Renal clearance of $^{14}$C-oxalate: comparison of constant-infusion with single-injection techniques

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Summary
1. The renal clearance of $^{14}$C-oxalate was assessed by the constant-infusion technique and single-injection technique (plasma sampling only: one-compartment and two-compartment model; plasma and urine sampling). Healthy volunteers and patients with renal stones were studied.
2. Results with the constant-infusion techniques (with and without urine sampling) were not significantly different from each other.
3. The renal clearance of $^{14}$C-oxalate measured with the single-injection technique as compared with the constant-infusion technique was overestimated in the single-injection one-compartment model (52%) as well as in the two-compartment model (30%).
4. The calculated level of plasma oxalate in the healthy volunteers ranged from 1.04 to 1.78 µmol/l (mean 1.39).
5. The biological half-life of $^{14}$C-oxalate, estimated by the cumulative excretion of $^{14}$C in urine after equilibrium had been established, was 128 min (range: 113–142).
6. The oxalate/creatinine clearance ratio in the healthy volunteers ranged from 1.73 to 2.22 (mean 2.01).

Key words: clearance, kidney, oxalate.

Introduction
Until recently the levels of oxalate in plasma determined in normal subjects by various chemical methods were nearly ten times the calculated levels obtained by the radioisotopically measured renal clearance of oxalate and chemically determined urine oxalate [1, 2]. However, in a recent study [3] an enzymatic method for measuring plasma oxalate was introduced that yielded slightly higher values in normal subjects (mean 2.26 ± SD 1.67 µmol/l) than those found by radioisotopic methods in vivo.

The reliability of the calculated values depends on the radionuclide clearance technique and the chemical urinary oxalate method employed. Though in general overestimation of the renal clearance with the single-injection methods as compared with the constant-infusion methods is well known [4–7], the former methods are widely used because they offer practical advantages [8–11].

In this study we compared results from the constant-infusion and single-injection methods in the same subjects (ten healthy volunteers and two patients with renal stones).

Methods and subjects

Subjects
Ten healthy students and two patients with renal stones, in one case normo-oxaluric and the other with primary hyperoxaluria type I, were investigated during a 7 day period under conditions of a strictly low oxalate diet. Informed consent was obtained from all persons participating in this study. The protocol was approved by the Committee on Ethics of the Utrecht University Hospital.
Materials

$[^{14}\text{C}]$ Oxalate (specific radioactivity 75 mCi/mmol or 2.78 GBq/mmol; The Radiochemical Centre, Amersham, U.K.) was dissolved in a 0.9% sodium chloride solution and sterilized by passage through a Millipore filter (0.22 μm).

Analytical methods

The determination of urine oxalate was performed with a spectrophotometric method [12], after precipitation in the cold (4°C) for 5 days. The decrease in absorbance effected by oxalate of the red uranium(IV)-4-(2-pyridylazo)resorcinol (PAR) complex at 515 nm is linear in the range 0–25 μmol/l in the final solution. All determinations were performed in duplicate. Corrections for incomplete precipitation of calcium oxalate were made separately by calculating the precipitated fraction of $[^{14}\text{C}]$ oxalate added as a tracer and counting the radioactivity in both supernatant and precipitate. Urinary and serum creatinine were determined by the alkaline picrate method of Jaffé as adapted for the Technicon Auto-Analyzer.

Clearance experiments

In subjects nos. 5–12 the constant-infusion technique was preceded by the single-injection technique, whereas in subjects nos. 1–4 only the constant-infusion technique was performed [6, 13].

Constant-infusion technique (subjects nos. 1–4). After an intravenous priming dose of 2 μCi (74 kBq) of $[^{14}\text{C}]$ oxalate, a constant infusion containing approximately 0.06 μCi/ml (2.2 kBq/ml) of $[^{14}\text{C}]$ oxalate was started at a rate of 5 ml/h; the total infusion period was 6 h. Blood samples drawn at 30 min intervals (at 15 min intervals during the first hour) were collected in heparinized syringes and centrifuged. A constant plasma level of $[^{14}\text{C}]$ oxalate was reached about 2 h after the start of the infusion (equilibration time) and could be maintained for 4 h. Urine was collected at 60 min intervals by spontaneous voiding; adequate diuresis was ensured by having the subjects drink 1.5 litres of water during this period. The renal clearances of $[^{14}\text{C}]$ oxalate were calculated by means of the single-injection one-compartment and two-compartment models and single-injection with plasma and urine sampling respectively. Two hours after the first priming dose ($P_1$), a second priming dose of 1 μCi (37 kBq) of $[^{14}\text{C}]$ oxalate ($P_2$) was injected intravenously and a constant infusion containing $[^{14}\text{C}]$ oxalate (approximately 0.06 μCi/ml (2.2 kBq/ml)) was applied for 6 h at a rate of 5 ml/h. Plasma radioactivity levels showed the same pattern as described above for the constant-infusion technique.

