Sequential studies of oxalate dynamics in primary hyperoxaluria

R. W. E. WATTS¹, N. VEALL² AND P. PURKISS¹

¹Division of Inherited Metabolic Diseases and ²Division of Radioisotopes, Clinical Research Centre, Harrow, Middlesex, U.K.

Summary

1. We have measured the total plasma clearance, renal clearance and equilibrium distribution volume of [¹⁴C]oxalate, and the urinary oxalate excretion rate and plasma oxalate levels at approximately 6 month intervals for up to 2.5 years in five patients with primary hyperoxaluria. The renal clearance and distribution volumes of [⁹⁹ᵐTc]DTPA (diethylenetriaminepenta-acetate) were measured simultaneously to provide estimates of glomerular filtration rate (GFR) and extracellular fluid volume (ECF). The same measurements were made on each of five normal volunteers.

2. Clearances and distribution volumes were measured with a modified single injection technique.

3. The oxalate clearance was two to three times the simultaneously measured GFR in the patients and control subjects. The renal clearance of oxalate was less than the total plasma clearance in the patients. The oxalate distribution volume was approximately 1.5 times the ECF in both the patients and controls. Only small changes were observed over a 2.5 years period in these particular patients.

4. The plasma oxalate concentration was derived from the urinary oxalate excretion rate and the plasma [¹⁴C]oxalate clearance. It was raised in the patients. The oxalate removal rate was derived from the total plasma clearance and the plasma oxalate concentration.

Key words: diethylenetriaminepenta-acetate, extracellular fluid volume, glomerular filtration rate, oxalate, oxalosis, oxaluria, primary hyperoxaluria, renal clearance.

Abbreviations: ECF, extracellular fluid; GFR, glomerular filtration rate; ODV, oxalate distribution volume; Pox, plasma oxalate concentration; PCox, plasma oxalate clearance; RCox, renal oxalate clearance.

Introduction

Type I primary hyperoxaluria is an inborn error of metabolism in which there is a sustained increase in urinary oxalate and glycylate excretion, and the lack of cytosolic glyoxylate:2-oxoglutarate carboligase activity has been described as the underlying metabolic lesion [1]. In type II primary hyperoxaluria [2], the hyperoxaluria is accompanied by L-glyceric aciduria and the deficient enzyme is D-glycerate dehydrogenase. The high level of urinary oxalate excretion cannot usually be modified to a therapeutically useful degree by dietary or other means [3], except that in some type I cases it can be reduced by pharmacological doses of pyridoxine [4]. This effect has not been sought in type II. The patients present with urolithiasis, develop renal and extra-renal oxalosis, and die from renal failure [5]. The terminal illness often evolves rapidly and the present work was initiated to see if this could be predicted by studying some dynamic aspects of oxalate handling in vivo.

We measured the total plasma (PCox) and renal (RCox) clearances and the oxalate distribution volume (ODV) simultaneously with the glomerular filtration rate (GFR), extracellular fluid space...
volume (ECF) and plasma oxalate concentration (Pox), sequentially over a period of up to 2.5 years. The total oxalate removal rate (PCox x Pox) has been calculated and compared with the simultaneously measured urinary oxalate excretion rate.

Some of these results have been the subject of a preliminary communication [6].

Materials and methods

Subjects

Patients. Background data on the patients at the beginning of the study and on the control subjects are presented in Table 1. Urinary oxalate excretion and blood urea values were essentially unaltered during the study. All of the patients were advised to exclude oxalate rich foods and beverages, and citrus fruits, from their diet and to limit their calcium intake to about 18 mmol/day. Patients nos. 1, 2 and 3 are typical cases of primary hyperoxaluria and have been referred to previously as patients nos. 1, 2 and 3 [3].

Patient no. 1 was clinically stable during the study although she had bilateral urinary stones. Her urinary oxalate excretion increased from about 0.6 mmol/day at the beginning of the study to about 1.1 mmol/day at the end of the study.

Patient no. 2 passed several small stones spontaneously before the first study. He was asymptomatic between the first and third studies. His stones increased in size and he experienced a left-sided obstructive uropathy after the last study.

