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Dietary Citrate Treatment of Polycystic Kidney Disease in Rats

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Key Words
Autosomal-dominant polycystic kidney disease · Calcium citrate · Sodium citrate · Transforming growth factor-β · Han:SPRD rat

Abstract
Progression of autosomal-dominant polycystic kidney disease (ADPKD) in the heterozygous male Han:SPRD rat is dramatically slowed by ingestion of potassium or sodium citrate. This study examined the efficacy of delayed therapy with sodium citrate, the effect of sodium citrate therapy on kidney cortex levels of transforming growth factor-β (TGF-β), and the response to calcium citrate ingestion. Rats were provided with citrate salts in their food, and renal clearance, blood pressure, blood chemistry, and survival determinations were made. Sodium citrate therapy was most effective when started at age 1 month, and delay of therapy until age 3 months produced no benefit. Kidney cortex TGF-β levels were elevated in 3- and 8-month-old rats with ADPKD, but not in 6-week-old rats. Sodium citrate treatment, started at age 1 month, lowered TGF-β levels to normal in 3-month-old rats, but this is probably not the primary mechanism of citrate's beneficial effect. Calcium citrate had only a modest effect in preserving glomerular filtration rate. Effective treatment of ADPKD in this rat model requires early administration of a readily absorbed alkalinizing citrate salt. Existing data on ADPKD patients on vegetarian diets or with kidney stones should be studied in light of these findings.

Introduction

Autosomal-dominant polycystic kidney disease (ADPKD) is the fourth leading cause of renal failure in the world. In this disorder, numerous fluid-filled epithelial cysts develop from nephrons or collecting ducts and grow in both kidneys. These processes ultimately lead to greatly enlarged kidneys and a fall in glomerular filtration rate (GFR). Treatments to slow or halt the progression of this disease are extremely limited [1].

Torres et al. [2] first tested dietary treatment of ADPKD with alkali (sodium or potassium bicarbonate) in an animal model, the heterozygous (cy/+) Han:SPRD rat. They found that treated animals had less kidney enlargement, cystic dilation, and interstitial disease, but ingestion of concentrated bicarbonate solutions resulted in precipitation of calcium phosphate in the kidney medulla. We subsequently treated cy/+ rats with potassium citrate, starting when the rats were 1 month of age [3, 4]. An advantage of using citrate is that, unlike bicarbonate, it

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furnishes alkali without producing kidney stones. This treatment led to dramatic preservation of GFR. The GFR of treated rats at age 6 months was essentially normal, in contrast to untreated litter mates in which GFR had declined to one-third of normal. Furthermore, the untreated rats died at an average age of 10 months, but the treated rats survived to an average age of 17 months. Sodium citrate was as effective as potassium citrate in preserving GFR in 3-month-old cy/+ rats [4].

In the present work, we explored further the effects of citrate on the progression of PKD in the Han:SPRD cy/+ rat. First, we investigated whether delaying treatment until late in the disease, when GFR has already been compromised, could confer some benefit. Second, we investigated the possibility that citrate may be acting by influencing transforming growth factor-β (TGF-β) levels in the kidney. TGF-β is a multifunctional cytokine involved in the regulation of cell proliferation, differentiation, angiogenesis, and extracellular matrix production [5]. It is thought to play a major role in the production of interstitial fibrosis in kidney disease [6]. Recently, it was reported that treatment of doxorubicin (adriamycin)-induced nephropathy in rats with sodium bicarbonate solutions for drinking water reduces renal injury, renal production of TGF-β, and urinary excretion of TGF-β [7]. We predicted that TGF-β might similarly play a role in the prominent interstitial fibrosis seen in PKD [3, 4, 8, 9], and hypothesized that kidney cortex TGF-β levels would be elevated in untreated rats with cystic disease and decreased with citrate treatment. Third, we determined whether calcium citrate might be effective in ameliorating PKD; these experiments were actually prompted by the question of a PKD patient, who noted that calcium citrate is a commonly used dietary supplement.

Citrate is a normal dietary constituent. Large doses of potassium citrate are used to treat kidney stone disease in patients [10, 11] and might possibly be of benefit in slowing the progression of ADPKD. Therefore, within the confines of our current knowledge, we compare the characteristics of the rat model with those of human ADPKD, and discuss the extent to which our findings may be applicable to patients.

