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Turbidimetric Carbamazepine Immunoassay on the ADVIA® 1650 and 2400 Analyzers is Free From Interference of Antihistamine Drugs Hydroxyzine and Cetirizine

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A recent report indicates that hydroxyzine and its active metabolite cetirizine interfere with the particle-enhanced turbidimetric inhibition immunoassay (PENTINA) carbamazepine assay. We studied potential interference of hydroxyzine and cetirizine with the turbidimetric carbamazepine immunoassay on ADVIA® 1650 and ADVIA 2400 (Bayer Diagnostics, Tarrytown, NY) analyzers. Aliquots of drug-free serum pools were supplemented with various concentrations of hydroxyzine and cetirizine representing therapeutic, moderate toxic, as well as very toxic concentrations. These samples were assayed by the turbidimetric carbamazepine immunoassay on two analyzers. To study the interference in presence of the analyte, aliquots of a serum pool prepared from patients receiving carbamazepine were further supplemented with various amounts of hydroxyzine and or cetirizine and apparent carbamazepine concentrations were measured again in order to compare with the value of original pool. No apparent carbamazepine concentration was observed when aliquots of drug-free serum were supplemented with hydroxyzine or cetirizine. Moreover, in the carbamazepine pool, the original carbamazepine concentration compared well when aliquots of this serum pool were further supplemented with hydroxyzine or cetirizine. We conclude that the turbidimetric carbamazepine immunoassay is free from interference of hydroxyzine and cetirizine. J. Clin. Lab. Anal. 21:188–192, 2007.

Key words: hydroxyzine; cetirizine; carbamazepine; turbidimetric

INTRODUCTION

Hydroxyzine is a commonly prescribed first-generation antihistamine with sedative properties and has a benzhydrylpiperazine structure. Hydroxyzine is also one of the drugs used in the first-line therapy for the treatment of allergic rhinitis and chronic idiopathic urticaria (1,2). This drug is also used in the treatment of generalized anxiety disorder (3). Another article indicates that hydroxyzine has a potential as an adjuvant in the treatment of multiple sclerosis (4). Hydroxyzine is metabolized to cetirizine, which has antihistamine property but is devoid of sedative effect. Cetirizine is also used as a second generation H_1-antagonist and has a lesser tendency to cross the blood-brain barrier compared to hydroxyzine and therefore does not cause drowsiness like the first-line drug hydroxyzine.

Carbamazepine is an iminostilbene derivative used in the treatment of epilepsy as well as various psychiatric disorders. The current uses of carbamazepine include treatment of partial seizure with complex symptoms, generalized tonic-clinic seizure, and mixed seizure. Carbamazepine, along with phenytoin, is considered as the drug of choice for treating these seizure disorders (5). Carbamazepine, like lithium, may help some individuals with episodic behavioral lack of control and aggression even in the absence of epileptic, affective,

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or organic features (6). Carbamazepine has a narrow therapeutic index and therapeutic drug monitoring is essential for efficacy of therapy as well as for avoiding adverse drug reactions. Even in developing countries where a limited number of drugs are targeted for therapeutic drug monitoring, carbamazepine is one of those drugs (7).

Recently Parant et al. (8) reported that hydroxyzine interferes with particle-enhanced turbidimetric inhibition immunoassay (PENTINA) of carbamazepine marketed by Dade-Behring (Deerfield, IL) for application on the Dimension® analyzer and concluded that such interference could significantly affect proper interpretation of serum carbamazepine concentrations for therapeutic drug monitoring of carbamazepine. The turbidimetric carbamazepine immunoassay on the ADVIA 1650 and ADVIA 2400 analyzer (Bayer Diagnostics, Tarrytown, NY) is a relatively new carbamazepine immunoassay but is widely used in clinical laboratories for therapeutic drug monitoring of carbamazepine. However, potential interference of hydroxyzine or cetirizine on this carbamazepine immunoassay has never been studied before. We studied the potential interference of both hydroxyzine and cetirizine on the turbidimetric assay of carbamazepine on both ADVIA 1650 and ADVIA 2400 analyzers. Here we report our findings.

