HPLC Determination of Thiopurine Nucleosides and Nucleotides in Vivo in Lymphoblasts following Mercaptopurine Therapy

Thierry Dervieux,1 Yaqin Chu,1 Yi Su,1 Ching-Hon Pui,2,3 William E. Evans,1,3 and Mary V. Relling1,3*

Background: Mercaptopurine is a prodrug requiring intracellular activation to thiopurine nucleotides to exert antileukemic effect. We developed a reversed-phase liquid chromatographic assay for the quantification of mercaptopurine, thioguanine, and methylmercaptopurine nucleoside and nucleotide concentrations in the target tissue, the leukemic lymphoblast.

Methods: Leukemic blasts were isolated from peripheral blood and bone marrow by a standard Ficoll-hypaque procedure. Proteins were removed by ultrafiltration in the presence of dithiothreitol. Thiopurine ribonucleotides were converted into their respective ribonucleosides by treatment of ultrafiltrate with acid phosphatase. Thiopurine nucleosides and bases were measured by direct injection of ultrafiltrate into the chromatographic system. Thiopurine nucleotide concentrations were calculated by subtracting the thiopurine nucleoside concentrations measured after treatment with acid phosphatase from those measured after direct injection of ultrafiltrate in the chromatographic system. Analytes were separated on a C18 Supelco column with ammonium phosphate-methanol eluent coupled with ultraviolet detection.

Results: CVs for intra- and interday precision were 1.1–14% (median, 4.9%), and recovery of added analyte was 89–126% (median, 105%) at low and high concentrations of analytes, except for mercaptopurine riboside. The median signal for each of the five metabolites in lymphoblast samples was 98% (range, 80–106%) of that in water. Detection limits for thiopurine bases and nucleosides ranged from 0.5 to 4.5 pmol/5 × 10^6 cells.

Conclusions: This method is suitable for measurement of thiopurine metabolite concentrations in lymphoblasts in children with acute lymphoblastic leukemia following a single dose of intravenous mercaptopurine.

© 2002 American Association for Clinical Chemistry

Mercaptopurine (MP),4 the analog of hypoxanthine, is an antileukemic agent widely used to treat acute lymphoblastic leukemia (ALL) (1). MP is a prodrug requiring intracellular activation to thiopurine nucleotides to exert cytotoxicity. Intracellularly, MP is converted by hypoxanthine guanine phosphorybosyltransferase into thioinosine monophosphate (TIMP) and subsequently into thioguanosine monophosphate by a two-step process involving inosine monophosphate dehydrogenase and guanosine monophosphate synthetase (2). This process is in competition with methylation by thiopurine methyltransferase, an enzyme subject to genetic polymorphism (3, 4). Thiopurine methyltransferase converts MP into inactive methylmercaptopurine (MMP), but also metabolizes TIMP into active methyl-TIMP (5-7). Each of the thiopurine riboside monophosphates (TIMP, thio-guanosine monophosphate, and methyl-TIMP) can be converted into thiopurine ribonucleotide triphosphates (8, 9) and dephosphorylated into thiopurine ribonucleosides [mercaptopurine riboside (rMP), mercaptoguanosine riboside (rTG), and MMP riboside (rMMP)] by 5’-nucleotidase (10, 11).

In clinical practice, studies have reported that the
antileukemic effect of MP is related to the formation of thioguanine nucleotides (TGNs) (12–14), which are incorporated into genomic DNA in their reduced form (deoxythioguanosine) (15). However, MMP nucleotides (MeMPNs) are potent inhibitors of de novo purine synthesis in vitro and, therefore, can contribute to the effects of MP (16, 17). The monitoring of MP therapy with quantification of thiopurine metabolite concentrations by HPLC in plasma (18, 19) or erythrocytes (14, 20, 21) has been advocated by several groups as a strategy to optimize therapy. Although erythrocytes are a convenient tissue to study the intracellular metabolism of MP, very little is known about the disposition of thiopurine metabolites in the target of MP therapy, the leukemic blast. Several chromatographic methods for the determination of intracellular thiopurine nucleotide concentrations in erythrocytes (22–25) or peripheral lymphoblasts (8, 9, 26, 27) have been developed. Many of these methods are based on acid hydrolysis of the thiopurine nucleotide moieties to thiopurine bases, making them incapable of distinguishing among nucleobases, nucleosides, and nucleotides (23–26). In contrast, other methods have proposed direct quantification of thiopurine mono-, di-, or triphosphate nucleotides by ion-pairing chromatography (8, 9, 22), but they require calibrators that are not available commercially. Here we report a HPLC method sensitive enough to measure thiopurine nucleosides and metabolites in the target of MP therapy, the leukemic lymphoblast, after MP therapy given to children with ALL.

