Summary:

Carbamazepine (CBZ) is widely used in the treatment of epilepsy, frequently in combination with other anticonvulsants. Its metabolite, carbamazepine-10,11-epoxide, is pharmacologically active and is increased with concurrent use of valproate and other anticonvulsants. This pharmacokinetic interaction may be particularly important because CBZ, its epoxide, phenytoin, and lamotrigine all act on fast voltage-dependent sodium channels. Over a 2-month period, routine serum requests for CBZ (n = 47) (excluding known cases of overdose) were also analyzed for CBZ epoxide, phenytoin, and lamotrigine using a simultaneous high performance liquid chromatographic (HPLC) method. Valproate was measured using fluorescence polarization immunoassay (FPIA). With concurrent phenytoin and lamotrigine administration, there was a relative increase in CBZ epoxide and a significant decrease in the ratio of CBZ to epoxide (from more than 5 to 3). If valproate was also present, the concentration of parent and metabolite increased significantly, causing potential toxicity. Two patients in this latter group had significant clinical toxicity, with parent CBZ concentrations in the reference range; a third patient suffered from poor control of seizures. This study illustrates the importance of awareness of the contribution of active metabolites in therapeutic drug monitoring and raises questions about the role of the routine monitoring of such metabolites.
are very difficult to control, four or five anticonvulsants may be used concurrently. The potential pharmacokinetic and
pharmacodynamic interactions are complex (1-3). This current work was stimulated by a clinical request concerning a patient with
difficult epilepsy and the possibility that an accumulation of carbamazepine-10,11-epoxide (CBZ epoxide) might be contributing to
marked clinical toxicity. CBZ epoxide has been shown to contribute to both seizure control and toxicity (4,5).

MATERIALS AND METHODS
Carbamazepine-10,11-epoxide was a gift of Ciba-Geigy Australia Ltd (Pendle Hill NSW 2145, Australia); lamotrigine was a gift of
Glaxo Wellcome Aust Pty Ltd. (Boronia, Victoria, Australia). CBZ, phenobarbitone, and phenytoin were purchased from Sigma-
Aldrich Pty, Ltd. (Castle Hill, NSW, Australia); and alphenal from Alltech Australia (Balkham Hills, NSW, Australia). Acetonitrile
was HPLC grade (Mallinckrodt, Notting Hill, Victoria, Australia) and potassium dihydrogen phosphate (KH₂PO₄) was analytic
reagent grade (Ajax Chemicals, Auburn, NSW, Australia).

All anticonvulsants except valproate were measured using high performance liquid chromatography (HPLC). A 100-µL aliquot of
plasma or serum was added to 300 µL acetonitrile containing alphenal (5 mg/L) as the internal standard. Precipitated protein was
removed by placing the mixture in a centrifuge. The sample (25 µL) was injected into a C18 Novapak 4µ 8mm-cartridge using a
Wisp 710B (Waters, Australia Pty Ltd, Rydalmere, NSW, Australia 2116), a 6000A pump, and 484 variable wavelength detector
with Millennium software (version 2.15, Waters, Australia Pty Ltd, Rydalmere, NSW, Australia 2116). The mobile phase was
0.05M KH₂PO₄ pH 7.0 and acetonitrile ([70% to 30%], flow rate 2.0 ml/minutes). The detector was set at 309 nm until 3.2 minutes
postinjection and then reset to 215 nm, thus avoiding potential interference from sulthiame, which elutes with a similar retention
time to lamotrigine but has little absorption at 309 nm. A typical elution profile of the anticonvulsants detected in this system is
shown in Figure 1; Performance characteristics are detailed in Table 1. The assays are linear over the standard ranges shown, with
between-run coefficients of variation (CVs) of less than 8% for all assays. Valproic acid was measured by fluorescence polarization
immunoassay (Abbott Laboratories; Abbott Park, IL, U.S.A.).
FIG. 1. Separation of anticonvulsants using HPLC. Carbamazepine, carbamazepine-10,11-epoxide, lamotrigine, phenobarbitone, and phenytoin were separated by high-performance liquid chromatography (HPLC) using alphenal as the internal standard, as described in the article.

