.

**Statistical Analyses**

Data presented are means ± SD. They were analyzed by an ANOVA, after a preliminary test for homogeneity of variances. Individual groups were compared with the Bonferroni method. If variances were heterogeneous, the Welch F’ test or Welch–Satterthwaite t’ test was used to compare means.

**Results**

Table 1 and Figure 1 summarize the functional data in normal rats and rats with PKD. There were no statistically significant differences in rat age, body weight, or urine flow rate (V) among the four groups of rats. The rats with PKD had mean arterial BP significantly higher than the normal rats ($P < 0.01$), and these pressures were not affected by KCitr ingestion. Rats with PKD that drank tap water had significantly lower hematocrit levels than normal rats drinking tap water ($P < 0.001$); KCitr ingestion yielded a higher average hematocrit level in rats with PKD, but the difference was not significant.

Figure 1 shows the most striking results of this study. In rats with PKD that drank tap water, GFR was approximately half of normal, i.e., $242 ± 56$ versus $503 ± 78 \mu\text{L/min per 100 g body wt}$ ($P < 0.001$). By contrast, in rats with PKD that ingested KCitr, GFR was normal ($534 ± 103 \mu\text{L/min per 100 g body wt}$). This finding was the case even though KCitr did not affect GFR in the normal rats ($562 ± 123 \mu\text{L/min per 100 g body wt}$). The increase in kidney size in rats with PKD, however, was not prevented by KCitr. Cystic kidneys of both treated and untreated 3-mo-old rats weighed about twice as much as normal kidneys (Figure 1).

Table 1 also shows that KCitr ingestion had no effect on plasma $[K^+]$ in normal rats; however, in rats with PKD, KCitr ingestion produced a lower plasma $[K^+]$ ($P < 0.001$), despite a higher potassium intake. Plasma $[K^+]$ in the KCitr-treated rats with PKD was also lower than in either group of normal rats. The low plasma $[K^+]$ in the KCitr-treated rats with PKD could be explained by a high GFR, efficient potassium excretion, and a relatively alkaline blood pH. In five 2- to 3-mo-old rats with PKD that drank tap water, arterial blood pH averaged $7.33 ± 0.04$; in four 2- to 3-mo-rats with PKD that drank KCitr since 1 mo of age, pH averaged $7.38 ± 0.03$. Although the means are statistically not significantly different ($P = 0.12$), they suggest that blood pH may be closer to normal in rats with PKD when they are treated with KCitr. Urinary potassium excretion rate was clearly elevated on the KCitr diet ($P < 0.01$).

Figure 2 shows representative sections from normal kidneys and from cystic kidneys of untreated (tap water) and treated (KCitr) rats. The histology of normal kidneys was not affected
by KCitr ingestion. In the rats with PKD, KCitr ingestion was associated with a less abnormal interstitium and smaller cysts. The histograms in Figure 2 show the distribution of tubule/cyst lumen sizes in normal kidneys (pooled data), and in treated and untreated rats with PKD. The histograms reveal a clear tendency for lumen sizes to be larger in the cystic animals, especially in the untreated (tap water) group. In the normal animals, only 0.3% of the lumen areas were greater than 6000 μm²; whereas in the cystic kidneys, 15% (KCitr-treated rats) and 24% (untreated rats) of lumen areas were greater than 6000 μm².

Table 2 summarizes the histologic assessment of the kidneys in animals with PKD. Interstitial changes were significantly fewer in the KCitr-treated animals than in the untreated animals. Both the median and mean lumen areas were significantly smaller in the rats treated with KCitr than in untreated rats. The untreated rats also had a significantly greater number of very large cysts compared with the KCitr-treated rats. Finally, the number of BrdU-positive interstitial cells was significantly higher in the untreated rats than in the KCitr-treated rats with PKD. This last result is consistent with the finding that interstitial changes are more prominent in the untreated rats and suggests increased proliferation of interstitial fibroblasts.

Discussion

This study demonstrates that early treatment of Han:SPRD rats with KCitr prevents the fall in GFR that normally accompanies PKD. This prevention is an important finding, because it is the first treatment that preserves GFR in an animal model of PKD without untoward side effects. This treatment also lowers the plasma [K⁺] in rats with PKD, but does not prevent hypertension or enlargement of the kidneys in 3-mo-old rats. Unlike effects produced by ingesting 200 or 300 mM KHCO₃ (4), KCitr ingestion has no detrimental effect on body weight. Histologic evaluation of cystic kidneys reveals fewer interstitial changes and smaller cysts in rats with PKD treated with KCitr.

The remarkable beneficial effect of KCitr ingestion on renal function in rats with PKD raises several important issues: What is the mechanism of action of KCitr? How can we explain the changes in GFR? Would KCitr have beneficial effects with longer treatments, with lower doses, and in other species?

