assay, leading to falsely high values FT$_3$ values. FT$_3$ values in the 2 patients with antibodies to T$_2$, T$_3$, or their conjugates were 12.3 and 32.6 pmol/L for the Vitros FT3II assay.

With the Vitros FT3 assay, FT$_3$ concentrations in 2-fold diluted control sera were 96% (1.1%) [mean (SD)] of those in the nondiluted sera, and in 2-fold diluted sera from 2 patients taking diclofenac, FT$_3$ concentrations were 95.9% and 96.3% of those in the nondiluted sera (Fig. 1). With the Vitros FT3II assay, FT$_3$ concentrations in 2-fold diluted control sera were 97% (0.7%) of those in the nondiluted sera. For 2-fold diluted sera from 2 patients taking diclofenac, FT$_3$ concentrations were 98% and 97% of those in the nondiluted sera, indicating that with the Vitros FT3 assay the dilution effects on FT$_3$ concentrations did not differ substantially in sera from patients taking diclofenac and control sera. This result suggests that the concentration of free diclofenac in the bloodstream does not change with dilution, because diclofenac is strongly bound to serum proteins and thus obeys mass action dilution criteria.

With the Vitros FT3II assay, the FT$_3$ concentrations in sera from 2 patients with antibodies to T$_2$, T$_3$, or their conjugates decreased 71% and 55% with dilution. This result suggests that, in the Vitros FT3II assay, antibodies to T$_2$ or its conjugate interfered with binding of the T$_2$-conjugate. It should be noted that the converse phenomenon may occur in the presence of large quantities of weaker binding drugs or lower, nonsaturating quantities of autoantibodies.

In conclusion, because the serum concentration of free drug (diclofenac) in serum is not altered by dilution, according to the mass action dilution criteria, the measured FT$_3$ concentration in the serum remains falsely high. When the interfering substance (autoantibody) leading to falsely high FT$_3$ concentrations is diluted, the interference diminishes and measured serum FT$_3$ concentration decreases. This is the first report to show that dilution tests can differentiate between drugs and interfering substances in serum.

This study was supported in part by a Grant-in-Aid for Encouragement of Scientists (18925003) from the Japan Society for the Promotion of Science (JSPS).

References

Kunihiro Iwahara
Chizuko Tanabe
Masato Maekawa*

Department of Laboratory Medicine
Hamamatsu University School of Medicine
Hamamatsu, Japan

*Address correspondence to this author at: Department of Laboratory Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan. Fax 81-53-435-2794; e-mail mmaekawa@hama-med.ac.jp.

DOI: 10.1373/clinchem.2006.072272

Dipyrone (Metamizole) Metabolites Interfere with HPLC Analysis of Plasma Catecholamines but Not with the Determination of Urinary Catecholamines

To the Editor:
Catecholamines and their metabolites are measured for the diagnosis of pheochromocytoma (1, 2) and neuroblastoma (3) and for various other reasons (4–6). The widely used HPLC assay, which uses electrochemical detection (ECD), combines high selectivity and sensitivity (7). The internal standard, 3,4-dihydroxybenzylamine (DHBA), is added before analysis. After pretreatment with a sample clean-up column, catecholamines are separated and quantified by HPLC-ECD. Commercial methods (Chromsystems) elute nor-epinephrine, epinephrine, DHBA, and dopamine at 8, 9, 12–13, and 17–19 min, respectively. The next sample is injected after 25 min.

We sometimes observed an unknown peak in the plasma catecholamine chromatogram that interfered with the internal standard DHBA (Fig. 1A). When we repeated the analysis, the spurious extra peak disappeared (Fig. 1B). When we evaluated the medical histories of these patients, we found that for a preceding analysis they had received dipyrone orally or intravenously within 12 h before blood collection. Therefore, we hypothesized that administration of dipyrone and/or its metabolites may have led to the interference with DHBA in the subsequent analysis.

Dipyrone (metamizole) is widely used and has effective analgesic, antipyretic, and antispasmodic properties. After oral or intravenous administration, dipyrone is rapidly hydrolyzed to the active moiety 4-methylaminoantipyrine (MAA) (8, 9). MAA is further metabolized to 4-formylaminoantipyrine (FAA) and 4-aminoantipyrine (AA), which is acetylated to 4-acetylaminoantipyrine (AAA). These 4 major metabolites account for ~60% of the administered dose excreted in urine.

To determine whether dipyrone and/or its metabolites interfere with the measurement of plasma catecholamines, we prepared solutions of dipyrone (1 mg/mL) and MAA, AA, FAA, and AAA (50 μg/mL) corresponding to 5 times the expected maximum concentration obtained after a 1-g dose of dipyrone (9). Dipyrone caused a peak at the expected retention time of dopamine (Fig. 1C) and another broad peak in the next chromatogram at the retention time of DHBA when an aqueous injection was started after 25 min (not shown). The bioactive metabolite MAA also had a retention time of 13 min in the next analysis, and thus it interfered with the internal stan-
Fig. 1. HPLC chromatograms of plasma catecholamines.

