References

1. Opatrny K Jr, Bouda M, Kohoutkova L, Sefrna F. A clinical study to assess the effect of heparin in dialyser rinsing solutions. Int J Artificial Organs 1997; 20: 112-118.
2. Bartels PCM, Schoorl M, Schoorl M. Activatie van stolling tijdens hemodialyse is afhankelijk van de wijze van antistolling. Ned Tijdschr Klin Chem Labgeneesk 2005; 30: 282-284.
3. Sebekova K, Spustova V, Optatrny K Jr., Dzurik R. Serotonin and 5-hydroxyindole-acetic acid. Bratisl Lek Listy 2001;102:351-356.
4. Frank RD, Weber J, Dresbach H, Thelen H, Weiss C, Floege J. Role of contact system activation in hemodialyzer-induced throbogenicity. Kidney Int 2001; 60: 1972-1981.
5. Sirolli V, Ballone E, Di Stante S, Amoros L, Bonomini M. Cell activation and cellular-cellular interactions during hemodialysis: Effect of dialyzer membrane. Int J Artif Organs 2002; 25: 529-537.
6. Bartels PCM, Schoorl M, Schoorl M, Nubé MJ. Deviations in coagulation activation due to treatment with different haemodialysis membranes. Scand J Clin Lab Invest 2003; 63: 417-424.
7. Apsner R, Buchmayer H, Lang T, Unver B, Speiser W, Suner-Plassmann G, Hörl WH. Simplified citrate anticoagulation for high-flux hemodialysis. Am J Kid Dis 2001; 38: 979-987.
8. Pivac N, Mück-Šeler D, Barišic I, Jakovljević M, Puretić Z. Platelet serotonin concentration in dialysis patients with somatic symptoms of depression. Life Sciences 2001; 69: 2423-2433.
9. Bos JC, Grooteman MPC, Houte AJ van, Schoorl M, Limbeek J van, Nubé MJ. Low polymorphonuclear cell degranulation during citrate anticoagulation: a comparison between citrate and heparin dialysis. Nephrol Dial Transplant 1997; 12: 1387-1393.

Ned Tijdschr Klin Chem Labgeneesk 2006; 31: 238-239

Lamotrigine in dried blood spots by HPLC

J.W.P.H. SOONS, M.L. van BREE, J.H. COUMOU and J.A.R.J. HULSMAN

Lamotrigine is an anti epileptic drug which blocks voltage-dependent sodium channels, thereby stabilizing neuron membranes. Moreover, it acts as a glutamate antagonist thereby preventing excitatory neurotransmitter release. Lamotrigine is as effective as carbamazepine and phenytoin against partial and secondarily generalised tonic-clonic seizures as well as idiopathic (primary) generalised epilepsy (1, 2). Lamotrigine can be used as monotherapy, but is particularly effective and generally well tolerated as a broad spectrum agent for adjunctive treatment of both partial epilepsy and idiopathic generalised epilepsy in adults and children (3).

Lamotrigine, is standard monitored in plasma by HPLC. Therapeutic drug monitoring of anti epileptic drugs from blood spots is far from general practice. Diagnosis of inborn errors of metabolism from blood spots in stead of plasma or whole blood in containers on the other hand is well known (4,5). Dried blood spots have the advantage of a smaller volume and are easy to obtain by the patient self. The sample can be transported by mail without any precaution.

We designed an assay in which lamotrigine is measured in dried blood spots and compared this assay with the standard plasma analysis.

Methods

Lamotrigine is measured in plasma or dried blood spots obtained from patients blood by HPLC. Discs of ¼ inch were purched of paper (Schleicher and Schuell, RC 55, 0.45 µm). Samples (10 µL) of blood, lamotrigine and/or internal standard (A725C 78, Wellcome) were added to the disc. The sample size of the plasma analysis is 200 µL. Lamotrigine and the internal standard were extracted out of the plasma sample or the dried blood spot by 200 µL dichloromethane. The extraction is performed at a pH of 10.4 and 3% propanol-2 is added to the dichloromethane to optimize the extraction-recovery. After evaporation and reconstitution of the residue in 200 µL mobile phase solution 20 µL is injected to the HPLC system. The reversed phase HPLC system (Agilent 1100) contains a stationary phase of an ODS Hypersil, 5µm, 100 x 4,6 mm column and a mobile phase of a phosphate buffer pH = 7,0 containing 32% methanol. The lamotrigine concentration is measured by a 4-point standard curve of lamotrigine and is internal standardized with A725C 78.

