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Short communication

Serum lamotrigine analysis by capillary electrophoresis

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Abstract

Lamotrigine, a new antiepileptic drug, is analyzed by capillary zone electrophoresis. Samples were deproteinized with acetonitrile containing an internal standard, acidified with dilute acetic acid and injected into the capillary. The drug migrated rapidly with the cationic compounds in about 3.5 min far from any interfering substances. The test was linear between 0.5–10 mg/l. The analysis time was about 5 min. The CE values correlated well with an HPLC method ($r=0.97$; $n=35$). The mean serum concentration of 121 patients on this drug was 3.7 mg/l. Incubating the serum with β-glucuronidase for 1 h increased the peak height of lamotrigine by about 24%.

Keywords: Lamotrigine

1. Introduction

Lamotrigine (Lamictal, Burroughs Wellcome, Research Triangle Park, NC, USA) is a new antiepileptic drug [(3,5 diamino-6-dichlorophenyl)-1,2,4-triazine; Fig. 1]. In serum, about 55% of the drug is bound to proteins. The half-life of the drug is about 24 h, being shorter in children, and increased in patients with renal failure. The peak level occurs 2 h after an oral dose [1–3]. The drug is metabolized by glucuronide conjugation and excreted in the urine. The therapeutic level is not well established but patients who respond to this drug have levels of about 1–4 mg/l. However, as we show in this study many patients who respond well to this drug have values higher than this range.

Basic drugs, in general, are difficult to measure by GC or HPLC because of the binding to the silica surface of the columns. Several methods for measurement of lamotrigine by HPLC have been described. These methods require large amounts of organic solvents for elution [4–7] which are expensive and environmentally hazardous, and many of these methods require sample extraction for clean-up before injection on the column. Here, we describe a simple and rapid assay for this drug by capillary electrophoresis (CE) which avoids the previous problems and compare it to an HPLC method.

2. Experimental

2.1. Chemicals

Tyramine and β-glucuronidase were obtained from Sigma (St. Louis, MO, USA) and acetoacetanilide from Eastman Kodak (Rochester, NY, USA).

2.2. CE instrument

A Model 2000 capillary electrophoresis instrument (Beckman Instruments, Fullerton, CA, USA) was set at 10 kV, 24°C and 214 nm. The capillary was 42 cm×50 μm (I.D.). Initially, the capillary was

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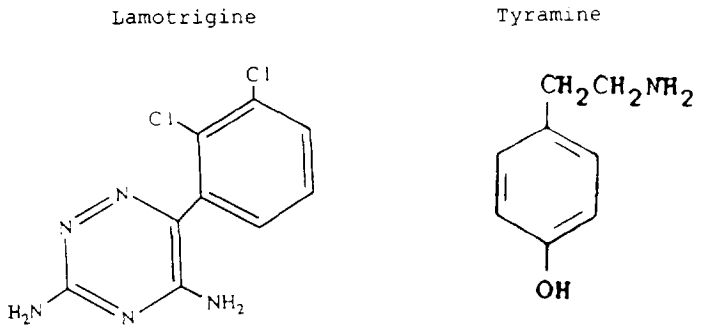

Fig. 1. Structure of lamotrigine and tyramine.

washed with sodium hydroxide (0.2 mol/l for 30 min). After each sample the capillary was washed for 1 min each with the sodium hydroxide solution and the electrophoresis buffer (sodium acetate, 130 mmol/l, adjusted to pH 4.8 with acetic acid, 10 mol/l). Samples were introduced by pressure injection for 25 s.

2.3. CE method

Serum, standard, or control (50 μl) was mixed with 100 μl acetonitrile containing 40 mg/l tyramine as an internal standard. The mixture was centrifuged for 30 s at 14 000 *g* and the supernatant was mixed with 100 μl acetic acid 0.9 mol/l and injected into the capillary.

2.4. HPLC method

We used the same column and same conditions described earlier for a serum felbamate assay with the exceptions that the wavelength of the detector was set at 312 nm, the internal standard acetoacetanilide concentration was 50 mg/l, and the acetonitrile concentration in the mobile phase was increased to 180 ml/l [8].