The mechanism of action of KCitr ingestion remains to be defined. Potassium, citrate, and citric acid could be beneficial for many reasons. First, citric acid is a metabolic substrate in the kidneys (9) and so could increase energy production by kidney cells. Second, citrate is a base and is converted, at least in part, to bicarbonate in the body. It has the effect of alkalinizing the body fluids, including the urine. The alkalinizing effect of citrate might counteract the metabolic acidosis that frequently accompanies renal failure. Third, citrate/citric acid administration results in decreased renal ammonia synthesis by two mechanisms: a direct metabolic effect of citric acid (10) and the alkalinizing effect of citrate. Increased ammonia synthesis per nephron is thought to result in interstitial fibrosis in a variety of renal diseases, possibly by activating the alternative complement pathway (11) or by favoring the formation of long-lived oxidants such as chloramines (12). Interstitial changes are a prominent feature of the cystic kidney, and cyst fluid samples from patients with PKD have elevated ammonia levels (13). Ammonia increases the growth rate of cultured cells (14). Alterations of metabolism associated with renal ammoniagenesis could also have an impact on PKD (4). Fourth, reduced citrate excretion is commonly seen in patients with PKD. This reduction could contribute to the increased incidence of renal stone disease in these patients (1) and to development of obstruction, a factor that contributes to cyst enlargement (15). In Han:SPRD rats, renal citrate excretion is, surprisingly, higher in rats with PKD than in normal animals (16). Therapy with citrate, by complexing calcium in the urine or in renal tissue, could diminish renal stone formation and
citrate is an antioxidant; oxidant injury may play a role in the deposition of insoluble calcium salts in the kidneys. Fifth, citrate is an antioxidant; oxidant injury may play a role in the deposition of insoluble calcium salts in the kidneys. sixth, ingestion of citrate influences intestinal absorption of iron and trace elements (e.g., aluminum, zinc) (18); whether this could affect the expression of PKD is not known. seventh, administration of a potassium salt might alleviate intracellular acidosis and the associated increase in renal ammonia synthesis.

Shohl (19) first demonstrated, more than 60 yr ago, that administration of citrates to rats on a rachitogenic diet could prevent rickets. Interestingly, sodium citrate alone or citric acid alone did not cure the rickets, but a combination of citrate and citric acid in the diet did. he concluded that the beneficial effects of citrates were related to their specific properties and not to acid-base effects alone. although the study of Shohl was done on a different disease, it seems reasonable to suggest that the beneficial effects of KCitr in the present study, likewise, are not only due to acid-base effects but also reflect the many other actions of citrates in the body.

The maintenance of a normal GFR in the KCitr-treated rats with PKD may seem puzzling, because these animals clearly had prominent changes in renal structure. Both treated and untreated rats with PKD had kidneys that were about twice the normal size. There was less cystic enlargement and fewer interstitial changes in the KCitr-treated animals than in the untreated rats with PKD. It should be noted that GFR may be normal, despite markedly enlarged cystic kidneys in both rats (15, 20) and people (21). The reason for the decline in GFR in the untreated rats with PKD is most likely related to the severe anatomical changes observed. these rats had kidneys with marked interstitial damage and greatly enlarged cysts. With widening of the interstitial spaces and more large cystic nephrons per unit mass of kidney, normal nephrons must be lost, explaining the reduced GFR. Indeed, when tubule/cyst lumen areas were measured (Figure 2), more regions had to be surveyed to count 250 lumens per kidney. Thus, there is clearly a loss of patent nephrons in untreated cystic kidneys.

There are several important differences between this study and an earlier study by Torres et al. (4) on the effects of alkali therapy in Han:SPRD rats. this study used animals at 3 mo of age, whereas Torres et al. examined 2-mo-old animals. The onset of treatment was about the same in both studies (3 to 4 wk of age). The different ages at which measurements were made may explain why in the present work the treatment did not result in a difference in kidney size (Figure 1). Studies in this laboratory on five 2-mo-old rats with PKD show that KCitr treatment is indeed associated with a smaller kidney size (unpublished data). this finding is consistent with the findings of Torres et al. with other alkalinizing agents. Cowley et al. (2) reported that kidney size in heterozygous male Han:SPRD rats with PKD reaches a peak value at 8 wk (2 mo) of age and then declines. Thus, the effect of a treatment on kidney size may depend on rat age. Moreover, overall kidney size does not indicate the functional state of cystic kidneys. Also, in the present study, the histology of treated and untreated cystic kidneys was different despite their being the same size.

