Metabolic Studies on Folic Acid in Malignancy

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Summary

Plasma clearance of folic acid was studied in 25 patients, 19 of whom were selected early cases of malignancy with unimpaired general condition; 23 of these patients, including 17 with early tumors, had an accelerated clearance rate. On an average, the clearance of folic acid in plasma was more rapid in patients with low serum iron or hemoglobin concentration.

Pathologic values for the FIGLU excretion were recorded in 10 out of 22 patients; there was no correlation between the excretion of FIGLU and the serum concentration of folic acid.

Six days after administration of large doses of folic acid the SFAA was well above the value for the normal controls, and the FIGLU excretion was considerably diminished in 6 out of 7 patients examined; the clearance rate of folic acid was, however, only slightly changed and not normalized.

The changes in folic acid metabolism observed in cases of malignancy are probably due to an increased demand for folic acid.

Introduction

A low level of SFAA has been found in leukemia (15, 21), malignant lymphoma (21), and carcinoma (11, 20, 21). The purpose of the present investigation was to examine the plasma clearance rate of folic acid and the urinary excretion of FIGLU in malignancy and to ascertain whether the plasma clearance and excretion of FIGLU are altered after administration of large doses of folic acid.

FIGLU is a derivative of histidine, and it is catabolized in the presence of tetrahydrofolic acid—chiefly in the liver. In folic acid deficiency FIGLU is excreted with the urine, and an early test for the deficiency is to determine the urinary excretion of FIGLU after histidine load (19).

Materials and Methods

The plasma clearance of folic acid was determined in 25 patients with malignant tumors (mean age, 51 years) and in 10 healthy controls (mean age, 41 years). Three of the tumor patients had Hodgkin's disease and 22 had epithelial tumors; 19 selected patients were early cases with no known impairment of nutrition or the general condition. In 6 of the cases of malignant tumors—5 of them selected early cases—the clearance test was repeated after large doses of folic acid were administered.

The urinary excretion of FIGLU after histidine load was determined in 22 other patients (mean age, 53 years), 3 of whom had Hodgkin's disease and 19 epithelial tumors. Fifteen were selected patients whose nutritional state and general condition were unimpaired. In 7 cases the FIGLU test was repeated after large doses of folic acid were given.

None of the patients had known or suspected liver metastases or had received antibiotics or chemotherapy during the preceding 60 days. None of the patients or controls had received antiinflammatory drugs, butazolidine, or steroids. Any vitamin administration had been discontinued at least 7 days before the tests. All the patients were hospitalized throughout the investigation.

Plasma clearance of folic acid (2). This test was performed after an overnight fast. Immediately after a blood sample was drawn, 15 µg of pteroylglutamic acid (Polvite, Lederle) per kg of body weight was injected into a brachial vein. On blood samples taken from the other arm 3, 15, and 30 min after the injection, the folic acid activity was determined by the Lactobacillus casei (21). The values given as clearance are the differences between the activities before and after the injection.

Immediately after the clearance test, 6 of the patients were given 20 mg of pteroylglutamic acid daily for 4-5 days by i.v. injection (cf. Ref. 10). A new folic acid plasma clearance test was performed 6 days after the last injection.

Urinary excretion of FIGLU after histidine load. Following an overnight fast 15 gm of l-histidine were given p.o. After an additional 2-hr fast, food was permitted ad libitum. To ensure high diuresis the patients were required during the test period to drink 500-1000 ml of liquid in excess of their normal mealtime intake. Sixty min after administration of histidine the bladder was emptied and the voided urine discarded; during the next 8 hr all urine was collected. To prevent decomposition of FIGLU in alkaline urine, 10 ml of 5 N hydrochloric acid were added to the container. Seven of the patients were then given 20 mg of pteroylglutamic acid daily for 4-5 days by i.v. injection. The urinary excretion of FIGLU was determined again 6 days after the last injection.

Urinary excretion of FIGLU was determined in the Växjö Central Hospital according to a high-voltage electrophoresis method (16). Electrophoresis was performed on Whatman No. 3 paper at 2600 volts, about 45 ma, and -7°C for 1 hr in a buffer (pH 5.4) consisting of 20 ml of acetic acid, 50 ml of pyridine, and 5 liters of distilled water. For each patient, 25 cu mm of urine were applied in duplicate to a 3 cm wide strip and, parallelly, 25 cu mm of FIGLU standard (4.4 mm in 0.1 N hydrochloric acid) and glutamic acid standard (4.0 mm in 0.1 N hydrochloric acid). After drying for 30 min at 65°C, each paper was cut into 2, and 1 half was exposed to ammonia fumes in a closed vessel for 1 hr. After drying for 45 min at 53°C, both halves were passed through a mixture of acetic acid and acetic acid (23:2 by volume), dried for

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The following abbreviations are used: SFAA, folic acid activity in serum; FIGLU, formimino glutamic acid; PGA, pteroyl glutamic acid.