3. Results and discussion

Lamotrigine has two maxima of light absorption; a weak one at 312 nm and a strong one at 200 nm. However, also many drugs and endogenous compounds absorb at 200 nm and thus can interfere. For HPLC, detection at 312 nm decreases the interference especially from phenobarbital. In CE, with a pK_a of 5.7, lamotrigine migrates in the acetate buffer rapidly with the cationic compounds emerging at about 3.5 min (Fig. 2). In serum, very few cationic compounds with strong ultraviolet absorption can be detected on the electropherogram. These separation conditions eliminate many of the possible interferences, including the majority of the antiepileptic drugs and allow for detection at 214 or 200 nm. Few of the anti-arrhythmic drugs migrate in that region. No interferences were detected from the common drugs such as quinidine, quinine, procainamide, lidocaine, or phenytoin. We did not encounter any interferences in ten samples from patients admitted to the cardiology unit. Lamotrigine, elutes after tyramine, the internal standard and slightly ahead of an unknown endogenous substance (peak x) which can act as another marker on the electropherogram, (Fig. 2). Since the glucuronide metabolite is not available commercially, we did not test the migration of this compound. However, the addition of β-glucuronidase (10 000 units/ml) for 60 min at 37°C, for seven serum samples increased the peak height of lamotrigine on the average 24% indicating that the metabolite(s) migrate(s) in serum differently from the parent drug (Table 1). Furthermore, five serum samples extracted with 0.5 ml chloroform three times after adjusting the pH to 7.8 with phosphate buffer showed the absence of any lamotrigine in the aqueous phase, also indicating that the metabolite (which is water soluble) does not co-migrate with parent drug.

In CE, sample matrix affects greatly the separation and the peak height, much more than in chromatographic techniques [9]. Serum proteins and inorganic