### Materials and Methods

#### CHEMICALS AND PREPARATION OF CALIBRATORS

MP, rMP, rTG, MMP, rMMP, d,l-dithiothreitol (DTT), and acid phosphatase (type VII from white potato) were obtained from Sigma. TIMP and thioguanosine diphasphate (TGDP) were kindly provided by the National Cancer Institute (Bethesda, MD). Sodium citrate and monobasic ammonium phosphate were purchased from Fisher Scientific. HPLC-grade methanol was purchased from Baxter Healthcare. Microcon 10 ultrafiltration concentrators were from Amicon.

MP was dissolved in 0.1 mol/L sodium hydroxide, rTG was dissolved in 0.001 mol/L sodium hydroxide, rMMP was dissolved in 0.1 mol/L phosphate buffer (pH 7), and rMP and MMP were dissolved in 0.1 mol/L hydrochloric acid. Thiopurine nucleotides (TIMP and TGDP) were dissolved in water containing 10 mmol/L DTT. After the compounds were dissolved, the final concentrations of MeMPNs present in the ultrafiltrate were determined by an acid hydrolysis procedure as described (25). Briefly, 300 μL of cell extract was heated for 1 h at 100 °C in the presence of 30 μL of 700 mL/L perchloric acid to allow the conversion of MeMPNs to 4-amino-5-methylthiocarbonyl imidazole (29). Concentrations were determined by comparison with a calibration curve for rMMP treated using the same conditions. The final concentration of MeMPNs in the cell extract was 54 μmol/L. MMP and rMMP were undetectable in the prepared ultrafiltrate.

#### HPLC INSTRUMENT AND CHROMATOGRAPHIC SEPARATION OF COMPOUNDS

The liquid chromatography system consisted of two Shimadzu LC-10AS liquid chromatography pumps, a SCL-10A system controller, a SIL-10A autoinjector, a sample cooler kept at 4 °C, and a SPS-10A ultraviolet–visible (UV-Vis) detector. Chromatograms were acquired and analyzed with the Shimadzu Class VP 5.0 software running on a Dell computer.

The HPLC separation (30) was performed on a 25 cm × 4.6 mm Supelco column (5-μm particle size), protected by a Supelco guard column. Mobile phase A consisted of 98% monobasic ammonium phosphate (30 mmol/L, pH 2.90) and 2% methanol containing 0.26 mmol/L DTT. Mobile phase B consisted of 60% monobasic ammonium phosphate (30 mmol/L, pH 2.90) and 40% methanol. The samples were isocratically eluted at a flow rate of 1 mL/min, with 12% mobile phase B for 15 min and a 25-min linear gradient to 100% phase B. After 40 min, the mobile phase was returned to 12% mobile phase B. One sample was injected every 60 min. Detection was at 330 nm for the first 30 min (detection of MP, rMP, and rTG) and at 290 nm (MMP and rMMP) thereafter.

#### CALIBRATORS AND CALIBRATION CURVES

Calibration curves were prepared by adding known amounts of MP, rMP, rTG, MMP, and rMMP to a pool of lymphoblasts isolated from patients with ALL before any chemotherapy. We anticipated low metabolite concentrations. The range for these enriched calibrators were as follows: MP, 0.8–13.1 pmol/5 × 10⁶ cells; rMP, 0.9–14.1 pmol/5 × 10⁶ cells; rTG, 0.8–13.4 pmol/5 × 10⁶ cells;
MMP, 7.5–120.3 pmol/5 × 10⁶ cells; and rMMP, 4.2–67.0 pmol/5 × 10⁶ cells. All enriched calibrators were assayed in duplicate. Calibration curves were fit by linear regression using peak heights vs concentrations.