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### TABLE 1
**Elimination of PGA after i.v. Administration of 15 µg/kg of Body Weight**

The values given are the differences between the SFAA activities before and after the injection. The S.E. of the mean is indicated.

<table>
<thead>
<tr>
<th></th>
<th>Clearance after injection of PGA (µg/ml)</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 min</td>
<td>15 min</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>320 ± 24</td>
<td>136 ± 15</td>
</tr>
<tr>
<td><strong>Malignant tumors</strong></td>
<td>179 ± 36</td>
<td>52 ± 5</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### TABLE 2
**Elimination of PGA in Healthy Controls with SFAA ≤ 3.0 µg/ml and in Cases of Malignant Tumor with SFAA ≥ 9.0 µg/ml**

<table>
<thead>
<tr>
<th>SFAA before and clearance after injection of PGA (µg/ml)</th>
<th>Before</th>
<th>3 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls with low SFAA</td>
<td>2.9</td>
<td>537</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>337</td>
<td>100</td>
</tr>
<tr>
<td>Mean of 10 controls</td>
<td>4.6</td>
<td>320</td>
<td>136</td>
</tr>
<tr>
<td>Malignant tumors with SFAA ≥ 9.0 µg/ml</td>
<td>9.5</td>
<td>135</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>165</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>250</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>15.5</td>
<td>159</td>
<td>39</td>
</tr>
</tbody>
</table>

5 min at 53°C, and treated in a shallow dish with 0.5% Ninhydrin in n-butanol saturated with a mixture of water, pyridine, and acetic acid (95:10:1, pH 4.5). Each strip was then placed between 2 filter papers and was pressed and dried for 30 min with a fan and for 120 min at 53°C. The area of each spot was then measured. The strips were eluted in the dark for 1 hr in 5 ml of a mixture of 1000 ml of 95% ethanol with 13 ml of acetic acid and 4 ml of 1% copper sulfate. Densitometry was performed in a Beckman model B instrument (wave length, 5150 Å; slit, 0.05; elution solution from 2 pieces of blank paper, 20 x 50 ml). A FIGLU standard specimen was examined for each half strip, and the extinctions of these and the specimens were corrected for area and blank. From the corrected extinctions the concentration was calculated.

The dose of 15 gm of L histidine, which suffices for maximum FIGLU production in normals (8), was well tolerated by all the patients. At this laboratory the normal range for urinary excretion of FIGLU after histidine load is 0-225 µmoles in 8 hr (≤ 28 µmoles/hr).

### Results

The injected folic acid was eliminated more rapidly in the patients than in the healthy controls, the difference being significant for all 3 tested times (Table 1). In all 6 patients in poor general condition the folic acid clearance was accelerated. Seventeen of the 19 selected patients with early cases of malignancy also had an accelerated clearance, the SFAA range among them being 8-70 µg/ml 15 min after the injection; these values were far below the minimum for the control group (85 µg/ml). An accelerated folic acid clearance is more characteristic of malignancy than is a low SFAA; the clearance was more rapid for patients with SFAA < 9 µg/ml than for healthy controls with a fairly low SFAA (Table 2). For the 6 patients given large doses of folic acid the mean SFAA was 3.6 µg/ml before administration and 10.9 µg/ml 6 days later; the folic acid clearance, however, was only slightly changed, and the mean clearance after large doses of folic acid was significantly different from the mean for the control group at all 3 times tested (P < 0.01-0.001). (Table 3).

The relationships between the clearance rate and the serum iron, hemoglobin concentration and degree of wasting are given in Table 4.

The urinary excretion of FIGLU after histidine load was elevated in 10 of the 22 tested patients with tumor, but there was little evidence of a relation to the serum folic acid activity (Chart 1). Four of the 5 patients in poor general condition had high FIGLU values, and so had 4 of the 13 selected patients with early tumors. For the 7 patients given large doses of folic acid the mean SFAA was 2.0 µg/ml before administration and 6.8 µg/ml 6 days later; the urinary excretion of FIGLU was greatly diminished in all but 1 of the patients (Table 5).

