Metabolic Effects of A-Methopterin in Man

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Although folic acid antagonists have been used in clinical medicine for 10 years, the metabolic effects of these agents have not been systematically evaluated in man. Since the patients who receive these drugs are generally poor subjects for metabolic balance studies, the lack of data is understandable. Furthermore, the few balance studies that have been reported with the anti-metabolites have been concerned with the catabolism of tumor tissue and have thus provided little information regarding the response of the host.

During the course of treatment with A-methopterin of patients with histologically proved metastatic choriocarcinoma, we have had the opportunity of examining its metabolic effects. Since the subjects of this study had only minimal disease, it was hoped that any metabolic alterations would reflect the action of A-methopterin on the host. A-methopterin in high doses proved to have only a minimal catabolic effect, and the findings suggested that this effect was the result of the action of A-methopterin on normal tissue.

MATERIALS AND METHODS

The patients received a constant weighed diet and unrestricted fluids. Sample diets were analyzed. The study was divided into three periods of 5 days each, with a control period preceding and following the treatment period. Each patient received daily intramuscular doses of 25 mg. A-methopterin for 5 days.

Daily urine specimens were analyzed for nitrogen, calcium, phosphorus, sodium, potassium, chloride, creatine, creatinine, uric acid, 17-ketosteroids, and Porter-Silber chromogens. Stools were analyzed for nitrogen and inorganic constituents in 5-day pools. Methods used in this laboratory have been reported (1).

Patients.—Patient 1, a 27-year-old white woman, had two small metastatic lesions visible by chest x-ray. The study was carried out during her sixth course of A-methopterin, 15 days following the previous course. The urine gonadotropin titer increased during the study period, and the chest x-ray remained stable.

Patient 2, a 23-year-old white woman, had only an elevated urine gonadotropin excretion as residual evidence of disease. The study was conducted during the sixth course of therapy, 10 days after the fifth course. The gonadotropin titer did not change during the study.

Patient 3, a 26-year-old white woman, had an increased excretion of urine gonadotropin as the only persisting evidence of disease. The study was carried out during the third course of therapy, 5 days following the previous course. The gonadotropin titer did not change during the study.

Patient 4, a 28-year-old white woman, was studied during her first course of A-methopterin. Evidence of metastatic choriocarcinoma at this time was the urinary gonadotropin titer of 400,000 mouse units. Following this course of A-methopterin, the gonadotropin titer was 25,000 mouse units.

RESULTS

The daily urinary nitrogen excretion of each patient has been plotted in Chart 1, and the urinary nitrogen excretion during each period is recorded in Table 1. Although the values during the initial control period were somewhat erratic, an increase in urine nitrogen of 1 gm. daily was noted in patients 1 and 2. In patient 3, the study was complicated by emesis on the second day of the treatment period. Toward the end of this period, the nitrogen excretion rose. The fourth patient (patient 4) displayed a gradual fall in nitrogen excretion during the initial control period extending into the A-methopterin period. During the last 2 days of treatment and the following 2 days, the urinary nitrogen rose. In each case a decrease in urinary nitrogen occurred in the post-treatment period. There were no consistent changes in fecal nitrogen attributable to A-methopterin (Table 1).

The urinary phosphorus excretion displayed the same pattern as the urinary nitrogen (Chart 2). The excretion of phosphorus increased during therapy and decreased markedly in the subsequent...
control period. Fecal phosphorus excretion was not consistently affected (Table 1). The balance data (Table 1) consequently are qualitatively well portrayed by the urine phosphorus excretion.

Potassium balances (Table 1) demonstrated changes similar to those for nitrogen and phosphorus. In all the patients, the largest positive potassium balances occurred during the final control period. This was correlated with the period of nitrogen anabolism.

The data relating to uric acid excretion are presented in Chart 3 and Table 1. During the period of A-methopterin therapy, there was a consistent increase in the average uric acid excretion, varying from 35 mg/24 hours to 385 mg/24 hours. In each case, uric acid excretion showed a pronounced decrease in the subsequent control period.

There were no alterations in the balances of sodium chloride or calcium resulting from the administration of A-methopterin. Body weights varied only slightly throughout the studies, suggesting that water balance was not affected. Urinary excretion of creatine, creatinine, and amino acid nitrogen was constant. Adrenal function, as estimated by the 24-hour urine excretion of 17-ketosteroids and Porter-Silber chromogens, was unaltered. Serum electrolyte concentrations, serum creatinine, and uric acid levels remained constant throughout the study.

**DISCUSSION**

Satisfactory interpretation of balance data generally requires either that the alteration in the balance of any constituent be greater than 10 per cent of the control value or that a prolonged stable control period be achieved. Owing to the exigencies of the clinical situation, we were unable to prolong the control period before the administration of A-methopterin. The variability of the excretion of nitrogen, phosphorus, and uric acid during the initial control period reflects the inadequacy of this time period both for dietary adjustment and for attainment of equilibrium following previous courses of therapy.

However, there are adequate reasons for believing that the changes in nitrogen balance are significant, although they are no greater than the possible cumulative errors of the balance technic. Li et al. (3), in a similar study, reported a negative nitrogen balance of 0.5–1.0 gm. daily as a result of A-methopterin therapy. In each of our subjects, the urinary nitrogen increased either throughout the period of therapy or toward the end of the period. The evidence is strengthened by the finding that, in the post-treatment period, the urinary nitrogen fell and the nitrogen balance became more positive in three of the patients. Such a phase of nitrogen anabolism is commonly seen after any catabolic episode.

