A Possible Etiological Role for Ascorbate in Calculi Formation


Studies of recurrent stone formers indicated that they have significantly increased urinary oxalate and decreased ascorbate excretions. Results of oral and intravenous administration of ascorbate indicate an enhanced production of oxalate from ascorbate in recurrent calcium stone formers as compared with normal persons and that most of this oxalate is generated in the gut.

In most western civilisations, calcium oxalate-containing stones comprise about 80% of all renal calculi (1). Calcium oxalate is extremely insoluble and its daily output in the urine is perhaps the most critical factor to renal stone formation (2).

Urinary oxalate is derived essentially from three major sources: ascorbate, which contributes about 40% of urinary oxalate output (3, 4); 2-carbon metabolites (glyoxylate, glycocolate), which also contribute about 40% (5, 6), and dietary oxalate itself, which contributes about 5 to 10% (7).

The route from ascorbate to oxalate does not involve generation of 2-carbon metabolites such as glyoxylate (4), but is thought to involve the following sequence:

Ascorbate → dehydroascorbate → diketogulonic acid → oxalate + L-threonate

This pathway is both enzymic and non-enzymic in that alkaline pH conditions will result in production of oxalate from ascorbate (8).

Most of the data linking ascorbate to increased urinary oxalate excretion are confusing and contradictory in that some (9, 10) but not all (11, 12) workers find that ascorbate may increase urinary oxalate output. In an attempt to clarify this confusion, we undertook a detailed study of the role of ascorbate in oxalate excretion in patients with renal stone disease. The result of this investigation is presented here, and ascorbate is discussed as a possible etiological factor in the pathogenesis of this disease state.

Patients and Methods

Urine analyses: Oxalate analyses were done by a specific-enzyme method (13, 14) on 24-h urine specimens collected over disodium ethylenediaminetetraacetate (20–25 mmol) or HCl (0.25 mol). Ascorbate was measured by a variation (15) of the 2,4-dinitrophenylhydrazine method of Roe (16). Urines were collected over disodium EDTA to inhibit the degradation of ascorbate and formation of oxalate from oxalate precursors (17).

Plasma analyses: Ascorbate was measured by the 2,4-dinitrophenylhydrazine colorimetric method (16) in plasma from EDTA-anticoagulated blood.

Patients: The 17 patients (10 men, seven women) initially investigated for urinary ascorbate and oxalate excretions had a history of recurrent calcium nephrolithiasis. All patients had radio-opaque stones demonstrable on abdominal roentgenograms. Nine of these stones, obtained from the patients and analyzed by a wet chemical method (18), were mixed calcium oxalate/phosphate calculi.

The ages of the patients ranged from 21 to 56 years, with a mean of 42 years. All were investigated as outpatients and had normal values for plasma creatinine.

The 11 control subjects (five men, six women) ranged in age from 21 to 46, with a mean of 34 years; all were laboratory staff. None had a history of renal calculi or were taking any ascorbic acid preparation.

In subsequent studies involving ascorbate loading tests, the patients selected had a history of recurrent mixed calcium oxalate/phosphate nephrolithiasis.

Ascorbate dosages: Oral ascorbate was given as four 500-mg orange-flavored tablets (Protea Pharmaceuticals, Sydney, Australia). Ascorbate (500 mg/5 mL, for intravenous injection; David Bull Laboratories, Melbourne, Australia) was infused intravenously, in 150 mL of isotonic saline, during 2 h. This test was done initially on normal persons.

There were no clinical side effects and the ascorbate concentrations in the plasma remained within the normal reference interval (10–140 μmol/L) throughout the infusion. These experiments on recurrent stone formers were approved by the Mater Public Hospital Ethics Committee. Bolus intravenous 1-g doses of ascorbate have been described in the literature (19).

Statistics: In correlating data we used the product moment correlation and paired t-test (20). In comparing urinary excretions of normals and stone formers we used the Wilcoxon nonparametric two-sample statistic (21). For comparison of oxalate excretion by stone patients given oral and intravenous ascorbate we used the Wilcoxon signed rank test for paired observations (21).

