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Short communication

Liquid chromatographic determination of plasma lamotrigine in pediatric samples

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1. Introduction

Lamotrigine (Fig. 1) is a new antiepileptic drug unrelated to the major drugs in current use. It is an inhibitor of release of the excitatory transmitter glutamate, which might contribute to its mechanism of action and give a new possibility for the treatment of epilepsy.

In adults, the elimination half-life of lamotrigine has been shown to vary widely owing to interaction with other co-administered antiepileptic drugs [1,2]. The interaction with valproate has been shown to be an inhibited liver metabolism, while carbamazepine, phenytoin and phenobarbital induce increased lamotrigine metabolism. Lamotrigine is currently under clinical investigation for use in pediatric patients suffering from intractable epilepsy [3,4]. In order to develop optimized drug therapy for children on polytherapy and to determine pharmacokinetic parameters, there was a need for the continuous measurement of plasma lamotrigine levels.

Both chromatographic and immunological methods have been presented for plasma lamotrigine measurements [5–10]. This paper describes the development of a liquid chromatographic method based on reversed-phase high-performance liquid chromatography and the optimization of its usefulness for application to samples from pediatric patients.

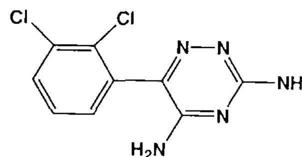

Fig. 1. Structure of lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine).

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2. Experimental

2.1. Chemicals

Lamotrigine (3,5-diamino-6-(2,3-dicholorphenyl)-1,2,4-triazine) was obtained from the Wellcome Foundation (London, UK). Other chemicals were obtained from commercial sources and were of analytical purity.

2.2. Plasma samples

Plasma samples were obtained from children and young adults (7 months–23 years) suffering from intractable epilepsy. The dose range of lamotrigine in the patients was 1–12 mg kg^{-1} day^{-1}. Venous blood was collected in heparinized Vacutainer tubes. Plasma was prepared within 12 h by centrifugation at 1000*g* for 5 min. Analysis was performed on the same day and the remaining plasma was stored at −20 °C.

2.3. Preparation of samples

Aliquots of plasma (200 μl) were pipetted into 10 ml glass test-tubes, followed by the addition of 1 ml of 1 M NaOH and 3 ml of ethyl acetate. The samples were shaken for 2 min and the layers separated by centrifugation at 1000*g* for 10 min. A measured volume of 1.0 ml of the organic layer was transferred into a new glass test-tube and evaporated to dryness under a stream of nitrogen gas with gentle heating (35°C). The residues were dissolved in 200 μl of the mobile phase. In routine use, samples were injected every 25 min.

2.4. Chromatographic analysis

The chromatographic system consisted of a ConstaMetric 3200 pump (LDC, Riviera Beach, FL, USA), a Kontron 360 Autosampler (Kontron Instruments, Zürich, Switzerland) with a 50 μl loop, a 250 mm × 4.6 mm i.d. 5 μm C_{18} Nucleosil column (ChromTech, Stockholm, Sweden), a Spectromonitor 3200 UV detector (LDC) set at 270 nm and a Hitachi D-2500 Chromato-Integrator (Hitachi, Tokyo, Japan).

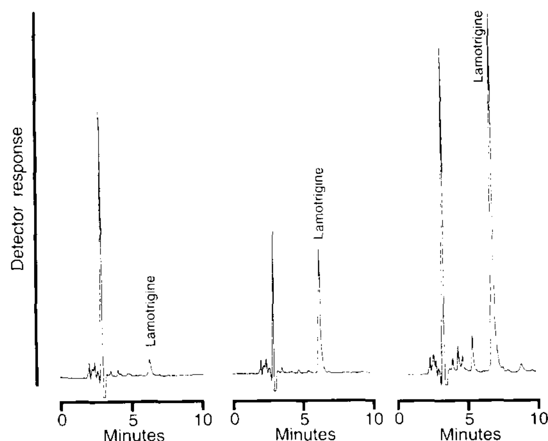

Fig. 2. Chromatograms obtained from the determination of lamotrigine in plasma from three pediatric patients. The retention time was between 6 and 7 min. The following concentrations were found: trace A (left), 0.16 μg ml^{-1}; trace B (middle), 1.3 μg ml^{-1}; trace C (right), 3.2 μg ml^{-1}.

The mobile phase was a solution of 25% (v/v) acetonitrile in 30 mM KH_2PO_4 (pH 3.7) and was degassed by ultrasonication. The flow-rate was 1.2 ml min^{-1}.

2.5. Quantitation

Quantitation was based on peak areas using external standard calibration. Calibration samples were prepared by spiking blank plasma with lamotrigine at levels from 0 to 15 μg ml^{-1}.

