THE EFFECT OF ETHANE-1-HYDROXY-1,1-DIPHOSPHONATE (EHDP) ON CALCIUM OXALATE CRYSTALLURIA IN RECURRENT RENAL STONE-FORMERS

W. G. ROBERTSON, M. PEACOCK, R. W. MARSHALL AND F. KNOWLES

M.R.C. Mineral Metabolism Unit, The General Infirmary, Leeds

(Received 21 January 1974)

SUMMARY

1. The volume, size and type of calcium oxalate crystals excreted in the urine of a group of patients with recurrent ‘idiopathic’ stones were studied on a controlled basal diet, after an oral supplement of sodium oxalate and after oral administration of ethane-1-hydroxy-1,1-diphosphonate (EHDP) for 4 weeks.

2. Before administration of EHDP the stone-formers passed the large crystals and aggregates of calcium oxalate dihydrate characteristic of recurrent calcium oxalate stone-formers. For the same level of urine saturation and crystalluria EHDP caused a significant reduction in the proportion of large crystals and aggregates excreted. Studies by light-microscopy confirmed that EHDP caused a striking change in the size and habit of calcium oxalate crystals in some but not all of the urine samples examined.

3. The decrease in average crystal size during the administration of EHDP was attributed to the observed increase in the ability of urine to inhibit the growth and aggregation of calcium oxalate crystals as measured by a growth system in vitro.

4. The possible use of EHDP as a therapeutic agent in the treatment of calcium oxalate stone-formation is discussed.

Key words: calcium, oxalates, crystallization, urinary calculi, phosphonic acids.

The cause and treatment of calcium-containing renal calculi have been long-standing subjects of controversy. In recent years, however, a hypothesis of stone-formation has been developed which attributes the formation of stones to the combination of two main factors: first, the propensity of urine to promote frequent formation of crystals of one of the stone-forming salts (Vermeulen, Ellis & Hsu, 1966; Vermeulen, Lyon, Ellis & Borden, 1967; Robertson,
Peacock & Nordin, 1969, 1971); secondly, the deficiency of an inhibitor of crystallization in urine [thought to be an acidic mucopolysaccharide (Robertson, Peacock & Knowles, 1974)], which normally prevents the formation of crystals and aggregates of calcium salts large enough to become trapped at some narrow point in the urinary tract (Dent & Sutor, 1971; Robertson & Peacock, 1972). It is the formation and retention of large particles which is thought to be the critical phase in the initiation of calcium stone-formation (Robertson et al., 1969, 1971).

This increased understanding of some of the chemical factors involved in the formation of stones has made it possible to tackle the treatment of the disease from a sounder theoretical standpoint. First, treatment may be aimed at preventing the formation of crystals in urine by lowering urinary supersaturation, either by restricting the dietary intake of calcium and/or oxalate (Marshall, Cochran & Hodgkinson, 1972; Nordin, Robertson, Barry, Bulusu & Speed, 1974) or by ingestion of water (Robertson, Peacock & Nordin, 1972). An alternative form of treatment is to allow crystals to form in the urine but to increase the inhibitory activity in order to block the growth and aggregation of the primary crystals at the embryo stage. By this mechanism any crystals formed would be excreted harmlessly in the form of small particles as in normal urine (Robertson et al., 1969; Robertson & Peacock, 1972). Attempts have been made to increase the concentration of various natural and synthetic inhibitors of crystallization in urine such as pyrophosphate (Edwards, Russell & Hodgkinson, 1965), magnesium (Moore & Bunce, 1964) and methylene blue (Boyce, McKinney, Long & Drach, 1967), with varying degrees of success on the rate of stone-formation. More recently Fraser, Russell, Pohler, Robertson & Fleisch (1972) have shown that ethane-1-hydroxy-1,1-diphosphonate (EHDP), which is structurally related to pyrophosphate and is a powerful inhibitor of the growth and aggregation of calcium phosphate (Francis, 1969; Fleisch, Russell, Bisaz, Muhlbauer & Williams, 1970; Robertson, 1973) and of calcium oxalate (Robertson, Peacock & Nordin, 1973), significantly decreased experimentally induced calcium oxalate stone-formation in rats.

The object of this paper is to examine the possibility that EHDP might be applied to the treatment of calcium oxalate stone-formation in man through its ability to inhibit the growth and aggregation of calcium oxalate crystals.

