Urinary Adenosine and Aminoimidazolecarboxamide Excretion in Methotrexate-Treated Patients With Psoriasis

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Background: We hypothesized that low-dose methotrexate treatment for patients with psoriasis would block purine biosynthesis at the step catalyzed by aminoimidazolecarboxamide (AICA) ribotide transformylase and would inhibit adenosine metabolism as evidenced by increased urinary levels of AICA and adenosine, respectively. Eight patients collected a 24-hour urine specimen on the day before their methotrexate dose and the next day during their methotrexate dose. Eight age- and sex-matched controls also collected a 24-hour urine sample. Urinary AICA and adenosine were assayed by spectrophotometric and radioimmune assays, respectively; means are reported as micromole per millimole of creatinine and were compared by the paired t test (1-tailed).

Observations: Mean AICA excretion increased from 1.30 µmol/mmol on the day before to 1.85 µmol/mmol on the day during methotrexate dosing (P<.01). Mean adenosine values increased from 0.68 to 1.07 µmol/mmol, (P<.03). Controls had mean AICA and adenosine levels of 1.29 and 0.50 µmol/mmol, respectively. During the day of methotrexate dosing, patients had higher mean AICA and adenosine levels when compared with controls (P<.01). Mean AICA levels increased from 1.36 to 2.06 µmol/mmol (P<.025), and mean adenosine levels increased from 0.72 to 1.25 µmol/mmol (P<.025) in 5 patients showing improvement in clinical disease activity. In contrast, 3 patients with no change or worsening in clinical disease activity had smaller increases.

Conclusions: Methotrexate treatment of patients with psoriasis inhibits AICA ribotide transformylase and adenosine metabolism. Since adenosine is a T-lymphocyte toxin, it may be partially responsible for the immunosuppressive effect.

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Günther was the first to use an antifolate in the treatment of an autoimmune disease when he reported the beneficial effect of aminopterin in the treatment of psoriasis. Several years later, Edmundson and Guy reported the successful treatment of psoriasis with methotrexate, which possessed a similar antifolate activity but had less toxic effect. Low-dose methotrexate therapy has been a mainstay in the treatment of psoriasis for several decades.

Methotrexate in high doses is used successfully in chemotherapy. The relatively low doses used to treat autoimmune disease do not provide the cell kill necessary for cancer therapy; therefore, the mechanisms of action of methotrexate in these 2 diseases are likely to be different. The folate-dependent enzyme of de novo purine nucleotide biosynthesis, aminoimidazolecarboxamide ribotide (AICAR) transformylase, is sensitive to methotrexate inhibition (Figure 1). Methotrexate therapy was shown by Luhby and Cooperman to increase urinary aminoimidazolecarboxamide (AICA) excretion in patients with leukemia. Since AICA is a metabolite of AICAR, an increase in urinary AICA indicates that AICAR transformylase is inhibited.

We are especially interested in these findings since AICA-riboside (another metabolite of AICAR) inhibits adenosine deaminase and S-adenosyl-L-homocysteine hydrolase, is cytotoxic to cultured T lymphocytes, and potentiates the cytotoxicity of methotrexate added to cultured T lymphocytes (Figure 1). In the rat adjuvant arthritis model, an increase in urinary AICA is associated with methotrexate efficacy. In addition, in mice, methotrexate treatment has been shown to increase cellular AICAR concentration in concert with an increase in adenosine in exudates from carageenan-inflamed air pouches. Interference with normal adenosine metabolism in T lymphocytes should be immunosuppressive. In severe com-
PATIENTS AND METHODS

STUDY DESIGN

Patients and controls gave informed consent, and this study was approved by the Institutional Review Board at the University of Alabama at Birmingham. A paired design was used to determine if methotrexate therapy increases urinary AICA and adenosine levels. Patients with psoriasis were asked to collect a 24-hour urine sample in a bottle containing sulfuric acid (to make the final pH 2.5 to 3.5) the day before methotrexate dosing and on the day during methotrexate dosing and to return the urine samples on a subsequent clinic visit. The urine collection was completed within 5 days of their next clinic visit. This procedure was performed twice with a 3-month interval. We reasoned that if methotrexate therapy increases urinary AICA and adenosine levels, the increase should be most apparent on the day of methotrexate dosing compared with the day before. Blood folate and B12 levels were determined in the patients during each clinic visit. Urinary AICA and adenosine levels and blood folate and B12 levels were also measured in 8 age- and sex-matched healthy controls. Patient evaluation and laboratory data were not communicated among the investigators during the study.