3. Results and discussion

Sample preparation was performed by a single-step solvent extraction with a total recovery of 98% in the organic phase. The use of ethyl acetate as extraction solvent from a basic aqueous phase with almost quantitative recovery is consistent with earlier reported results [6]. The high recovery allowed quantitation to be achieved without an internal standard. Other reported preparation procedures that have been used for plasma lamotrigine are C_{18} solid-phase extraction [4] and acetonitrile precipitation with sodium carbonate saturation of the aqueous phase [5]. The latter of

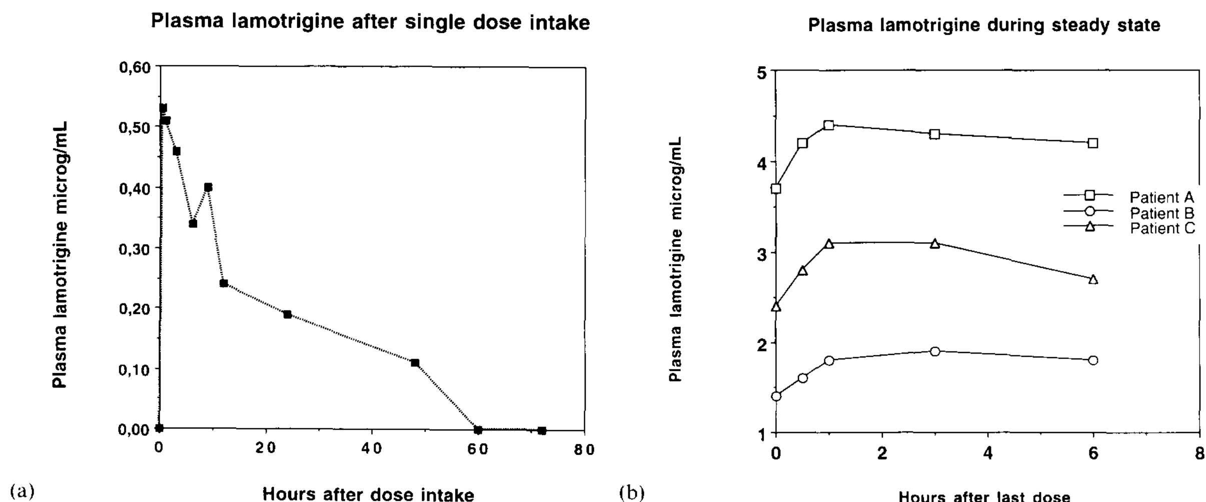

Fig. 3. (a) Plasma concentrations of lamotrigine in a pediatric patient (5 years old) during 3 days after a single oral dose of 1 mg kg^{-1}. (b) Plasma concentrations of lamotrigine in three pediatric patients during steady-state conditions. Doses of lamotrigine were (A) 1.1 and (B, C) 1.2 mg kg^{-1}.

these gives a high recovery of lamotrigine and might also be suitable with quantitation based on external standard calibration.

The analytical procedure gave chromatograms free of interfering peaks in clinical samples (Fig. 2) with a retention time for lamotrigine between 6 and 7 min. The method has been in routine use in connection with clinical studies of pediatric patients, and about 2400 samples have been analysed. Interferences have been observed only rarely, and only at the beginning of lamotrigine treatment when concentrations are low. No commonly co-administered therapeutic drug (valproic acid, carbamazepine, clonazepam, vigabatrin, ethosuximide, phenytoin, phenobarbital and nitrazepam) has caused analytical interference. A further validation of the method was achieved by participation in external quality control for lamotrigine. The results showed a high degree of concordance with other participants in the Wellcome Lamotrigine EQA Scheme (administered by Cardiff Bioanalytical Services, Cardiff, UK).

The calibration graphs showed a linear relationship between spiked concentration and peak area over the range 0–15 $\mu g\ ml^{-1}$. The limit of detection (signal-to-noise ratio = 3) was about 0.02 $\mu g\ ml^{-1}$ and 0.1 $\mu g\ ml^{-1}$ was used as the limit of quantitation. Precision in quantitation was obtained by careful pipetting during transfer of the organic extraction solvent. The within-day repeatability was 6.1% at 0.2 $\mu g\ ml^{-1}$ ($n = 8$), 1.5% at 2.0 $\mu g\ ml^{-1}$ ($n = 10$) and 3.3% at 7.3 $\mu g\ ml^{-1}$ ($n = 10$). The between-day reproducibility was 6.2% at 1.9 $\mu g\ ml^{-1}$ and 2.7% at 7.0 $\mu g\ ml^{-1}$ ($n = 18$).

With the proposed method it is possible to determine plasma levels of lamotrigine both after single doses and under steady-state conditions during repetitive intake in pediatric patients. A typical plasma concentration versus time curve is shown for one patient after a single-dose intake of lamotrigine (Fig. 3(a)). The plasma peak concentration was well below 1.5 $\mu g\ ml^{-1}$, which is the mean peak concentration reported in adult healthy volunteers after a 120 mg dose [5]. The slow terminal elimination is in agreement with the reported elimination ($t_{1/2}$) of about 24 h. Even at these low levels plasma concentrations can be followed for about 48 h with the proposed method. Also shown are plasma concentrations during steady-state conditions (Fig. 3(b)), where higher levels of lamotrigine accumulate. These three patients in the steady state show only small fluctuations in lamotrigine concentration. Therapeutic drug monitoring can be used to optimize

lamotrigine dosing in order to obtain stable plasma levels and avoid side-effects.

Most other bioanalytical procedures for lamotrigine have used polar chromatographic columns [8–10]. One previous method employed reversed-phase ion-pairing chromatography [7]. A reversed-phase system was found to be adequate for clinical application. The sensitivity of the proposed method is higher than that of previous methods [5–10]. This was needed for the measurement of plasma concentrations in the doses given to pediatric patients.

In conclusion, a validated procedure has been presented for plasma lamotrigine determinations which is suitable for use in clinical laboratories wanting to offer an on-line analytical service.

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