Methylated Purines in Urinary Stones

KRZYSZTOF SAFRANOW * and ZYGMUNT MACHOY

Background: The aim of the study was to measure the content of methylated purines that appear as admixtures in uric acid stones.

Methods: We analyzed urinary calculi from 48 residents of Western Pomerania who underwent surgery at the urology ward in Szczecin. Stone samples were dissolved in 0.1 mol/L NaOH. Extracts were diluted in 50 mmol/L KH2PO4 and analyzed by reversed-phase HPLC with ultraviolet detection and use of a gradient of methanol concentration and pH.

Results: Uric acid was the main component of 9 stones. All 9 showed admixtures of 9 other purine derivatives: endogenous purine breakdown products (xanthine, hypoxanthine, and 2,8-dihydroxyadenine) and exogenous methyl derivatives of uric acid and xanthine (1-, 3-, and 7-methyluric acid; 1,3-, 1,7-, and 3,7-dimethyluric acid; and 1-, 3-, and 7-methylxanthine). Amounts of these purine derivatives ranged from the limit of detection to 12 mg/g of stone weight and showed a strong positive correlation (Spearman rank correlation coefficients, 0.63–0.94) with the uric acid content of the samples. The main methylated purine in the stones was 1-methyluric acid.

Conclusions: Urinary purines at concentrations below their saturation limits may coprecipitate in samples supersaturated with uric acid and appear as admixtures in urinary stones. The amount of each purine depends on its average urinary excretion, similarity to the chemical structure of uric acid, and concentration of the latter in the stone. These findings suggest that purines in stones represent a substitutional solid solution with uric acid as solvent. Methylxanthines, which are ubiquitous components of the diet, drugs, and uric acid calculi, may be involved in the pathogenesis of urolithiasis.

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Many purines are poorly soluble in aqueous solutions and may precipitate from urine, forming urinary calculi, if their concentrations exceed the limit of saturation. Uric acid (UA)1 is the end product of human purine metabolism and is excreted mainly in the urine. Acidic urine is often a supersaturated solution of uric acid, even when the daily output remains in the normal range, and uric acid stones may form. Crystallization is usually prevented by inhibitors (citrates, glycosaminoglycans, and some proteins) (1), although not always effectively enough, particularly at low urine pH. Calculi composed of UA also form in males with hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) deficiency (complete or partial). Xanthine (Xan) may appear in xanthinuria as well as during treatment with allopurinol (2). 2,8-Dihydroxyadenine (2,8-DHA) is excreted in urine and can precipitate in renal tissue and the urinary tract in patients with dihydroxyadeninuria attributable to adenine phosphoribosyltransferase (EC 2.4.2.7) deficiency (3).

Limited attention has been paid to the possible role of metabolites of methylxanthines (caffeine, theophylline, and theobromine) in the pathogenesis of urolithiasis, although their average daily output in urine, depending on dietary intake, is ~500 mg/day (4), which equals the output of UA. The main metabolites—methyluric acids—have properties similar to those of UA, including low solubility in aqueous solutions. Detection of their presence in stones by use of traditional analytical techniques (x-ray diffraction, infrared spectroscopy, and semiquantitative wet tests) has not been reported. We have recently developed a gradient HPLC method for separation of 16 purines, including 10 methyl derivatives of Xan and UA, in urinary calculi (5). We present here results of analysis of purine derivatives in urinary calculi by this method.

Materials and Methods

We examined urinary stones from 48 adult patients (31 men and 17 women) of Caucasian descent [mean (SD) age

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1 Nonstandard abbreviations: UA, uric acid; Xan, xanthine; 2,8-DHA, 2,8-dihydroxyadenine; Hyp, hypoxanthine; 1-, 3-, 7-, and 9-MUA, 1-, 3-, 7-, and 9-methyluric acid; 1,3-, 1,7-, and 3,7-DMU, 1,3-, 1,7-, and 3,7-dimethyluric acid; and 1-, 3-, and 7-MX, 1-, 3-, and 7-methylxanthine.
of 49 (15 years) who underwent surgery at the urology ward in Szczecin. If the stones were heterogeneous, i.e., their respective parts or layers differed in color or structure from each other, separate samples were taken from each part, e.g., nucleus and outer layer. In total, 65 samples were analyzed.