Patient no. 3 passed one small stone on the day of the first study. A further stone was passed midway between the first and second studies. Pyridoxine treatment (1.2 g daily) was begun at the time of the second study. She had an attack of left-sided renal colic with ureteric obstruction between the second and third studies; this was treated successfully by conservative measures. She remained clinically stable between the third and fourth studies.

Patient no. 4 had her first symptoms of urolithiasis at age 44 years, nephrolithotomies when she was 49 and 54 years old and a left pyeloplasty for pelviureteric junction obstruction at 57 years. She had three small stones in the left renal pelvis throughout the period of study. Their size did not change, and she remained clinically stable. This patient has sustained hyperoxaluria but normal glycollate excretion. She is not therefore a classical case of type I primary hyperoxaluria nor is she a case of type II primary hyperoxaluria, her excretion of glycerate being normal.

Patient no. 5 is also atypical in that although she has been a recurrent calcium oxalate stone former since age 13 years and has hyperglycollic aciduria (5.5–7.6 mmol/day, normal value less than 1 mmol/day) her urinary oxalate excretion, which was 0.58 mmol day⁻¹ 1.73 m²⁻¹ at age 17 years decreased to a level within the normal range when her diet was modified as described above. She does, however, have an abnormally high oxalate removal and her plasma oxalate levels are at or just above the upper limit of normal (Table 2). These points support the correctness of the diagnosis of a mild variant form of primary hyperoxaluria even though the urinary oxalate excretion can be reduced to near normal by dietary restriction. She has been followed closely for about 12 years and has never had any symptoms suggesting intestinal disease which could cause oxalate hyperabsorption.

Control subjects. The control subjects were volunteers (two males, three females) aged 23–62 years from the staff.

Radioactive tracers

[⁹⁹ᵐTc]DTPA was prepared from a commercial kit (DRN 4362, Byk-Mallinkrodt (U.K.) Ltd) by the addition of [⁹⁹ᵐTc]pertechnetate from a sterile generator (Amersham International, code MCC 4) and used within 1 h of preparation. Ascending
TABLE 2. Plasma and renal clearances of $^{99m}\text{Tc}\text{DTPA}$ and $^{14}\text{C}\text{oxalate}$, extracellular fluid (ECF) and oxalate distribution volume (ODV) values in five patients and five controls

