Routine therapeutic monitoring of lamotrigine in epileptic patients using a simple and rapid high performance liquid chromatographic technique

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SUMMARY. We have developed a simple and rapid high performance liquid chromatographic technique to determine lamotrigine concentrations in epileptic patients and validated it using external quality control material. The method has been used to monitor the lamotrigine concentration in 70 specimens from 61 patients. Only 50% of the specimens had concentrations within the proposed target range of 1 mg/L to 4 mg/L, and there was no relationship between lamotrigine concentration and age, sex, other anti-epileptic treatments, or dose, although this could in part be explained by concomitant anti-epileptic therapy. We suggest that lamotrigine should be monitored therapeutically in order to assess its efficacy and audit its use as an anti-epileptic treatment, especially with the introduction of this relatively new drug as monotherapy.

Additional key phrases: epilepsy; anticonvulsant drugs; therapeutic drug monitoring

Lamotrigine (Lamictal) is a phenyltriazine compound, originally synthesized as a folic acid antagonist, which is chemically unrelated to any established anticonvulsant drugs in use. Lamotrigine was originally indicated for the adjunctive treatment of partial and generalized seizures, but has recently become licensed as monotherapy for epilepsy in adults and children. It may also be useful in other neurological conditions such as Parkinson's disease and motor neurone disease, Huntington's chorea, and neuralgia. The exact mode of action of lamotrigine is not fully understood, although it is thought to act by inhibiting the release of excitatory amino acid neurotransmitters (e.g. GABA) which results in the stabilization of neuronal membranes. Lamotrigine has been found to be readily absorbed (oral bioavailability = 98%) with a mean clearance after oral dosing of 2.5 L/h, and a mean plasma elimination half-life of 20-30 h. It is estimated that around 90% of absorbed lamotrigine is eliminated as glucuronide metabolites. Concomitant therapy with other anticonvulsant drugs has a substantial effect on the clearance of lamotrigine. Sodium valproate decreases the rate of metabolism, resulting in reduced lamotrigine clearance and doubling of the elimination half-life to around 48 h, due to competition for the rate limiting process of glucuronidation, whilst enzyme inducing drugs such as carbamazepine, phenobarbitone and phenytoin accelerate the elimination of lamotrigine, reducing its half-life to approximately 12 h.

Initiation of lamotrigine therapy may lead to a skin rash, but if mild, this may spontaneously subside without drug withdrawal. However, the likelihood of skin rashes is diminished if lamotrigine therapy commences on a low dose, for example 25 mg daily for 2 weeks followed by 50 mg daily for 2 weeks. The dose of lamotrigine prescribed also depends on concomitant anti-epileptic treatments because of the effects of other drugs on lamotrigine clearance. In addition, there is some evidence that adverse relations to lamotrigine could be concentration dependent, with nausea arising at concentrations around 6 mg/L, vomiting around 8 mg/L and headaches around 10 mg/L. However, studies also indicate that high concentrations can be well tolerated with concentrations up to 26 mg/L being used to
HPLC monitoring of lamotrigine in epileptic patients

**Treat intractable epilepsy without adverse effects** (Patsalos PN, personal communication.)

In view of the interactions described above, therapeutic drug monitoring of lamotrigine may prove beneficial in the management of epileptic patients. A tentative therapeutic range of 1-4 mg/L (SI conversion factor, 1 mg/L = 4 μmol/L) has already been suggested.1,2 Therapeutic monitoring of lamotrigine may also be important for optimizing and auditing its efficacy as an anti-epileptic treatment, especially with the introduction of this relatively new drug as monotherapy.

Several methods have been described for the analysis of lamotrigine, using either normal-phase or reverse-phase high performance liquid chromatographic (HPLC) techniques. However, specimen volumes (200–300 μL) tend to be large (especially for paediatric cases), and the retention times tend to be around 10-15 min per injection. We report a simple and rapid method for the determination of plasma lamotrigine concentrations that uses only 100 μL of specimen and a 5 min injection time. The results of routine use of this method in the analysis of 70 specimens from 61 patients for lamotrigine are presented.

**MATERIALS AND METHODS**

**HPLC assay**
Lamotrigine and 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (internal standard) were kindly donated by the Wellcome Foundation, and Cardiff Bioanalytical Services Ltd, respectively. The HPLC equipment used comprised:

1. Spherisorb 5 μm nitrile column (150 mm × 4 mm; Jones Chromatography, Mid-Glamorgan, UK)
2. Kontron LC 414 pump, and MSI 660 autosampler (Kontron Electricals Ltd, Ashford, UK)
3. Pye Unicam LC-UV detector (Unicam Ltd, Cambridge, UK)

The working method is as follows: one hundred microlitres patient specimen/standard/quality control, 100 μL internal standard (20 mg/L in methanol), 500 μL 1·0 M sodium hydroxide, and 2·5 mL ethyl acetate are pipetted into a 12 mL polypropylene tube. The tubes are then stoppered and mechanically shaken for 10 min, followed by centrifugation for 5 min at 4500 rpm. Using a clean polypropylene pipette, all of the organic phase is transferred into a clean 12 mL polypropylene tube, and evaporated to dryness under air at 40°C. The residue is then reconstituted in 200 μL ethyl acetate, and 50 μL are injected onto the column. The mobile phase is 80% acetonitrile in 0·04 M ammonium acetate buffer (pH 3·5), used at a flow rate of 2·5 mL/min. Detection is at 305 nm, range 0·0-0.8 AUFS.