Urinary stone samples were dissolved in 0.1 mol/L NaOH as described previously (6). The extracts were diluted in 50 mmol/L KH2PO4 and analyzed by HPLC (5). Using this method, we separated 16 compounds: UA; Xan; hypoxanthine (Hyp); 2,8-DHA; allopurinol; oxypurinol; 1-, 3-, 7-, and 9-methyluric acid (1-, 3-, 7-, and 9-MUA); 1,3-, 1,7-, and 3,7-dimethyluric acid (1,3-, 1,7-, and 3,7-DMU); and 1-, 3-, and 7-methylxanthine (1-, 3-, and 7-MX). Limits of detection for individual compounds ranged from 6 to 35 μg/g of stone weight, and imprecision (CV) was 0.5%–2.4%. Other components of the stones were quantified by wet methods (7).

When multiple samples were taken from a stone, the arithmetic mean was calculated for the content of each compound. Normality of distributions was checked with the Shapiro–Wilk test. Correlations were measured with Spearman rank correlation coefficient (rs).

### Results

The purine compound found as the main stone constituent was UA; its content exceeded 50% in 9 (19%) stones. Of the 48 stones analyzed, 14 (29%) contained no UA, 14 (29%) contained detectable UA that constituted <1% of stone mass, 8 (17%) contained 1%–25% UA, 3 (6%) contained 25%–50% UA, 1 (2%) contained 50%–75% UA, and 8 (17%) contained >75% UA.

In all UA-containing calculi, we found 9 other purines in addition to UA: Xan; Hyp; 2,8-DHA; 1-, 3-, and 7-MUA; 1,3-DMU; and 3- and 7-MX (Table 1 and Fig. 1). Allopurinol, oxypurinol, 9-MUA, 1,7- and 3,7-DMU, and 1-MX were not detected in any sample. Other main constituents of the calculi were calcium oxalates in 26 (54%) stones, calcium phosphates in 6 (13%), struvite (magnesium ammonium phosphate) in 6 (13%), and cystine in 1 stone (2%).

The distributions of UA and other purines in the stones were far from gaussian; therefore, the results were not expressed as mean (SD). The median was not a useful parameter either because most of the compounds were detected in less than one-half of the stones.

Several purines were detected in stones containing at least 4% UA (1-MUA was detectable even when UA constituted 1% of stone mass) but were never found in stones containing no UA. There were strong positive correlations (P < 0.0001) between the UA content and each purine (Table 1).

The correlation plot of UA and 1-MUA in the stones is shown in Fig. 2. For other purines, correlation plots also suggested a linear regression, with the exception of 3-MX, for which the highest concentrations were observed in stones containing 10%–20% UA.

The strong linear correlation of UA with admixtures of other purines justifies expressing their quantities as purine/UA quotients, calculated as the ratio of masses of any purine to UA in a stone. The quotients could not be calculated for stones containing no UA; for calculi containing little UA and accompanying purines, purine/UA quotients would be very imprecise. That is why only 19 quotients calculated for stones containing >4% UA were analyzed further (Table 1). The distributions of quotients were gaussian with the exception of the 3-MX/UA and Xan/UA quotients. The mean of the quotients (MQ) is an optimal estimate of the regression coefficient in the equation formulating dependence between content of UA and other purines: predicted purine content = MQ × (UA content).

