An Alternative Method for Assaying Cerebrospinal Fluid Protein in the Presence of Methotrexate

Linda M. Kasper, Wells R. Moorehead, Tijen O. Oel, and Marianne Markanich

Therapeutic concentrations of methotrexate can cause significant positive interference in cerebrospinal fluid (CSF) protein values when assayed in the Du Pont acu. Conversely, our modified turbidimetric method, in which trichloroacetic acid (TCA) plus a sample blank containing dilute hydrochloric acid is used in place of TCA, exhibits little or no interference from methotrexate. This was verified by assaying solutions that contained a constant amount of protein (−430 mg/L) and various amounts of methotrexate (0.0-2.3 × 10⁻⁴ mol/L) by both the Du Pont acu and the manual turbidimetric method. As expected, the acu results showed increasing protein values with increasing methotrexate, whereas the manual method gave results approximating the expected protein value irrespective of the methotrexate concentration.

Additional Keyphrases: analytical error · turbidimetry

When therapeutic concentrations of methotrexate are being maintained in cerebrospinal fluid (CSF) by intrathecal injection, protein in CSF should be assayed by a method other than the Du Pont acu. Zweig (1, 2) reported that methotrexate concentrations in the 10⁻⁴ to 10⁻⁵ mol/L range produced a positive interference in determinations of total protein in CSF when assayed by the Du Pont acu. He also noted some degree of interference with the Folin-Lowry method at methotrexate concentrations of 10⁻⁴ mol/L, but no interference with an automated immunoprecipitin method. In this study we used a manual turbidimetric method with a dilute HCl sample blank that demonstrated little or no interference when protein in CSF was measured in the presence of methotrexate.

Materials and Methods

Reagents

Stock methotrexate solution. Low-sodium methotrexate, cryodesiccated powder, 20 mg per vial (Lederle Parenterals, Inc.), was reconstituted with 20 mL of sterile sodium chloride solution (9 g/L) to yield a methotrexate concentration of 1 g/L (2.2 × 10⁻³ mol/L).

Phosphate-buffered saline, pH 7.4. Place 7.65 g of NaCl, 0.742 g of Na₃HPO₄, and 0.210 g of KH₂PO₄ into a 1-L beaker and dissolve in ~500 mL distilled water. Adjust to pH 7.4 with 1 mol/L NaOH, transfer to a 1-L volumetric flask, dilute to volume with distilled water, and mix.

Hydrochloric acid, 0.25 mol/L. Place 20.8 mL of concentrated hydrochloric acid in a 1-L volumetric flask. Dilute to volume with distilled water and mix.

Stock protein solution. A serum sample containing 48 g of protein per liter was diluted 100-fold with phosphate-buffered saline, pH 7.4, to yield a protein solution containing 480 mg/L.

Test solutions. Eight test solutions containing the same amount of protein and various amounts of methotrexate (0.0-2.3 × 10⁻⁴ mol/L) were prepared from the stock protein solution. All eight solutions had a final protein concentration of ~430 mg/L.

Methods

Quantification of protein. We modified the method of Henry et al. (3) for quantifying protein in CSF and urine. In the original method the reaction mixture contained 1.0 mL of CSF, 3.0 mL of 8.5 g/L sodium chloride reagent, and 1.0 mL of 125 g/L trichloroacetic acid reagent, resulting in a final trichloroacetic acid concentration of 0.153 mol/L. Absorbance measurements were taken between 5 and 10 min against a water blank at 420 nm.

The reaction mixture in our modified method contains 0.5 mL of sample and 1.5 mL of 50 g/L trichloroacetic acid reagent, resulting in a final trichloroacetic acid concentration of 0.23 mol/L. The modified method also includes a sample blank for each specimen: 0.5 mL of sample and 1.5 mL of 0.25 mol/L HCl reagent instead of trichloroacetic acid. Absorbance measurements were taken between 5 and 10 min against the appropriate sample blank at 420 nm.

Protein was also quantified with the Du Pont acu.

Quantification of methotrexate. The Abbott TDx was used to quantify methotrexate.

Results

Figure 1 illustrates the effect of various methotrexate concentrations on the two quantitative methods for protein in CSF (Du Pont acu and manual turbidimetry). When no methotrexate was present, the protein values as determined by the acu and by manual turbidimetry were similar. With the manual method, increasing methotrexate concentrations were accompanied by relatively small changes in observed protein values. However, the magnitude of interference in the acu method in the presence of therapeutic...
concentrations of methotrexate is striking (Figure 1). Our data indicate that such concentrations of methotrexate can produce an increase of as much as fivefold in the value for apparent protein in CSF as assayed in the acq.

