Cross-Reactivity of Fosphenytoin in Four Phenytoin Immunoassays

To the Editor:

Fosphenytoin, 5,5-diphenyl-3-[(phosphonooxy)methyl]-2,4-imidazolidine-dione disodium salt (Cerebyx®; Parke-Davis), is a phosphorylated form of the anticonvulsant drug phenytoin. Fosphenytoin itself has no pharmacological activity but is dephosphorylated in vivo by phosphorylases to the active drug, phenytoin. The elimination half-life of fosphenytoin in plasma is 8–15 min in healthy subjects [1, 2]. Fosphenytoin can be used for parenteral or intramuscular administration, where its superior aqueous solubility results in less severe side effects than does phenytoin [2, 3]. Few data, however, are available on the interference of fosphenytoin in phenytoin immunoassays, which are currently the most common method used to monitor patients’ phenytoin concentrations. In an abstract [4], Kugler et al. reported that the TDx® Phenytoin assay (Abbott Labs.) was interfered with by fosphenytoin. We report here the results of detailed cross-reactivity studies for fosphenytoin in four phenytoin immunoassays: ACS:180® Automated Chemiluminescent Immunoassay System (Chiron Diagnostics), TDx Phenytoin and Phenytoin II, and AxSym® Phenytoin II (also from Abbott Labs.).

A therapeutic total plasma phenytoin concentration (>10 mg/L) is attained within 10–30 min after administration of fosphenytoin. Conversion of fosphenytoin to phenytoin is reported to be complete in 2–4 h, depending on mode and rate of administration [5]. The plasma concentration of fosphenytoin depends on the route of administration and length of time between administration and patient sampling. The ordinary half-life of fosphenytoin is reduced by ~50% in patients with hepatic or renal diseases, apparently because of less protein binding of the prodrug [5].

A stock solution of fosphenytoin (a gift from Parke-Davis) in methanol was added to two separate serum pools—one without phenytoin (pool A), and the other containing 12.4 mg/L of phenytoin (pool B)—to give final fosphenytoin concentrations of 52, 39, 26, and 13 mg/L. Apparent phenytoin concentrations in both sets of samples were measured by all four assays according to the manufacturers’ directions.

The TDx and AxSym assays use homogeneous fluorescence polarization (FPIA) technology and were run on the TDxFlx® and AxSym automated analyzers, respectively. The TDx Phenytoin (TDx) assay uses a polyclonal sheep anti-phenytoin antiserum, whereas the TDx Phenytoin II (TDx-II) and AxSym Phenytoin II (AxSym-II) assays use murine monoclonal antisera. The ACS:180 Phenytoin (ACS:180) is a heterogeneous chemiluminescent immunoassay that uses murine monoclonal antibody; it was run on the automated, random-access ACS:180 chemiluminescent system [6–8]. The analytical range (0.5–40 mg/L) and detection limit (1 mg/L) for the TDx and ACS:180 assays are similar [8], but the AxSym-II assay has a lower detection limit (0.5 mg/L). Results of the ACS:180 assay agree well with both TDx and TDx-II assays [7, 8], whereas the AxSym-II assay correlates with the TDx-II assay (AxSym-II package insert).

The results for fosphenytoin cross-reactivity in pool A for the four phenytoin assays (Table 1) show that the TDx-II cross-reacted very strongly (>250%) with fosphenytoin; such cross-reactivity would result in seriously high TDx-II assay results, even in samples containing very low concentrations (<5 mg/L) of fosphenytoin. Fosphenytoin cross-reactivity in the absence of phenytoin for the other three assays exhibited the following order: ACS:180 > TDx > TDx-II > AxSym-II. However, although the ACS:180 cross-reactivity was independent of fosphenytoin concentration, that of TDx and AxSym-II decreased with increasing concentrations of fosphenytoin.

Table 1 also presents our data on the interference of fosphenytoin in the four assays in serum pool B, containing 12.4 mg/L phenytoin. The fosphenytoin cross-reactivity in TDx and AxSym-II assays was ~2- and 3-fold greater, respectively, in the presence of phenytoin than in its absence. Given that the rate of fosphenytoin metabolism may differ from person to person or in different disease states, the higher cross-reactivity in presence of phenytoin is of concern. Cross-reactivity in the ACS: 180 assay, however, was indepen-
dent of the presence of phenytoin. Similar data have also been found in interference studies of oxaprozin in the TDx-II assay, in which cross-reactivity to oxaprozin increased by 5% in the presence of 10 mg/L phenytoin [9].

In summary, different phenytoin immunoassays may cross-react differently with fosphenytoin, resulting in discordant results for samples containing fosphenytoin. Because of this problem, serum specimens taken from patients treated with fosphenytoin before all of the prodrug is metabolized (2–4 h after the drug administration is complete) may yield misleading phenytoin immunoassay results. Finally, whereas the ACS:180 assay shows consistent cross-reactivity to fosphenytoin in samples with or without phenytoin, the TDx assay cross-reactivity is dependent on both fosphenytoin and phenytoin concentrations. This underlines the importance of cross-reactivity determination in both the presence and the absence of the primary analyte.

References

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Table 1. Cross-reactivity of fosphenytoin in TDx Phenytoin, TDx Phenytoin II, AxSym Phenytoin II, and ACS:180 Phenytoin assays in serum pools A (no phenytoin) and B (containing phenytoin).

<table>
<thead>
<tr>
<th>Fosphenytoin concn., mg/L</th>
<th>TDx</th>
<th>TDx-II</th>
<th>Axsym-II</th>
<th>ACS:180</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0.0</td>
<td>ND</td>
<td>12.4</td>
<td>ND</td>
<td>12.5</td>
</tr>
<tr>
<td>13.0</td>
<td>5.5 (42)</td>
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<td>26.6 (71)</td>
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<tr>
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<td>30.1 (68)</td>
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<td>39.0</td>
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<td>35.4 (59)</td>
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</tr>
<tr>
<td>52.0</td>
<td>16.4 (32)</td>
<td>&gt;</td>
<td>&gt;</td>
<td>6.7 (17)</td>
</tr>
</tbody>
</table>

a Cross-reactivity (%) in absence of phenytoin (pool A) = 100 x (apparent phenytoin concentration observed)/(fosphenytoin concentration in sample).

\[ \text{Cross-reactivity (\%) in presence of phenytoin (pool B) = 100 x (apparent phenytoin concentration observed in presence of fosphenytoin)} - \text{ (phenytoin concentration in absence of fosphenytoin)/(fosphenytoin concentration in sample).} \]

ND, not detected; >, greater than the range of the assay (40 mg/L); NA, not available.