**PRECISION, RECOVERY, AND EXTRACTION EFFICIENCY**

Intra- and interday precision and recoveries were determined by analyzing enriched low and high concentrations of thiopurines added to pretreatment lymphoblasts prepared independently from the calibrators. Intraday analysis was performed with 10 enriched replicates, whereas interday evaluation was assessed with 3 replicates on 5 different days. Recovery was calculated as the measured concentrations in the enriched samples relative to the target concentration (measured concentration/target concentration × 100%). Imprecision was determined by the CV. Precision and recovery were also determined from three replicates of unknown samples enriched with low, medium, and high concentrations of thiopurines with the analyst blinded to the sample preparation and concentrations.

To estimate extraction efficiencies of MP, rMP, rTG, MMP, and rMMP, we compared the peak heights from enriched lymphoblast samples with those from samples prepared with water at the same concentrations within the validated range.

The conversion of thiopurine nucleotides to thiopurine nucleosides was determined with available thiopurine nucleotide calibrators (TIMP and TGDP) and with MeMPNs that were formed after incubating CEM cells with rMMP, as described above. TIMP, TGDP, and MeMPNs were added to lymphoblasts at concentrations of 100–500 pmol/5 × 10⁶ cells. Precision and recovery were determined after quantification of rMP, rTG, or rMMP concentrations formed after enzymatic treatment (with five replicates at each concentration 4 different days). The activity of each lot of acid phosphatase was tested using a pool of patient lymphoblasts following treatment with MP.

**SAMPLE TREATMENT PROCEDURE**

Cell pellets (5 × 10⁶) resuspended in a final volume of 200 μL of 10 mmol/L sodium citrate (pH 4.5) were sonicated for 10 s, and 10 μL of a 10 mmol/L DTT solution was added. The sample mixture was transferred to a Microcon-10 and centrifuged 30 min at 12 000 g. Ultrafiltrates were separated into two aliquots of 90 μL each. To quantify thiopurine nucleotides, a total of 2.5 μL of acid phosphatase was added to the first aliquot, and the mixture was heated for 2 h at 56 °C to convert thiopurine ribonucleotides into thiopurine ribonucleosides. Thionosine nucleotides were converted into rMP, TGNs into rTG, and MeMPNs into rMMP. After incubation and cooling, 5.0 μL of 10 mmol/L DTT was added to the mixture and centrifuged for 2 min. A total volume of 80 μL was injected into the HPLC system. To quantify thiopurine nucleosides and bases (MP, rMP, rTG, MMP, and rMMP), the second aliquot of ultrafiltrate was not treated with acid phosphatase. A total of 2.5 μL of water and 5.0 μL of 10 mmol/L DTT were added to the ultrafiltrate, which was then centrifuged for 2 min; a total volume of 80 μL was injected into the HPLC system.

Thiopurine nucleotide concentrations (MPNs, TGNs, and MeMPNs) were calculated by subtracting the thiopurine nucleoside concentrations measured after direct injection of ultrafiltrate into the chromatographic system from those measured after treatment with acid phosphatase.

**APPLICATION TO PATIENT SAMPLES**

Blood samples (5 mL) were collected into heparin-containing tubes before and 6 and 20 h after the initiation of a 6-h intravenous infusion (1 g/m²) of MP in children with newly diagnosed ALL after written informed consent was obtained from the guardians or parents. Bone marrow aspirates were drawn 20 h after initiation of MP infusion. Lymphoblasts were isolated using a Ficoll-hypaque density gradient procedure and washed three times with a solution of HEPES, Hank’s buffered salt solution, and heparin. The final cell yield was determined by hemocytometer and the percentage of viability by trypan blue exclusion. The mononuclear cells layer contained, on average, 10% red blood cells. Peripheral blood red cell lysates were prepared as described previously (31). Thiopurine metabolites were measured as described for blasts in 100 samples. The correlation coefficient between total concentrations measured in the Ficoll-hypaque mononuclear layer and those calculated after subtracting the concentrations arising from red blood cells was 0.967 (slope, 1.079) for TGNs and 0.929 (slope, 1.14) for MeMPNs, demonstrating that the residual red blood cells had little effect on the estimate of blast concentrations. Mononuclear cells were pelleted (5.0 × 10⁶ to 20 × 10⁶ cells depending on cell yield) and stored at −80 °C until analysis. Results were normalized to 5 × 10⁶ mononuclear cells. Patient results are expressed as mean ± SE. Intrapatient comparisons of thiopurine metabolite concentrations were assessed using the Wilcoxon test.