Methods

Animals

Experiments were performed on male heterozygous rats with PKD (cy/+) and their normal littermates (+/+), except for one group of homozygous rats with PKD (cy/cy) of both sexes in which we measured kidney TGF-β levels. The breeding colony was originally obtained in 1995 from the Polycystic Kidney Program at the University of Kansas. The rats were provided with a standard diet containing 24% protein, 6% fat, and 0.52% sodium (Teklad 6% mouse/rat diet 7002; Harlan, Madison, Wisc., USA). All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Delayed Treatment

Rats with PKD were provided with sodium citrate (trisodium citrate dihydrate, 3.2%) in pelleted feed starting at 1, 2, or 3 months of age or were continued on the standard feed. The sodium citrate-supplemented feed contained 2.4× the sodium of the standard feed. Consumption of the sodium citrate-supplemented feed, measured in 3-month-old rats with PKD, averaged 7.0 ± 1.0 g/100 g body weight per day (n = 5) and provided an extra daily intake of 0.76 mmol citrate/100 g body weight. Normal rats were provided with the sodium citrate-supplemented feed at 1 month of age or were continued on the standard diet. In all animals, at age 6 months, systolic blood pressure was measured by tail cuff plethysmography and a tail vein blood sample was collected for measurements of plasma urea and creatinine concentrations. The rats with PKD were then continued on the sodium citrate-supplemented feed, and survival times were noted.

TGF-β Experiments

Normal (+/+) male rats and male rats with PKD (cy/+) were started on 6.0% sodium citrate-supplemented feed at age 4 weeks and were studied at age 6 weeks or 3 months. This diet had 3.7× the sodium content of the standard feed. Consumption of the 6% sodium citrate-supplemented feed, measured in 3-month-old normal rats and rats with PKD, averaged 7.5 ± 0.4 g/100 g body weight per day (n = 10), and provided an extra daily citrate intake of about 1.5 mmol/100 g body weight. Untreated groups, on the standard diet, included (1) cy/cy rats of both sexes, 3–4 weeks old; (2) +/+ and cy/+ male rats, 6 weeks old; (3) +/+ and cy/+ male rats, 3 months old; and (4) cy/+ male rats, 8 months old.

TGF-β was measured in kidney cortex tissue using a commercially available sandwich ELISA kit (Quantikine, human TGF-β1, R & D Systems, Minneapolis, Minn., USA). The nonfasted rats were anesthetized with Inactin (130 mg/kg body weight), and the kidneys were exposed. Tail vein blood was collected for measurements of plasma urea and creatinine concentrations, and bladder urine was sampled for measurement of pH. Slices of cortex were then cut from the kidneys and rapidly frozen in liquid nitrogen. Samples were stored at –80°C until use. A weighed amount of kidney tissue, about 200 mg, was homogenized in five times its volume of pH 7.4 buffer containing 20 m*M* Trizma-HCl (Sigma, St. Louis, Mo., USA), 255 m*M* sucrose, 1 m*M* EDTA, and protease inhibitor cocktail (Sigma Cat. No. P-8340). The homogenates were briefly centrifuged and TGF-β was acid-activated by mixing 0.2 ml homogenate with 0.2 ml solution of 0.2 *M* HCl, 0.1% Tween, and 0.2% non-fat dry milk blocker (Bio-Rad, Hercules, Calif., USA) in phosphate-buffered saline (PBS) for 10 min at room temperature. The pH of the sample was then returned to 7.2–7.6 by adding 0.4 ml wash solution (0.1% Tween-20, 0.2% blocker, in PBS) and 43–45 μl 1.2 *M* NaOH/0.5 *M* HEPES. TGF-β standards (125, 250, 500, and 1,000 pg/ml) were prepared in the wash solution. Subsequent steps followed the kit recommendations. The samples were read in a microplate reader at a wavelength of 450 nm. Recovery of TGF-β, added as an internal standard to kidney cortex samples, averaged 99 ± 11% (n = 9).