**Patients’ samples**
A total of 70 specimens from 61 patients (32 male; 29 female) were studied. These had been submitted for therapeutic monitoring of lamotrigine concentrations. Three specimens were requested as urgent from patients admitted to casualty departments with seizures. In addition to the City Hospital, specimens were received from 18 other hospitals (outpatient departments and general practitioners) and from three local general practitioners. The age range of the patients was 2–75 years, arithmetic mean 30 years (geometric mean 24 years). The other drugs taken by these patients are shown in Table 1. Lamotrigine dosage regimens stated on the request form ranged from 25 to 250 mg bd.

**RESULTS**
Initial assay development work was performed using drug free plasma spiked with lamotrigine over the concentration range 0.5–20 mg/L. The assay recovery for lamotrigine over this range was determined to be 91%, and 10 replicate analyses at 1·0 mg/L and 5·0 mg/L yielded

<table>
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<th>Therapy</th>
<th>No. of requests</th>
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<td>Single additional anti-epileptic drug</td>
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<tr>
<td>Carbamazepine</td>
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<td>Phenytoin</td>
<td>10</td>
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<td>Valproic acid</td>
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<tr>
<td>+ phenytoin + phenobarbitone</td>
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<td>Number of requests</td>
<td>22</td>
</tr>
</tbody>
</table>

**TABLE 1. Details of the concomitant medication for the 70 requests received for therapeutic monitoring of lamotrigine**

coefficients of variation of 3.7% and 4.0%, respectively. Other commonly administered anticonvulsant drugs (carbamazepine, ethosuximide, phenobarbitone, phenytoin, primidone and valproic acid) were found not to interfere with the performance of the assay. The retention times for lamotrigine and internal standard are 1.5 and 3.1 min, respectively (Fig. 1).

The method was validated using 11 external quality control specimens donated by Cardiff Bioanalytical Services Ltd. A linear regression analysis performed on the quality control specimens yield a regression coefficient of 0.9953, and the line of best fit was represented by the equation

\[ y = 0.93x + 0.47 \]

where \( y \) is the stated concentration, and \( x \) was the determined concentration.

The method was made routinely available from June 1994, and in the first 8 months was used for the analysis of 70 specimens. The concentrations determined are shown in Fig. 2. Of these 70 specimens, only 50% were within the provisional target range of 1 mg/L—4 mg/L.

**DISCUSSION**

Several methods have been published for the analysis of lamotrigine. However, the specimen volumes required by these methods are between 200 and 300 \( \mu \)L. This volume may be too large, especially for paediatric cases and patients who are difficult to bleed. In addition, the retention times are long at around 10–15 min per injection. The method of Ramachandran has the advantage of simultaneous determination of multiple analytes, but at the expense of specimen volume and run-time. In the light of an increasing tendency towards immunoassay for the therapeutic monitoring of established antiepileptic drugs, the advantages of this method may be lost.

Our method has been used to determine the plasma lamotrigine concentration in 70 specimens from 61 patients, where it was found that
only 35 (50%) were within the target range of 1–4 mg/L. Of the three urgent requests received from casualty departments, two were from patients not complying with their anti-epileptic treatment and one case was subsequently thought to be fitting due to a head injury.

It was found that 31 of the 70 specimens were above the target range, with concentrations reaching as high as 15·5 mg/L. These results show a 30-fold interindividual variation in 'steady-state' lamotrigine concentration, which is indicative of the possible need to monitor its concentration, and thereby optimize anti-epileptic treatment. It should, however, be stated that adverse reactions were never cited as a reason for the original requests.

The concomitant therapy of the 61 patients monitored (Table I) appeared to have no relationship to the concentration of lamotrigine in the specimens. It was found that the mean lamotrigine concentration determined in the eight patients concomitantly administered valproic acid was 5·3 mg/L, with a range of 1·0–10·0 mg/L. In the 13 patients given lamotrigine and carbamazepine, the mean lamotrigine concentration was found to be 3·8 mg/L (range <0·5–7·2 mg/L), and the 10 patients prescribed lamotrigine with phenytoin had a mean lamotrigine concentration of 2·7 mg/L (range 0·6–5·7 mg/L). The mean concentrations of lamotrigine therefore appear to follow the expected trend, showing higher levels when given with valproic acid and lower levels when given with the enzyme-inducing anti-epileptic drugs carbamazepine and phenytoin. However, the range of lamotrigine concentrations determined in these patients was so large that any simple predictions regarding either lamotrigine dose or concentration based on concomitant therapy would be unreliable.

There also appeared to be no relationship between the dose of lamotrigine administered and concentration determined. The dose/concentration data for patients aged 16 years and above (n = 35) are shown in Fig. 3. With a total daily dose of 100 mg the range of concentrations determined was found to be 1·1–5·5 mg/L (mean 2·6 mg/L), the range for 200 mg/day was 2·0–11·2 mg/L (mean 6·1 mg/L), whilst that for 400 mg/day was 2·8–8·9 mg/L (mean 4·9 mg/L). These data again show considerable interindividual variation in plasma lamotrigine concentration in patients receiving similar dose regimens, which could, in part, be explained by concomitant therapy and fulfill one of the criteria for drug monitoring.

There was no relationship between lamotrigine concentration and the sex or age of the patients. Lamotrigine has recently become licensed as monotherapy for epilepsy in adults and children over 12 years, and has been found to be as effective and better tolerated than carbamazepine. Since there is some evidence that adverse effects of lamotrigine could be concentration dependent, and in view of the effects of polypharmacy on the clearance of lamotrigine, therapeutic monitoring could be beneficial to patient management. More studies are required, and this article describes a simple method to facilitate these.

**CONCLUSION**

A simple and rapid method for the quantitation of lamotrigine in epileptic patients has been developed that requires only 100 μL of specimen and takes 5 min per injection. To date, no other drugs have been found to interfere with the quantitation of lamotrigine using this method. This method has been validated using external quality control material and used successfully to monitor the lamotrigine concentrations in 61 patients.
REFERENCES


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