Xanthine Oxidase Deficiency: Studies of a Previously Unreported Case

Edward W. Holmes, Jr., David H. Mason, Jr., Leonard I. Goldstein, Robert E. Blount, Jr., and William N. Kelley

Xanthinuria is a familial disorder of purine metabolism that results from a marked deficiency of xanthine oxidase (EC 1.2.3.2) activity. We report here the clinical and biochemical features of a new case of xanthinuria. Serum urate concentration was 0.8 mg/100 ml, urinary uric acid excretion was 16 mg per day, urinary oxypurine excretion was 1630 μmol per day, and total purine excretion was 314 mg per day. After allopurinol was administered, total purine excretion was 323 mg per day and erythrocyte phosphoribosylpyrophosphate content was unchanged. The ratio (by wt) of xanthine to hypoxanthine in the urine was 4.6 before and 9.6 after allopurinol was administered to this patient. Both allopurinol and oxipurinol were detectable in urine. Orotic acid and orotidine excretion increased from undetectable amounts (<2 mg per day) to 47 and 86 mg per day, respectively. These data suggest that this xanthinuric subject has a markedly decreased xanthine oxidase activity, although some residual activity may be functional in vivo. It is probable that he re-utilizes purine so extensively that hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) is virtually saturated with hypoxanthine and xanthine in vivo. In addition, these data indicate that the increase in orotic acid and orotidine seen in normal and gouty subjects taking allopurinol is not a direct consequence of xanthine oxidase inhibition, but probably an effect of allopurinol or one of its metabolites on pyrimidine biosynthesis.

Additional Keyphrases: inherited disorders • purine and pyrimidine metabolism • xanthinuria

Xanthinuria is a disorder of purine metabolism that results from a marked deficiency of xanthine oxidase (EC 1.2.3.2) activity in hepatic tissue, small-intestinal mucosa, colostrum, and rectal mucosa (1). Although half the patients with this disorder are asymptomatic, about a third of them have nephrothiasis and 10% have myopathy as a consequence of the derangement in purine metabolism (1). Because some individuals with this disorder are only discovered incidentally through the finding of hypouricemia and because the available data suggest that the disorder is inherited as an autosomal recessive, it is impossible to estimate the prevalence of the mutant gene in the general population. However, xanthinuria must be very uncommon; one prospective survey for hypouricemia in a large general hospital failed to detect a single case in 6629 consecutive uric acid determinations (2). To date, only 18 cases of xanthinuria have been reported (1). Interest in this disorder has not been stifled by its rarity, however, because the absence of xanthine oxidase provides a unique opportunity to understand several aspects of purine metabolism in man. We have recently studied several features of purine metabolism in a previously unreported case of xanthinuria and in this report we describe the results of these studies.

Case Report

J.P. is a 21-year-old healthy male of Italian descent, an only child, who gives no history of renal calculi or myopathy. He was incidentally noted to be hypouricemic during evaluation for thalassemia minor while in the U.S. Army. The results of physical ex-
amination, including a detailed neurological examination, were within normal limits, except for mild icterus. Values for all laboratory data were normal except for the findings associated with hemolytic anemia and xanthinuria. Routine urine analysis revealed a moderate number of crystals, which were digested by xanthine oxidase but not uricase. An intravenous pyelogram was normal. The patient refused a muscle biopsy but an electromyogram was normal. There was no clinical evidence of hemochromatosis, pheochromocytoma, or polyarthritis in this patient. There was no family history of renal calculi or myopathy. There was no history of consanguinity.

Methods

J.P. was hospitalized on the Clinical Research Unit at Duke Hospital after obtaining informed consent. He was given a 2600-calorie, 70-g protein, purine-free diet, and no drugs were administered other than those described in this protocol. All urine samples were collected at room temperature in a clear plastic container under 3 ml of toluene and aliquots were stored at −20 °C. Routine blood samples were obtained by Vacutainer and processed at room temperature. Blood samples for analysis of phosphoribosylpyrophosphate (PP-ribose-P) were collected in tubes with ethylenediaminetetraacetate as anticoagulant and immediately placed in a 4 °C ice bath.

Rectal biopsy was performed with a Quinton rectal biopsy kit, jejunal biopsy with a Quinton small bowel biopsy kit (3). These tissue specimens were washed in sodium pyrophosphate buffer (67 mmol/liter, pH 8.0), homogenized, and dialyzed against the same buffer before assay. Xanthine oxidase activity was determined by a radiochemical assay in which the conversion of [14C]hypoxanthine to [14C]xanthine and [14C]uric acid (3) was measured.

PP-ribose-P was extracted from the erythrocytes and assayed by use of adenine phosphoribosyltransferase (EC 2.4.2.7) (4).