Results

Figure 1 shows the urinary ascorbate and oxalate excretion by 11 normal individuals and 17 stone formers. The mean daily excretion of oxalate by stone formers was 0.43 (SD 0.21) mmol/day, significantly (p <0.05) higher than for normals, 0.30 (SD 0.10) mmol/day. The mean ascorbate excretion by stone formers, 0.17 (SD 0.15) mmol/day, was about fivefold less than that for the normal persons: 0.96 (SD 0.76) mmol/day (p <0.002).

To compare the metabolism of ascorbate in stone formers and controls, we performed the following ascorbate load test on four normal individuals and five recurrent formers of calcium oxalate/phosphate stones. Urine was voided before 7:00 a.m. and collections made between 7:00 a.m. and 9:00 a.m. to give a baseline ascorbate/oxalate excretion. We then administered 2 g of ascorbate orally at 9:00 a.m. and collected three-hourly urines for the next 12 h, then a final 12-h (overnight) collection. The results of this study are summarized in Figure 2. The hourly oxalate excretion per given load of ascorbate appears to be greater for the stone formers, and the excretion reached significance 3–6 h after the oral dose. In contrast, the stone formers excreted less ascorbate. Oxalate excretion reached a maximum about 3 h later than did ascorbate excretion.

Table 1 summarizes the increase in ascorbate and oxalate
excretions after the 2-g oral ascorbate load. These data were derived by assuming that the oxalate/ascorbate excretions before the oral ascorbate intake and in the final overnight 12-h collection were baseline and that outputs in the interim periods above that baseline were ascribable to the oral ascorbate load (Figure 2). Ascorbate excretion by the normal individuals agreed well with previously published data (22). Our results indicate a significantly \( p = 0.01 \) increased excretion of oxalate by recurrent stone formers after the oral ascorbate load.

To understand more fully the mechanism responsible for the ascorbate-derived hyperoxaluria in stone patients, we intravenously infused 500 mg of ascorbate as described. Urine collections were made as in the oral ascorbate experiments and excretion of oxalate and ascorbate was measured. The ascorbate infused represented approximately the average amount of the oral dose excreted by the normals (Table 1). The results (Figure 3) indicate no significant difference in the oxalate output in response to the intravenously administered ascorbate, although the ascorbate excretion was once again lower in the stone formers. Excretion of ascorbate was similar to that in the oral loading study, confirming that the intravenous load of ascorbate was similar to the ascorbate absorbed in the oral-dosage experiments. Oxalate excretion by the stone formers was significantly increased in the oral

---

**Table 1. Urinary Excretion of Ascorbate and Oxalate by Controls and Recurrent Calcium Stone Formers Given a 2-g Oral Ascorbate Load**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Ascorbate</th>
<th>Oxalate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal persons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>♀</td>
<td>29.3</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>♀</td>
<td>23.3</td>
<td>0.56</td>
</tr>
<tr>
<td>3</td>
<td>♀</td>
<td>27.5</td>
<td>0.28</td>
</tr>
<tr>
<td>4</td>
<td>♀</td>
<td>19.9</td>
<td>0.37</td>
</tr>
<tr>
<td>Stone formers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>♀</td>
<td>17.4</td>
<td>2.47</td>
</tr>
<tr>
<td>2</td>
<td>♀</td>
<td>17.5</td>
<td>1.14</td>
</tr>
<tr>
<td>3</td>
<td>♀</td>
<td>0.5</td>
<td>1.29</td>
</tr>
<tr>
<td>4</td>
<td>♀</td>
<td>0.5</td>
<td>0.87</td>
</tr>
<tr>
<td>5</td>
<td>♀</td>
<td>31.1</td>
<td>1.90</td>
</tr>
</tbody>
</table>

loading experiments as compared with the stone-former intravenous study in the 3 to 6 h \( p = 0.06 \) and 6 to 9 h \( p = 0.03 \) collection periods (cf. Figures 2 and 3).
Discussion

