Discordant Carbamazepine Values Between Two Immunoassays: Carbamazepine Values Determined by ADVIA Centaur Correlate Better With Those Determined by LC-MS/MS Than PETINIA Assay

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Discordant carbamazepine values as determined by two different immunoassays may be due to different cross-reactivities with the active metabolite carbamazepine 10, 11-epoxide and may cause confusion in interpreting carbamazepine serum levels. In this study, we compared carbamazepine values in samples containing carbamazepine and the epoxide metabolite, as determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and by two commercial carbamazepine immunoassays: the PETINIA and the ADVIA Centaur carbamazepine. Clinical specimens were used for the comparative studies wherein we determined carbamazepine concentrations using the PETINIA, ADVIA Centaur, and LC-MS/MS assays. We observed an excellent correlation

between carbamazepine concentrations determined by the ADVIA Centaur and LC-MS/MS methods while carbamazepine values were overestimated using the PETINIA assay. When aliquots of drug-free serum were supplemented with clinically relevant concentrations of the carbamazepine epoxide metabolite, we observed negligible cross-reactivity of epoxide with the ADVIA Centaur assay but over 90% cross-reactivity with the PETINIA assay. We conclude that the ADVIA centaur assay accurately measures carbamazepine concentrations in plasma or serum and that the PETINIA assay significantly overestimates true carbamazepine concentration. Such discordance may cause confusion in interpreting serum carbamazepine levels. *J. Clin. Lab. Anal.* 25:212–216, 2011. © 2011 Wiley-Liss, Inc.

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Carbamazepine is an antiepileptic drug that was approved in the United States in the 1970's for the management of seizure disorders in adults and in children over 6 years of age and remains widely used today (1). Carbamazepine is structurally similar to the tricyclic antidepressant imipramine and is also indicated for the treatment of trigeminal or glossopharyngeal neuralgia. Investigational indications include resistant schizophrenia, bipolar disorder, ethanol withdrawal, aggression, and post-traumatic stress disorder (2–5). Similar to many antiepileptic drugs, therapeutic drug monitoring (TDM) of carbamazepine is routinely used to optimize dosing, with a recommended therapeutic range of 4–12 µg/mL. The carbamazepine 10,11-epoxide, commonly known as

the “epoxide” metabolite, is equipotent to carbamazepine and contributes significantly to therapeutic benefits of carbamazepine therapy, but the epoxide concentration is not usually measured or considered in routine TDM.

Carbamazepine TDM is performed commonly using commercially available immunoassays that demonstrate a wide range of cross-reactivities with the epoxide

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metabolite. The Vitros carbamazepine reagent system (Ortho Clinical Diagnostics, Rochester, MN) is reported to have 0% cross-reactivity with the epoxide metabolite, whereas cross-reactivity of epoxide varies significantly between various carbamazepine immunoassays (6,7). For most patients, the concentration of the epoxide metabolite at steady state is approximately 15–20% of the total carbamazepine concentration. Although the concentration of epoxide metabolite is typically less than 2 µg/mL, concentrations of 6–10 µg/mL are not uncommon. These higher concentrations are likely to be observed with renal impairment or failure, and in patients who have received excessive dosing of carbamazepine, either intentionally or accidentally, such as a consequence of co-administered interacting drugs such as valproic acid (8,9). In these patients, there would be significant discrepancy between carbamazepine values determined by a carbamazepine immunoassay with zero or low cross-reactivity with epoxide metabolite and another carbamazepine immunoassay with high cross-reactivity with the epoxide metabolite. Therefore, if a patient is transferred from one healthcare facility to another where two different methods are used for TDM, discrepancy between two carbamazepine values may cause serious confusion in interpreting the carbamazepine value. Although package inserts may provide cross-reactivity data for the epoxide, such data are generally based on a single concentration and is not evaluated in the context of authentic patient specimens. As such, we investigated in detail, two carbamazepine assays (PETINIA and ADVIA Centaur) with widely different reported cross-reactivity with the epoxide, for the concentration relationships associated with detecting both carbamazepine and the active epoxide metabolite. Results were compared with values obtained by a liquid chromatography/tandem mass spectrometric (LC-MS/MS) method, the gold standard. In this study, we report our findings.