Derived values for plasma oxalate and total and non-renal removal rates are shown. All values except $P_{Ox}$ are corrected to a body surface area of 1.73 m$^2$.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years, months)</th>
<th>RC (ml/min)</th>
<th>PC (ml/min)</th>
<th>$P_{Ox}$ (ml/min)</th>
<th>$R_{C_{Ox}}$ (ml/min)</th>
<th>ECF (l)</th>
<th>ODV (l)</th>
<th>P$_{Ox}$ (µmol/l)</th>
<th>Oxalate removal rate (mmol/day)</th>
<th>Non-renal clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18,2</td>
<td>75.6</td>
<td>72.8</td>
<td>162</td>
<td>147</td>
<td>12.0</td>
<td>16.8</td>
<td>3.18</td>
<td>0.742</td>
<td>15</td>
</tr>
<tr>
<td>*18,6</td>
<td>47.7</td>
<td>40.9</td>
<td>181</td>
<td>168</td>
<td>14.4</td>
<td>19.1</td>
<td>5.39</td>
<td>1.40</td>
<td>1.928</td>
<td>4</td>
</tr>
<tr>
<td>19,0</td>
<td>77.4</td>
<td>56.5</td>
<td>172</td>
<td>151</td>
<td>13.2</td>
<td>22.6</td>
<td>3.29</td>
<td>0.815</td>
<td>0.479</td>
<td>21</td>
</tr>
<tr>
<td>19,2</td>
<td>84.8</td>
<td>85.3</td>
<td>182</td>
<td>178</td>
<td>11.9</td>
<td>18.7</td>
<td>3.52</td>
<td>0.923</td>
<td>0.178</td>
<td>4</td>
</tr>
<tr>
<td>19,10</td>
<td>74.6</td>
<td>74.5</td>
<td>167</td>
<td>10.8</td>
<td>13.1</td>
<td>20.1</td>
<td>1.78</td>
<td>0.479</td>
<td>0.047</td>
<td>20</td>
</tr>
<tr>
<td>20,3</td>
<td>68.3</td>
<td>76.4</td>
<td>149</td>
<td>10.8</td>
<td>7.10</td>
<td>1.59</td>
<td>3.18</td>
<td>0.742</td>
<td>1.928</td>
<td>7</td>
</tr>
<tr>
<td>20,6</td>
<td>78.5</td>
<td>77.7</td>
<td>170</td>
<td>12.9</td>
<td>20.8</td>
<td>6.78</td>
<td>1.66</td>
<td>0.742</td>
<td>1.928</td>
<td>0</td>
</tr>
<tr>
<td>15,0</td>
<td>67.2</td>
<td>60.2</td>
<td>139</td>
<td>12.0</td>
<td>20.6</td>
<td>3.16</td>
<td>7.78</td>
<td>32</td>
<td>11.73</td>
<td>32</td>
</tr>
<tr>
<td>15,7</td>
<td>53.0</td>
<td>52.4</td>
<td>159</td>
<td>11.8</td>
<td>20.6</td>
<td>26.5</td>
<td>6.07</td>
<td>10</td>
<td>3.03</td>
<td>10</td>
</tr>
<tr>
<td>16,3</td>
<td>65.0</td>
<td>66.3</td>
<td>153</td>
<td>11.0</td>
<td>19.2</td>
<td>26.9</td>
<td>5.93</td>
<td>21</td>
<td>16.85</td>
<td>21</td>
</tr>
<tr>
<td>12,2</td>
<td>58.5</td>
<td>57.1</td>
<td>152</td>
<td>12.4</td>
<td>19.6</td>
<td>21.2</td>
<td>4.64</td>
<td>13</td>
<td>7.14</td>
<td>13</td>
</tr>
<tr>
<td>13,2</td>
<td>63.2</td>
<td>65.6</td>
<td>139</td>
<td>11.5</td>
<td>19.2</td>
<td>13.8</td>
<td>2.96</td>
<td>10</td>
<td>1.85</td>
<td>10</td>
</tr>
<tr>
<td>13,8</td>
<td>61.7</td>
<td>60.0</td>
<td>121</td>
<td>12.3</td>
<td>20.3</td>
<td>17.4</td>
<td>3.66</td>
<td>25</td>
<td>1.68</td>
<td>25</td>
</tr>
<tr>
<td>13,10</td>
<td>44.1</td>
<td>44.9</td>
<td>90</td>
<td>11.0</td>
<td>15.9</td>
<td>20.7</td>
<td>2.68</td>
<td>8</td>
<td>1.23</td>
<td>8</td>
</tr>
<tr>
<td>61,0</td>
<td>53.4</td>
<td>49.6</td>
<td>120</td>
<td>11.1</td>
<td>17.6</td>
<td>5.36</td>
<td>0.926</td>
<td>13</td>
<td>0.926</td>
<td>13</td>
</tr>
<tr>
<td>61,5</td>
<td>54.1</td>
<td>61.0</td>
<td>123</td>
<td>11.6</td>
<td>19.4</td>
<td>5.44</td>
<td>0.964</td>
<td>10</td>
<td>0.964</td>
<td>10</td>
</tr>
<tr>
<td>62,1</td>
<td>50.3</td>
<td>52.3</td>
<td>123</td>
<td>10.2</td>
<td>16.9</td>
<td>6.43</td>
<td>1.14</td>
<td>4</td>
<td>0.63</td>
<td>4</td>
</tr>
<tr>
<td>62,6</td>
<td>43.0</td>
<td>37.0</td>
<td>114</td>
<td>11.2</td>
<td>15.9</td>
<td>5.43</td>
<td>0.891</td>
<td>9</td>
<td>0.891</td>
<td>9</td>
</tr>
<tr>
<td>62,8</td>
<td>51.7</td>
<td>50.1</td>
<td>109</td>
<td>9.7</td>
<td>14.8</td>
<td>4.08</td>
<td>0.640</td>
<td>8</td>
<td>0.640</td>
<td>8</td>
</tr>
<tr>
<td>27,6</td>
<td>83.5</td>
<td>71.3</td>
<td>178</td>
<td>13.9</td>
<td>24.8</td>
<td>2.05</td>
<td>0.525</td>
<td>33</td>
<td>0.525</td>
<td>33</td>
</tr>
<tr>
<td>28,1</td>
<td>72.4</td>
<td>60.3</td>
<td>185</td>
<td>10.4</td>
<td>16.1</td>
<td>1.62</td>
<td>0.440</td>
<td>7</td>
<td>0.440</td>
<td>7</td>
</tr>
<tr>
<td>28,6</td>
<td>91.4</td>
<td>91.1</td>
<td>204</td>
<td>11.3</td>
<td>19.6</td>
<td>2.01</td>
<td>0.590</td>
<td>46</td>
<td>0.590</td>
<td>46</td>
</tr>
<tr>
<td>29,1</td>
<td>62.8</td>
<td>58.4</td>
<td>188</td>
<td>10.4</td>
<td>17.1</td>
<td>1.15</td>
<td>0.311</td>
<td>12</td>
<td>0.311</td>
<td>12</td>
</tr>
</tbody>
</table>