Discussion

We became interested in this problem when we received a CSF specimen with a request for total protein assay. In our laboratory, each CSF protein specimen is assayed without delay in the acq (normal range = 150–450 mg/L). In this case the measured protein concentration was 670 mg/L. Suspecting that this was an inappropriate value for a clear, colorless CSF specimen, we assayed the protein by our manual turbidimetric method and obtained a value of 140 mg/L. After checking the patient's diagnosis and current therapy regimen, we found that the patient was being treated with intrathecal injections of methotrexate for Burkitt's lymphoma. Three days later a second specimen of CSF requiring protein determination was received from the same patient. The following results were obtained: protein by acq, 680 mg/L; protein by the manual turbidimetric method, 120 mg/L; and methotrexate by the Abbott TDx, \( 8.6 \times 10^{-5} \) mol/L. As reported by Zweig (2), the interference by methotrexate in the acq assay for protein in CSF is due to the significant absorbance of methotrexate at the measuring wavelength of 340 nm. The drug does not absorb at the correction wavelength of 540 nm.

Thus we designed the experiment described above. Our data indicate that the manual turbidimetric method, unlike the Du Pont acq, is unaffected by methotrexate concentrations of \( 10^{-4} \) to \( 10^{-5} \) mol/L. Consequently, we propose this manual turbidimetric assay with dilute HCl sample blank as an acceptable alternative method for quantifying protein in CSF in the presence of therapeutic concentrations of methotrexate. We recommend dilute hydrochloric acid instead of saline as the diluent for the sample blank, to correct for occasional interfering substances that behave as acid-base indicators, i.e., that may produce added color in the mixture finally measured, and also to detect and correct for interference caused by certain drugs that produce turbidity when subjected to an acidic environment.

We have used this same manual method for the past six years to assay urinary total protein for patients who have undergone a renal transplant. Because these patients are on multiple drug regimens, each urine specimen must serve as its own blank. This manual turbidimetric method is recommended because the inclusion of the dilute HCl sample blank eliminates many of the interferences previously associated with the use of trichloroacetic acid methodology.

References


Age-Related Reference Values for Free Amino Acids in First Morning Urine Specimens

P. R. Parvy, J. I. Bardet, D. M. Rablier, and P. P. Kamoun

We determined age-related reference values for urinary free amino acids (in mmol/mol creatinine) in first morning urine specimens from 360 control subjects who were divided into nine age groups: birth to 1 month, 1–6 months, 6–12 months, 1–2 years, 2–4 years, 4–7 years, 7–10 years, 10–13 years, and older than 13 years. Except for taurine and 3-methylhistidine, the concentration of all the amino acids decreased with increasing age. The use of these results to detect aminoacidopathies and tubulopathies is discussed.

Additional Keyphrases: pediatric chemistry • aminoacidopathies • taurine • 3-methylhistidine

Identification and measurement of free amino acids in urine is crucial for the diagnosis and the treatment of aminoacidopathies, and has been the subject of numerous reports on newborns, children, and adults (1–7). All these reported results except one (6) were obtained with 24-h urine sampling and were expressed as \( \mu \text{mol/day} \) (1), \( \mu \text{mol/kg per day} \) (2), \( \mu \text{mol/g creatinine} \) (2, 4, 6, 7), or \( \mu \text{mol/min per 1.73 m}^3 \) of body surface area (5).

Because it is often very difficult to obtain 24-h urine samples from patients, we previously developed the quantification of aminoaciduria in samples of first morning urine specimens and reported age-related reference values for 13 amino acids (6). The increased sensitivity of amino acid analysis and the better resolution of the new ion-exchange resins motivated us to determine normal values for 26 amino acids, total aminoaciduria, and creatinine in first morning urine specimens from 360 new control subjects divided into nine age groups (40 patients each).

Materials and Methods

Control subjects. All the subjects studied were hospitalized. We excluded premature infants (8, 9) and patients with kidney diseases (10) or liver failure (11), known metabolic diseases, or other disorders for which variations of aminoaciduria have been described: e.g., muscular, bone, skin, and eye diseases (12). We also excluded treated patients whose therapy is known to induce abnormal aminoaciduria or methodological interferences (13). All patients were in good nutritional state with a normal protein intake.

Sample collection. The first morning urine specimen (~10-h urine) was collected, with no added preservative, by voluntary bladder voiding or with collection bags if necessary. Creatinine was promptly determined in the Astra 8 ( Beckman Instruments, Gagny, 93220, France).

Preparation and storage of samples. We added 1 mL of the...

Laboratoire de Biochimie Médicale B, Hôpital Necker–Enfants Malades, 149 Rue de Sèvres, 75743 Paris Cedex 15, France. Received March 30, 1988; accepted June 14, 1988.