**Results**

**SEPARATION OF COMPOUNDS, CALIBRATION CURVES, AND DETECTION LIMIT**

Typical chromatograms of 5 × 10⁶ pretreatment bone marrow lymphoblasts to which thiopurines had been added are presented in Fig. 1. Calibration curves demonstrated a linear relationship between peak height and concentration for all analytes, with correlation coefficients >0.995 for all. Equations describing the calibration curves were as follows: MP, y = 0.0169x – 0.2442; rMP, y = 0.0226x – 0.6170; rTG, y = 0.0232x – 0.2919; MMP, y = 0.0207x + 0.0720; rMMP, y = 0.0173x – 0.0849, where y is
the peak height and \( x \) is the concentration of added thiopurine.

The limits of detection, defined as three times the signal-to-noise ratio, were 0.5 pmol/5 \( \times 10^6 \) cells for MP, rMP, and rTG and 4.5 and 2.5 pmol/5 \( \times 10^6 \) cells for MMP and rMMP, respectively. The precolumn was changed every 30 injections, and the analytical column demonstrated no deterioration in performance after up to 300 injections.

### Precision, Recovery, and Extraction Efficiencies

The intra- and interday precision and recoveries for the assay are summarized in Table 1. The CVs for intra- and interday precision were <15 at low and high concentrations of analytes. Recovery of analytes added to samples was 89–126% for MP, rTG, MMP, and rMMP. However, at low concentrations of rMP (1.1 pmol/5 \( \times 10^6 \) cells), interday recoveries were poor because of an interfering peak in some pretherapy samples. Mean extraction efficiencies were 97.0%, 99.7%, 96.5%, 80.3%, and 85.3% at low concentrations and 103.3%, 105.8%, 98.8%, 105.9%, and 105.5% at high concentrations of MP, rMP, rTG, MMP, and rMMP, respectively. For three replicates of "unknown" lymphoblast samples enriched with different concentrations of compounds, with the analyst blind to the sample concentrations, median imprecision (as CVs) and recovery were 4.2% (range, 0.4–14%) and 102% (range, 87–114%), respectively.

The efficiency of dephosphorylation of TIMP, TGDP, and MeMPNs after treatment with acid phosphatase was determined at concentrations of 100–500 pmol/5 \( \times 10^6 \) cells. After dephosphorylation, TIMP and TGDP peaks were undetectable in the chromatograms, whereas rMP and rTG appeared (not shown). Similarly, no rMMP peak was detectable before dephosphorylation of MeMPN calibrators added to lymphoblasts, whereas the rMMP peak appeared after conversion with acid phosphatase. Complete conversion of TIMP, TGDP, and MeMPNs to rMP, rTG, and rMMP, respectively, was demonstrated by the recoveries >90% and CVs <15% for TIMP, TGDP, and MeMPNs (Table 2).

The activity of the acid phosphatase was tested, for each lot purchased, against a pool of lymphoblasts from patients treated with MP. Over a period of 1 year, the variability of rTG and rMMP measured after enzymatic