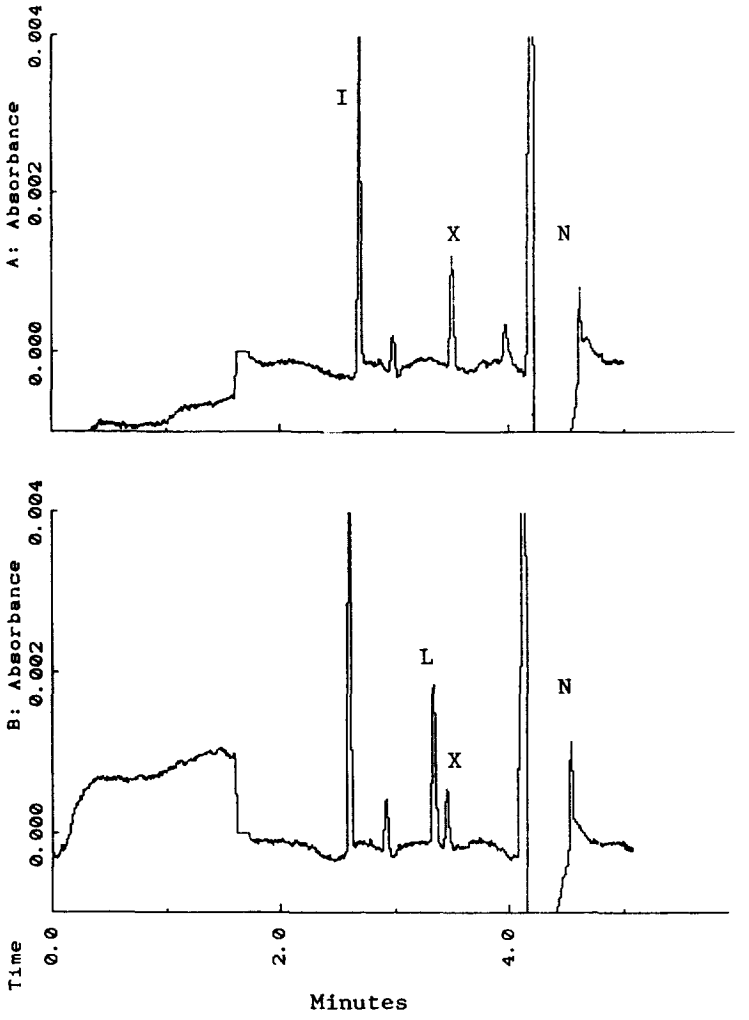

Fig. 2. Electropherogram of: (top) patient free from the drug; and (bottom) patient receiving lamotrigine (7.8 mg/l) (L=lamotrigine, I=tyramine, X=unknown endogenous compound, N=neutral compounds).

Table 1
Percentage increase in the lamotrigine peak after incubating the serum with β-glucuronidase for 60 min

Patient	Lamotrigine (initial value) (mg/l)	Increase (%)
1	3.1	26
2	5.1	32
3	4.6	10
4	2.5	36
5	7.2	28
6	7.1	8
7	4.8	28
Mean	4.9	24

ions greatly affect the peak height and electrophoretic mobility [9]. The peak height of lamotrigine in aqueous standards (Fig. 3A) is much higher than that in saline (Fig. 3C) or serum (Fig. 3B). If the sample is not acidified with acetic acid the lamotrigine peak is not detected (Fig. 3D). To lessen the effects of salts and proteins, serum is treated first with acetonitrile, then diluted with acetic acid yielding a final dilution of about 5-fold. The acetonitrile treatment also liberates the drug from binding to serum proteins. The average recovery of 8 mg/l standard into the serum compared to that in the acetonitrile

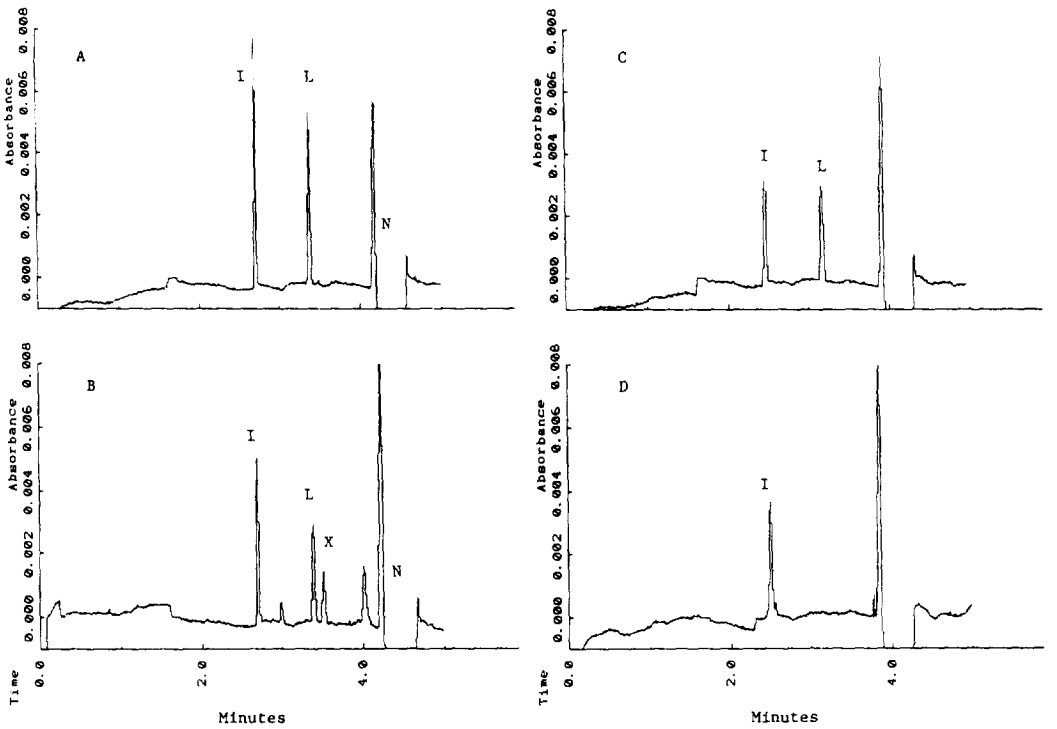

Fig. 3. Effects of sample matrix on lamotrigine peak height: (A) 10 mg/l in water with the addition of acetic acid; (B) 10 mg/in serum with the addition of acetic acid; (C) 10 mg/l in saline with the addition of acetic acid; and (D) 10 mg/l in water without the addition of acetic acid. (L=lamotrigine, I=tyramine, N=neutral compounds).