(A) Chromatographic separation of plasma catecholamines from patients yielded chromatograms with an additional peak (arrow) interfering with the internal standard DHBA. (B) When the same sample was rerun, the interfering peak was no longer present. (C) Chromatographic separation of 1 mg/mL dipyrone, i.e., 5 times the expected maximum after a 1-g dose of dipyrone yielding an extra peak at 18 min (arrow). (D) Chromatographic separation of an aqueous sample blank (without adding the internal standard) analyzed after a sample containing 50 μg/mL MAA (i.e., 5 times the expected maximum after a 1-g dose of dipyrone) yielding an extra peak at 13 min (arrow) that may interfere with the internal standard. Plasma catecholamines were analyzed according to the manufacturer's instructions. In brief, a reversed-phase C18 column was used for isocratic separation. Assay conditions included a 0.8 mL/min flow rate; the buffer contained salts, methanol, and an ion pair reagent. Quantification was performed with the electrochemical detector L 3500 A (Recipe) at a potential of +500 mV.
dard (Fig. 1D). These findings indicate that the interfering peak is MAA, which is readily formed from the chemically labile drug dipyrone (9). AA eluted after 23 min, and no signal was observed for FAA or AAA.

To evaluate whether this interference is relevant in vivo, we studied 6 patients who routinely received 1 g of dipyrone intravenously for analgesia after surgery. Informed written consent was obtained from all participants, and the local ethics committee approved the protocol. Blood samples were obtained before drug administration and 1, 2, 4, 6, 8, 10, and 12 h after dipyrone administration. Urine was collected during the periods 0–4, 4–8, 8–12, and 12–24 h after drug administration. HPLC chromatograms of plasma catecholamines showed an extra peak after 13 min in the subsequent analysis—consistent with the elution time of the metabolite MAA—up to 7 h after dipyrone administration. No interference was detected before or 12 h after administration of dipyrone, in accordance with the plasma half-life of ∼3 h for MAA and ∼5 h for AA (8). Furthermore, we observed no interference of dipyrone with dopamine at any time point.

Urinary catecholamine measurements were not affected by dipyrone administration. To investigate this unexpected finding, we treated MAA and AA solutions according to the protocol for urine samples, including column pretreatment. HPLC-ECD analysis showed no signal in the chromatogram, but a marked disturbance was seen when the pretreatment was omitted, indicating that pretreatment eliminates the interference caused by MAA and AA. This unexpected observation may be attributable to the different binding methods: for plasma samples the relative nonspecific adsorption of catecholamines on Al₂O₃ is used, whereas urinary catecholamines are bound to immobilized phenylboronic acid by formation of cyclic esters. This specific interaction is possible only for cis-diol compounds and cannot occur with dipyrone or its metabolites. A previous study of pretreatment procedures revealed that the Al₂O₃ method is superior for plasma samples because they are less diluted (specificity was not examined) (10). Interference with HPLC-ECD-based catecholamine analysis by paracetamol (11) and labetalol (12), but not by dipyrone, has been reported.

In conclusion, we found that (a) the metabolite MAA may interfere with the next analysis if the runtime is shorter than 40 min; (b) MAA and AA do not interfere with urinary catecholamine analysis if phenylboronic acid pretreatment is used; and (c) dipyrone may interfere with plasma dopamine determination if dipyrone is administered intravenously.

The authors thank Sanofi-Aventis for providing the dipyrone metabolites, Ms. Steffi Hasanovic for excellent technical assistance, and Drs. R. Lehmann (Clinical Chemistry Unit, University of Tuebingen, Germany) and K. Gempel (Institute for Clinical Chemistry, Schwabing-City Hospital, Munich, Germany) for critical discussion. Meanwhile, Chromsystems has referred the described disturbance for plasma catecholamine analysis.

References


Karsten Müßig†
Albrecht Pfäfflin†
Hans-Ulrich Häring
Erwin D. Schleicher

Department of Internal Medicine IV
Clinical Chemistry Unit
University Hospital
University of Tuebingen
Tübingen, Germany

†The authors contributed equally to this work.
* Address correspondence to this author at: Department of Internal Medicine IV, Clinical Chemistry Unit, University Hospital, University of Tuebingen, Hoppe-Seyler-Str. 3, D-72076-Tuebingen, Germany. Fax: 49-07071-29-4696; e-mail Erwin.Schleicher@med.uni-tuebingen.de.

DOI: 10.1373/clinchem.2006.071662