---

**Table 1. Precision and recoveries for thiopurine metabolites in leukemic blasts.**

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Target concentration, pmol/5 ( \times 10^6 ) cells</th>
<th>Mean observed concentration, pmol/5 ( \times 10^6 ) cells</th>
<th>CV, %</th>
<th>Mean recovery, %</th>
<th>Mean observed concentration, pmol/5 ( \times 10^6 ) cells</th>
<th>CV, %</th>
<th>Mean recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP</td>
<td>1.0</td>
<td>1.1</td>
<td>11</td>
<td>108</td>
<td>1.1</td>
<td>3.9</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>11.0</td>
<td>1.1</td>
<td>111</td>
<td>11.1</td>
<td>2.4</td>
<td>111</td>
</tr>
<tr>
<td>rMP</td>
<td>1.1</td>
<td>1.3</td>
<td>9.3</td>
<td>126</td>
<td>2.7</td>
<td>5.9</td>
<td>254</td>
</tr>
<tr>
<td></td>
<td>10.6</td>
<td>10.1</td>
<td>8.3</td>
<td>96</td>
<td>9.4</td>
<td>5.6</td>
<td>89</td>
</tr>
<tr>
<td>rTG</td>
<td>1.0</td>
<td>1.1</td>
<td>14</td>
<td>106</td>
<td>1.0</td>
<td>11</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.6</td>
<td>5.1</td>
<td>106</td>
<td>9.1</td>
<td>1.9</td>
<td>91</td>
</tr>
<tr>
<td>MMP</td>
<td>9.0</td>
<td>10.5</td>
<td>12</td>
<td>116</td>
<td>10.7</td>
<td>4.5</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>90.2</td>
<td>87.9</td>
<td>2.4</td>
<td>97</td>
<td>85.8</td>
<td>2.1</td>
<td>95</td>
</tr>
<tr>
<td>rMMP</td>
<td>5.0</td>
<td>5.1</td>
<td>4.7</td>
<td>102</td>
<td>4.8</td>
<td>6.8</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>50.3</td>
<td>46.8</td>
<td>1.4</td>
<td>93</td>
<td>46.8</td>
<td>2.8</td>
<td>93</td>
</tr>
</tbody>
</table>
conversion in this control pool was 13.4% and 6.2%, respectively (n = 7).

APPLICATION TO PATIENT SAMPLES

The method was applied to the in vivo assessment of lymphoblast thiopurine metabolite concentrations 6 and 20 h after initiation of a 1 g/m² intravenous MP infusion given over 6 h to children with newly diagnosed ALL. Typical chromatograms of bone marrow lymphoblasts of a patient at 20 h after initiation of MP infusion are presented in Fig. 2.

The concentrations of TGNs, MeMPNs, rTG, and rMMP measured in bone marrow mononuclear cells are shown in Fig. 3A. At 20 h, TGNs were undetectable in only 2 of 41 samples. Thiopurine base, nucleoside, and nucleotide concentrations measured at 6 and 20 h in bone marrow mononuclear cells from 20 patients with peripheral lymphoblasts are presented in panels B, C, and D of Fig. 3, respectively. At 6 h, MPN metabolites predominated over TGN and MeMPN metabolites, whereas at 20 h, a decrease in MPN concentrations (P < 0.001) was associated with an increase in MeMPN concentrations (P < 0.001) without significant change of TGN concentrations (P = 0.16). The total thiopurine nucleotide concentrations (MPN + TGN + MeMPN) were similar at 6 and 20 h (P = 0.48).

**Discussion**

Although MP has been administered for more than 40 years in the treatment of ALL, very little is known about the in vivo intracellular disposition of thiopurine metabolites in the target of MP therapy, the leukemic blast. Two different strategies have been reported for the quantification of thiopurine nucleotide concentrations in lymphoblasts or peripheral mononuclear cells (8, 9, 26, 27). These strategies use protein precipitation with acid in the presence of DTT, followed by acid hydrolysis and heating to convert thiopurine nucleotides into thiopurine bases (26), or protein-free samples are directly injected and thiopurine nucleotides are detected by ion-pairing chromatography (8, 9, 27). Each of these approaches has limitations. The acid hydrolysis procedure cannot establish which

