Ultrafiltration of Urine Specimens for Electrophoresis of Mucopolysaccharides, Monica C. Hsieh and Helen K. Berry (Metabolic Disease Center, Children's Hosp. Med. Center, Eiland and Bethesda Aves., Cincinnati, OH 45229)

Mucopolysacchariduria is a feature of mucopolysaccharide (MPS) storage diseases: Hurler's (MPSI), Hunter's (MPSII), and Sanfilippo's (MPSIII) syndromes. The first step in differential diagnosis is identification of MPS by electrophoresis on cellulose acetate membranes (1).

Before electrophoresis, MPS is concentrated by precipitation with quaternary salts such as cetylpyridinium chloride or a dye such as Alcian blue (1). These tedious and time-consuming methods require periods of standing, extensive washing, and centrifugation. Low-molecular-mass species may not be isolated by these procedures.

We describe a procedure for simple, rapid ultrafiltration in a 10-mL stirred "Omegacell" (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) to concentrate urine for MPS electrophoresis. After preliminary screening (2), 3 mL of each MPS-positive specimen is placed in an Omegacell and ultrafiltered through a membrane with a 10,000-Da cutoff. The cell is connected to an air line to exert a pressure of approximately 2.7 kg/cm². With magnetic stirring, 3 mL of urine can be concentrated to 0.3 mL in 10 to 15 min. Filtrate and retentate can be retested for presence of MPS (2). For most specimens, MPS was retained by the membrane; in a few, MPS was found in both filtrate and retentate. When the process was repeated with use of a 3000-Da cutoff membrane, about 45 min was required to reduce the volume of the specimen to 0.1 of the original.

Electrophoresis was carried out in a 0.1 mol/L barium acetate buffer system (pH 6.0) with constant current, 5 mA per 15 x 2.5 cm strip. After staining the strips in Alcian blue (1) and destaining in water, we transferred them to glycerol/water (1/9 by vol) and soaked them for 1 h. This removes residual dye and prevents breaking of the strips.

The electrophoretic pattern was identical regardless of molecular size. In a patient with MPS I, dermatan sulfate retained by the 10-kDa-cutoff membrane migrated with the dermatan sulfate that was filtered through the 10-kDa-cutoff membrane but retained by the 3-kDa-cutoff membrane. The procedure may be useful in monitoring patients for MPS of decreased molecular size after bone-marrow transplant.

The stirred cell can be re-used if it is cleaned and stored in glycerol/water (1/19 by vol) at 4°C. Distilled water (5 mL) should be filtered through the membrane before re-use.

References
Fig. 1. Typical standard curve of synthetic AVP (C) and serial twofold-dilution curves of human urine (D). 

Analytical recovery (n = 10) of authentic AVP added to a twofold-diluted urine to give 5, 10, and 15 ng/L was at least as good as that obtained with the comparison method and ranged from 97% to 101%.

Correlation between the direct assay (y) and the comparison assay (x) yielded the following results: y = 1.06x + 3.16, r = 0.95, n = 17.

Thus we found direct assay of AVP in urine to be at least as precise and accurate as the comparison procedure involving extraction but more rapid and cost efficient.

Cross-Reactivity of Cyclosporine Metabolites in Two Different Radioimmunoassays In Which the Same Specific Monoclonal Antibody is Used, Pierre E. Wallemacq, Sung C. Lee, Georges Lhoest, and Alexandre Hassoun

Monitoring cyclosporine (CsA) in whole blood is essential for maintaining adequate immunosuppressive effects and avoiding toxicity. Two radioimmunoassays (RIA) currently are available for measuring the concentration of the parent drug: the RIA Sandimmune kit (Sandoz) (1) and the RIA CYCLO-Trac SP kit (INCSTAR) (2). In both, the same specific monoclonal antibody, developed by Sandoz, is used, but analytical conditions differ between the two kits. The tracers are labeled at different sites with different isotopes: Sandimmune with 3H at amino acid position 1 and CYCLO-Trac SP with 125I at amino acid position 2. Also, separation procedures involve use of either charcoal (Sandimmune) or pre-formed second-antibody complex (CYCLO-Trac SP). Therefore, we studied the cross-reactivities of the major CsA metabolites in the two methods.

CsA metabolites were isolated from human bile after extraction with diethyl ether. Purification involving two HPLC procedures and characterization by mass spectrometry and nuclear magnetic resonance have been described (3, 4). Each identified metabolite was quantified by weighing on an electronic balance (Precisa 40 SM-200A). The amount weighed averaged 0.5 mg each.

These dried metabolites were reconstituted in 20 μL of methanol and either 500 μL of human drug-free serum or EDTA-treated whole blood and further diluted with their respective matrix to give six different concentrations, aliquots of which were assayed in two different runs with both RIAs, in serum and whole blood, according to the respective kit protocols. The concentrations of metabolites used presented a suppression line ranging from 90% to 10% B/Bo. All metabolites were examined in the same run to eliminate error due to run-to-run variability. In addition, to check the validity of each run, we included a reference set of Sandoz CsA calibrators in each run of CYCLO-Trac SP.

Table 1 lists percentage cross-reactivities of metabolites. Results are expressed as the ratio (CsA concentration/metabolite concentration) x 100 at 50% inhibition of maximum binding. Analyses of the suppression lines confirmed that all metabolite lines paralleled the CsA line. The cross-reactivities never exceeded 3% in either method. Among the metabolites tested, metabolite 1 showed the greatest (2.5%), whereas metabolite 5 showed the least (<0.1%). In serum, the cross-reactivity appears greater in CYCLO-Trac SP than in Sandimmune. However, all cross-reactivities are so low as to be of doubtful clinical significance.

Evidently CYCLO-Trac SP RIA should be considered equivalent to Sandimmune in cross-reactivities with major metabolites of CsA.

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References