MATERIALS AND METHODS

The patients studied were three recurrent idiopathic calcium oxalate stone-formers (as defined by Robertson et al., 1969, 1971), each of whom had formed at least two stones during the previous year; their full consent was obtained for the performance of this study. Apart from having renal stones all were healthy at the time of study. There was no evidence of urinary tract infection and all had normal renal function. They were investigated in a metabolic ward on a constant basal intake of calcium 40 mmol/day (1000 mg/day), oxalate 1.3 mmol/day (120 mg/day) and phosphate 39 mmol/day (1200 mg/day). Fluid intake was controlled at 1300 ml/day and given at fixed times. All subjects emptied their bladders before going to bed and on the following day urine samples were collected into warm Dewar flasks at 09.00, 11.30, 14.30, 17.00 and 23.00 hours. Similar collections were made on the following day to study the effect on calcium oxalate crystalluria of a single oral dose of disodium oxalate 37 μmol (5 mg)/kg body weight added to the basal diet and given at 10.00 hours.
Effect of EHDP on crystalluria

The patients were allowed home and given EHDP orally for 4 weeks: 80 µmol (20 mg)/kg body weight. To maximize intestinal absorption of the drug the tablets were given in three doses per day between meals. The patients were asked to maintain as closely as possible the same dietary and fluid intake as in the ward. After 4 weeks the patients were re-investigated in the metabolic ward to study the effect of EHDP on their calcium oxalate crystalluria and urinary inhibitory activity.

Calcium oxalate and calcium phosphate crystalluria was measured in each sample immediately after voiding as described by Robertson (1969). The samples were maintained at 37°C during the crystal-counting procedure. The type of crystal counted was identified by light-microscopy and photomicrograph records made with a Zeiss Photomicroscope II (Carl Zeiss, Jena).

The saturation of urine with respect to calcium oxalate, as defined by its activity product, was measured as described by Robertson, Peacock & Nordin (1968). The ability of urine to inhibit the growth and aggregation of calcium oxalate crystals in vitro was measured as described by Robertson & Peacock (1972) and modified by Robertson et al. (1973). The total concentration of acidic mucopolysaccharides in urine was measured by the method of Whiteman (1973).

RESULTS

Saturation of urine with calcium oxalate

The mean values of the various ion concentrations measured in the urines of the stone-formers on the various regimes are compared in Table 1 by the Wilcoxon ranking method.

<p>| Table 1. pH, creatinine and concentrations of ions in urine of stone-forming subjects on various regimes |
| Mean values ±SEM are shown. P was assessed by the Wilcoxon ranking test. NS = not significant. |</p>
<table>
<thead>
<tr>
<th>(1) Basal diet (n = 18)</th>
<th>(2) Basal diet + oxalate (n = 13)</th>
<th>(3) Basal diet + EHDP (n = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.79±0.14</td>
<td>5.78±0.13</td>
<td>5.92±0.11</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>11.8±1.3</td>
<td>10.8±1.3</td>
<td>10.4±0.7</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>5.2±0.5</td>
<td>5.0±0.6</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td>Magnesium (mmol/l)</td>
<td>2.6±0.3</td>
<td>2.6±0.3</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>89±9</td>
<td>89±9</td>
<td>87±11</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>38±3</td>
<td>36±3</td>
<td>32±2</td>
</tr>
<tr>
<td>Ammonium (mmol/l)</td>
<td>30±4</td>
<td>27±3</td>
<td>31±4</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>19.1±1.9</td>
<td>18.1±1.5</td>
<td>20.6±1.9</td>
</tr>
<tr>
<td>Oxalate (10^{-4} mol/l)</td>
<td>3.3±0.2</td>
<td>5.4±0.8</td>
<td>4.8±0.3</td>
</tr>
<tr>
<td>Citrate (mmol/l)</td>
<td>1.8±0.2</td>
<td>2.1±0.2</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Sulphate (mmol/l)</td>
<td>17±1</td>
<td>15±1</td>
<td>17±1</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>93±9</td>
<td>87±9</td>
<td>78±5</td>
</tr>
</tbody>
</table>

This shows that the oral supplements of sodium oxalate significantly increased the urinary concentration of oxalate (P<0.05) as found previously. Administration of EHDP for 4 weeks
increased the urinary concentration of oxalate \((P<0.01)\) to a level comparable with that observed during the period of oxalate supplements. At the same time there was a significant fall in the urinary concentration of magnesium \((P<0.01)\).