Table 1. Effects of 3.2% sodium citrate (NaCitr)-supplemented diet on normal (+/+) male rats and heterozygous (cy/+) male rats with PKD

	Plasma urea mmol/l	Plasma creatinine µmol/l	Systolic blood pressure mm Hg	Survival time days
+/+ rats, standard feed	8.1 ± 0.8 (n = 21)[b]	62 ± 12 (n = 21)[a]	105 ± 6 (n = 20)[b]	
+/+ rats, NaCitr at 1 month	8.3 ± 0.7 (n = 6)	66 ± 5 (n = 6)	107 ± 6 (n = 6)	
cy/+ rats, standard feed	22.0 ± 6.5 (n = 24)	118 ± 41 (n = 24)	126 ± 17 (n = 12)	294 ± 50 (n = 14)
cy/+, NaCitr at 1 month	12.3 ± 1.2 (n = 14)[b]	71 ± 11 (n = 14)[a]	111 ± 6 (n = 14)[a]	429 ± 54 (n = 13)[b]
cy/+, NaCitr at 2 months	14.9 ± 1.4 (n = 6)[b]	99 ± 11 (n = 6)	108 ± 5 (n = 6)[a]	296 ± 40 (n = 6)
cy/+, NaCitr at 3 months	18.1 ± 3.7 (n = 7)	120 ± 47 (n = 7)	123 ± 16 (n = 7)	249 ± 58 (n = 8)

Values are means ± SD (n = number of rats). Rats were started on NaCitr-supplemented feed at age 1, 2, or 3 months or were kept on standard feed. Plasma urea, plasma creatinine, and systolic blood pressure were measured when the rats were 6 months old.

[a] $p < 0.01$ and

[b] $p < 0.001$ compared with cy/+ rats on standard feed.

Calcium Citrate Experiments

To test whether calcium citrate would be effective in maintaining GFR, the standard diet was supplemented with 3.0% tricalcium citrate tetrahydrate in pelleted feed, starting at age 1 month. This increased the calcium level of the feed from 1.91% to 2.54%. Daily food intake by the rats on 3.0% calcium citrate-supplemented feed averaged 7.3 ± 1.0 g/100 g body weight (n = 5), corresponding to an extra daily intake of 0.77 mmol citrate/100 g body weight.

Clearance measurements were done on treated and untreated male cy/+ rats at age 3 months, exactly as described before [3]. Briefly, the rats were deprived overnight of food, but not drinking fluids, and were anesthetized with Inactin. They were placed on a heated animal board, and trachea, femoral artery, femoral vein, and left ureter were cannulated. After a priming dose, the rats were infused with a 3% solution of polyfructosan in 0.9% NaCl at 3.0 ml/h. Three timed 20-min urine collections were obtained with mid-period arterial blood sampling. GFR for the left kidney was calculated from the renal clearance of polyfructosan using standard formulas, and expressed per 100 g body weight. An anaerobic arterial blood sample and three ureteral urine samples were collected for measurements of pH.

Chemical Methods

Polyfructosan in urine and plasma filtrates was measured by the anthrone method [12], plasma urea concentration with urease and the Berthelot reaction [13], plasma creatinine by the alkaline picrate method, plasma and urine calcium by atomic absorption spectrophotometry, and plasma phosphate by the Fiske-SubbaRow method.

Statistical Methods

Data are presented as means ± SD. They were analyzed by ANOVA, after a preliminary test for homogeneity of variances. Individual groups were compared with the Bonferroni method. If variances were heterogeneous, the Kruskal-Wallis test and Dunn's test were used to compare means. $p < 0.05$ was considered significant.

Results

Delayed Treatment

In normal rats, ingestion of 3.2% sodium citrate-supplemented feed for 5 months had no significant effect on plasma urea or creatinine concentrations or on systolic blood pressure (table 1). Untreated 6-month-old cy/+ rats had elevated plasma urea and creatinine concentrations and elevated systolic blood pressure when compared to normal rats on standard feed. Treatment of cy/+ rats with sodium citrate-supplemented feed from 1 month of age resulted in significantly lower plasma urea and creatinine concentrations and lower systolic blood pressure than in untreated 6-month-old cy/+ rats. When treatment was delayed until age 2 months, cy/+ rats had a significantly lower plasma urea concentration and systolic blood pressure, but plasma creatinine was not significantly reduced, compared to untreated 6-month-old cy/+ rats. When treatment was delayed until age 3 months, no beneficial effects were seen.