MATERIALS AND METHODS

Carbamazepine and carbamazepine 10, 11-epoxide was obtained from the Sigma Chemical Company (St. Louis, MO). Stock solutions (1 mg/mL) were prepared in HPLC grade methanol. Residual patient plasma or serum samples were obtained and deidentified according to protocols approved by the University of Utah Institutional Review Board.

Two different immunoassay reagent systems designed to quantify carbamazepine were included: PETINIA and ADVIA Centaur. Both the PETINIA assay, performed using a Dimension Vista 1500 analyzer, and the ADVIA Centaur assay performed using a ADVIA Centaur instrument were obtained from Siemens

Healthcare Diagnostics (Deerfield, IL). The PETINIA assay is a homogenous-particle-enhanced turbidimetric inhibition immunoassay that is based on monoclonal antibody. The reported analytical measurement range (AMR) is 0.5–20 g/mL and the cross-reactivity with the epoxide metabolite exceeds 90% according to the package insert. The ADVIA Centaur carbamazepine assay is a competitive immunoassay using direct chemiluminescent technology. This assay also employs a monoclonal antibody, and the reported AMR is 0.25–18 µg/mL. The reported cross-reactivity with the epoxide metabolite is 7.3% in the ADVIA Centaur assay.

To evaluate the cross-reactivity and approximate limits of quantification for the carbamazepine epoxide metabolite with the immunoassays, aliquots of a drug-free plasma pool were supplemented with various amounts of the epoxide metabolite to achieve final epoxide concentrations in the plasma ranging from 0.0 to 25.0 µg/mL. In order to ensure that methanol originating from the stock solution does not interfere with the carbamazepine immunoassays, we added microliter quantity of the stock solution in a dry test tube and methanol was evaporated under nitrogen at the room temperature. Then the dry residue was reconstituted with an aliquot of the drug-free serum and apparent carbamazepine concentration was measured using both immunoassays. Each measurement was performed in triplicate.

In addition, concentrations of carbamazepine and the epoxide metabolite were determined for 50 residual patient specimens using the two immunoassays, and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method. The LC-MS/MS method was designed to detect and quantify five anticonvulsant drugs and metabolites with one internal standard. The method was performed using a Waters Acquity UPLC/TQD system, Masslynx software, and a Waters HSS T3 1.8 µm, 2.1 × 50 mm column (Waters, Milford, MA). Injection volume was 5 µL and chromatography was performed at room temperature with mobile phases of 75% water and 25% acetonitrile, both with 0.1% formic acid, at a flow rate of 0.65 mL/min. At 1.4 min, the mobile phase proportions were reversed for 1 min. An ESI (electrospray) interface, a multiple reaction monitoring, and a positive ion mode were employed. The resolution of both quadrupoles was maintained at unit mass resolution with a peak width at a half height of 0.7 amu. Two transitions for each analyte, and the single internal standard were included in the method: carbamazepine (retention time: 0.92 min, 237.16 > 194.1 and 179.0); carbamazepine 10,11-epoxide (0.70 min, 253.11 > 236.1 and 210.1); oxcarbazepine (0.76 min, 253.12 > 236.1 and 208.1) and its mono-hydroxy

metabolite (0.58 min, 237.1>194.0 and 179.0); lamotrigine (0.42 min, 256.08>211.1 and 165.7); and internal standard (UCB17025, 0.31 min, 185.06>140.0 and 69.0). Data were processed using the Water Quanlynx software. The LC-MS/MS method was considered as the reference method for accurate determination of both carbamazepine and the epoxide metabolite in a specimen. The AMR of the LC-MS/MS method was 0.5–30 µg/mL. The LC-MS/MS method was considered as the reference method for accurate determination of both carbamazepine and the epoxide metabolite.