### Table 1. Content of purines detected in the examined urinary stones.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. (%) of stones in which detected</th>
<th>Content range, a %</th>
<th>Correlation with UA content (rs) b</th>
<th>Content relative to UA content, c Mean (MQ) d</th>
<th>Median</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>UA</td>
<td>34 (71)</td>
<td>0–90.7</td>
<td>0.88</td>
<td>1.43</td>
<td>0.83</td>
<td>1.81</td>
<td>0.31–8.55</td>
</tr>
<tr>
<td>Xan</td>
<td>20 (42)</td>
<td>0–0.12</td>
<td>0.87</td>
<td>0.35</td>
<td>0.37</td>
<td>0.17</td>
<td>0–0.82</td>
</tr>
<tr>
<td>Hyp</td>
<td>19 (40)</td>
<td>0–0.04</td>
<td>0.84</td>
<td>0.09</td>
<td>0.07</td>
<td>0.06</td>
<td>0–0.19</td>
</tr>
<tr>
<td>2,8-DHA</td>
<td>16 (33)</td>
<td>0–0.012</td>
<td>0.94</td>
<td>9.91</td>
<td>9.48</td>
<td>3.97</td>
<td>2.60–18.74</td>
</tr>
<tr>
<td>1-MUA</td>
<td>24 (50)</td>
<td>0–1.20</td>
<td>0.94</td>
<td>9.91</td>
<td>9.48</td>
<td>3.97</td>
<td>2.60–18.74</td>
</tr>
<tr>
<td>3-MUA</td>
<td>14 (29)</td>
<td>0–0.05</td>
<td>0.80</td>
<td>0.23</td>
<td>0.23</td>
<td>0.19</td>
<td>0–0.59</td>
</tr>
<tr>
<td>7-MUA</td>
<td>22 (46)</td>
<td>0–0.31</td>
<td>0.92</td>
<td>2.38</td>
<td>2.65</td>
<td>1.03</td>
<td>0.20–3.63</td>
</tr>
<tr>
<td>1,3-DMU</td>
<td>18 (38)</td>
<td>0–0.08</td>
<td>0.87</td>
<td>0.52</td>
<td>0.48</td>
<td>0.25</td>
<td>0–0.92</td>
</tr>
<tr>
<td>3-MX</td>
<td>13 (27)</td>
<td>0–0.04</td>
<td>0.63</td>
<td>0.60</td>
<td>0.07</td>
<td>1.00</td>
<td>0–2.90</td>
</tr>
<tr>
<td>7-MX</td>
<td>18 (38)</td>
<td>0–0.16</td>
<td>0.85</td>
<td>0.98</td>
<td>0.86</td>
<td>0.73</td>
<td>0–2.69</td>
</tr>
</tbody>
</table>

a Content of purines detected in the 48 examined stones is expressed as percentage of total stone mass.
b Correlations with UA content are calculated as Spearman rank correlation coefficients (rs).
c Statistics of purine/UA quotients were calculated for each purine in 19 stones containing >4% UA.
d Mean purine content in a stone (in mg) = MQ × UA content (in g).

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Discussion

All of the UA calculi examined contained N-methyl derivatives of UA (1-MUA, 3-MUA, 7-MUA, and 1,3-DMU) and Xan (3- and 7-MX). Methylated purines were not present only as the result of contamination of the stone surface by urine; they were often present in larger amounts in the stone nuclei than in the outer layers. The maximum total content of methylated purines in the calculi examined was 1.7% of stone mass. This amount is relatively small but considerably greater than the sum of endogenous purines (Xan, Hyp, and 2,8-DHA), which amounted to 0.16% of the weight.

The methylated purines detected in the stones are excreted in urine as metabolites of ingested methylxanthines: 1,3,7-trimethylxanthine (caffeine); 1,3-dimethylxanthine (theophylline); and 3,7-dimethylxanthine (theobromine). These compounds are present in popular dietary items: coffee and some carbonated beverages (caffeine), tea (caffeine and theophylline), and cocoa and chocolate (theobromine). Theophylline and its derivative aminophylline are frequently used as drugs. A simplified diagram of the metabolism of methylxanthines in humans is shown in Fig. 3. Activities of particular enzymes show great variability in the population, being determined genetically and modified by numerous factors such as age, smoking, alcohol, drugs, and liver diseases (4, 8–10).

The order of MUAs arranged according to their content
in urine (1-MUA > 7-MUA > 3-MUA) (4) is consistent with their content relative to UA in stones (MQ values in Table 1). The amount of 3- and 7-MX was proportionally much less in stones than reported in urine. Neither 1-MX nor 1,7-DMU were detected in stones, although they are present in urine. The absence of 9-MUA is consistent with the assumption that MUAs in stones are metabolites of methylxanthines originating from plants, which never contain a methyl group at N-9 of the purine ring.

Urinary concentrations of methyl derivatives of UA and Xan probably never exceed the saturation point of the solution, with the exception of 1-MUA, for which the upper limit of excretion is \(\sim 800-900\) mg/day (4). To our knowledge, only 1 patient with urinary stones consisting mainly of 1-MUA has been reported (unpublished data; personal communication from Dr H. Anne Simmonds, Purine Research Laboratory, London, UK). That patient drank at least 8 cups of coffee per day. This unusual case supports the hypothesis that precipitation of 1-MUA may be the cause of urolithiasis in people ingesting excessive amounts of caffeine.

Precipitation of any compound may occur when its concentration in urine exceeds its solubility (11). However, the results of the present work suggest that compounds present in urine at concentrations much below the saturation point of their solutions may coprecipitate during UA stone formation. Methylated purines were present exclusively in the stones containing UA, and their content showed a linear correlation with UA content. This finding supports the notion that in an environment of UA supersaturation, coprecipitation with UA from undersaturated solution occurs.

