Biochemical and clinical effects of ethane-1-hydroxy-1,1-diphosphonate in calcium nephrolithiasis

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Summary

1. The short- and longer-term effects of ethane-1-hydroxy-1,1-diphosphonate (EHDP), an inhibitor of crystal growth and potential preventive agent against urinary tract stones in man, have been studied.

2. Measurement of urinary excretion of EHDP was used to define the best dosage regimen. When 4·4 mmol of EHDP was given daily in four divided doses the urinary concentration of EHDP achieved was high enough (10⁻³ mol/l) to inhibit the crystallization of calcium crystals throughout the day.

3. Nine patients with recurrent calcium stones were given this dose of EHDP daily for 12 months and seven were then studied for a further 12 months under placebo. During treatment with EHDP, inhibitory activity in urine towards precipitation of calcium phosphate was restored from low values to greatly above normal. This could be accounted for by the inhibitory effect of EHDP itself, coupled with an increase in urinary inorganic pyrophosphate. After stopping EHDP the excretion of EHDP rapidly fell to undetectable levels but the excretion of pyrophosphate remained elevated throughout the 12 months of placebo treatment. EHDP also induced a rise in plasma phosphate and an increase in the urinary excretion of oxalic acid and uric acid, but these changes were all fully reversible when EHDP was stopped.

4. The average rate of stone formation per patient per year decreased from 2·4 to 0·2 during treatment with EHDP and remained low during the following 24 months. However, the dose needed for this effect is known to affect bone turnover and mineralization.

Key words: bone, calcium, ethane-1-hydroxy-1,1-diphosphonate, diphosphonate, oxalate, phosphate, pyrophosphate, uric acid, urine, urolithiasis.

Abbreviation: EHDP, ethane-1-hydroxy-1,1-diphosphonate.

Introduction

Several treatments exist for recurrent calcium stone disease, but none is beneficial in all patients, indicating that stone formation can arise from a variety of pathogenic mechanisms. Among these the increase in urinary saturation of the stone-forming salts (Robertson, Peacock & Nordin, 1968; Pak, 1969; Marshall, Cochran, Robertson, Hodgkinson & Nordin, 1972) and the lack of crystallization and aggregation inhibitors in the urine (Russell & Fleisch, 1969; Robertson, Peacock, Marshall, Marshall & Nordin, 1976) are probably the most important. Citrate, magnesium and inorganic pyrophosphate (Fleisch & Bisaz, 1962; Smith, Meyer & McCall, 1973) are the main inhibitors of crystallization of calcium phosphate, whereas aggregation of calcium phosphate crystals is inhibited not only by citrate and pyrophosphate but also by some
unidentified high-molecular-weight compounds, possibly glycosaminoglycans (Hansen, Felix, Bisaz & Fleisch, 1976). Among inhibitors of crystallization and aggregation of calcium oxalate, glycosaminoglycans and to a lesser degree pyrophosphate are the most important (Fleisch & Bisaz, 1964; Robertson, Peacock & Nordin, 1973; Meyer & Smith, 1975; Robertson, Knowles & Peacock, 1976; Felix, Monod, Broge, Hansen & Fleisch, 1977).

Ethane-1-hydroxy-1,1-diphosphonate (EHDP), a synthetic compound related in structure to pyrophosphate but with P-C-P instead of P-O-P, has been studied as an inhibitor of crystallization. Low concentrations of EHDP inhibit the formation of apatite (Fleisch, Russell, Bisaz, Mühlbauer & Williams, 1970; Meyer & Nancollas, 1973), brushite (Ohata & Pak, 1973) and calcium oxalate (Fräser, Russell, Pohler, Robertson & Fleisch, 1972) in vitro. It also interferes with the conversion of amorphous calcium phosphate into apatite (Francis, 1969) and with the aggregation of calcium oxalate (Robertson et al., 1973; Felix et al., 1977) and hydroxyapatite crystals (Hansen et al., 1976). EHDP, which is not degraded in the body, prevents experimentally induced soft tissue calcification in vivo (Fleisch et al., 1970) and inhibits to some extent the formation of calcium oxalate stones, induced by feeding with ethylene glycol, and also the formation of brushite concretions, induced by vitamin D in the rat (Fraser et al., 1972). In man EHDP has been claimed to slow down the progression of myositis ossificans progressiva (Geho & Whiteside, 1973) and to restore increased bone turnover in Paget's disease to normal (Altman, Johnston, Khairi, Wellman, Serafini & Sankey, 1973; Russell, Smith, Preston, Walton & Woods, 1974). Recently this compound has been approved by the Food and Drug Administration in the U.S.A. for use in Paget's disease.