RESULTS

The cross-reactivity of epoxide with the PETINIA carbamazepine assay varied from 93 to 101% with an average cross-reactivity of 95.7% based on nine specimens tested containing various amounts of epoxide. In contrast, the cross-reactivity of epoxide with the ADVIA Centaur carbamazepine assay varied from only 5–7.6% with an average cross-reactivity of only 6.3% (Table 1).

We observed an excellent correlation between the carbamazepine concentrations measured by the ADVIA Centaur assay and the LC-MS/MS assay. Using the *x*-axis as the carbamazepine values obtained by the reference LC-MS/MS method and the *y*-axis as the carbamazepine values obtained by using the ADVIA Centaur carbamazepine assay, we observed the following regression equation:

$y = 1.01x + 0.26 \quad (r = 0.97, n = 50)$

However, with the PETINIA assay, we observed significant overestimation of carbamazepine value compared with the values obtained by the LC-MS/MS method. Using the *x*-axis as the carbamazepine values obtained by the reference LC-MS/MS method and the

y-axis as the carbamazepine values obtained by using the PETINIA carbamazepine assay, we observed the following regression equation:

$y = 1.12x + 0.30 \quad (r = 0.89, n = 50)$

When we compared a combined carbamazepine and active metabolite epoxide concentration as obtained by the LC-MS/MS with carbamazepine values obtained by the PETINIA, we observed an excellent correlation. Using the *x*-axis as the carbamazepine plus the epoxide values obtained by the reference LC-MS/MS method and the *y*-axis as the carbamazepine values obtained by using the PETINIA carbamazepine assay, we observed the following regression equation:

$y = 0.93x + 0.26 \quad (r = 0.95, n = 50)$

However, using the *x*-axis as the carbamazepine plus the epoxide values obtained by the reference LC-MS/MS method and the *y*-axis as the carbamazepine values obtained by using the ADVIA Centaur carbamazepine assay, we observed the following regression equation indicating poor correlation:

$y = 0.79x + 0.75 \quad (r = 0.86, n = 50)$

On the basis of these results, it is expected that there would be a discordant between carbamazepine values obtained by the PETINIA assay and the ADVIA Centaur assay in patients with elevated epoxide concentrations. In Table 2, we listed such patients along with their epoxide concentrations as determined by the LC-MS/MS method. In general when epoxide concentration is less than 15% of total carbamazepine concentration, there is a good agreement between carbamazepine concentrations determined by two different assays but in

TABLE 1. Cross-Reactivity of Carbamazepine 10, 11-Epoxide With PETINIA and ADVIA Centaur Carbamazepine Immunoassays

Epoxide (µg/mL)	Apparent carbamazepine (µg/mL), mean of triplicate determinations per assay, and percent cross-reactivity			
	PETINIA		ADVIA Centaur	
0.0	ND	—	ND	—
0.5	0.5	100%	ND	—
1.0	1.0	100%	ND	—
1.5	1.4	93%	ND	—
3.0	2.7	90%	ND	—
5.0	4.5	90%	ND	—
7.5	7.3	97%	0.5	6.6%
10.0	10.1	101%	0.6	6.0%
20.0	19.2	96%	1.0	5.0%
25.0	23.4	94%	1.9	7.6%

ND, none detected.

TABLE 2. Discordant Carbamazepine Values Between PETINIA and Centaur Carbamazepine Assay in Patients With Accumulation of Epoxide

Patient ID	Carbamazepine (µg/mL)			Epoxide (µg/mL)	
	LC/MS	ADVIA Centaur	PETINIA	% Change*	LC/MS
#5	7.3	7.9	10.0	27	4.8
#13	10.9	11.2	14.9	33	4.8
#14	7.3	8.1	9.8	21	3.0
#16	9.2	8.9	11.3	27	2.8
#23	6.4	6.0	12.9	115	8.1
#28	8.0	7.8	13.9	78	7.2
#29	12.4	12.3	15.9	29	5.0
#31	6.3	5.9	9.3	57	3.2
#35	8.9	9.0	11.6	29	4.6
#36	2.2	2.4	3.6	50	1.1