Other differences from the study by Torres et al. (4) should be noted. First, in the present study, KCitr treatment produced a highly significant improvement, in fact, a normalization of GFR. By contrast, bicarbonate therapy did not yield any statistically significant improvement in creatinine clearance (4). Second, Torres et al. used metabolic cages to collect urine and calculated GFR from the endogenous creatinine clearance. In the present study, GFR was determined by direct collection of ureteral urine and by use of a synthetic inulin, polyfructosan, allowing this determination to be more accurate (22). Third, the absence of data on normal animals in the article by Torres et al. precludes judging how sick the animals with PKD were and how much of an improvement the treatments produced. Finally, the KCitr treatment, unlike some treatments with bicarbonate salts (4), produced no untoward effects on the animals, such as impaired growth or precipitation of calcium salts in the kidneys.
Although treatment with KCitr proved to be remarkably effective in preventing a decline in GFR in young Han:SPRD rats with PKD, it remains to be seen whether this treatment would be effective over a more extended period of time, and whether it would be beneficial in patients with PKD. Oral potassium citrate and citric acid therapy is not new; this combination is widely used for treating patients with renal calculi or renal tubular acidosis (RTA). Igarashi and coworkers (23,24) found that renal cysts are a common complication in patients with primary distal RTA. They reported that 5 yr of alkali therapy with sodium and potassium citrate was effective in stabilizing the number and size of renal cysts in one patient (23). In a later report (24), however, they state that alkali therapy did not prevent an increase in number of renal cysts in three patients with distal RTA. The effect of KCitr ingestion on the progression of autosomal dominant PKD in patients deserves study.

The dose of KCitr administered in the present rat experiments produced no detrimental effect on body growth or appearance and no obvious gastrointestinal or BP disturbances. Assuming a daily fluid intake of 50 ml/d for a 300-g rat (body surface area = 0.039 m²), the rats consumed 23 g of potassium citrate and 16.5 g of citric acid per m² body surface area per day. In adult patients, the usual daily oral dose of potassium citrate and citric acid, for use as an antiurolithic or systemic or urinary alkalizer, is approximately 7 to 14 g of potassium citrate and 2 to 4 g of citric acid per m² body surface area (25), assuming an average body surface area of 1.73 m². Whether such lower doses would be beneficial in slowing renal cystic disease is an open question. One caveat is that administration of potassium citrate to patients with advanced PKD may be dangerous because of the risk of hyperkalemia. It is important to note that in the present study KCitr treatment was begun at a young age, at a time when GFR seems to be normal in heterozygous cystic rats, as judged from measurements of serum creatinine and urea nitrogen concentrations (2,3). It is
Table 2. Histologic evaluation of kidneys from KCitr-treated and untreated rats with PKD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tap Water (n = 6)</th>
<th>KCitr (n = 7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intersitial damage score</td>
<td>3.0 ± 0</td>
<td>2.3 ± 0.49</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cyst/tubule lumen area (μm²)^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median</td>
<td>2340 ± 530</td>
<td>1734 ± 220</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>mean</td>
<td>6529 ± 892</td>
<td>3534 ± 527</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of large cysts per 26-mm² cortex</td>
<td>15 ± 6.6</td>
<td>6 ± 2.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BrdU-positive nuclei per 2.54-mm² cortex</td>
<td>9 ± 12</td>
<td>2 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>cyst/tubule cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>interstitial cells</td>
<td>51 ± 30</td>
<td>13 ± 8.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

^a BrdU, bromodeoxyuridine. Other abbreviations as in Table 1.
^b In kidney sections from 14 normal rats, median tubule lumen area averaged 887 ± 170 μm², and mean tubule lumen area averaged 1170 ± 227 μm²; no effect of KCitr ingestion was seen.
^c Large cysts were defined as having a lumen cross section greater than 50,000 μm².
^d In kidney sections from 14 normal rats, the number of BrdU-positive nuclei per 2.54-mm² cortex averaged 1 ± 2.1 for tubule epithelial cells and 1 ± 2.0 for interstitial cells. No effect of KCitr ingestion was seen in the normal rats.

not known whether KCitr treatment of animals with more advanced disease would be beneficial.

In conclusion, chronic intake of KCitr in young Han:SPRD rats with PKD prevented the fall in GFR that is usually seen in these animals, thus helping to maintain good renal function. The kidneys, although enlarged, had less severe interstitial damage and smaller cysts. If such striking effects on GFR were to be seen in other species, this might prove to be a valuable treatment for patients, because it is the decline in GFR that puts PKD patients at peril. The mechanisms involved in the action of KCitr remain to be elucidated.

Acknowledgments

I am indebted to Dr. Judith A. Tanner for her encouragement and help in analyzing the data, to Dr. James A. McAteer for preparing the histologic slides and critiquing the manuscript, to Dr. Lynn R. Willis for use of his atomic absorption spectrophotometer, to Dr. H. Glenn Bohlen for use of his Image 1 system, and to the Polycystic Kidney Research Foundation for grant support.

References