MATERIALS AND METHODS

Hydroxyzine hydrochloride was purchased from Sigma Chemical Company (St. Louis, MO) and cetirizine was extracted from Zyrtec tablets as described by Parant et al. (8). The turbidimetric carbamazepine assay was run on the ADVIA 1650 and ADVIA 2400 analyzers according to the manufacturer's instructions.

Aliquots of drug-free serum were supplemented with hydroxyzine or cetirizine to achieve final concentrations of 500 ng/mL, 1 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL, 40 μg/mL, and 100 μg/mL, then apparent carbamazepine concentrations were measured using carbamazepine immunoassay on both the ADVIA 1650 and ADVIA 2400 platform. The various final concentrations of hydroxyzine or cetirizine selected for these study represents subtherapeutic, therapeutic, moderate toxic, very toxic, and potentially fatal concentrations of this drug based on the concentration range studied by Parant et al. (8) as well as other published studies.

Because cross-reactivity of an interfering substance should be studied in the presence of the primary analyte (9), in another experiment a serum pool was prepared from patients receiving carbamazepine. These specimens are routinely submitted to our laboratory for therapeutic drug monitoring of carbamazepine and specimens are discarded after 1 week after performing all tests ordered by the clinicians and reporting all results. We used leftover specimens for this study after 1 week of storage at 4°C. These specimens would otherwise be discarded. Aliquots of this serum pool were further supplemented with various amounts of hydroxyzine, cetirizine (concentration range: 500 ng/mL to 100 μg/mL) as well as both hydroxyzine and cetirizine. Then carbamazepine concentrations were measured again for comparison with the original carbamazepine concentration of the pool.

Statistical analysis was performed using the independent *t*-test. We considered a difference significant using a two-tailed test only at a 95% confidence level or higher ($P < 0.05$).

RESULTS

When aliquots of drug-free serum pool were supplemented with various concentrations of hydroxyzine or cetirizine as well as a combination of both hydroxyzine and cetirizine, we observed no apparent carbamazepine concentration even in the presence of reported life-threatening toxic concentrations of both drugs (100 μg/mL hydroxyzine or cetirizine). Our results indicate that, unlike the PENTINA carbamazepine assay, the turbidimetric carbamazepine assay is completely free from interference of both hydroxyzine and cetirizine (Table 1).

Because cross-reactivity should be tested in the presence of the primary analyte (9), we prepared a serum pool from patients receiving carbamazepine and then aliquots of that pool were further supplemented with hydroxyzine, cetirizine, or a combination of hydroxyzine and cetirizine. Carbamazepine concentrations were measured using the turbidimetric carbamazepine assay. The concentrations selected for supplementation are based on expected in vivo concentration of hydroxyzine or cetirizine after therapeutic use as well in life-threatening overdose. Because hydroxyzine is metabolized to cetirizine, we also supplemented several aliquots of carbamazepine pool with both hydroxyzine and cetirizine, mimicking in vivo concentrations in both therapeutic use and accidental overdose with hydroxyzine. We observed no statistically significant change in observed carbamazepine concentration in the presence of hydroxyzine and cetirizine in all concentrations studied. For example, when an aliquot of carbamazepine pool was supplemented with 50 μg/mL of hydroxyzine and 50 μg/mL of cetirizine, the observed carbamazepine concentration of 10.32 μg/mL (ADVIA 1650 analyzer) or 10.58 μg/mL (ADVIA 2400) did not differ significantly from the carbamazepine concentration (10.54 μg/mL by the ADVIA 1650 and

TABLE 1. Effect of supplementing hydroxyzine and cetirizine in aliquots of drug-free serum pool and measuring carbamazepine using the turbidimetric assay