<table>
<thead>
<tr>
<th>Anticonvulsant</th>
<th>Range (mg/l)</th>
<th>CV (%)</th>
<th>Limit of detection (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>0–30</td>
<td>7.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Carbamazepine epoxide</td>
<td>0–10</td>
<td>6.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>0–20</td>
<td>4.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>0–60</td>
<td>5.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>0–50</td>
<td>6.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; HPLC, high-performance liquid chromatography.

TABLE 1. Summary of performance characteristics of anticonvulsants measured using...
Patient History
The patient was a 27-year-old woman with ataxia whose movements had become uncoordinated after lamotrigine was added to her anticonvulsant regime. When she was first seen, she was taking multiple medications. The plasma concentrations of these were as follows: Phenytoin 21 mg/L (reference range, 10-20 mg/L), CBZ 7.7 mg/L (6-12 mg/L), lamotrigine 3.6 mg/L (1-12 mg/L), and valproate 58 mg/L (50-100 mg/L). Plasma CBZ epoxide concentration was 5.8 mg/L. She was also taking nitrazepam but the concentration was not measured.

Study Details
Carbamazepine epoxide concentrations were measured in samples, which were submitted for therapeutic drug monitoring of CBZ from both outpatients and inpatients. The samples were selected randomly during a 2-month period. A summary of demographic details from these patients (n = 47) is shown in Table 2. Most patients were taking two or more anticonvulsants. Eleven patients were taking other medications, particularly phenothiazines, antidepressants, or lithium. Data are presented in groups according to combinations of the major anticonvulsants.

<table>
<thead>
<tr>
<th>Anticonvulsant regime</th>
<th>Total (n)</th>
<th>Age range (y)</th>
<th>Other anticonvulsants</th>
<th>Psychotropic medication</th>
<th>Other medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>17 (M: 9, F: 8)</td>
<td>2-72</td>
<td>Vgalbucristin (1)</td>
<td>Lithium haloperidol</td>
<td>Amiodarone, Digoxin*</td>
</tr>
<tr>
<td>Carbamazepine + lamotrigine</td>
<td>9 (M: 3, F: 6)</td>
<td>2-48</td>
<td>Chlordiazepone (1)</td>
<td>CBZ epoxide</td>
<td>Metoclopramide*</td>
</tr>
<tr>
<td>Carbamazepine + phenobarbital</td>
<td>1 (F: 1)</td>
<td>31</td>
<td></td>
<td>Fluoxetine*</td>
<td>Osapipine</td>
</tr>
<tr>
<td>Carbamazepine + valproate</td>
<td>6 (M: 5, F: 1)</td>
<td>3-37</td>
<td>Gabapentin (1)</td>
<td>Lithium</td>
<td>Ergotamine, Methadone</td>
</tr>
<tr>
<td>Carbamazepine + valproate + phenytoin</td>
<td>1 (M: 1)</td>
<td>30</td>
<td></td>
<td>Thyroidaline</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine + lamotrigine + phenytoin</td>
<td>4 (M: 2, F: 2)</td>
<td>2-25</td>
<td>Chlordiazepine (1)</td>
<td>Fluoxetine*</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine + lamotrigine + valproate</td>
<td>6 (M: 3, F: 3)</td>
<td>12-51</td>
<td>Gabapentin (1)</td>
<td>Lithium</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine + lamotrigine + valproate + phenytoin</td>
<td>3 (M: 1, F: 2)</td>
<td>25-36</td>
<td>Acetaminophen (1)</td>
<td>Naltrexone (1)</td>
<td></td>
</tr>
</tbody>
</table>

* Total number of subjects, with number of males (M) and females (F) are shown as a ratio.  
† Anticonvulsants used in addition to carbamazepine, lamotrigine, phenobarbital and phenytoin; the number of patients is identified (x).  
§ Patients taking psychotropic medication. Six patients were being treated for psychiatric conditions such as bipolar affective disorder and were taking combinations of the antipsychotic drugs shown.  
¶ Inhalational medications such as glucocorticoids were not included. One patient was being treated for cardiac arrhythmias, one patient for chronic, intractable pain.