### Discussion

Disturbance of the folic acid activity in cases of malignant disease have been reported by a number of workers. In advanced cases Girdwood (6) found a lowering of the urinary excretion on administering folic acid, and reduced SFAA levels have been recorded in malignancy (11, 15, 20, 21). Hansen (8) reported increased excretion of FIGLU in 24 out of 28 cases of malignancy, and Carey et al. (1) in 35 out of 70 patients with extensive malignant disease. Dymock (4) reported increased excretion of FIGLU in 24 out of 28 cases of malignancy, but he does not indicate the extent of the disease or the general condition of the patients.

Of the tests for folic acid deficiency examined in the present study, folic acid clearance differentiated the most clearly between patients with malignancy and healthy controls. For 90% of the selected patients with early tumors who were in good general condition the folic acid clearance rate was higher than the lowest value for the healthy controls. This conflicts with the results re-
TABLE 4

SFAA and Elimination of PGA after i.v. Administration of 15 μg of PGA/kg of Body Weight in Various Groups of Cases of Malignant Tumor

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>SFAA before and clearance after injection of PGA (μg/ml)</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;12.2 gm/100 ml</td>
<td>Before: 4.8 ± 1.1, 215 ± 34, 59 ± 6, 36 ± 4</td>
<td>16</td>
</tr>
<tr>
<td>≤12.2</td>
<td>15 min: 4.2 ± 1.0, 115 ± 17, 37 ± 7, 29 ± 8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>30 min: &gt;0.05, &lt;0.05, &gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Serum iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Before: 6.3 ± 1.5</td>
<td>13</td>
</tr>
<tr>
<td>Low*</td>
<td>15 min: 3.3 ± 0.7</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>30 min: &gt;0.05</td>
<td></td>
</tr>
<tr>
<td>General condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>Before: 4.5 ± 0.8</td>
<td>19</td>
</tr>
<tr>
<td>Poor</td>
<td>15 min: 4.9 ± 2.1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>30 min: &gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

* Serum iron: men <0.090 mg/100 ml; women <0.075 mg/100 ml.

Increase in the urinary excretion of FIGLU has been observed also in conditions other than folic acid deficiency, and the absence of a correlation between the urinary excretion of FIGLU and the SFAA (Chart 1) is remarkable. However, the decrease in the excretion of FIGLU following administration of pteroylglutamic acid (Table 5) suggests that the increase observed in neoplastic disease is a manifestation of folic acid deficiency.

The decreased folic acid activity in serum, accelerated rate of plasma clearance, and increased excretion of FIGLU all point to folic acid deficiency or an increased demand for folic acid, or both, in neoplastic disease. That there is a considerable reduction in intake is unlikely in early cases of tumor when the patient is in good general condition, and judging from the results obtained by Girdwood (6) an increased urinary excretion of folic acid in malignancy is no more probable. Although there are other conceivable explanations for the folic acid deficiency observed in early tumor cases, the most plausible one would seem to be an increase in the demand for folic acid. This explanation is also strongly favored by the fact that the plasma clearance was not normalized 6 days after administration of large doses of pteroylglutamic acid (Table 3), even though at that time there was no longer evidence of folic acid deficiency; the SFAA was well above the mean for normal controls (4.6 μg/ml), and the higher than the value reported for Streptococcus faecalis (38–45 μg/ml) (2, 9, 22).

TABLE 5

Urinary Excretion of FIGLU Following Histidine Load before and 6 Days after Administration of 20 mg of PGA Daily for 5 Days

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Before folic acid administration</td>
<td>110</td>
<td>230</td>
<td>290</td>
<td>130</td>
<td>40</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>After folic acid administration</td>
<td>0</td>
<td>30</td>
<td>160</td>
<td>0</td>
<td>290</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
urinary excretion of FIGLU had diminished in 6 out of 7 patients examined (Table 5).

Whether this increase in the demand for folic acid takes place in the tumor or whether the tumor elicits the increased demand in other tissues is not known, but it is probable that both occur. The cell proliferation in the tumor causes a greater consumption of folic acid; the hematopoietic demand for folic acid is probably also higher in malignant disease. Erythropoiesis is often enhanced in neoplastic disease, and the metabolism of iron is accelerated even in early cases of tumor (14, 18). A tendency for a greater rate of clearance in patients with low serum iron and hemoglobin concentration was also observed (Table 4). It has also been shown that in acute leukemia the intracellular folic acid is not decreased in erythrocytes (21) and is probably increased in the white blood cells (5, 21, 23), even though the folic acid concentration in the serum lowers.

Acknowledgments

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References

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