PATIENT SELECTION

Patients with psoriasis being treated with low-dose methotrexate were recruited from the Dermatology Clinic at the University of Alabama and did not have liver disease, renal disease, or cancer. Demographic data are presented in Table 1. One female and 7 male age-matched controls were recruited from among University of Alabama at Birmingham employees and other volunteers (mean ± SD age, 47 ± 16 years).

PATIENT EVALUATION

The patients were evaluated by 1 clinician (W.M.S.) using a skin score (1-4) that took into account the area of the psoriatic lesion and its severity. A decrease of 1 or more or an increase of 1 or more in the skin score indicated clinical improvement or worsening, respectively.

LABORATORY METHODS

Samples (100 mL) of each 24-hour urine specimen were stored at −70°C prior to the assays. Extraction of AICA from the urine and a spectrophotometric assay for AICA were performed. A mean of at least 4 determinations for each sample was used. A radioimmunoassay (mean of 4 assays) of urinary adenosine was used. Plasma B12 and blood folate levels were measured for each sample using a radioisotope competition assay (Ciba Corning, East Walpole, Mass) and a methotrexate-resistant Lactobacillus casei assay, respectively. Urinary creatinine levels were measured in duplicate using the Sigma Diagnostics Creatinine Kit (Procedure 535; Sigma Chemical Co, St Louis, Mo).

STANDARDIZED ANALYSIS

Differences in mean urinary AICA and adenosine excretion were detected by the paired t test (1-tailed) either using each patient as his or her own control or using age- and sex-matched controls. Differences in mean plasma B12 and blood folate levels were also detected by the paired t test (1-tailed).

The means ± SDs of urinary creatinine excretion (in millimoles) for the four 24-hour urine samples obtained from each of the 8 patients with psoriasis were 14.4 ± 2.3,

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12.7 ± 1.1, 11.6 ± 0.6, 11.7 ± 1.2, 10.3 ± 1.3, 11.9 ± 2.3, 21.9 ± 2.3, and 16.5 ± 1.3. The relatively small SDs indicate that there was little variation in the amount of urine collected. Urinary creatinine excretion in the controls varied from 12.8 to 22.9 mmol with a mean of 17.1.

Urinary AICA excretion data are given in Table 2. A statistically significant increase (43%) in mean urinary AICA excretion (in micromoles per millimole of creatinine) was observed in patients with psoriasis when comparing the day-before methotrexate dosing with the day-during methotrexate dosing (1.30 vs 1.85 µmol/mmol; \( P < .01 \)). All patients with psoriasis showed this increase in urinary AICA excretion (Figure 2, left). A 52% increase in mean urinary AICA excretion (1.36-2.06 µmol/mmol, \( P < .025 \)) was greater in those patients who showed clinical improvement compared with the 23% increase in those who did not show clinical improvement (1.15-1.41 µmol/mmol; \( P < .025 \)) (Table 2). Mean urinary AICA excretion during the day of methotrexate dosing (1.85 µmol/mmol) was found to be greater than mean urinary AICA excretion in controls (1.29 µmol/mmol) (Table 2). McGeer et al\(^2\) reported a mean urinary AICA excretion of 0.63 µmol/mmol for male and female subjects (age, >16 years), which is somewhat lower than the means we report for our controls and the patients with psoriasis.