	Carbamazepine, μg/mL, mean (SD), n = 3	
Specimen	ADVIA 1650	ADVIA 2400
Drug free serum pool	None detected	None detected
+500 ng/mL hydroxyzine	None detected	None detected
+1 μg/mL hydroxyzine	None detected	None detected
+5 μg/mL hydroxyzine	None detected	None detected
+10 μg/mL hydroxyzine	None detected	None detected
+20 μg/mL of hydroxyzine	None detected	None detected
+40 μg/mL of hydroxyzine	None detected	None detected
+100 μg/mL of hydroxyzine	None detected	None detected
+500 ng/mL cetirizine	None detected	None detected
+1μg/mL cetirizine	None detected	None detected
+5μg/mL cetirizine	None detected	None detected
+10 μg/mL cetirizine	None detected	None detected
+20 μg/mL of cetirizine	None detected	None detected
+40 μg/mL of cetirizine	None detected	None detected
+100 μg/mL of cetirizine	None detected	None detected
+500 ng/mL hydroxyzine+500 ng/mL cetirizine	None detected	None detected
+5 μg/mL hydroxyzine+5 μg/mL cetirizine	None detected	None detected
+10 μg/mL hydroxyzine+10 μg/mL cetirizine	None detected	None detected
+20 μg/mL hydroxyzine+20 μg/mL cetirizine	None detected	None detected
+50 μg/mL hydroxyzine+50 μg/mL cetirizine	None detected	None detected

10.82 μg/mL by the ADVIA 2400) of the original pool (Table 1). Similarly, when another aliquot of this carbamazepine pool was supplemented with 100 μg/mL of hydroxyzine, the observed carbamazepine concentration of 10.69 μg/mL (ADVIA 1650) or 10.85 μg/mL (ADVIA 2400) did not differ significantly from the carbamazepine concentration of the original pool (Table 2). Because no statistically significant difference was observed even in the presence of such high toxic concentrations of hydroxyzine and cetirizine, our in vitro results indicate that, unlike the PENTINA carbamazepine assay, the turbidimetric carbamazepine assay on both the ADVIA 1650 and ADVIA 2400 platform is free from interference of hydroxyzine and cetirizine.

DISCUSSION

Expected in vivo concentration of hydroxyzine after a standard dose is less than 100 ng/mL and toxicity is common at serum hydroxyzine level of 1 μg/mL. Simons et al. (10) reported that the mean serum peak hydroxyzine level was 116.5 ng/mL in healthy volunteers when they ingested a single dose of 0.7 mg/kg of bodyweight (mean dose 43.9 mg). Johnson (11) reported a case in which a 43-year-old woman who committed suicide with hydroxyzine had a blood level of 39 μg/mL. Magera et al. (12) published a case of hydroxyzine toxicity following accidental ingestion in a 13-month-old female infant in which a plasma hydroxyzine concentration reached 102.7 μg/mL after 8.5 hr. The baby recovered in 72 hr. Cetirizine is the main metabolite of hydroxyzine and its concentrations tend to exceed the hydroxyzine concentration. Cetirizine overdose has also been reported in a 18-month-old boy whose serum cetirizine concentration was 2.4 μg/mL 14 hr after overdose. The boy survived the episode (13).

Parant et al. (8) reported two cases in which hydroxyzine in serum caused false-positive carbamazepine levels using the PENTINA assay. A 22-year-old female with a hydroxyzine concentration of 1.77 μg/mL and cetirizine concentration of 2.1 μg/mL showed an apparent carbamazepine level of 5.3 μg/mL. Another patient with a hydroxyzine level of 520 ng/mL and cetirizine level of 2.18 μg/mL demonstrated a carbamazepine level of 25.4 μg/mL. However, EMIT 2000 assay showed no cross-reactivity (8). Our results indicate that the turbidimetric assay for carbamazepine is completely free from interference of both hydroxyzine and cetirizine as evidenced by observing no apparent carbamazepine concentration when aliquots of drug-free serum pools were supplemented with 100 μg/mL of hydroxyzine or cetirizine. Moreover, in the presence of high concentrations of both hydroxyzine and cetirizine, the observed carbamazepine values in aliquots of the carbamazepine pool further supplemented with hydroxyzine and