*The transient decrease in renal function observed on this occasion may have been due to the recent passage of a stone.
chromatography on Kieselgel 60 (Merck, Darmstadt) plates with acetone/water (95:5) and 0.9% sodium chloride solution was used to confirm the absence of appreciable amounts of free pertechnetate and reduced unbound technetium.

$^{14}$C Oxalic acid was supplied by Amersham International (code CFA84). The specific radioactivity of each batch was adjusted to a standard value of $1 \mu Ci (37 \text{ kBq})$ /$\mu g$ by the addition of unlabelled carrier oxalate.

The two tracers were mixed immediately before administration to provide about 100 $\mu Ci (3.7 \text{ MBq})$ of $^{99m}$Tc-DTPA and 20 $\mu Ci (0.7 \text{ MBq})$ of $^{14}$Coxalate in a single 5.0 ml intravenous injection.

**Radiation dose**

There is no evidence of significant uptake or retention of either of the two radioactive tracers in any organ apart from the bladder. The effective dose equivalent as defined by the International Commission on Radiological Protection [9] is estimated to be 0.024 mSv (2.4 mrem) from $^{99m}$Tc and 0.006 mSv (0.6 mrem) from $^{14}$C. The latter figure and to a lesser extent the contribution due to $^{99m}$Tc will tend to increase as renal function decreases; even so the radiation dose commitment for a single study is well within the variations of natural background radiation received by the subject during 1 year [10].

**Samples**

The bladder was emptied so far as was possible by voluntary voiding immediately before intravenous administration of the tracer dose and a blank blood sample was taken. All urine passed during the period of the study was collected and pooled, the bladder again being emptied just before the last blood sample was taken. Accurately timed blood samples were taken through an indwelling intravenous Butterfly or Venflon cannula at about 4, 20, 60, 120, 180, 240, 300, 360 and 420 min after injection of the tracers, into lithium-heparin tubes and the plasma was separated by centrifugation. The cannula was kept patent using heparin-saline solution and the first 2 ml of each blood sample were discarded.

**Measurements**

**Radioactivity.** The aqueous standard was an accurate 1:500 dilution of the mixed dose solution with a few milligrams of sodium oxalate as carrier. Two or more plasma standards were prepared by adding about 5 $\mu l$ of dose solution to 1 ml of blank plasma.

Duplicate samples (exactly 1 ml) of plasma, urine and standards were pipetted into suitable 5 ml plastic minivials, from the same grade A 1 ml pipette, washed and dried, for every sample. The $^{99m}$Tc activities were measured in an LKB-Wallac 1280 gamma counter with automatic correction for background and decay, each sample being measured for 20 min or 40,000 counts. The activity of each sample as well as the plasma standards was expressed as a percentage of the dose/l.

After allowing 2–3 days for the $^{99m}$Tc to decay, 4 ml of scintillator fluid (NE 262, Nuclear Enterprises Ltd) was added to each sample and the $^{14}$C activities were measured with an LKB-Wallac Rackbeta liquid scintillation spectrometer with external standard channels ratio quench correction. To minimize quench corrections the aqueous standard was used for the urine samples and the $^{99m}$Tc measured values of the plasma standards were used for the plasma samples.