Serum and urinary uric acid were determined by the uricase method (5). Total oxypurines in the urine were determined as the difference between the uric acid concentration before and after xanthine oxidase was added to the sample (6).

Urine samples (2 ml) were applied to the Mark II ultraviolet analyzer as previously described (7). The compounds that were eluted from the column were identified by their relative elution volume and their absorption characteristics at two different wavelengths. The solute concentrations were calculated by a FOCAL 8 program on a PD8/E computer.

Protein was determined by the method of Lowry et al. (8), with bovine serum albumin as standard.

All chemicals were of the highest grade commercially available and their purity was based on data supplied by the manufacturer.

Results

Uric acid and oxypurine determinations. Table 1 lists the uric acid and oxypurine determinations performed while the patient was on the purine-free diet. Serum urate by the uricase method averaged 0.8 mg/dl, with the highest value being 1.3 mg/dl. The daily excretion of uric acid averaged 16 mg, with a maximum of 26 mg. Total urinary oxypurines, determined as the amount of uric acid produced from hypoxanthine and xanthine present in the urine, averaged 1630 μmol per day. If we assume that hypoxanthine and xanthine had been totally converted to uric acid endogenously, this patient would excrete an average 314 mg of uric acid per day. This is less than the 418–469 mg of uric acid excreted per day, on the average, by the normal adult male (9).

Xanthine oxidase activity. Enzyme activity was determined in both small intestinal and rectal mucosa. Xanthine oxidase activity in jejunal mucosa obtained from the patient was 5% as great as that found for a control subject. The xanthine oxidase activity in his rectal mucosa was 25% of that found for 15 control subjects. These data confirm the patient's deficiency of xanthine oxidase activity and establish the diagnosis of xanthinuria in this subject.

Effect of allopurinol on purine metabolism. As mentioned above, total purine excretion on a purine-free diet averaged 314 mg per day, a value well below that for the normal man (9). After 800 mg per day of allopurinol was administered, total purine excretion was unchanged (323 mg per day). This contrasts with the decreased purine excretion seen in many normal individuals after allopurinol administration, but is similar to the response seen in patients with a deficiency of hypoxanthine phosphoribosyltransferase (1).

The PP-ribose-P content of erythrocytes from the patient was 5.3 nmol/ml in the control specimen. It was unchanged (5.1 nmol/ml) after the administration of 800 mg of allopurinol per day for four days. This also is different from the response observed in control subjects, since in whom allopurinol normally produces a decreased PP-ribose-P content of erythrocytes. Although this lack of effect resembles the results obtained in subjects with a deficiency of the transferase (11), results of direct assay of this enzyme in erythrocytes and rectal mucosa from J.P. were within normal limits (3).

\[ ^1 \text{Also commonly called hypoxanthine-guanine phosphoribosyltransferase.} \]
Table 2. Urinary Excretion of Purines and Pyrimidines by the Patient (J.P.) and by Normal Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Xanthine mg/24 h</th>
<th>Hypoxanthine</th>
<th>Xanthine/hypoxanthine (ratio)</th>
<th>Orotic acid mg/24 h</th>
<th>Orotidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J.P. Controls</td>
<td>J.P. Controls</td>
<td></td>
<td>J.P. Controls</td>
<td>J.P. Controls</td>
</tr>
<tr>
<td>Control</td>
<td>137</td>
<td>4.0</td>
<td>44</td>
<td>5.9</td>
<td>0.70</td>
</tr>
<tr>
<td>Allopurinol*</td>
<td>344</td>
<td>41</td>
<td>37</td>
<td>12.5</td>
<td>9.3</td>
</tr>
</tbody>
</table>

* 800 mg of allopurinol per day for four days.

Urinary excretion of purines and pyrimidines. Table 2 gives results of studies performed with the analyzer. On a purine-free diet and in the absence of drugs, J.P. excreted 137 mg of xanthine and 44 mg of hypoxanthine per day. The xanthine/hypoxanthine ratio (by wt) in the urine during the control period was 4.6. The same ratio for three control subjects on a purine-free diet was 0.7 (7). After 800 mg of allopurinol per day had been administered for four days, urinary xanthine increased to 344 mg and hypoxanthine decreased to 37 mg. This resulted in a further increase in the xanthine/hypoxanthine ratio to 9.3. In three control subjects, allopurinol therapy was associated with a xanthine to hypoxanthine ratio of 3.3.

Allopurinol was identified in the urine of the patient during administration of this drug. In addition, oxipurinol, the major metabolite of allopurinol, has been tentatively identified in this urine sample.

Orotic acid and orotidine excretion were also quantitated in the patient's urine and these results are recorded in Table 2. No detectable (<2 mg per day) orotic acid or orotidine was found in his urine or in that of control subjects. However, after the administration of allopurinol, the patient's excretion of both orotic acid and orotidine markedly increased (47 and 86 mg per day, respectively). This response is qualitatively similar to that seen in normal subjects (12).