A statistically significant increase (58%) in mean urinary adenosine excretion was observed in patients with psoriasis when comparing the day-before methotrexate dosing with the day-during methotrexate dosing (0.68 vs 1.07 µmol/mmol; \( P < .025 \)) (Table 2). Most, but not all, patients showed this increase in urinary adenosine excretion (Figure 2, right). The 73% increase in mean urinary adenosine excretion was much greater in patients who showed clinical improvement (0.72-1.25 µmol/mmol; \( P < .025 \)) compared with the 22% increase in those who did not show clinical improvement (0.55-0.68 µmol/mmol) (Table 2). The latter increase of 22% failed to reach statistical significance (\( P > .20 \)). Mean urinary adenosine excretion during the day of methotrexate dosing (1.07 µmol/mmol) was greater than mean urinary adenosine excretion in controls (0.50 µmol/mmol) (Table 2). Hirshhorn et al\(^2\) have reported mean urinary adenosine excretion of 0.46 µmol/mmol in 5 infants and children. Mills et al\(^2\) have reported that mean urinary adenosine excretion was 0.64 µmol/mmol in 6 normal infants at birth. These means are in general agreement with means for our controls and our patients with psoriasis the day before methotrexate dosing (0.50-0.72 µmol/mmol) (Table 2).

Since both folate and B\(_12\) nutriture are known to influence urinary AICA excretion, blood levels of these vitamins were measured.\(^3,2\) Mean plasma folate and B\(_12\) levels were not statistically different when comparing the groups of patients with psoriasis and the controls. However, the mean red blood cell folate level in the controls was approximately twice that of the mean level in the patients with psoriasis. Four of the patients with psoriasis and 2 of the controls had red blood cell folate levels in the deficient range (<314 nmol/L). One patient with psoriasis with a deficient red blood cell folate level also had a deficient plasma folate level (<4.5 nmol/L).

**COMMENT**

This is the first clinical study to demonstrate that low-dose methotrexate therapy for psoriasis increases urinary excretion of both AICA and adenosine. The study design was selected because Hendel and Nyfors\(^2\) demonstrated that mean erythrocyte methotrexate levels reached a peak of about 200 nmol/L 2 to 3 hours after oral dosing in patients with psoriasis receiving their first treatment with methotrexate. Long-term methotrexate therapy produced a steady state mean of 64 nmol/L in the erythrocytes of these patients. Therefore, it is likely that the tissue methotrexate concentration and therefore the antimetabolic effect of methotrexate should be the greatest on the day during methotrexate dosing. For the following reasons, it is unlikely that these results are produced by errors in urine collection, by fluctuations in renal function, or by changes in vitamin nutriture: (1) There is a relatively small variation in the amount of urinary creatinine in the four 24-hour urine collections completed by each patient with psoriasis. (2) The paired study design makes it unlikely that renal function, vitamin nutriture, or other factors could substantially change throughout the 48 hours of 2 sequential 24-hour urine collections. (3) The slightly greater creatinine excretion by the controls suggests a slightly better renal function in this group. If there were no differences in in vivo AICA and adenosine production during methotrexate dosing,
the controls should actually have had slightly higher urinary levels of these metabolites. This was not the case.

Urinary levels of these metabolites in patients before methotrexate dosing and in controls are in reasonable agreement with previously published data. One possible explanation for the somewhat high urinary AICA excretion in our controls and patients with psoriasis the day before methotrexate dosing is that some controls and some patients with psoriasis had blood folate levels in the deficient range. Folate deficiency is known to increase urinary AICA excretion. The urinary adenosine excretion in the controls and patients with psoriasis on the day be-