TABLE 2. Effect of supplementing hydroxyzine and cetirizine in aliquots of carbamazepine serum pool and measuring carbamazepine using the turbidimetric assay

	Carbamazepine, μg/mL, mean (SD), n = 3	
Specimen	ADVIA 1650	ADVIA 2400
Carbamazepine serum pool	10.54 (0.26)	10.82 (0.34)
+500 ng/mL hydroxyzine	10.39 (0.21)	10.58 (0.12)
+1 μg/mL hydroxyzine	10.35 (0.32)	10.62 (0.41)
+5 μg/mL hydroxyzine	10.06 (0.51)	10.73 (0.39)
+10 μg/mL hydroxyzine	10.59 (0.15)	10.65 (0.25)
+20 μg/mL of hydrozyzine	10.86 (0.14)	10.33 (0.22)
+40 μg/mL of hydroxzyzine	10.14 (0.22)	10.71 (0.55)
+100 μg/mL of hydrozyzine	10.48 (0.28)	10.36 (0.15)
+500 ng/mL cetirizine	10.03 (0.54)	10.66 (0.36)
+1μg/mL cetirizine	10.90 (0.41)	10.48 (0.52)
+5μg/mL cetirizine	10.33 (0.20)	10.63 (0.44)
+10 μg/mL cetirizine	10.65 (0.24)	10.53 (0.16)
+20 μg/mL of cetirizine	10.38 (0.11)	10.63 (0.37)
+40 μg/mL of cetirizine	10.33 (0.20)	10.89 (0.50)
+100 μg/mL of cetirizine	10.69 (0.08)	10.85 (0.42)
+500 ng/mL hydroxyzine+500 ng/mL cetirizine	10.01 (0.43)	10.67 (0.23)
+5 μg/mL hydroxyzine+5 μg/mL cetirizine	10.48 (0.12)	10.72 (0.36)
+10 μg/mL hydroxyzine+10 μg/mL cetirizine	10.28 (0.26)	10.56 (0.28)
+20 μg/mL hydroxyzine+20 μg/mL cetirizine	10.25 (0.14)	10.44 (0.13)
+50 μg/mL hydroxyzine+50 μg/mL cetirizine	10.32 (0.18)	10.58 (0.39)

cetirizine did not differ significantly from the original carbamazepine concentration of the pool. However, we are unable to confirm these findings in vivo because even with the PENTINA carbamazepine assay the interference was observed only with a moderate toxic level of hydroxyzine. We decided not to even approach our Institutional Review Board with such request because of the inherent danger of such levels of hydroxyzine in normal subjects.

Despite the fact that carbamazepine immunoassays are subjected to interference, these assays are used through out the world for therapeutic monitoring of carbamazepine due to ease of automation and quick output of results. Various carbamazepine assays are affected by their major active metabolite carbamazepine 10, 11-epoxide (14). Interestingly, the PENTINA carbamazepine assay also has about 90% cross-reactivity with the active metabolite of carbamazepine, the carbamazepine 10, 11-epoxide (15). On the other hand, the new turbidimetric carbamazepine immunoassay on ADVIA 1650 analyzed has only 16% cross-reactivity with the epoxide metabolite (16).

We conclude that the turbidimetric carbamazepine immunoassay assay is free from the interference of hydroxyzine and cetirizine and this assay can be used for therapeutic drug monitoring of carbamazepine in patients receiving either hydroxyzine or cetirizine. However, this finding requires confirmation using specimens from patients accidentally overdosed with hydroxyzine or cetirizine to establish firmly that the turbidimetric carbamazepine assay is free from interference of both hydroxyzine and cetirizine.

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