The aim of this study was firstly to find a dose and mode of administration of EHDP that would result in a relatively constant daily urinary excretion, sufficient to inhibit calcium salt crystallization and aggregation. The drug was then given to a small number of recurrent calcium stone-forming patients and its biochemical and clinical effects were examined under standardized conditions. Because of the potential effects of long-term administration on the skeleton the drug was given for 1 year only. A preliminary report of this work has been published (Baumann, Ganz, Bisaz, Fleisch & Rutishauser, 1974).

**Methods**

**Patients**

Nine patients (eight men, one woman) with idiopathic recurrent stones, described in Table 1, were observed before, during and after EHDP administration.

**Mode and duration of EHDP administration**

In a preliminary study the concentration of EHDP in urine samples collected over 24 h was measured after giving EHDP at various doses in various ways. In order to adequately inhibit the formation, growth and aggregation of calcium oxalate and calcium phosphate crystals, the aim was to attain a minimal urinary concentration of EHDP above $10^{-3}$ mol/l in all urine samples throughout the 24 h. This had been found to be the lowest concentration that consistently inhibited calcium phosphate (Fleisch et al., 1970) and oxalate (Fraser et al., 1972; Robertson et al., 1973; Felix et al., 1977) crystallization and aggregation. It was found that this could be achieved by the administration of 4.4 mmol of EHDP/day, divided into four doses. EHDP was therefore given as follows, to nine patients over 12 months: 0.8 mmol 30 min before breakfast, 1.2 mmol before lunch, 0.8 mmol before dinner and 1.6 mmol before going to bed. Seven of the patients were studied for a further 12 months when receiving a placebo. All patients gave informed consent.

**Examination protocol**

Patients were reviewed every 6 months with a physical examination, an X-ray examination of the

<table>
<thead>
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<th>No.</th>
<th>Sex</th>
<th>Year of birth</th>
<th>Stone analysis</th>
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<tr>
<td>1</td>
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<td>1939</td>
<td>Oxalate</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
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<td>Oxalate/urate</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>1935</td>
<td>Oxalate</td>
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<tr>
<td>4</td>
<td>M</td>
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</tr>
<tr>
<td>6</td>
<td>F</td>
<td>1949</td>
<td>Oxalate/phosphate</td>
</tr>
<tr>
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<td>M</td>
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<td>Oxalate</td>
</tr>
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<td>8</td>
<td>M</td>
<td>1924</td>
<td>Oxalate/phosphate</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>1936</td>
<td>Oxalate</td>
</tr>
</tbody>
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abdomen and hands and determinations of blood Ca$^{2+}$, inorganic phosphorus (P$_i$), urea, uric acid, alkaline phosphatase, creatinine, haemoglobin, erythrocyte count, differential leucocyte count and erythrocyte sedimentation rate, as well as urine Ca, P$_i$, oxalic acid, uric acid, pH, acetone, glucose, protein, sediment, pyrophosphate and inhibitors of calcium phosphate precipitation. These measurements were made during a 2 day stay in hospital while the patients received a diet of known Ca, P and oxalate content, but which was otherwise typical for this country. Fluid intake was unrestricted but confined to distilled water with flavouring. The exact protocol has been described previously (Baumann, Bisaz, Felix, Fleisch, Ganz & Russell, 1977).

**Analytical techniques**

Calcium was measured by atomic absorption spectrophotometry (Perkin Elmer Atomic Absorption Spectrophotometer, model 290 B), phosphorus (P$_i$) by the method of Bisaz, Russell & Fleisch (1968), uric acid by the method of Kageyama (1971), oxalic acid by the method of Hodgkinson & Zarembski (1961), pyrophosphate by the method of Fleisch & Bisaz (1963), creatinine with a Greiner Selective Analyser II (Greiner Electronic Ltd, CH-4900 Langenthal) according to a modified method of Jaffe (1886), and EHDP by the techniques of Bisaz, Felix & Fleisch (1975). Inhibitors of calcium phosphate precipitation were determined as described by Baumann et al. (1977) in urine samples diluted to an equivalent of 200 ml/h. The test consists in assessing the influence of about 3% of this diluted urine on the minimum [Ca] x [P$_i$] necessary to induce calcium phosphate precipitation in a standard solution of fixed composition.

