Urinary Excretion of Thymine and Uracil in a Two-Year-Old Child with a Malignant Tumor of the Brain

Gunilla Berglund, Joachim Greter, Sven Lindstedt, Göran Steen, Johan Waldenström, and Urban Wass

A two-year-old boy with a malignant tumor of the brain (medulloblastoma) excreted large amounts of thymine and uracil in his urine. The excretion was related to progress and regress of the disease, and reached a maximum of 3.0 mol of thymine per mole of creatinine and 2.6 mol of uracil per mole of creatinine. The excretion by 20 apparently normal children was less than 0.01 mol/mol of creatinine for each of the two pyrimidines. Three children with brain tumors, two with leukemias, and one with neuroblastoma were also studied; two of them had a moderate increase in urinary pyrimidine excretion, but only up to 0.07 mol/mol of creatinine. The activity of dihydrouracil dehydrogenase (NADP⁺) (EC 1.3.1.2) in cultured fibroblasts from the patient was somewhat lower than in control fibroblasts. The tumor was considered to be the likely cause of the increased excretion of pyrimidines, but an impaired degradation of pyrimidines in the liver could not be ruled out.

Additional Keyphrases: cancer • medulloblastoma • pyrimidine excretion • neurological disorders

Children with unexplained neurological disorders should routinely be examined for abnormalities in the metabolism of amino acids, organic acids, and carbohydrates, preferably with the use of chromatographic multi-component methods. The major objective of these laboratory investigations is the diagnosis of inborn errors of metabolism. One may, however, sometimes make unexpected findings not related to the presence of a genetic disease. Increased knowledge of the excretion patterns of different classes of compounds will possibly also give useful diagnostic information about intoxications, infections, and malignancy. As an example of this, we report on a two-year-old boy who developed a hemiparesis and excreted large amounts of thymine and uracil. Soon afterwards, he was diagnosed to have a malignant tumor of the brain.

Case Report

The boy was the second child in a healthy farm family with no known malignancy in the last two generations. He was breast fed for nine months. His development was normal and he had not had any diseases until the age of 16 months. He developed walking difficulties with a tendency to fall to the right side. Neurological symptoms progressed rapidly during the following weeks. He was admitted to the Children’s Hospital at the end of September 1977. Encephalography and computer tomography demonstrated a very large expansive process in the anterior part of the posterior fossa. The tumor, which was inaccessible for surgery, was treated with radiotherapy and chemotherapy with lomustine and vincristine. The patient improved rapidly, and in January 1978 only a slight weakness of his right hand remained of his earlier right-sided hemiparesis. At that time computer tomography still demonstrated a large tumor but with some calcifications. A few weeks later his condition deteriorated rapidly and he died at the end of February.

The post mortem examination demonstrated a soft grayish-white tumor in the pons region that had destroyed large parts of the pons and cerebellum. The histologic picture was that of a medulloblastoma with monomorphic tumor cells with round or oval cell nuclei, mostly very rich in chromatin. The tumor cells were mainly arranged like a diffuse carpet of cells, but in some areas they formed trabecules and rosettes around vessels and stroma. There was no sharp border between tumor and normal brain tissue.

Methods

Determination of Urinary Pyrimidines

The method used for determination of uracil and thymine was designed for the analysis of organic acids in urine, but gave reproducible results for the two pyrimidines. We mixed 2 mL of urine sample with 2 mL of saturated sodium chloride solution, added 100 μg of 2-hydroxy-3-methylbenzoic acid as internal standard, and adjusted to pH 1 with 6 mol/L hydrochloric acid. After extracting the sample three times with 3 mL of ethyl acetate, we separated the phases by centrifugation and dried the combined organic phases under a stream of nitrogen. We derivatized the oxo groups with 100 μL of methoxymine hydrochloride in pyridine (20 g/L) for 1 h at room temperature; other functional groups were derivatized with 100 μL of N,O-bis(trimethylsilyl)trifluoroacetamide for 1 h at room temperature.

For gas chromatography, we used a Model 5750 instrument (Hewlett-Packard Instrument, Avondale, PA 19311), equipped with a hydrogen flame ionization detector. The glass column (length 2 m, i.d. 3 mm) was packed with 3% OV-17 on Gas Chrom Q, the carrier gas was helium at a flow rate of 60 mL/min, and the oven temperature was programmed from 70
The volume of work obtained photometer native centrifuged phosphate liter. Scotland) spectrometry mmol/L. for known to 60 nm. We prepared standard curves by adding known amounts of thymine and uracil to urine from apparently healthy individuals. The coefficient of variation was 9% for a single determination in the concentration interval 0.2–2.0 mmol/L. Values reported later (Figure 3) are the mean of duplicate determinations. For gas chromatography–mass spectrometry we used an LKB 9000 instrument (LKB-Cliniton, Bromma, Sweden), to record electron impact spectra, at an electron energy of 70 eV, an ion source temperature of 270 °C, and an acceleration voltage of 3.5 kV.