Results

The concentration of lamotrigine in 17 patients is measured in the following different blood components: whole blood, lysed whole blood, red blood cells, lysed red blood cells and plasma (reference). No significant difference in the lamotrigine concentration is found in these blood components when compared to the plasma concentration. Comparison of whole blood versus plasma according to Passing and Bablock shows the following equation: whole blood value = 1.09 x plasma value -0.07. This indicates that whole blood can be used in the assay in

Epilepsy centre, Kempenhaeghe, Heeze, The Netherlands

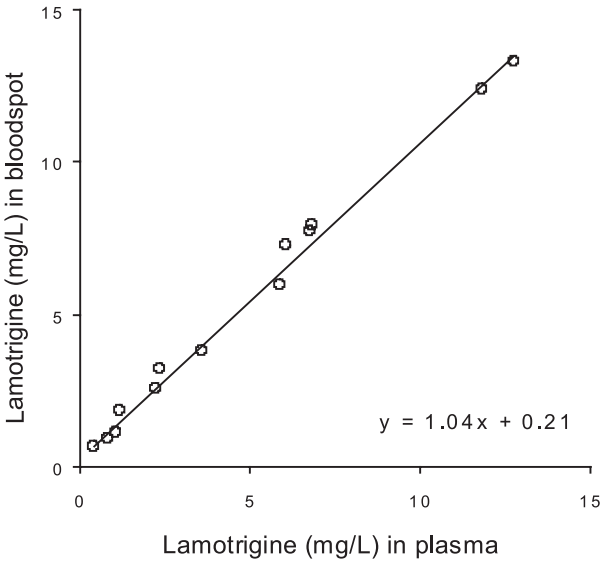

Figure 1. Lamotrigine concentration measured in 13 patients in dried blood spots and in plasma. Comparison of the results according to Passing Bablock analysis.

stead of plasma. The standard volume of patient serum in the reference assay is 200 µl. The dried blood spots contain only 10 µl blood. The reproducibility (n=10) of the plasma assay is measured at three concentrations in a plasma volume of 10µl. The between run CV is 20.9%, 9.1% and 9.9% at a lamotrigine concentration of respectively 1.2 mg/l, 3.2 mg/l and 7.3 mg/l.

The reproducibility (n=10) of the lamotrigine analysis using the dried blood spots is measured in three patients. The patient blood (10 µl) and internal standard (10 µl) are added to a dot and allowed to dry for one day at room temperature. The between dot CV obtained is 10.7%, 8.9% and 10.6% at a lamotrigine concentration of respectively 3.8 mg/l, 6.5 mg/l and 9.3 mg/l.

Comparison of the lamotrigine concentration in dried blood spots and in plasma is obtained by measuring the concentration in 13 patients receiving lamotrigine. The concentration in serum is measured according the reference assay. The patients blood (10 µl) and the internal standard (10 µl) is also added to the dots. After drying for one day at room temperature the analysis is performed. Figure 1 shows the comparison according to Passing and Bablock of these data.

Conclusion

Lamotrigine can be measured both in plasma as well as in whole blood. Samples of plasma and blood can be exchanged in the same assay. A 20-fold reduction in the patient sample volume still gives an admissible reproducibility in the therapeutic range. This indicates that the assay can handle a sample volume (10 µl) that is generally used in dried blood spots. The inter dot CV obtained in blood of three patients receiving lamotrigine were 10% or less. This indicates that lamotrigine can be measured in dried blood spots in stead of plasma or blood in containers. Moreover, the lamotrigine concentration of patients measured in the dried bloodspots correlates well with the concentration measured in plasma.

Literature

1. Brodie MJ, Richens A, Yuen AWC. Double-blind comparison of lamotrigine and carbamazepine in newly diagnosed epilepsy. Lancet 1995; 345: 476-479.
2. Steiner TJ, Silveira C, Yuen AWC, et al. Comparison of lamotrigine (Lamictal) and phenytoin monotherapy in newly diagnosed epilepsy. Epilepsia 1994; 35 Suppl. 7: 61.
3. Fitton A, Goa KL. Lamotrigine, an update of its pharmacology and therapeutic use in epilepsy. Drugs 1995; 50: 691-713.
4. Zytkovicz TH, Fitzgerald EF, Marsden D, et al. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. Clin Chem 2001; 47: 1945-1955.
5. Vollmer DW, Jinks DC, Guthrie R. Isocratic reverse-phase liquid chromatography assay for amino acid metabolic disorders using eluates of dried blood spots. Anal Biochem 1990; 189: 115-121.