TABLE 2. Patient details including concurrent medication

RESULTS
The mean CBZ concentrations were similar in all therapeutic groups (Table 3), but there were significantly higher concentrations of CBZ epoxide in samples from patients on combined regimes that incorporated both lamotrigine and valproate. The net result was a mean increase in the combined concentration of CBZ plus epoxide of approximately 40% (p < 0.025). The effect was even more apparent when the data were expressed as the ratio of parent CBZ to CBZ epoxide: the mean ratio fell from more than 5.0 in patients not taking lamotrigine and valproate to less than 2.0 in those taking the multiple regime. The mean lamotrigine concentration was lower in the group of patients taking CBZ and phenytoin (Table 4). Although only CBZ and lamotrigine data are shown, the doses of all other anticonvulsants were similar between groups.
It is interesting to consider the CBZ data briefly for efficacy and clinical toxicity. Six patients with epilepsy had poor control of the condition. Four patients were in the carbamazepine-only group (concentrations were 3.4/0.7 mg/L, 6.5/1 mg/L, 9.5/1.7 mg/L, and 9.5/1.8 mg/L for CBZ and epoxide, respectively) and one was taking valproate as well (concentrations, 3.5/1.7 mg/L). The sixth patient with epilepsy had very poor control and had a seizure during sampling; therapy was a combination of CBZ, valproate, and lamotrigine (concentrations, 7.3/9.7 mg/L). It is apparent that the majority (27/47) of CBZ concentrations in this study fell below the quoted reference range in this laboratory (6-12 mg/L, similar to the concentrations quoted in many standard texts of 6 mg/L). Using an alternative and lower therapeutic range (4-11 mg/L) (7), the number of subtherapeutic concentrations was 9/47. Taking into account the epoxide concentrations, 15 of 47 patients had total concentrations of less than 6 mg/L; 3 of 47 patients had less than 4 mg/L. In defining the upper limit of the therapeutic range, the occurrence of toxicity is important. There were two cases with clinical toxicity identified as the reason for analysis (CBZ/total [parent + metabolite] concentrations of 8.0/13.8 mg/L and 5.3/9.3 mg/L, respectively). Both patients were on multiple therapy, including valproate and lamotrigine.

**DISCUSSION**

The mean CBZ concentrations were similar in all groups. This outcome might be anticipated because medical practitioners have been encouraged to prescribe dosages of anticonvulsants to a target concentration (within the defined therapeutic range) and to use clinical judgment about the efficacy of the therapy (i.e., control or toxicity). It is interesting that in this study, clinicians tended to target concentrations at the lower end of the therapeutic range; some of these patients might have benefited from a more aggressive monotherapy rather than the combination of anticonvulsants used for the majority of patients in this study (33 of 41 patients with epilepsy). The reference ranges quoted in this article (6,7) are representative of those in use in various laboratories and are useful for demonstrating a general principle.

Although the CBZ concentrations were comparable between treatment groups, the relative amount of epoxide present in the patients receiving CBZ alone in this study is higher than that reported by authors such as Kudriakova and colleagues (8) at similar and
relatively low plasma CBZ concentrations. These authors demonstrated significant and rapid autoinduction, which was dose and concentration dependent. The influence of valproate is apparent with increased concentrations of CBZ epoxide in those patients receiving valproate, particularly in combination with lamotrigine. This finding is consistent with previous reports (9-12) and is attributed to inhibition of epoxide hydrolase and decreased production of CBZ-diol. Phenytoin and phenobarbitone induce both cytochrome P450 3A4 (12) and epoxide hydrolase, resulting in increased clearance of CBZ; however, induction of epoxide hydrolase is relatively less and there is an accumulation of CBZ epoxide (9,11,12). Lamotrigine has been reported to produce an increase in epoxide, compared with the parent drug, of between 10% (14) and 45% (15), and resultant clinical toxicity. Interestingly, in this cross-sectional study, although there was a slight increase in CBZ epoxide concentration in patients taking valproate concurrently, the effect was not statistically significant unless lamotrigine was used as well. There are only anecdotal reports concerning other medications taken by patients (Table 2); for instance, the epoxide-to-CBZ ratio was not altered in patients receiving fluoxetine (16).