% Change represents percent increases in carbamazepine value in PETINIA assay compared with carbamazepine value obtained by the Centaur ADVIA carbamazepine assay. Assuming 20% or more difference as the definition of discordance, all patient specimens listed here showed discordant carbamazepine values between two assays studied.

patients where epoxide level is 20% or more of the carbamazepine concentration, we observed clinically significant discordance between carbamazepine values with the values determined by the PETINIA assay, which is always significantly greater than the value observed by the ADVIA Centaur carbamazepine assay. Assuming 20% of more difference in values between two assays as the definition of discordant specimens, we observed between 21 and 115% increase in carbamazepine values obtained by the PETINIA assay compared with ADVIA Centaur carbamazepine assay (Table 2).

DISCUSSION

In our report, we demonstrate that discordance in carbamazepine values determined by the ADVIA Centaur assay and PETINIA assay is due to the accumulation of epoxide metabolites in certain patients by determining true carbamazepine and epoxide concentration using a reference LC/MS/MS method, which is the novel aspect of this study. The overestimation of carbamazepine concentrations using the PETINIA assay was due to high cross-reactivity of the epoxide metabolite with this assay. The ADVIA Centaur carbamazepine assay correlated better with carbamazepine values obtained by using LC-MS/MS due to its poor cross-reactivity with the epoxide metabolite. It is recognized that patients with renal insufficiency who are treated with carbamazepine may exhibit disproportionately high concentrations of carbamazepine 10,11-epoxide and an increased risk of dose-related carbamazepine toxicity. Potter and Donnelly suggested that routine monitoring of the epoxide metabolite could be clinically useful (8). In addition to renal patients, elderly patients have demonstrated changes in pharmacokinetics of carbamazepine (10), and patients with genetic polymorphisms in microsomal epoxide hydroxylase, the enzyme primarily attributed with the production of the epoxide metabolite, which may exhibit an abnormal metabolic phenotype and may require epoxide determinations to optimize the dose (11). Moreover, drug-drug interactions may place a patient at risk for inappropriate epoxide concentrations requiring therapeutic monitoring of the metabolite. For example, lamotrigine may contribute to toxicity of carbamazepine through increasing the epoxide concentration by as much as 45% (12). Co-administration of quetiapine also increases the concentration of epoxide metabolite possibly by inhibiting the epoxide hydroxylase enzyme (13). Finally, valproic acid increases the incidence of carbamazepine-related toxicity due to accumulation of epoxide metabolite in the plasma, even when total plasma carbamazepine concentrations fall within the therapeutic range (7).

An immunoassay with low cross-reactivity with the epoxide metabolite such as the ADVIA Centaur carbamazepine assay shows an excellent correlation with carbamazepine concentrations measured by LC-MS/MS, and is useful for TDM of most patients. Because epoxide is an active metabolite, there may be value in determining epoxide level in addition to carbamazepine in patients where accumulation of epoxide metabolite is likely. Tutor-Crespo et al. commented that in patients with moderate to severe renal insufficiency where accumulation of epoxide metabolite is likely, carbamazepine concentrations obtained by an immunoassay with low cross-reactivity with epoxide may have an inadequate diagnostic efficiency (9). Currently, there is no immunoassay for determining epoxide concentration and only chromatographic methods are available. The PETINIA carbamazepine assay is capable of measuring a combined carbamazepine and epoxide level but in the package insert of PETINIA only carbamazepine therapeutic range is mentioned which could be misleading. To our knowledge, no therapeutic range of carbamazepine plus epoxide concentration has been established but if such range can be established, PETINIA assay may be very useful in TDM of carbamazepine in patients with elevated epoxide levels. However, without such information, PETINIA assay can provide an estimation of carbamazepine plus epoxide concentration and it will be preferable to send the specimen to a reference laboratory for determining both carbamazepine and epoxide concentration by using a reference chromatographic method for comparison with the value obtained by the PETINIA assay. For death investigation involving carbamazepine overdose, chromatographic methods are preferable (14).

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