We previously reported that untreated male cy/+ rats in our colony live for 288 ± 39 days [4], compared to 294 ± 50 days in the present study (table 1). Hence, there has been no change in survival time in our colony over a period of several years. Rats with PKD eventually develop severe uremia, and hence we presume they die because of end-stage renal disease. Animals in our colony are free of pulmonary disease. Ingestion of sodium citrate significantly ($p < 0.001$) prolonged survival of rats with PKD by about 4.5 months when treatment was started at age

Table 2. Effects of 6% sodium citrate (NaCitr)-supplemented feed on kidney function and TGF-β levels in normal (+/+) rats and in homozygous (cy/cy) and heterozygous (cy/+) rats with PKD

	Body weight g	Plasma urea mmol/l	Plasma creatinine μmol/l	Urine pH	Kidney cortex TGF-β ng/mg tissuee
cy/cy, 3–4 weeks old, standard feed (n = 9)	53 ± 51	24 ± 18[d]	280 ± 34[d]		8.0 ± 1.7
6-week-old rats					
+/+, standard feed (n = 5)	223 ± 53	7.9 ± 1.2	45 ± 10	6.56 ± 0.34	7.8 ± 2.6
cy/+, standard feed (n = 8)	209 ± 25	10.1 ± 1.3	74 ± 12[g]	6.24 ± 0.24[a]	6.0 ± 2.7
+/+, NaCitr (n = 5)	215 ± 20	8.2 ± 1.1	57 ± 12	7.72 ± 0.23[g]	8.5 ± 4.5
cy/+, NaCitr (n = 6)	206 ± 18	9.3 ± 0.6	60 ± 17	7.52 ± 0.37[h]	7.2 ± 0.8
3-month-old rats					
+/+, standard feed (n = 5)	384 ± 25	7.8 ± 0.8	52 ± 10	6.66 ± 0.22[b]	6.3 ± 1.7
cy/+, standard feed (n = 6)	371 ± 16	21.5 ± 2.1[d]	89 ± 22[c]	6.04 ± 0.30	16.2 ± 3.3[d]
+/+, NaCitr (n = 5)	375 ± 24	9.1 ± 0.9	66 ± 6	7.29 ± 0.47	7.7 ± 2.2
cy/+, NaCitr (n = 5)	406 ± 12	9.1 ± 0.9[f]	59 ± 9[e]	7.31 ± 0.53[d]	6.0 ± 2.7[f]
8-month-old cy/+ rats, standard feed (n = 5)	545 ± 24	34.3 ± 12.5[d]	161 ± 65[d]		15.6 ± 2.5[d]

Values are means ± SD (n = number of rats). Treatment with NaCitr-supplemented feed was started at 4 weeks of age. All rats were males, except for the cy/cy group which included both males and females.

[a] n = 7.

[b] n = 4.

[c] $p < 0.01$ and

[d] $p < 0.001$ compared with 3-month-old +/+ rats on standard feed.

[e] $p < 0.05$ and

[f] $p < 0.001$ compared with 3-month-old cy/+ rats on standard feed.

[g] $p < 0.001$ compared with 6-week-old +/+ rats on standard feed.

[h] $p < 0.001$ compared with 6-week-old cy/+ rats on standard feed.

1 month, but had no significant effect if started at 2 or 3 months (table 1). The life span with sodium citrate therapy started at 1 month (429 ± 54 days) is shorter than what we had previously seen [4] with potassium citrate (506 ± 33 days), but the average daily citrate intake was higher in the potassium citrate experiments (0.92 vs. 0.76 mmol/100 g body weight).

TGF-β Experiments

Table 2 reports functional measurements, together with kidney cortex TGF-β measurements, on rats of different ages. Sodium citrate treatment had no significant effect on body weights in 6-week-old and 3-month-old rats, and produced an expected increase in urine pH. Plasma urea concentration (in 3-month-old rats) and creatinine concentration (in 6-week-old and 3-month-old rats) were significantly higher than normal in rats with PKD on the standard feed, but were significantly decreased by citrate treatment. Daily water intake by 3-month-old rats on the 6% sodium-citrate feed averaged 24 ± 2 ml/100 g body weight (n = 5) in normal animals, and 23 ± 2 ml/100 g body weight (n = 5) in rats with PKD; compared to published water intakes on standard feed [14], these values are significantly ($p < 0.001$) higher for the normal rats but not for the rats with PKD.