**Results**

**Urinary excretion of EHDP**

The administration of a single dose of 0.04 mmol of EHDP/kg body weight 30 min before breakfast to four healthy members of the staff revealed that only 1.04 (SE 0.34)% of the total dose was excreted within 24 h. When EHDP was given together with breakfast, the excretion, although not significantly less at 0.22 (SE 0.06)% was, however, rapid. As early as 4 h after the ingestion, 80.9 (SE 8.54)% of the total EHDP excreted during 24 h had appeared in the urine.

When EHDP was given four times a day, 30 min before the meals and before going to bed, the excretion was fairly constant in the various collections (Fig. 1). By testing various daily doses, it appeared that a daily administration of 4.4 mmol of EHDP was necessary to reach a concentration above 10$^{-3}$ mol/l in every urine sample during the day. On this regimen 0.4 (SE 0.08)% of the administered drug was excreted in the first 24 h. Excretion increased to 1.32 (SE 0.21)% after 6 months' treatment and to 1.39 (SE 0.15)% after 12 months. Six weeks after changing to placebo administration, only small amounts of EHDP were found in the urine, and after 12 months the amounts of EHDP excreted were not significantly different from the value in urine samples without EHDP (Fig. 2).

**Biochemical effects of EHDP**

Serum calcium, uric acid and creatinine were not different in stone-forming patients and control.
subjects and did not show significant alterations during EHDP and placebo treatment. Serum phosphate increased significantly with EHDP, from 1.07 mmol/l (SE 0.09) before treatment to 1.3 mmol/l (SE 0.04) after 12 months' therapy.

The findings for the urine of eight male patients are shown in Fig. 3. The only female is not included, since normal control values differ between males and females. The initial hypercalciuria of the stone-forming patients was partially corrected under EHDP therapy and recurred after it was stopped. Urinary pyrophosphate, which was lower than normal \((P < 0.005)\) before treatment, increased significantly after 12 months' treatment with EHDP \((P < 0.005)\), reaching normal values and, interestingly, remaining so up to 12 months after EHDP was discontinued. A similar effect was observed for the inhibitory activity on calcium phosphate precipitation, which was markedly diminished in stone-formers \((P < 0.005)\), increased above normal values during EHDP therapy, and after 12 months' placebo treatment was still significantly higher than the initial values \((P < 0.02)\). Fig. 4 shows that, during EHDP therapy, inhibitory activity was due mainly to inhibitors other than pyrophosphate, i.e. EHDP. However, on placebo it
could be attributed entirely to the urinary pyrophosphate. Both urinary oxalate and uric acid excretion increased during EHDP medication and after 12 months' treatment were significantly higher than the normal range ($P < 0.001$) as well as the pretreatment values ($P < 0.001$). At the end of the placebo administration both returned to pretreatment values. pH and volume of urine did not change under EHDP.

Effect on stone recurrence

As shown in Fig. 5, two of the six patients (no. 6 and no. 8) who were first treated with lower doses of EHDP had to be operated on for recurrence of stones. One patient (no. 4) also passed further small concretions spontaneously. However, during the administration of the higher dose, 4.4 mmol of EHDP/day, only one patient (no. 3) passed two more stones spontaneously and this close to the start of the treatment. The other eight patients revealed no clinical or radiological evidence of new stone formation during therapy. In the year after discontinuation of EHDP, during which the patients received a placebo, only one patient (no. 5) produced a new calcium oxalate stone. It is interesting that in this patient the excretion of pyrophosphate had decreased again to 0.68 μmol/h and the urinary inhibitory activity to 4.4 (mmol/l)$^2$, whereas in the other six patients these values remained high. In the period after discontinuation of placebo administration one more patient (no. 7) produced a further new concretion, the stones operated on in patient no. 9 having been present before. Thus although the average rate of stone passage during 2 years before treatment was 2.4 per patient per year, it decreased to 0.2 on EHDP and remained at this figure for 2 years after discontinuation of EHDP treatment.
Side effects