**Urinary oxalate.** The method used depends on the enzymic decarboxylation of oxalate followed by enzymic oxidation of the formate produced and measurement of the change in absorbance at 340 nm due to the reduction of NAD$^+$ to NADH [7].

**Urinary glycollate and glycerate.** These metabolites were determined by combined gas chromatography–mass spectrometry [11].

**Calculations**

The total plasma clearance and equilibrium distribution volumes for both tracers were calculated by the method described by Veall & Gibbs [12]. An input pulse followed by a series of regular unit pulses is regarded as being mathematically equivalent to a priming dose followed by a continuous infusion. By using the CRC DEC-20 computer the observed plasma activity–time function was convolved with this input function, the size of the initial pulse being determined by iteration to minimize the slope of the terminal or plateau section of the notional plasma–activity function. Having thus obtained a computer simulation of the data which would have been obtained from a continuous infusion study with an almost perfectly optimized priming dose, the clearance and distribution volumes are derived in the normal way [13].

The renal clearance averaged over the period of the study was given by the fraction of the tracer dose in the pooled urine (%) divided by the area
under the part of the plasma activity-time curve corresponding to the urine collection period (% of dose·1⁻¹·min).

The integration of the plasma curve and calculation of renal clearances were also done on the DEC-20 computer.

Results

Table 2 shows the values for the simultaneously determined GFR, ECF, PCox, RCox, oxalate distribution volume, plasma oxalate concentration, oxalate removal rate and urinary oxalate excretion for the patients and controls.

There was no appreciable difference between the renal clearance of [⁹⁹ᵐTc]DTPA (RC) and the total plasma clearance (PC). The regression equation of RC on PC is RC = 1.02 PC - 3.37 (n = 31). The total plasma clearance of oxalate, however, was significantly higher than the renal clearance (RCox = 0.95 PCox - 6.37, n = 31).

Discussion

Although [⁶¹Cr]EDTA is now widely regarded as a reference substance for the measurement of GFR [14] because it gives very similar results to inulin [15] we have preferred to use [⁹⁹ᵐTc]DTPA for this purpose in order to avoid the contribution to the experimental error on the data points resulting from the need to correct the ¹⁴C counts for ⁶¹Cr interference. ⁹⁹ᵐTc preparations from different suppliers can give discordant results when compared with [⁶¹Cr]EDTA [16]. We have confirmed that the preparation used for this work gives results in close agreement with those obtained with [⁶¹Cr]EDTA (unpublished data). The close agreement between the total plasma clearance and the renal clearance provides further evidence that [⁹⁹ᵐTc]DTPA is a satisfactory agent for GFR measurements without urine collection, and that the extrarenal clearance of oxalate which we have observed is unlikely to be an experimental or analytical artifact.

Previous investigations have used either a loading dose of [¹⁴C]oxalate followed by a slow infusion to keep the plasma [¹⁴C]oxalate constant during the study [13, 17, 18], or a single injection method with extrapolation of the slow compartment of the plasma disappearance curve [19, 20]. The latter analysis gives erroneous estimates of pool size when the plasma clearance is finite [21]. The method developed for the present investigation combines the theoretical advantages of a continuous infusion method with the simplicity needed for a routine clinical test which can be repeated on the same patient.

Attempts to treat patients with primary hyperoxaluria and advanced renal failure by any form of renal replacement have generally been unsuccessful in the long term [5]. The careful selection, vigorous peri-operative dialysis and the use of pyridoxine may improve the outlook for pyridoxine-sensitive patients who are treated by a related donor transplantation [22]. However, the problem of selecting the optimum time for grafting in these patients remains unresolved. The excessive oxalate biosynthesis causes extrarenal calcium oxalate deposition (oxalosis). The kidneys are destroyed by recurrent urolithiasis and renal oxalosis and when overall renal function decreases to a critical level, the ability to excrete oxalate is greatly reduced. This accelerates the evolution of systemic oxalosis [23].

From these data it is possible to derive an estimate of the rate of oxalate deposition in the tissues. This is the product (Pox x extrarenal clearance). It remains to be seen whether this is a better predictor than Pox alone.