The phosphorus balances consistently mirrored the nitrogen balances. It is improbable that these parallel variations could arise from random errors. This and the consistency of the results within the group support the view that the observed alterations in nitrogen balance were due to the administration of A-methopterin.

The source of the increment of urine nitrogen has not been defined. In two of the three patients, the gonadotropin titer did not fall during this course of therapy, suggesting that the amount of functioning tumor had not changed. Furthermore, patient 4, whose nitrogen balance and uric acid excretion were least affected by A-methopterin, was studied during her first course of treatment as a result of which there was a marked drop in the gonadotropin titer. If the increase in urine nitrogen resulted from breakdown of tumor tissue, the metabolic changes should have been most pronounced in this subject.
A prominent site of action of A-methopterin is the bone marrow. In Chart 4, the variations in reticulocyte count and hemoglobin concentration in three cases are diagrammed. The fall in the reticulocyte count reflects the depression of erythropoiesis occurring 2–4 days earlier, since this is the approximate maturation time of the reticulocyte (4). Thus, during most of the period of A-methopterin therapy, virtual suppression of red cell synthesis occurred. If the disposal of red cells throughout this period continued at the normal rate of 0.8 per cent per day, then, with the use of average values for total red cell mass and red cell nitrogen (5), the loss of nitrogen due to red cell destruction can be estimated. For these subjects, this was 0.4–0.8 gm. daily, values of the same magnitude as those observed.

Following A-methopterin therapy there was prompt resumption of bone marrow activity. This was temporally correlated with the period of nitrogen and phosphorus retention. It has been similarly noted that, immediately following the administration of vitamin B₁₂ to the patient with pernicious anemia in relapse, resumption of erythropoiesis is accompanied by positive nitrogen and phosphorus balances (2). Therefore, as periods of bone marrow suppression and activation can be correlated with periods of negative and positive nitrogen and phosphorus balances, the hypothesis is warranted that the metabolic effects of A-methopterin were due to its impact on the bone marrow.

The minimal metabolic sequelae of treatment with high doses of A-methopterin were notable. This is in accord with the clinical impression that A-methopterin toxicity is limited primarily to bone

1 Assumptions: Blood volume = 8.0 per cent body weight; red cell breakdown = 0.83 per cent per day; protein nitrogen/100 ml rbc = 5.9 gm.; mean corpuscular hemoglobin concentration, normal.

Patient No. 3: 57 kg. body weight, hematoctrit 37 per cent; 57 × 0.8 × 37 = 1688 ml of rbc; 1685 × 5.9 = 995 gm. rbc nitrogen; and 995 × 0.83 = .83 gm. rbc nitrogen liberated by red cell breakdown daily.

### TABLE 1

<table>
<thead>
<tr>
<th>PATIENT NO.</th>
<th>THERAPY*</th>
<th>NITROGEN Urine stool balance (Gm/5-day period)</th>
<th>Phosphorus Urine stool balance (Gm/5-day period)</th>
<th>Potassium Urine stool balance (mEq/5-day period)</th>
<th>Uric Acid Urine (Mg/5-day period)</th>
</tr>
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<td>5 Control</td>
<td>A-methopterin</td>
<td>48.5 4.8 -5.8</td>
<td>3.50 1.8 0</td>
<td>212 29 + 59</td>
<td>3280</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>50.5 4.2 -4.7</td>
<td>2.86 1.8 + .2</td>
<td>213 23 + 59</td>
<td>3250</td>
</tr>
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<td>1 Control</td>
<td>A-methopterin</td>
<td>42.0 5.9 +5.1</td>
<td>3.01 2.8 0</td>
<td>274 56 + 50</td>
<td>2800</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>40.5 5.4 +7.1</td>
<td>2.16 1.64 +.8</td>
<td>215 44 +100</td>
<td>2075</td>
</tr>
<tr>
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<td>47.5 5.2 +5.8</td>
<td>3.43 .88 +1.09</td>
<td>252 33 + 60</td>
<td>1600</td>
</tr>
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<td></td>
<td>49.5 5.7 +7.4</td>
<td>2.55 .90 +1.95</td>
<td>270 21 + 84</td>
<td>2275</td>
</tr>
<tr>
<td>4 Control</td>
<td>A-methopterin</td>
<td>49.0 6.1 +5.9</td>
<td>3.59 1.43 +1.81</td>
<td>271 20 0</td>
<td>2885</td>
</tr>
<tr>
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<td>45.0 4.0 +7.8</td>
<td>3.46 1.12 +1.42</td>
<td>279 20 + 2</td>
<td>2505</td>
</tr>
</tbody>
</table>

*For each patient, the first row of control values represents the 5-day period prior to treatment, and the second row of control values is for the 5-day period following treatment.
† Vomitus, 4 gm.
‡ Vomitus, 174 mg.
§ Vomitus, 10 mEq.
A-METHOPTERIN 25 mg. IM. Daily

CHART 3.—24-hour urine uric acid excretion. Figures on the ordinate represent mg/hr.

CHART 4.—Hemoglobin and reticulocyte counts

The metabolic effects of large doses of A-methopterin have been examined in four women with metastatic choriocarcinoma. Evidence was presented suggesting that the observed effects were due to the action of A-methopterin on the host. Small increases in the excretion of nitrogen, phosphorus, and potassium were induced. Uric acid excretion increased with A-methopterin therapy. There were no alterations in electrolyte balance or adrenal function. The changes in nitrogen excretion have been tentatively ascribed to suppression of erythropoiesis.

ACKNOWLEDGMENTS

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REFERENCES

5. ———. Ibid., p. 184.
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