**TABLE 2.** *Measured values of calcium oxalate saturation, crystalluria and the proportion of large crystals in urine of stone-forming subjects on various regimes*

<table>
<thead>
<tr>
<th>Regime</th>
<th>Basal diet (1)</th>
<th>Basal diet + oxalate (2)</th>
<th>Basal diet + EHDP (3)</th>
<th>(1) vs (2)</th>
<th>(1) vs (3)</th>
<th>(2) vs (3)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log (\left[Ca^{2+}\right]) (\times) (\left[Ox^{2-}\right])</td>
<td>7.45 ± 0.05 7.48 ± 0.05</td>
<td>7.45 ± 0.07 7.48 ± 0.05</td>
<td>7.45 ± 0.05 7.48 ± 0.05</td>
<td>0.05</td>
<td>&lt;0.02</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>3.3 ± 2.2</td>
<td>8.0 ± 2.2</td>
<td>6.2 ± 1.4</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>crystalluria (mm(^3)/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>21.2 ± 2.3</td>
<td>46.2 ± 5.6</td>
<td>25.2 ± 4.3</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>crystals ≥12.2 (\mu)m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The distributions of calcium oxalate activity products in the urines of the stone-formers on the various regimens are shown in Fig. 1 in relation to the solubility and formation products of that salt. The means and standard errors of the groups are compared in Table 2. As found previously (Robertson *et al.*, 1971) the recurrent stone-formers on the basal diet passed urine which frequently exceeded the formation product of calcium oxalate above which spontaneous precipitation of the salt takes place. Crystals of calcium oxalate were observed in all urines which exceeded the formation product. The oral supplement of sodium oxalate significantly increased \((P<0.05)\) the level of supersaturation. This significantly increased level of supersaturation was accompanied by a significant decrease in the urinary concentration of magnesium.
Effect of EHDP on crystalluria

saturation was maintained during the period of treatment with EHDP (Table 2), owing to the observed increase in the urinary excretion of oxalate (Table 1).

Calcium oxalate crystalluria

Since these stone-formers rarely excreted calcium phosphate crystals in their urine only the calcium oxalate crystalluria measurements are presented. The distributions of the total volume of calcium oxalate crystals per unit volume of urine on the various regimens are shown in Fig. 2 in relation to the normal range of calcium oxalate crystalluria. The means and standard errors of the groups are compared in Table 2. As found previously the recurrent stone-formers persistently excreted calcium oxalate crystals on the basal diet and this crystalluria was markedly increased after the oral supplement of sodium oxalate (Robertson et al., 1969). The stone-formers continued to pass large amounts of crystals during treatment with EHDP, as would be expected from their elevated supersaturation values (Fig. 2).

The distributions of the percentage of large crystals of calcium oxalate (defined as being greater than 12 μm in diameter) are shown in Fig. 3 in relation to the normal range of large crystals. The means and standard errors of the groups are compared in Table 2. As found previously the recurrent stone-formers persistently excreted an abnormally high proportion of large crystals while on the basal diet (Robertson & Peacock, 1972). The percentage of large crystals was markedly increased after the oral supplement of sodium oxalate (Fig. 3). During the treatment with EHDP the percentage of large crystals decreased significantly (Table 2), in spite of the continuing high level of urinary supersaturation and crystalluria. Many of the values fell into the normal range.
Inhibition of calcium oxalate crystal growth and aggregation

The percentage inhibitory activity, as defined by Robertson et al. (1973), was measured \textit{in vitro} in a standard metastable solution containing 1\% of urine. The distributions of inhibitory activities in urine samples before and during treatment with EHDP are shown in Fig. 4 in relation to the normal range of inhibitory activity (Robertson et al., 1974). The mean inhibitory activity during treatment was significantly higher ($P<0.02$) than that before treatment (Table 3). Whereas less than one-half of the values lay in the normal range before treatment, this increased to over two-thirds during treatment. The mean value of $71 \pm 4\%$ during treatment was, however, still significantly less ($P<0.02$) than that found in normal subjects ($87 \pm 4\%$) (Robertson et al., 1974). The increase in inhibitory activity during treatment with EHDP was achieved in spite of a fall ($P<0.05$) in the concentration of acidic mucopolysaccharides in the urine (Table 3).

Light-microscopy studies

Fig. 5(a) shows a photomicrograph of calcium oxalate dihydrate crystals and aggregates in the fresh, warm urine of one of the recurrent stone-formers while on the basal diet. These large particles, about 20–40 \(\mu\)m in diameter, are typical of the crystals passed regularly by this group of patients (Robertson et al., 1969). Fig. 5(b) shows smaller crystals of calcium oxalate dihydrate often seen during treatment with EHDP. In some of the urine samples during treatment with EHDP the crystal size and habit was strikingly changed to the form shown in Fig. 5(c). These small ellipsoidal biconcave particles, similar to erythrocytes in appearance, were
Effect of EHDP on crystalluria

Fig. 5. Calcium oxalate crystals and aggregates in fresh urine from a recurrent stone-forming subject (a) on the basal diet, and (b) and (c) during treatment with EHDP. (All $\times 320$.)