The drug was generally well tolerated. Except for the rise in plasma phosphate no changes in the various laboratory blood investigations were observed. X-rays of the spine and X-rays of the hands, with measurement of cortical bone thickness of the second metacarpal (Nordin, 1976), showed no alterations. Two patients, however, complained of pains and weakness in the legs and swollen ankles. One of them (no. 3) discontinued treatment. The second (no. 2) was free of symptoms 14 days after stopping EHDP but the same disturbances appeared during the completion of the 12 months' treatment.

Discussion

The recovery of 80% of the daily urinary excretion of EHDP within 4 h of a single oral dose points to a rapid urinary elimination. High renal clearances of EHDP, even exceeding glomerular filtration rates, have been demonstrated in rats (Troehler, Bonjour & Fleisch, 1975). This rate of elimination makes it necessary to administer the drug several times a day, four times a day appearing to be sufficient for a steady urinary output.

It has been reported that about half of the absorbed drug is excreted in urine, almost all of the rest being deposited in the bone (Michael, King & Wakim, 1972). This relation seems to alter during long-term treatment since urinary excretion increased markedly during therapy. This effect may be explained by progressive saturation of bone with EHDP.

All stone-formers receiving 4-4 mmol of EHDP/day showed a marked increase of an initially diminished urinary inhibitory activity towards calcium phosphate crystallization. A similar effect has been described for calcium oxalate crystals (Robertson, Peacock, Marshall & Knowles, 1974) and for the formation of brushite (Ohata & Pak, 1974). The increase in our study was due both to EHDP and to an increase in pyrophosphate excretion. Interestingly, the increase due to the latter remained high in six of seven patients 12 months after stopping the treatment. Furthermore, the only patient whose urinary pyrophosphate and inhibitory activity had dropped to pathologically low values after stopping the diphosphonate produced new concretions, the others remaining stone-free. The protracted increase in pyrophosphate excretion and concentration might perhaps partly explain the surprising finding that stone formation is lowered even after discontinuation of the drug, despite the fact that EHDP excretion has dropped to undetectable levels. However, it is likely that another, yet unknown, factor is also responsible for this effect.

Besides increasing urinary pyrophosphate, EHDP led also to an increase in uric acid and oxalate, which reversed after stopping the drug. The effect on oxalate, which has been described previously (Robertson et al., 1974), might be due to increased intestinal absorption of oxalate, after a binding of calcium by EHDP in the gut. The increase in plasma phosphate has been seen in other studies and seems to be due to a change in the renal handling of phosphate (Recker, Hassing, Lau & Saville, 1973; Walton, Russell & Smith, 1975).

Two of the patients who had received EHDP for at least 7 months complained of pains and weakness in the legs. These symptoms were fully reversible on withdrawal of the drug. Similar side effects have been seen in children with myositis ossificans progressiva, who had been treated for long periods (H. Fleisch et al., unpublished observation). The cause of these symptoms could not be elucidated, but it could be related to an inhibition of mineralization by EHDP, similar to that reported both in animals (Schenk, Merz, Mühlbauer, Russell & Fleisch, 1973) and man (Russell et al., 1974). Indeed during these studies it was found that doses of 40 μmol day⁻¹ kg⁻¹ and above inhibit mineralization of bone in some patients with Paget's disease when given for more than 3 months. However, this effect is reversible after discontinuation of the drug. Doses of 20 μmol day⁻¹ kg⁻¹ for as long as 2 years showed no such effects (Smith, Russell, Bishop, Woods & Bishop, 1973; P. Meunier, personal communication).

Our results indicate the general possibility of using orally administered inhibitors of crystallization in the treatment of renal stones. Although EHDP itself is not the agent of choice in urolithiasis because of its effects on skeletal turnover and mineralization at the doses necessary to prevent stone formation, it may be of value if given over limited periods in severe cases resistant to other treatment. However, it is important that the principle of inhibition could be extended to the development of related compounds having similar physicochemical effects in urine but without effects on bone.

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References


Diphosphonate in calcium urolithiasis 515