Endogenous renal clearance of creatinine (subjects nos. 1–12). Three days preceding the infusion studies (subjects were ambulant), the 24 h urine was collected for determination of creatinine and oxalate and blood samples were taken for determination of the serum creatinine level in order to calculate the renal clearance of creatinine [14].

Plasma oxalate concentration. The plasma oxalate concentration ($P_{\text{ox.}}$) was calculated with the formula

$$P_{\text{ox.}} = \frac{\text{urinary oxalate excretion (μmol/min)}}{[^{14}\text{C}]\text{oxalate clearance (ml/min)}} \times 1000$$

where the numerator is the mean oxalate excretion on the 3 days preceding the infusion study, determined as described under Analytical methods, and the denominator is the mean $[^{14}\text{C}]$ oxalate clearance calculated with the standard clearance formula ($C_r$).

Biological half-life of $[^{14}\text{C}]$ oxalate. The
biological half-life of $[^{14}C]$_oxalate was calculated from the cumulative $^{14}$C radioactivity excreted after single injection (2 h period data) as well as after termination of the infusion (4 h period data).

**Results**

The mean urinary $^{14}$C recovery after a 4 days' collection period exceeded 95%. Table 1 shows the clearances of creatinine and oxalate, the latter calculated in five different ways. The mean values ($\pm$ SD) of the $[^{14}C]$_oxalate clearances obtained with the constant-infusion methods ($C_i$ and $C_c$) were nearly identical ($n = 10$): 240 ± 45 and 235 ± 47 ml/min respectively ($P > 0.1$ by paired t-test). Both the single-injection one-compartment model ($C_i$) and the two-compartment model ($C_c$) consistently overestimated the renal clearance of $[^{14}C]$_oxalate as compared with the renal clearance obtained with the constant-infusion model ($C_c$), the latter calculated with the standard formula ($n = 6$): 395 ± 36 and 339 ± 28 ml/min respectively ($P < 0.001$ and $P < 0.006$). Expressed as a percentage of the standard clearance values ($C_i$), overestimation amounted to 52% (range: 35–67) for the single-injection one-compartment model and 30% (range: 18–44) for the single-injection two-compartment model. $[^{14}C]$_oxalate clearance values ($\pm$ SD) obtained with single-injection and urine sampling were comparable with values obtained with the constant-infusion method ($C_i$) only in the last 30 min sampling period ($C_i$): 268 ± 31 and 261 ± 23 ml/min respectively ($P > 0.1$). For the preceding sampling periods the calculated clearance values were significantly higher. The mean 24 h urine oxalate excretion amounted to 0.47 mmol (range: 0.32–0.58) and the calculated plasma oxalate levels ($P_{ax}$) ranged from 1.04 to 1.78 $\mu$mol/l (mean 1.39). After single injection a mean plasma half-life ($\pm$ SD) of 51 ± 5 min (range: 45–59) was found. The mean biological half-life ($\pm$ SD) after single injection (2 h period data) was 58 ± 4 min (range: 54–64), whereas after termination of the infusion (4 h period data) it was 128 ± 10 min (range: 113–142), the latter half-life resulting in an estimated total body absorbed dose of 20–25 mg.

The mean oxalate/creatinine clearance ratio ($\pm$ SD) was found to be 2.01 ± 0.16 (range:

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sex</th>
<th>Body wt. (kg)</th>
<th>$C_{cr}$ (ml/min)</th>
<th>$[^{14}C]$_oxalate clearance (ml/min)</th>
<th>Plasma oxalate ($\mu$mol/l)</th>
<th>Urinary oxalate (mmol/24 h)</th>
<th>$t_1/2$ after single injection (min)</th>
<th>Biological $t_1/2$ (min)</th>
<th>$C_i/C_{cr}$ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>75.0</td>
<td>108</td>
<td>236 214</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>125 2.19</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>66.0</td>
<td>111</td>
<td>246 231</td>
<td>1.24 0.45</td>
<td>1.35 0.39</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>138 2.22</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>55.3</td>
<td>122</td>
<td>228 232</td>
<td>1.24 0.45</td>
<td>1.35 0.39</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>43.4</td>
<td>62</td>
<td>126 122</td>
<td>1.24 0.45</td>
<td>1.35 0.39</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>86.4</td>
<td>143</td>
<td>276 258</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>87.8</td>
<td>155</td>
<td>268 293</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>78.8</td>
<td>121</td>
<td>267 244</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>83.4</td>
<td>123</td>
<td>234 242</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>66.6</td>
<td>114</td>
<td>233 228</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>72.0</td>
<td>144</td>
<td>290 288</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>Mean (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td>236 214</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
<tr>
<td>Mean (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td>236 214</td>
<td>1.31 0.45</td>
<td>1.23 0.44</td>
<td>1.17 0.39</td>
<td>1.78 0.32</td>
<td>142 2.22</td>
</tr>
</tbody>
</table>
1.73–2.22) and a significant positive correlation between the two clearances was found: $C_{\text{ox}} = 1.81 C_{\text{cr}} + 16.2$ $(n = 12, r = 0.91, P < 0.001)$.