Discussion

The diagnosis of xanthinuria was documented in this patient by the demonstration that xanthine oxidase activity was markedly diminished in jejunal and rectal mucosa (3). The reactions catalyzed by xanthine oxidase convert hypoxanthine to xanthine and xanthine to uric acid (Figure 1). A significantly decreased xanthine oxidase activity would result in decreased production of uric acid, the normal end-product of purine metabolism. Consequently any patient with xanthinuria would be expected to have a low serum and urinary uric acid with a concomitant increase in oxytpurine (hypoxanthine + xanthine) excretion. As demonstrated here in J.P. (Table 1), and as previously reported for other patients with xanthinuria (1), hypo-uricemia, hypo-uricaciduria, and an increased oxytpurine excretion are the biochemical hallmarks of this disease. The increase in plasma and urinary oxypurines is responsible for the two clinical manifestations of xanthinuria—myopathy secondary to hypoxanthine and xanthine crystal deposition in the muscle and formation of renal calculi composed of xanthine.

Previous studies in other individuals with xanthinuria have suggested that there is extensive re-utilization of purine bases in this disorder (13-15). This is probably a result of the increased concentration of hypoxanthine. Both hypoxanthine and xanthine can be converted to purine ribonucleotides by the transferase enzyme, while uric acid cannot be salvaged by this pathway because it is not a substrate for it (Figure 1). The demonstration that hypoxanthine is a better substrate than xanthine for the transferase of human origin explains why it is re-utilized to a greater extent than is xanthine (13-15). This also explains why the excretion of xanthine is roughly fivefold that of hypoxanthine (Table 2).

It might be anticipated that the transferase would be saturated to a greater extent with hypoxanthine in the xanthinuric subject than in the normal subject because of the extensive re-utilization of this compound as described above. If the enzyme were almost completely saturated with hypoxanthine and xanthine in vivo, then there would be little binding of a relatively poor substrate such as allopurinol at this same site on the enzyme. These predictions appear to
be true, because allopurinol did not decrease total purine excretion or erythrocyte PP-ribose-P content in J.P. Both of these metabolic responses depend on functional activity of the transferase, as evidenced by the failure to observe either effect of allopurinol in patients with a genetic deficiency of this enzyme (10, 11). Activity of the transferase is normal in J.P., so one cannot attribute this failure to exhibit these effects of allopurinol to a deficiency of this enzyme. In accord with the reasoning presented above it seems more likely that the enzyme in this subject with xanthinuria is virtually saturated with hypoxanthine and xanthine in vivo and thus is relatively unavailable for the conversion of allopurinol to allopurinol ribonucleotide. It is interesting to note that administration of allopurinol to individuals with normal xanthine oxidase activity results in a change in xanthine and hypoxanthine excretion that is qualitatively similar to that observed in the subject with xanthinuria.

There are several lines of evidence to suggest that this patient has residual xanthine oxidase activity present in vivo. The administration of allopurinol to this subject caused a further increase in the excretion of xanthine relative to hypoxanthine. This could be the result of a reduction in the residual xanthine oxidase activity and an accentuation of the phenomenon described above. If the tentative identification of oxidase in the urine of J.P. can be confirmed, this would provide additional support for the presence of a residual amount of xanthine oxidase activity in vivo, because allopurinol is metabolized to oxipurinol by xanthine oxidase. However, we cannot exclude the possibility that the oxidation of allopurinol to oxipurinol was catalyzed by bacteria in the gut rather than by a low xanthine oxidase activity in vivo, because no parenteral preparation of allopurinol is currently available. The direct demonstration of a low degree of xanthine oxidase activity in extracts from jejunal and rectal mucosa provides the final evidence to support this hypothesis. Considering all of this evidence together, we think it likely that there is at least some functional residual xanthine oxidase activity present in this subject.

Administration of allopurinol to normal and gouty subjects results in a marked increase in orotic acid and orotidinuric excretion (12). The mechanism responsible for this is not completely understood, but it is thought to be a consequence of the inhibition of one or more of the steps in de novo pyrimidine biosynthesis, i.e., the reactions catalyzed by orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidylic decarboxylase (EC 4.1.1.23). One potential explanation for this effect of allopurinol is its inhibition of xanthine oxidase. The present study would seem to exclude this possibility, however, because J.P. did not exhibit orotic aciduria or orotidinuria before allopurinol was begun. In fact, the demonstration of orotic aciduria and orotidinuria in J.P. after allopurinol administration suggests that allopurinol or one of its metabolites is responsible for the increase in pyrimidine excretion observed under these circumstances.

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References