The finding that recurrent stone formers had consistently and significantly lower values for ascorbate excretions than did the controls was unexpected (Figure 1). From a biochemical viewpoint one would have expected increased urinary oxalate and ascorbate excretion to parallel one another, because ascorbate is a major precursor of oxalate (3, 4), and indeed there was a good correlation between oxalate and ascorbate excretions in the controls \( r = 0.85, p < 0.01 \). However, in stone formers the correlation was poor \( r = -0.19 \). These findings indicated that handling of ascorbate may be impaired in recurrent stone formers, and this was confirmed in the oral ascorbate load test. Here the increased oxalate and decreased ascorbate excretion found in the stone formers (Figure 2, Table 1) can be explained by two mechanisms:

- poor absorption of ascorbate from the gut, resulting in conversion of non-absorbed ascorbate to oxalate at this site and subsequent absorption of the oxalate, and (or)
- normal absorption of ascorbate from the gut but an increased metabolism of ascorbate to oxalate in the tissues or in the urinary tract.

Results of the intravenous infusion of ascorbate make it unlikely there was an increased uptake and metabolism of ascorbate to oxalate by internal tissues or the urinary tract, because the oxalate excretion was the same for controls and stone formers (Figure 3). More likely is that in stone formers there is an increased conversion of ascorbate to oxalate in the gut, resulting from the malabsorption of ascorbate. The data in Figure 2 confirm this idea, because in the stone formers the maximum oxalate output in urine was delayed as compared with the maximum ascorbate excretion. In addition, the maximum urinary excretion of oxalate reportedly occurs about 4 h after an oral oxalate dose (23, 24), and the profile of oxalate excretion in Figure 2 could be explained by oxalate being formed from ascorbate in the gut. The reported observation (23, 24) that there is no difference in oxalate absorption between normal persons and stone formers given an oxalate load would argue against an increase in the transport or uptake mechanism for ascorbate-derived oxalate across the gut mucosa in stone formers.

The results in Figure 2 also indicate that, in the stone formers, the rate of oxalate excretion averaged about two-fold that for the normal persons during the 3-12 h period after a 2-g ascorbate load. This transient doubling may be obscured when averaged over a 24-h period (11, 12), but it may contribute to stone formation because the urinary output of oxalate is a critical factor in this process (2). The results also suggest that high-dose ascorbate supplementation should be avoided by stone formers.

The decreased excretion of ascorbate by stone formers as compared with controls given oral or intravenous ascorbate suggests depleted stores of ascorbate in the tissues of the former group. Malabsorption of ascorbate would be consistent with this observation, and this was supported by the finding that values for plasma ascorbate measured a month after the oral ascorbate load were significantly \( (p = 0.025) \) lower in four of the stone formers studied (19, 26, 42, and 68 \( \mu \)mol/L) than in the controls (73, 77, 78, and 80 \( \mu \)mol/L). Whether the low urinary ascorbate excretion by stone formers has a relationship to stone formation independent of oxalate generation is not yet known. Studies on crystal growth and aggregation (25) may indicate whether ascorbate has a significant inhibitory activity in this test system.

In conclusion: our results suggest that, in stone formers, most of the oxalate is derived from malabsorbed ascorbate in the gastrointestinal tract and that most of this is generated during the first 12 h after an oral ascorbate load. The results from the oral ascorbate studies indicate that high-dose ascorbate intake should be avoided by recurrent stone formers. Currently we are investigating the possibility that there may be an inherent malabsorption of hydroxy aliphatic acids in some recurrent stone formers, because the preliminary data obtained with ascorbate would tend to support this hypothesis. We are also attempting to evaluate the effect of various amounts of ascorbate in the diet on urinary oxalate excretion by stone formers.

We thank the John P. Kelly Mater Research Foundation for a grant supporting this project.

References

4. Baker EM, Saari JC, Tolbert BM. Ascorbic acid metabolism in