**Table 2. Efficacy of acid phosphatase conversion of nucleotides to nucleosides.a**

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration added, pmol/5 × 10⁶ cells</th>
<th>Mean measured concentration of nucleoside, pmol/5 × 10⁶ cells</th>
<th>CV, %</th>
<th>Mean recovery, b %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP</td>
<td>100</td>
<td>rMP</td>
<td>97</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td></td>
<td>243</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td></td>
<td>492</td>
<td>7.7</td>
</tr>
<tr>
<td>TGDP</td>
<td>100</td>
<td>rTG</td>
<td>91</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td></td>
<td>230</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td></td>
<td>469</td>
<td>7.7</td>
</tr>
<tr>
<td>MeMPN</td>
<td>100</td>
<td>rMMP</td>
<td>114</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td></td>
<td>251</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td></td>
<td>491</td>
<td>12</td>
</tr>
</tbody>
</table>

a Presented as the imprecision and recovery for mercaptopurine riboside rMP, rTG, and rMMP measured after acid phosphatase conversion of TIMP, TGDP, and MeMPNs added into leukemic blasts. The table summarizes three to four different experiments from 3 to 4 different days with five replicates each day and at each concentration for each compound.

b Assuming 100% conversion by acid phosphatase.
portion of the thiopurine base originates from the thiopurine nucleotide or thiopurine nucleoside, or is present as the base. In contrast, direct detection of thiopurine nucleotides by ion-pairing chromatography has the advantage that it quantifies thiopurine nucleotide mono-, di-, or triphosphates; however, these calibrators are not available commercially. In addition, measuring each of the thiopurine mono-, di-, or triphosphates compromises the sensitivity to detect nucleotides vs other thiopurine metabolites.

Our method strikes a compromise in which we differentiate among thiopurine bases, nucleosides, and nucleotides, but do not separate nucleotides into mono-, di-, or triphosphates. We removed proteins by ultrafiltration and then enzymatically converted thiopurine nucleotides into thiopurine nucleosides with acid phosphatase. Cleavage of the ribonucleoside-phosphate bonds during the enzymatic treatment liberates the thiopurine nucleoside moiety, which is detected in our chromatographic system by an ultraviolet detector. Because the ultrafiltration step removes all proteins, it prevents the possible ex vivo catabolism of the thiopurine nucleotides by other purine enzymes, such as purine nucleotide phosphorylase. This method is rapid, robust, and accurate for the concentrations present in vivo in leukemic blasts, using only $5 \times 10^6$ cells. The method is linear for all analytes, with intra- and interday CVs <15% for both low and high concentrations. However, an endogenous peak that coelutes with rMP did
not allow an exact measurement of low concentrations of this metabolite.

The low number of cells required in this assay makes our method suitable for use in pediatric patients, in whom blood volume and bone marrow samples are usually limited. We measured bone marrow and peripheral blast thiopurine metabolites after administration of intravenous MP. To our knowledge, such a study has never been conducted in a large number of patients and never in bone marrow blasts. We observed substantial interpatient differences in thiopurine metabolite concentrations, which could have been attributable to interindividual differences in enzymes such as thiopurine methyltransferase or 5'-nucleotidase (10, 11, 31).

MPNs predominated over thioguanine and MeMPN concentrations at 6 h, but at 20 h, MPN concentrations were lower and MeMPN concentrations were higher relative to concentrations at 6 h. Total thiopurine nucleotide concentrations measured at 6 and 20 h were similar. This suggests that MPNs were preferentially methylated by thiopurine methyltransferase between 6 and 20 h, rather than converted by inosine monophosphate dehydrogenase and guanosine monophosphate synthetase into TGNs. Previous in vitro reports have shown that TIMP can inhibit inosine monophosphate dehydrogenase (32, 33). Our in vivo results are consistent with these observations: inhibition of inosine monophosphate dehydrogenase by TIMP or related nucleotides at 6 h may have limited the subsequent formation of TGNs, leaving TIMP available for methylation to methylthioinosine monophosphate.

In conclusion, we have developed a sensitive and specific reversed-phase chromatographic method for the determination of lymphoblast thiopurine bases, nucleosides, and nucleotides. The method is accurate and suitable for clinical pharmacologic studies of MP in children with ALL.

This work was supported by Grants CA 36401, CA 78224, and CA21765 from the NIH; by a Center of Excellence grant from the State of Tennessee; and by American Lebanese Syrian Associated Charities (ALSAC). We thank our clinical staff for scrupulous attention to patient care and management of blood sampling; our research nurses, Sheri Ring, Lisa Walters, Terri Kuehner, Margaret Edwards, and Paula Condy; and the patients and their parents for their participation in this study.

References