Determination of Dihydrouracil Dehydrogenase (NADP⁺) (EC 1.3.1.2) Activity in Fibroblasts

Fibroblasts were cultivated from skin biopsy. The cells were grown in McCoy 5a medium (Flow Laboratories Ltd., Irvine, Scotland) supplemented with 100 mL of fetal calf serum per liter. When harvested, the cells were washed three times in a phosphate buffer (25 mmol/L, pH 7.35). We suspended about 20 mg of cells (wet wt.) in 500 μL of phosphate buffer, subjected them three times to rapid freezing and thawing, then centrifuged them for 1 h at 100 000 × g at 4 °C. The supernatant liquid was used for the enzyme assay within the same day. We selected the conditions for the assay according to the work of Fritzon (1): 2.5 μmol of dihydrothymine, 0.1 μmol of NADP, 12 μmol of phosphate buffer (pH 7.35), and supernate corresponding to 0.1–0.2 mg of protein, in a total volume of 450 μL. We followed the formation of thymine for 60 min at 264.5 nm with a Model DMR 21 recording spectrophotometer (Carl Zeiss, Oberkochem, Württemberg, G.F.R.). The reaction rate was linear for 30 min. The blank cell contained all components except dihydrothymine. We measured the protein content of the fibroblast supernate according to Lowry et al. (2).

**Results**

**Identification and Determination of Thymine and Uracil in Urine**

Gas chromatography of the organic acids of the child's urine revealed two components not normally found (Figure 1). These components were identified by mass spectrometry as the trimethylsilyl derivatives of thymine and uracil (Figure 2). Retention times and mass spectra were in accordance with those obtained for the authentic compounds and with published mass spectra (3). Urine samples collected at an early stage of the disease contained about 0.22 mol of uracil and 0.12 mol of thymine per mole of creatinine. In 20 apparently healthy children of corresponding age, the excretion of these pyrimidines was less than 0.01 mol/mol of creatinine. The parents and a brother all had a normal pyrimidine excretion.

During the initial progress of symptoms the excretion of thymine and uracil increased (Figure 3). After two months of radiotherapy and chemotherapy, there was considerable clinical improvement, and the excretion of pyrimidines decreased. When the symptoms recurred, and deterioration was rapid, there was a very massive excretion: 3.0 mol of uracil and 2.6 mol of thymine per mole of creatinine. The uracil/thymine ratio, which had increased during therapy and remission, decreased considerably after the relapse shortly before death.

We also studied another three children with malignant brain tumors, two children with acute lymphatic leukemia, and one with neuroblastoma (Table 1). Two of them excreted the two pyrimidines in slightly greater than normal amounts.

**Excretion of Uric Acid**

The excretion of uric acid in the urine was 0.13–0.52 mol/mmol of creatinine, compared with a reference value of 0.2–1.0 mol/mol of creatinine (4).
Excretion of $\beta$-Alanine and $\beta$-Aminoisobutyric Acid

The concentration of $\beta$-alanine and $\beta$-aminoisobutyric acid in urine was less than 20 mmol/mol of creatinine for each of the compounds (normally expected value <50 mmol/mol of creatinine).

Determination of Dihydrouracil Dehydrogenase (NADP$^+$)

The activity of the dihydrouracil dehydrogenase (NADP$^+$) of cultured fibroblasts was about one-half the activity of control fibroblasts. The activity of the fibroblasts from our patient was 2.5 nmol of thymine produced per milligram of protein in 1 h (mean; range, 2.2–3.1). The activity in two control cases, five and two years old, was, respectively, 4.4 (3.9–5.3) and 5.6 (4.7–6.6) nmol/mg of protein in 1 h.

Discussion

At first we suspected that the massive excretion of thymine and uracil in this patient reflected a systemic enzyme deficiency affecting the first enzyme of pyrimidine catabolism, which is common for thymine and uracil (5). When the presence of a brain tumor had been established, the possibility remained that the child had a metabolic aberration, which might be coincidental with or predisposing for malignancy. However, in our studies with cultured fibroblasts we obtained no evidence for a complete deficiency in the activity of the dihydrouracil dehydrogenase.

It seems likely that the brain tumor caused the high excretion of pyrimidines, because there was a correlation between the excretion rate of pyrimidines and the clinical progress of symptoms, remission after therapy, and relapse. One possibility would be a deficiency of dihydrouracil dehydrogenase restricted to the tumor-cell population. From quantitative considerations, however, it seems unlikely that this could be the only explanation for an excretion of the observed magnitude. The daily synthesis of pyrimidines in a healthy adult has been estimated to be about 0.6 g (6); even if the tumor, which weighed about 50 g, was totally unable to metabolize pyrimidines, this would not result in an excretion of about 1 g of pyrimidines per day, as was the case in the final stage of the disease. One must assume, then, that there was a great increase in the formation of pyrimidines. The rate-limiting enzyme of pyrimidine biosynthesis in mammalian cells is considered to be carbamoyl-phosphate synthase II (EC 2.7.2.9) (7, 8). This enzyme is stimulated by phosphoribosylpyrophosphate and inhibited by uridine triphosphate. From available data it appears that if feed-back control of the enzyme is abolished, a synthesis of up to about 2.5 g/100 g of wet tissue might be achieved (recalculated from ref. 7 and 9).