supernatant is 86%, indicating that a small amount binds or co-precipitates with the serum proteins. For these reasons, the standard (5 mg/l) was prepared in serum.

The test was linear using both peak height and area between 0.5 and 10 mg/l (mg/l=0.33×mA+0.06, r=0.99) with 0.3 mg/l as the lowest detection level (3× baseline noise). Because of the limitation of the software we used peak height for quantitation although peak area in general gives better linearity and better precision [10]. The R.S.D. of a 10 mg/l sample was 4.8% (n=20). We compared 35 samples by this method to an HPLC assay (Fig. 4). The correlation is good, r=0.97. The advantages of the CE over HPLC (Fig. 5) are speed (5 versus 12 min by HPLC) with less interferences especially from phenobarbital. Organic solvents consumption is much less in CE compared to that for HPLC.

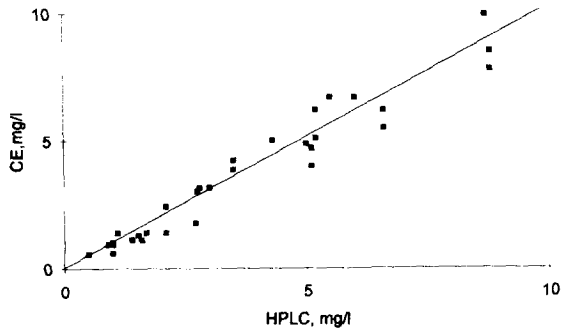

Fig. 4. Comparison of CE to HPLC patient results (r=0.97, n=35).

Most patients responding to this drug had values of 1–4 mg/l with some having values up to 17 mg/l without toxic symptoms indicating that the drug has low toxicity [4,11,12]. In this work, the mean serum level of 121 patients on this drug is 3.7±2.8 mg/l. Most of the patients with elevated levels were tolerating the drug well. For example, one patient

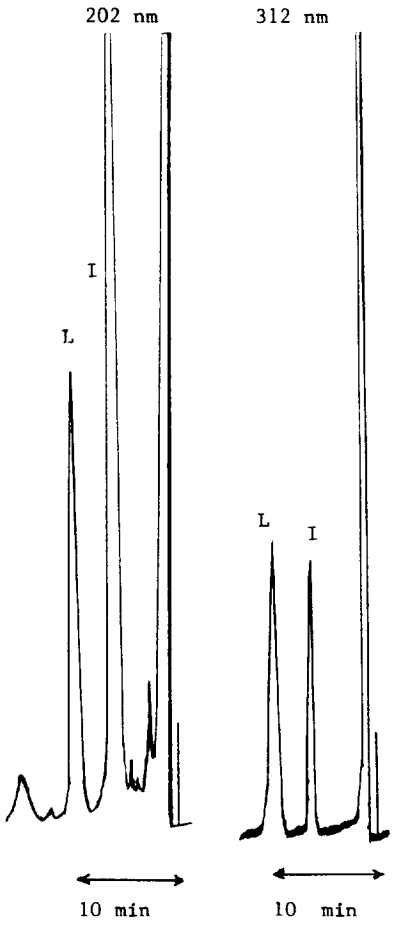

Fig. 5. Analysis of lamotrigine from a patient serum (7.3 mg/l) by HPLC at 312 nm (0.005 AUFS) and 202 nm (0.02 AFUS) (L=lamotrigine, I=internal standard).

had two levels of 13.3 and 17.2 mg/l. The patient's seizures were well controlled with slight side effects.

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