(Facing p. 18)
usually about 8–9 μm long and 5–6 μm wide and rarely found in aggregates. This form of calcium oxalate ‘crystal’, which may be akin to the classical ‘dumbell’ form sometimes found in urinary sediments, was not observed during the pre-treatment period.

**DISCUSSION**

The results observed on the basal diet and after the oral supplement of sodium oxalate confirm those found previously during a similar study on the factors affecting calcium oxalate crystalluria (Robertson *et al.*, 1969, 1971; Robertson & Peacock, 1972). In short, recurrent stone-formers pass urine sufficiently supersaturated with calcium oxalate to cause frequent spontaneous crystalluria. The high levels of crystalluria in conjunction with a low level of protective
inhibitor in the urine lead to the formation of abnormally large crystals and aggregates of calcium oxalate, which if retained in the urinary tract may lead to the formation of a stone.

Oral administration of EHDP over a period of 4 weeks caused an increase in calcium oxalate supersaturation and crystalluria but also increased the inhibitory activity of the urine sufficiently to correct at least partially the balance between crystalluria and inhibitory activity. Consequently for the same level of urine saturation and crystalluria EHDP caused a significant reduction in the proportion of large crystals and aggregates excreted (Table 2). This has been confirmed by light-microscopy (Fig. 5).

The increased level of calcium oxalate supersaturation and crystalluria observed during the administration of EHDP resulted from an increase in the urinary excretion of oxalate. One possible explanation for this is that most of the administered dose of EHDP is excreted unabsorbed in the faeces (Recker & Saville, 1973), and by competing with oxalate ions for formation of a complex with calcium could theoretically displace sufficient oxalate to cause an increase in the intestinal absorption and renal excretion of that ion.

It seems likely that the administered EHDP, about 1–3% of which is known to be absorbed from the intestine and excreted in the urine (Recker & Saville, 1973), is acting directly to increase the inhibitory activity of urine thereby causing the predicted decrease in the average size of crystals (Robertson et al., 1973). At the dose employed, however, EHDP did not inhibit the formation of large crystals and aggregates at all times. One possible explanation for this variable response could be extreme variability in the intestinal absorption of EHDP (Recker & Saville, 1973). It can be calculated from the known inhibitory activity of EHDP in vitro (Robertson et al., 1973) that if the intestinal absorption of EHDP falls below 1%, there may not be a sufficiently high concentration of EHDP in urine to inhibit completely the formation of large crystals and aggregates, particularly when crystalluria is maintained at a high level. A similar explanation might account for the failure of EHDP to block completely experimentally induced calcium oxalate stone-formation in rats, although it did cause a significant reduction in the weight of stones formed (Fraser et al., 1972).

An alternative explanation for the failure of EHDP to block completely the formation of large crystals may arise from some effects of EHDP on the naturally occurring inhibitor in urine. This inhibitor, at present thought to be an acidic mucopolysaccharide (Robertson et al., 1974), is significantly reduced in recurrent renal stone-formers. In the present study the total acidic mucopolysaccharide excretion was indeed low in all three subjects on the basal diet but was further significantly reduced during treatment with EHDP. Since the concentration of the naturally occurring inhibitor is reduced in this way more EHDP than otherwise would have been necessary is required to produce a net increase in inhibitory activity.

In conclusion this study shows that although EHDP does increase the net inhibitory activity of urine as predicted, thereby decreasing the average size of calcium oxalate crystals, it also raises calcium oxalate supersaturation and crystalluria by increasing the urinary excretion of oxalate. Moreover chronic administration of EHDP at the dose employed is known to produce significant changes in bone in man (Jowsey, Riggs, Kelly, Hoffman & Bordier, 1971) and animals (King, Francis & Michael, 1971; Schenk, Merz, Muhlbauer, Russell & Fleisch, 1973). Thus the balance of evidence suggests that administration of EHDP at these doses is not a feasible treatment for calcium oxalate stone-formation. Treatment with EHDP might become feasible if a more sustained increase in urinary inhibitory activity can be achieved without the accompanying rise in urinary oxalate excretion and changes in bone morphology.
Effect of EHDP on crystalluria

ACKNOWLEDGMENTS

We thank Miss Linda Brown and Mrs Wendy Jakeman for their technical assistance. We also express our appreciation to Dr W. R. King and Dr W. L. Hedglin of the Procter and Gamble Co., Cincinnati, Ohio, U.S.A., for kindly supplying us with EHDP and for many helpful discussions concerning the study.

REFERENCES