Kidney cortex TGF-β levels (table 2) were not elevated in untreated homozygous rats with PKD, despite massively enlarged cystic kidneys and severe renal failure (elevated plasma urea and creatinine concentrations). In 6-week-old rats, there was no difference in kidney cortex TGF-β concentration between normal rats and rats with PKD, and treatment with sodium citrate-supplemented feed had no effect. By contrast, in 3- and 8-month-old rats with PKD, TGF-β levels were increased to about 2.5 times normal. We looked at the effect of sodium citrate treatment up until age 3 months, and it prevented an increase in TGF-β level. All together, these results suggest that vigorous cyst development, as is seen in young cy/cy and cy/+ rats, occurs without an increase in kidney TGF-β level. It appears that elevated kidney levels of TGF-β are a relatively late event in PKD in Han:SPRD rats.

Table 3. Effects of 3.0% calcium citrate-supplemented feed in 3-month-old heterozygous (cy/+) male rats with PKD

	Standard feed (n = 4)	Calcium citrate-supplemented feed (n = 5)	p value
Body weight, g	357 ± 8	396 ± 13	<0.01
Left kidney weight, g	2.40 ± 0.30	2.47 ± 0.17	NS
MABP, mm Hg	120 ± 14	117 ± 5	NS
Left kidney GFR, µl/min/100 g body weight	229 ± 44	295 ± 30	<0.05
Plasma [Ca^{2+}], m*M*	2.16 ± 0.07	2.20 ± 0.09	NS
Plasma PO_4, mg/dl	8.87 ± 0.35	8.10 ± 0.69	NS
Urine Ca^{2+} excretion, nmol/min	9 ± 5	15 ± 7	NS
Arterial blood pH	7.32 ± 0.03	7.32 ± 0.03	NS
Urine pH	5.53 ± 0.22	5.86 ± 0.24	NS

Values are means ± SD.

Calcium Citrate Experiments

Rats with PKD were given 3.0% calcium citrate-supplemented feed, starting at age 1 month, and they were studied at age 3 months (table 3). GFR of rats with PKD treated with calcium citrate was significantly higher than that of untreated rats, but still far below values recorded in normal 3-month-old rats (about 500 µl/min/100 g body weight [3]). Body weight was 11% higher in the calcium citrate-treated rats, for unknown reasons. The effects of calcium citrate intake on plasma calcium concentration, plasma phosphate concentration, urine calcium excretion, and blood and urine pH did not reach statistical significance (table 3). The modest improvement in GFR and lack of significant changes in the other renal or acid-base parameters suggest that dietary calcium citrate is not an effective alkalinizing agent.

Discussion

The present study demonstrates that effective citrate therapy for PKD in the Han:SPRD rat requires administration of a readily absorbed alkalinizing citrate salt, starting before the disease has progressed too far. Early treatment with citrate is essential. When the start of treatment was delayed until the rats with PKD were 3 months old, a time when GFR is one-half of normal [3], no benefit was seen (table 1). Starting treatment with sodium citrate in the feed at age 2 months led to a lower plasma urea concentration and systolic blood pressure, when compared to untreated rats, but the plasma creatinine and survival time were not improved. Only when treatment was started at age 1 month was there a significant beneficial effect on all measured parameters: plasma urea, plasma creatinine, systolic blood pressure, and survival time.

The observation that citrate treatment works in the Han:SPRD rat only when applied early, at a time of vigorous cyst development, suggests that it might influence the process of cystogenesis. The exact mechanism of action is a mystery. Since cyst formation in the Han:SPRD rat occurs mainly in proximal tubules [8, 9], and citrate is selectively taken up by proximal tubule cells and metabolized to bicarbonate [15], it is tempting to suggest that the secret lies therein.

The extra intake of sodium in the rats on sodium citrate-supplemented feed did not result in an increase in systolic blood pressure in either the normal rats or rats with PKD (table 1). In fact, systolic blood pressures were significantly decreased in rats with PKD on the sodium citrate diet for 4 or 5 months, when compared to untreated rats with PKD. This is most likely related to improved renal function. Others have reported that even large doses of sodium citrate do not increase blood pressure in rats or people [16, 17]. On the other hand, Keith et al. [18] administered 3.3% NaCl-feed (1.3% sodium) to Han:SPRD rats with PKD, starting at age 3 weeks. This feed contains as much sodium as our 3.2% sodium citrate-supplemented feed. In contrast to our findings, they found that treatment with the chloride salt produced severe hypertension, enlarged kidneys, and worse renal histology. Apparently, sodium must be consumed as the chloride salt for it to produce hypertension. The beneficial effects of dietary sodium citrate on renal function clearly depend on citrate, not sodium.