The results presented here enabled us to identify 3 independent factors determining the content of compounds accompanying UA in stones:

- The UA content in the stone correlates positively with the amount of purine admixtures.
- The concentrations of particular compounds in urine are consistent with their content in the stones, e.g., 1-MUA > 7-MUA > 3-MUA.
- The greater the similarity of the structure of the derivative to the main constituent of the stone (UA), the higher the admixture. For example, 1,3-DMU with 2 methyl substituents was present in amounts \(\sim 5\)-fold less than those of monomethyl 7-MUA. Monomethyl derivatives of Xan, lacking an oxo substituent at C-8 of the purine ring, which is present in UA and its derivatives, were found in much lower amounts than N-1- and N-7-monomethyl UA derivatives. Xan was present in stones in amounts 2- to 4-fold greater on average than Hyp. This result can be explained by the presence of an oxo substituent in 2 positions of the Xan ring (C-2 and C-6) and in only 1 position of Hyp ring (C-6; Fig. 1).

These 3 factors may influence the incorporation of admixtures of components present in solution into the
growing crystal. In theory, the amount of admixture is directly proportional to its concentration in solution and to the amount of the main constituent of the crystal (12). Our results are consistent with this theory because the concentration of purines in the solid phase (or percentage of stone mass) was directly proportional to their concentration in urine and the UA content in the stone. The proportionality factor is determined by the similarity of the UA structure to the coprecipitating compound.

Processes of coprecipitation are related to the ability of crystallizing compounds to form a mixed crystal (13), which is a solid solution (14) containing ions or molecules of one compound in the crystal lattice of another. Crystals of purines are formed by molecules and classified as molecular crystals (15). Solid solutions of organic compounds are always substitutional, formed by replacing some solvent molecules with the admixture (16). The necessary condition for 2 or more organic compounds to form a solid solution is the similarity of shape and size of their molecules (13). This condition is fulfilled by purine derivatives identified in urinary stones (Fig. 1).

We propose a model of the UA stone molecular structure for which some purines may form a solid solution with UA as solvent. In the course of UA crystallization from a supersaturated solution, admixtures of other compounds are incorporated into the expanding crystal lattice. The proportion of substituted molecules is greater at higher concentrations of admixture in solution (which increases frequency of contact of its molecules with crystal surface) and when the difference between the structures of the main component and the admixture is smaller [which facilitates their incorporation into the crystal lattice (17)].

The model presented explains the linear correlation between the amounts of UA and admixtures of other purines in stones. Purines can incorporate into the crystal lattice formed by another purine (UA), but not into different crystal structures of stones. For verification of the presented model, it would be necessary to study the relationships between conditions of crystallization and the composition of crystals in vitro, using combination of various modern techniques (18).

Derivatives of methylxanthines were the dominant purines accompanying UA in stones in this study. Experiments by others regarding the role of various trace elements, or other organic compounds, in the processes of stone formation showed that they can have either inhibitory or promoting influences on crystal growth (19, 20). There are no such data for purines. Methylated purines in urine may also inhibit or promote UA crystallization.

Extensive prospective studies concerning the influence of beverages (e.g., tea and coffee with and without caffeine) were carried out in the United States (21, 22). The results showed that consumption of such beverages is associated with a lower frequency of urolithiasis, although this influence was relatively small. There was no difference between coffee containing and not containing caffeine. Unfortunately, the study did not take into consideration the composition of the stones. It is possible that the influence of methylated purines on calculi formation is limited to UA stones. Such calculi comprise as little as 5%–10% of all stones in the US population (23); therefore, the influence of methylxanthines on the occurrence of all kinds of urolithiasis could remain imperceptible.

In conclusion, the results of this study indicate that methyl derivatives of UA and Xan, being metabolites of methylxanthines originating from plants (caffeine, theophylline, and theobromine), are permanent constituents of urinary calculi containing UA. We developed a model in which admixtures of purines form substitutional solid solutions in UA. This model is consistent with the results presented but needs further experimental verification. It appears that 1-MUA may be the main constituent of urinary stones in patients with a history of excessive ingestion of methylxanthines. The frequency of such urolithiasis may be underestimated because of difficulties in diagnosis. Further studies are needed to establish the role of methylated purines in the pathogenesis of urolithiasis.

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References