Carbamazepine epoxide is pharmacologically active, having both anticonvulsant (4) and antineuralgic properties (17,18). However, clinicians do not agree about the importance of its contribution total anticonvulsant efficacy or the production of side effects, and few articles have addressed the benefits of monitoring epoxide as well as CBZ. Symptoms of CBZ intoxication occurred in five of six patients taking valnoctamide, which inhibited epoxide clearance in a concentration-dependent fashion (19). Valnoctamide is the isomer of valpromide, the amide derivative of valproic acid. In 1994, So and colleagues (5) demonstrated in a small group of patients that when significant toxicity and loss of seizure control were precipitated by the addition of valproic acid, epoxide concentrations were raised in the presence of unchanged CBZ levels. The epoxide concentrations published in a report about that group of patients ranged from 5.3 mg/L to 10.3 mg/L, with CBZ-to-epoxide ratios less than 1.7 (total concentrations, 12.5-21 mg/L). Importantly, the resulting seizures were resistant to phenytoin infusion. Reduction in the CBZ dose restored clinical control of the epilepsy in the presence of the low CBZ-to-epoxide ratio. The patient with epilepsy in this current study who had had a seizure and had poor control of the condition may be another such case. The observation of phenytoin resistance is important in our understanding of the mechanism of action of anticonvulsants and possible pharmacodynamic interactions, as discussed later. Although Lui and Delgado (9) supported epoxide monitoring, neither Theodore and colleagues (20) nor Semah and colleagues (21) considered there to be any benefit.

When considering any pharmacodynamic interaction, concentrations at the receptor or site of action are important. The free, unbound fraction of epoxide is approximately twice that of CBZ (9,12,22). The proposed mechanism of action of epoxide is identical to that of the parent, namely inhibition of the voltage-dependent fast sodium channel (2). If protein binding of epoxide is first order, as the concentration of epoxide increases (assuming equal potency), the contribution of epoxide to the anticonvulsant effect (and potentially, to the side-effect profile) will not be simply mole for mole but rather be amplified. Given that CBZ is often used in combination with other anticonvulsants, it is important to consider their mechanisms of action as well to anticipate potential pharmacodynamic interactions. In addition, there will be potential changes in the free, unbound fractions of anticonvulsants because of protein-binding dependent interactions. Phenytoin inhibits the same voltage-dependent fast sodium channels (2), although lamotrigine prolongs this inactivation (23) with marked inhibition of glutamine and aspartate release in conditions of sustained repetitive firing (24). The mechanism of action of valproate is less well understood and is considered to enhance sodium-channel inactivation and to reduce calcium currents (low threshold T-type [3]). Therefore, all of the drugs that have been demonstrated to be associated with a net increase in epoxide concentration are all acting on a limited number of active sites, particularly the fast sodium channels. The cases reported by So and colleagues (5) support this concept because the toxicity described mimics that seen with phenytoin, in which there can be recrudescence of seizures (25). In the cases reported in this current article, the pharmacokinetic and pharmacodynamic interactions are cumulative.

Is there a benefit to measuring the plasma concentration of epoxide? Given the data contained in this descriptive study, an increase in epoxide represented an increase in total anticonvulsant effect. In some cases, it is possible that the improvement in anticonvulsant control obtained by the addition of a second or third anticonvulsant drug may be partly caused by accumulation of the metabolite as well as inherent activity of the additional drug. To establish the role of CBZ epoxide and its monitoring, larger and more formal studies are required.

This article has raised a major point and two questions: First, awareness of the potential production of an active metabolite is important. Second, in cases in which active metabolites are produced, should the metabolite be monitored as well? (This obviously depends on the likely contribution of metabolite to the total efficacy or toxicity.) Third, what do reference ranges mean and should they be adjusted when multiple medications are being used? Although it makes therapeutic sense for the concept of monotherapy to be pursued to avoid or to minimize drug interactions if more than one agent is required, use of drugs with different mechanisms of action that may be synergistic is desirable. Indeed, in some patients, such synergy may allow apparently subtherapeutic concentrations to be efficacious and possibly avoid or limit side effects. In contrast, the anticonvulsants reported in this article have a predominant, common mechanism of action—namely, the inhibition of the fast voltage-dependent sodium channels; to some extent,
using them in combination may only be marginally more efficacious than an approach using monotherapy although it allows significant potential for concentration-related side effects.

REFERENCES


Key Words: Carbamazepine; CBZ-epoxide; Valproate; Lamotrigine; Phenytoin