The renal oxalate clearance exceeds the glomerular filtration rate (Table 2). This agrees with previous work in dogs [24] and in humans [13, 17] and indicates either net tubular secretion of oxalate or synthesis de novo of oxalate within the renal tubule epithelium and excretion into the tubule lumen. Micropuncture studies [25, 26] have shown bidirectional flux of the oxalate ion across the tubule epithelium. This agrees with the conclusion that the kidney handles oxalate by combination of glomerular filtration, tubular reabsorption and secretion, which was based on classical physiological studies in the whole animal [24]. The ratio of Cox/GFR is the same in the normal subjects and in the patients with primary hyperoxaluria, indicating that there is no selective decrease in the ability to excrete oxalate, or any evidence of a glomerulotubular imbalance, with respect to oxalate excretion at the stage of the disease represented by these patients.

Our values for Cox/GFR are higher than those reported for man by other workers [13, 17], who used the clearance of creatinine (COx) to measure GFR and this may account for the difference or it may reflect the fact that our normal subjects had GFR values in the lower part of the normal range.

The oxalate distribution volume is about 1.5 times the extracellular fluid space in both the normal subjects and the hyperoxaluric patients. This indicates the existence of an oxalate metabolic pool, which is impermeable to [⁹⁹ᵐTc]-DTPA, and therefore physiologically outside the ECF, but which contains oxalate in dynamic equilibrium with the oxalate in the ECF. We do
Table 3 shows the correlations between the variables that we have studied. The only correlations which reach conventional levels of statistical significance (P<0.05) are GFR with PCO\(_\text{X}\), and ECF with oxalate distribution volume.

The plasma oxalate concentration is raised in all of the patients, being highest in patients nos. 2 and 3, who have particularly rapidly advancing stone disease and were not receiving pyridoxine during the study. The high plasma oxalate concentration in the disease has been reported by other workers [17-19], as have less marked rises in patients with renal failure due to other causes.

The oxalate removal rate clearly exceeds the renal excretion measured over the same period in patients nos. 1, 2 and 3, whereas it was in the range of the normal urinary oxalate excretion rate in the control subjects. This effect was not seen in patient no. 4, in whom the disease has followed a particularly benign course. The findings in patients nos. 1, 2 and 3 are compatible with either deposition in subclinical oxalotic deposits or to excretion by a non-renal route. The mean percentage of oxalate removed non-renally compared with the total removal is 9.2 (range 0–23) and 5.7 (range 0–13) for the patients and controls respectively. Intravenously injected \[^{14}\text{C}]\text{oxalate}\ is virtually all recovered in the urine [19, 28], so there is no evidence for oxalate degradation \textit{in vivo}\ at sites which are accessible to oxalate ions given in this way. The absence of \[^{14}\text{C}\text{}}\text{ from the faeces does not exclude the possibility of increased loss of oxalate into the gut when the plasma concentration is raised. Possible degradation by the large intestine flora may cause excretion of \[^{14}\text{C}]\text{CO}_2\ in flatus. Observations in a patient with primary hyperoxaluria and advanced renal failure who was given \[^{14}\text{C}]\text{oxalate as in the present work, showed only minimal incorporation of isotope into either respiratory CO}_2\ or the faeces (unpublished data). It seems likely, therefore, that the discrepancy between the rates of oxalate removal and excretion in the patient is due to subclinical oxalosis. We have not felt justified in subjecting these patients to either muscle biopsy to look for calcium oxalate crystals in small muscular arterioles, or to bone and bone marrow biopsy, or to cardiac muscle biopsy, to search for crystals in these situations. These patients still have good residual renal function and no clinical features to suggest oxalotic involvement of the heart, digital arteries or peripheral nerves. We have therefore reserved these procedures for use in the assessment of patients later in the disease for whom renal replacement has to be considered. It is possible that the earlier use of biopsies would be of prognostic value.

Acknowledgments

These studies were approved by the Ethical Committee of Northwick Park Hospital and Clinical Research Centre. We are pleased to acknowledge the help of Dr K. Walton (Medical Registrar), the Nursing Staff of Haldane Ward and the skilled technical assistance of Miss R. Painter and Miss Yupa Chantachum. The computer programs for total plasma clearance and distribution volume and
for renal clearance were written by Mr G. P. Gibbs, Division of Computing and Statistics, Clinical Research Centre, who is prepared to provide Fortran listings on request.

References