Detailed results have been deposited as Clinical Science Table no. 82/1 with the librarian of the Royal Society of Medicine (1 Wimpole Street, London WIM 8AE), who will issue copies on request.

**Discussion**

In the present study, a consistent overestimation of the renal oxalate clearance was found by using both the single-injection one-compartment and two-compartment models, as compared with results from the constant-infusion methods. This overestimation was of the same order of magnitude as for example the one reported by Hall et al. [7], who found more than 30% overestimation of the glomerular filtration rate in dogs using the one-compartment model with single injection of $[^{125}]$iodohippurate and the standard inulin clearance technique.

Nevertheless Hodgkinson et al. [8] and Hautmann et al. [11] used the single-injection technique in their studies on oxalate metabolism. In the single-injection studies performed by Hodgkinson et al. [8], who injected 2 µCi (74 kBq) of $[^{14}]$oxalate into one healthy volunteer and seven patients with renal stones, a mean plasma half-life of 91 min (range: 68–114) was found. This is appreciably higher than the value we obtained in healthy volunteers. This discrepancy might be due to a slightly lower creatinine clearance in their patients compared with our healthy subjects, as well as to the difference in blood sampling: blood samples used for calculations were drawn between 120 and 300 min after the injection of $[^{14}]$oxalate, compared with 40–120 min in the present study. In the latter period the plasma radioactivity decreased linearly with time when plotted on semi-log paper. At the dose of $[^{14}]$oxalate used by Hodgkinson et al. [8], we thought that blood samples taken after 120 min have too low a count rate to be counted accurately.

On the other hand, Hautmann et al. [11] used the single-injection method in six normal subjects and one hyperoxaluric patient with a higher dose of $[^{14}]$oxalate (35 µCi of 1.3 MBq) and blood sampling up to 180 min. Yet they found a mean plasma half-life as calculated from the reported fractional disappearance rates of 92 min (range: 73–137).

In our study the biological half-life (calculated from the cumulative urinary $[^{14}]$excretion) estimated after single injection of $[^{14}]$oxalate (2 h period data) did not differ significantly from the plasma half-life estimated in the same period. However, the biological half-life estimated after termination of the infusion (4 h period data), when equilibrium had been reached, was appreciably longer. The low count rate of the blood samples drawn in the latter period did not permit calculation of the plasma half-life, but probably the value would approximate to the biological half-life. Elder & Wijngaarden [15] estimated the biological half-life of $[^{14}]$oxalate in three normal subjects by means of the isotopic dilution technique and arrived at a mean value of 148 min (range: 132–168), which is of the same order of magnitude as we found after termination of the infusion (4 h period data) when equilibrium had been established.

The amount of radioactivity cleared in the first minutes after single injection (when equilibrium has not been established) is probably not negligible, and a shorter plasma and biological half-life will be found, resulting in a falsely high clearance value. Hence it is preferable to calculate the plasma and biological half-life after equilibrium has been established. The oxalate clearance was found to be related to the creatinine clearance $(r = 0.91)$ and the oxalate/creatinine clearance ratio was about 2 in all subjects. Although this ratio could be influenced by minor errors because the clearances were not determined under the same conditions, the existence of such a relationship is of practical importance, making it possible to estimate the plasma oxalate concentration from the urinary oxalate excretion and creatinine clearance. The values of our calculated plasma oxalate levels are in good agreement with the data of Constable et al. [16], who also used the constant-infusion technique. In the hyperoxaluric patient (no. 12) the plasma oxalate level was, as might be expected, increased. From the present results we conclude that the constant-infusion techniques are superior to single-injection techniques for studies of oxalate metabolism.

**Acknowledgment**

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**References**