Table 2. Laboratory Data for Patients With Psoriasis and Controls*

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Urine Collection</th>
<th>Urinary Excretion, µmol/mmol of Creatinine</th>
<th>Folate Levels, nmol/L</th>
<th>Plasma Vitamin B12, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AICA</td>
<td>Adenosine</td>
<td></td>
</tr>
<tr>
<td>All patients with psoriasis (n = 8)</td>
<td>Day before methotrexate dosing</td>
<td>1.30 ± 0.50</td>
<td>0.68 ± 0.42</td>
<td>19 ± 12§ 347 ± 155¶ 276 ± 90</td>
</tr>
<tr>
<td></td>
<td>Day during methotrexate dosing</td>
<td>1.85 ± 1.02†‡ 1.07 ± 0.72†‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinically improved patients with psoriasis (n = 5)</td>
<td>Day before methotrexate dosing</td>
<td>1.36 ± 0.56</td>
<td>0.72 ± 0.45</td>
<td>20 ± 13 305 ± 130¶ 281 ± 97</td>
</tr>
<tr>
<td></td>
<td>Day during methotrexate dosing</td>
<td>2.06 ± 1.15†‡ 1.25 ± 0.78†‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinically worse or not improved patients with psoriasis (n = 3)</td>
<td>Day before methotrexate dosing</td>
<td>1.15 ± 0.24</td>
<td>0.55 ± 0.31</td>
<td>19 ± 12 417 ± 110¶ 268 ± 79</td>
</tr>
<tr>
<td></td>
<td>Day during methotrexate dosing</td>
<td>1.41 ± 0.35†‡ 0.68 ± 0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 8)</td>
<td></td>
<td>1.29 ± 0.28</td>
<td>0.50 ± 0.19</td>
<td>22 ± 15 724 ± 414# 329 ± 33</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD. AICA indicates aminoimidazolecarboxamide.
† Mean is greater than the mean on the day before methotrexate dosing, P < .025.
‡ Mean is greater than the mean of matched controls, P < .025.
§ One patient had a serum folate level lower than 4.5 nmol/L, in the deficient range.
¶ Four patients had red blood cell folate levels lower than 314 nmol/L, in the deficient range.
‖ Mean is less than the mean of matched controls, P < .05.
# Two controls had red blood cell folate levels lower than 314 nmol/L, in the deficient range.

Figure 2. Urinary excretion of aminoimidazolecarboxamide (AICA) (left) and adenosine (right) on the day before and the day during methotrexate dosing in patients with psoriasis and in age- and sex-matched controls. Each point represents the mean of at least 4 AICA or adenosine determinations. Lines connect paired data (solid circles) for patients with psoriasis, and open circles represent data for controls. Open squares indicate means, with attached 1 SD error bars. The data are given in the traditional per-gram creatinine unit.
fore methotrexate dosing are very similar to those reported for normal individuals by other investigators who used high-pressure liquid chromatography methods. This suggests that the high-pressure liquid chromatography and the radioimmunoassay methods for urinary adenosine give similar results.

The data suggest that inhibition of both AICAR transformylase and adenosine deaminase (Figure 1) result from methotrexate ingestion and that these inhibitions are causally linked to the efficacy of low-dose methotrexate therapy for psoriasis. This is reasonable because adenosine is a cytokotoxic and immunotoxic metabolite; synthetic inhibitors of adenosine deaminase are cytoxic and immunotoxic; and, most importantly, the inborn error of metabolism, adenosine deaminase deficiency, results in severe combined immunodeficiency disease. However, a note of caution is in order because increases in both urinary adenosine and AICA were relatively small. In human leukemia cells the Km of adenosine for adenosine deaminase was 54 µmol/L while the Ki of AICA-riboside was 540 µmol/L. Thus relatively high concentrations of AICA-riboside would be required to substantially inhibit the deaminase. The AICAR reached intracellular levels of approximately 30 µmol/L in cultured human leukemia cells exposed to 10 to 20 nmol/L of methotrexate. A 30-µmol/L AICA-riboside concentration would produce only a relatively small inhibition of the deaminase. In contrast to adenosine deaminase, S-adenosyl-L-homocysteine hydrolyase was substantially inhibited by 10 to 100 µmol of AICA-riboside. Thus the modest increase in urinary adenosine may reflect only modest inhibition of the deaminase, while the hydrolyase may be more substantially inhibited. Alternatively, a small increase in extracellular adenosine may produce a receptor-mediated immunosuppression. It is also important that methotrexate therapy blocks both purine nucleotide and thymidylate biosynthesis and depletes the cell of folate coenzymes. All of the above occur in a concerted fashion and the combination of these events plus interference with adenosine metabolism may be required to produce the full immunosuppressive effect. Our data suggest that low-dose methotrexate therapy interferes with normal adenosine metabolism, and this may be one of the mechanisms producing a beneficial immunosuppression in the treatment of autoimmune diseases such as psoriasis.

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REFERENCES