overlooked our suggestion in the final column of our paper [1] that “Possibly the PSA molecule of this patient has an alternative structure, leading to a diminished recognition by the anti-PSA antibodies used in the affected assays and a reduced binding to ACT.” If this indeed is the case, the entire discussion on (the lack of) equimolarity of response of different PSA assays may very well not apply. We have recently obtained preliminary evidence suggesting that indeed at least part of this patient’s PSA has a 41-amino acid deletion (AA 94–134) [2]. We are currently investigating the consequences that such a deletion may have on the reactivity of the protein in the different assays.

References

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Interference of Thioridazine (Mellaril) in Identification of Phencyclidine

To the Editor:
The use of immunoassays to detect abused substances is routine in many laboratories. Cost-cutting measures include using nonconfirmational techniques [1] and reporting a result as positive based solely on an immunoassay.

We have recently encountered two examples that demonstrate the interference of thioridazine in identification of phencyclidine (PCP) and serve to reinforce the importance of confirmation testing. The first case involves a 34-year-old man with pulmonary emboli, being treated with thioridazine and verapamil, who had no history of any illicit drug use. Urine was unavailable. His blood was first screened with Emit II reagents (Behring Diagnostics, Palo Alto, CA) on a Hitachi 704 analyzer (Boehringer Mannheim, Indianapolis, IN) for amphetamines, opiates, benzoylcegonine, barbiturates, methadone, cannabinoids, propoxyphene, and PCP. The PCP was calibrated at a 12.5 μg/L cutoff concentration. Positive and negative controls and cutoff calibrator were prepared in blood. The Emit II test for PCP gave a positive result in the patient’s blood prepared by the procedure of Lewellen and McCurdy [2] (acetone-extracted and buffer-reconstituted) for blood. However, this protocol is not approved by the manufacturer, Behring.

The raw data was a 2H (+2 high) as compared with the 12.5 μg/L blood PCP calibrator concentration with a defined reading of 0. Any reading equal to or greater than 0 on the Hitachi 704 is considered positive because the instrument assigns an arbitrary value of 0 to the change in absorbance (ΔA) recorded for the calibrator. The negative calibrators had a ΔA reading of −33.

The specimen was further tested as part of the laboratory’s drug screening protocol by an alkaline liquid–liquid extraction followed by gas chromatography with a nitrogen-phosphorus detector (GC-NPD; Hewlett-Packard, Avondale, PA) to test for drugs that are not detected with the Emit II assay [3]. The specimen tested positive for thioridazine and mesoridazine (its primary metabolite), but PCP was not detected. The specimen was then reextracted and quantified for thioridazine and metabolite. The blood concentration of thioridazine was 480 μg/L and that of mesoridazine was 700 μg/L by GC-NPD. GC-mass spectrometry (MS) (Hewlett-Packard) confirmation/quantification of PCP in selected-ion mode (SIM) produced three ions—m/z 242, 186, and 200—consistent with PCP at the [D 2 ]PCP retention time. The ratios were flagged as out of range, but the quantitative ion (m/z 200) produced a 5 μg/L concentration. A thioridazine calibrator (1000 μg/L) was then extracted and tested by the same GC-MS protocol as PCP; the result was quantified as 6.3 μg/L PCP, with a similar ion ratio response as that for the patient. Thioridazine and PCP share some of the same ions when analyzed by GC-MS in SIM, including those used for quantification.

A second case involved the screening of a urine sample with a 25 μg/L PCP cutoff concentration, which was also positive by the Emit II assay on the Hitachi 704 analyzer. This was an screen as well as a thin-layer chromatographic analysis, and the Emit methodology was performed in exact compliance with the manufacturer’s recommendations. The urine sample was positive for PCP, giving a Hitachi reading of 12H (the 30 μg/L control read 22H, the 20 μg/L control read −20, and the negative control read −89), but GC-MS did not confirm the presence of PCP. Thioridazine at 6.3 mg/L and mesoridazine at 18.9 mg/L were confirmed by dual capillary column nitrogen phosphorus detection and quantified in the urine. The combination of thioridazine and mesoridazine was apparently sufficient to produce a positive PCP result.

Although thioridazine has been reported to interfere with immunoassays for PCP, that interference was with the Emit d.a.u. reagents and not the newer Emit II reagents [4]. According to the manufacturer’s package inserts for Emit II (#J004UL3E, May 1992, and #J004UL7, February 1995), thioridazine gives a negative response at 20 mg/L for cross-reactivity in the PCP assay. The cases presented here were considered as therapeutic administration of thioridazine, and the blood concentrations were not unusually excessive.

These two cases demonstrate that both thioridazine and mesoridazine may interfere with the Emit II phencyclidine assay. Evaluation of interferences when the manufacturer’s method is modified (e.g., use of blood specimens in urine tests) is clearly needed to ensure proper testing, and positive immunoassay results require confirmation in every case.

References
Optimization of Serum Protein Separation by Capillary Electrophoresis

To the Editor:
Identification of serum paraproteins is important for diagnosing myeloma and monoclonal gammopathy of undetermined significance (MGUS), which occurs in >3% of the population age 70 years and older. In 1995 we described a prospective comparison of 1000 specimens for protein electrophoresis using high-resolution agarose gel electrophoresis (HRAGE) compared with capillary electrophoresis (CE). We reported two specimens (of a total of 362 paraproteins) in which capillary electrophoresis did not adequately quantify the paraprotein. At the time we noted that the retention time for albumin was markedly increased, and we used that factor to identify these "problem" specimens. Removal of calcium lactate from the borate buffer enabled correct separation of the monoclonal protein.

Since that time a further 5500 specimens have been processed by CE, with identification of a further 6 paraproteins that did not separate correctly on CE.

Of the eight paraproteins that did not separate correctly under the conditions described [1], five were IgM (1.4% of total IgM paraproteins screened) and three were IgG (0.3% of the total IgG paraproteins). We noted on HRAGE that all these proteins migrated in the very slow gamma region.

Isoelectric focusing of the paraproteins revealed that pI of the IgG proteins was higher than 8.5 while that of the IgM paraproteins was between 6.9 and 8.3 (see example in Fig. 1). By modification of the boric acid buffer system from 50 mmol pH 9.7 to 75 mmol pH 10.3, we were able to quantify all of the monoclonal bands.

We believe that by the use of increased ionic strength and increased pH, the modified buffer will correctly separate and quantify all monoclonal paraproteins.

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