Weber (9) has recently reviewed work by himself and co-workers on the enzyme patterns in rat hepatomas of different growth rates. They found that the activity of dihydrouracil dehydrogenase (NADP$^+$) of a rapidly growing hepatoma was 9% of the normal liver tissue activity, and that at the same time biosynthetic key enzymes also increased. There was a close correlation between growth rate and enzyme activity change, with reduction in enzymes of pyrimidine catabolism and increase in enzymes of pyrimidine synthesis.

Even though it seems possible that the tumor was able to

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**Table 1. Excretion of Thymine and Uracil in Six Children with Malignancy**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Present condition</th>
<th>Treatment</th>
<th>Thymine (mol/mol creatinine)</th>
<th>Uracil (mol/mol creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 14 yrs</td>
<td>Astrocytoma</td>
<td>Relapse</td>
<td>None</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>δ 17 yrs</td>
<td>Medulloblastoma</td>
<td>Post operation</td>
<td>Radiation</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>δ 5 yrs</td>
<td>Optic glioma Mb v Recklinghausen</td>
<td>Post operation</td>
<td>Cyclic chemotherapy</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>δ 3 yrs</td>
<td>Neuroblastoma</td>
<td>Relapse</td>
<td>Chemotherapy</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>δ 5 yrs</td>
<td>Acute lymph. leukemia</td>
<td>Remission</td>
<td>Maintenance chemotherapy</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>δ 5 yrs</td>
<td>Acute lymph. leukemia</td>
<td>Newly presented</td>
<td>Induction chemotherapy</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

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**Fig. 3. Excretion of thymine and uracil as related to the growth and remission of the brain tumor**

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CLINICAL CHEMISTRY, Vol. 25, No. 7, 1979 1327
synthetize a large amount of pyrimidines, it is puzzling that they were excreted to such an extent in the urine—one would have expected the pyrimidines to be metabolized. The activity of dihydouracil dehydrogenase was lower in cultures of the patient’s fibroblasts than in controls, but it was not reduced to an extent that would seem to explain the high excretion of unmetabolized pyrimidines. We have found no data for human liver, but rat liver is reported to be able to metabolize daily 0.7 g of both pyrimidines per 100 g of wet liver wt. (recalculated from ref. 9). One must then assume that the tumor produced considerably more than the excreted amount, or that the catabolism of pyrimidines was impaired in the liver of this child, or both.

An increased production of purines from the tumor may be considered unlikely, because the excretion of uric acid was not increased.

A massive excretion of thymine and uracil is not a constant or common finding in malignancy (see Table 1). The case reported may represent a unique example of a metabolic aberration in a rapidly growing tumor resembling in some aspects what has been reported by Weber (9) in experimental tumors. Or perhaps there is a subtype of medulloblastoma characterized by this excretion. Abnormal excretion of thymine and uracil in moderate amounts has been described in leukemia and in Hodgkin’s disease. In 1954 Horrigan (10) reported increased uracil excretion in patients with chronic myelocytic leukemia (approximately twice the normal excretion). This observation was confirmed by Adams and coworkers (11), who also reported that the excretion of thymine and uracil was slightly increased in patients with chronic lymphocytic leukemia; in acute leukemia, as much as 75 mg of uracil per day was excreted. 5-Ribosyluracil was of special interest, because it was increased in all of 40 patients observed (11). Weissman et al. (12), who also studied patients with leukemia, found 5-ribosyluracil excretion increased among most cases in chronic myelocytic and lymphocytic leukemia, and in a few cases with acute leukemia. Pinkard et al. (13) examined purines and pyrimidines in Hodgkin’s disease; half of the patients had increased 5-ribosyluracil excretion, which correlated with constitutional symptoms, late stage of disease, and poor prognosis. In a small group of patients (3/33), the 5-ribosyluracil excretion was below normal, but at the same time uracil excretion was increased. However, in adults with leukemia or Hodgkin’s disease, uracil excretion has never been reported to exceed 100 mg/24 h, which would correspond to 0.05–0.1 mol/mol of creatinine (11, 13). Furthermore, there is usually no simultaneous excretion of thymine and uracil among these patients.

In patients with Burkitt’s lymphoma, there has been reported a very large excretion of β-alanine and β-aminoisobutyric acid (14), both intermediary metabolites in the catabolism of pyrimidines (5). These two compounds were not detected in the urine from our patient, however.

This work was supported by a grant from the Swedish Medical Research Council (15X-585). We thank Dr. R. Jagenburg for analysis of β-alanine and β-aminoisobutyric acid.

References