Nakamura et al. [19] previously demonstrated that kidney TGF-β mRNA levels are elevated in 16- and 30-

week-old, but not in 8-week-old, pcy/pcy mice with slowly developing autosomal-recessive PKD. They observed focal interstitial inflammatory infiltrates and increased interstitial connective tissue in 16- and 30-week-old, but not in younger, mice. We found that kidney cortex TGF-β protein levels are not higher than normal in young (6-week-old) cy/+ rats or in (3- to 4-week-old) cy/cy rats with severe cystic disease, consistent with the report that interstitial fibrosis is not evident by light microscopy in such young rats [8]. In older rats, 3 or 8 months of age, when there is extensive interstitial fibrosis [3, 4, 8, 9], kidney cortex TGF-β levels were increased to about 2.5 times normal. Treatment with citrate decreased TGF-β levels in the 3-month-old rats (table 2) and decreased interstitial fibrosis [3]. These results suggest that elevated TGF-β levels are associated with renal fibrosis in PKD kidneys, but both are relatively late events in the disease. Our conclusions are tempered by the fact that we measured total TGF-β protein levels, and these may not reflect the active levels of TGF-β or its signaling [5, 6]. A reduction in kidney cortex TGF-β level is probably not the primary mechanism of citrate's amelioration of cystic disease, because beneficial effects were seen with citrate treatment started at age 1 month, before kidney TGF-β was elevated.

Calcium citrate is commonly consumed as a calcium supplement for prevention of osteoporosis. The usual human dose is about 800 mg elemental calcium (3.80 g calcium citrate) per day or about 3.8 mmol/day per m^2 body surface area for a 70-kg man. For comparison, our rats consumed an extra 32 mmol calcium citrate/day/m^2 body surface area. This high intake of calcium citrate produced only a modest improvement in GFR in our rats with PKD. The failure of calcium citrate to be more effective probably reflects relatively poor intestinal absorption of the calcium salt when compared to the potassium or sodium salts.

The Han:SPRD cy/+ rat is a useful model for studying possible treatments for ADPKD. There are, however, some significant differences between cystic disease in the Han:SPRD rat model and ADPKD in people. First, the abnormal gene that produces cystic disease in the rat is not homologous to the *PKD1* or *PKD2* genes in the human [20]. Second, the time courses of changes in kidney size differ in the rat and people. In the male cy/+ rat, kidney size increases markedly between ages 3 and 8 weeks, declines between ages 8 and 24 weeks, and subsequently increases [8]. In patients with ADPKD, the kidneys appear to enlarge progressively with age. Third, GFR declines appreciably early in the disease in the male cy/+ rat. In 3-week-old rats, plasma creatinine concentration is normal [unpubl. data], but by age 6 weeks, plasma creatinine is significantly increased (table 2). By age 3 months, GFR averages 50% of normal [3], and by age 6 months it is 37% of normal [4]. By contrast, in patients, GFR appears to be preserved for many years, but declines rapidly late in the disease [21, 22]. Fourth, cyst formation is primarily a proximal tubule event in the Han:SPRD rat, whereas it involves all nephron segments in human ADPKD [23, 24].

Despite these differences between the rat model and human disease, the basic pathophysiological mechanisms involved in cyst development and growth, interstitial inflammation and fibrosis, and glomerular failure may be similar. Thus our studies in rats could inform the protocols needed to establish whether citrate treatment will be useful in ADPKD patients. For example, from our studies on rats, the timing and form of dietary citrate treatment are important factors determining whether citrate is of benefit. Since the initial progression of ADPKD in people is usually slower than in the rat, there may be a longer window of time when citrate therapy might help patients.

The dramatic success of citrate therapy in rats, and the fact that the treatment poses no apparent harm to people whose GFR is not yet compromised, are compelling reasons to investigate this in patients. There are two populations of ADPKD patients who may be of particular interest for a retrospective study and have probably been followed for many years: those with nephrolithiasis and those who are vegetarians. Nephrolithiasis is common in patients with ADPKD [25], and potassium citrate has often been used to treat their stone disease. Vegetarian diets are more alkalinizing than mixed diets [26, 27]. A limitation of such a study, common to all population studies in ADPKD patients, would be the variable expression of the disease. Urine pH, however, would afford an easily measured index of alkalinization. Examination of the medical records from these two groups of ADPKD patients may reveal whether alkali can mitigate the ravages of this devastating disease.

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