Clinical Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Comparison between dried blood spot and plasma sampling for therapeutic drug monitoring of antiepileptic drugs in children with epilepsy: A step towards home sampling

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ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 12 December 2016
Accepted 22 December 2016
Available online xxxx

Keywords:
Red blood cell/plasma ratio
Conversion factor
Estimated plasma concentrations
Hematocrit
LC-MS/MS

ABSTRACT

Objectives: To investigate if dried blood spots could be used for therapeutic drug monitoring of the antiepileptic drugs, carbamazepine, lamotrigine and valproic acid in children with epilepsy.

Methods: Fingerprick blood samples from 46 children at a neuropediatric outpatient clinic was collected on filterpaper at the same time as capillary plasma sampling. A validated dried blood spot liquid chromatography tandem mass spectrometry method for carbamazepine, lamotrigine and valproic acid was compared with the routine plasma laboratory methods. Method agreement was evaluated and plasma concentrations were estimated by different conversion approaches.

Results: Strong correlation was shown between dried blood spot and plasma concentrations for all three drugs, with R2 values > 0.89. Regression analysis showed a proportional bias with 35% lower dried blood spot concentrations for valproic acid (n = 33) and concentrations were 18% higher for carbamazepine (n = 17). A ratio approach was used to make a conversion from dried blood spots to estimated plasma for these two drugs. Dried blood spot concentrations were directly comparable with plasma for lamotrigine (n = 20).

Conclusions: This study supports that dried blood spot concentrations can be used as an alternative to plasma in a children population for three commonly used antiepileptic drugs with the possibility to expand by adding other antiepileptic drugs. Clinical decisions can be made based on converted (carbamazepine, valproic acid) or unconverted (lamotrigine) dried blood spot concentrations. Dried blood spot sampling, in the future taken at home, will simplify an effective therapeutic drug monitoring for this group of patients who often have concomitant disorders and also reduce costs for society.

1. Introduction

The most commonly used antiepileptic drugs (AEDs) for the treatment of children with epilepsy in Sweden and elsewhere are carbamazepine (CBZ), lamotrigine (LTG), valproic acid (VPA) and levetiracetam [1,2]. Therapeutic drug monitoring (TDM) can assist in individualizing dosages in pediatric care due to pharmacokinetic variations between children on antiepileptic drug polytherapy, drug-drug interactions, subtle adverse drug reactions or co-morbidities in chronically disabled children [3,4].

Presently TDM is usually performed using venous or capillary blood sampling at out-patient clinics, measuring the trough drug concentrations in plasma before the morning dose [3]. Blood sampling at a clinic is time-consuming and sometimes stressful, especially for children with chronic diseases or disabilities. Self-collection at home of a capillary dried blood spot (DBS) sample should be of advantage for many of these patients and their guardians [5,6]. Although DBS has been used in different qualitative assays since the 60's, it is a new matrix for the TDM laboratory [7,8]. In quantitative bioanalysis based on DBS, hematocrit (HCT), spot homogeneity, blood-to-plasma ratio of the drug and sampling a correct volume are factors that may affect the result [9]. There has been a rapid development of assay technologies with much improved sensitivity such as liquid chromatography-tandem

Abbreviations: ADD, attention deficit disorder; ADHD, Attention deficit hyperactivity syndrome; AEDs, antiepileptic drugs; b/p, blood-to-plasma ratio; CBZ, carbamazepine; CI, confidence interval; DBS, dried blood spots; EMA, European Medicines Agency; HCT, hematocrit; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LTG, lamotrigine; RBC, red blood cell; RBC/p, red blood cell-to-plasma ratio; TDM, therapeutic drug monitoring; VPA, valproic acid.

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http://dx.doi.org/10.1016/j.clinbiochem.2016.12.008
0009-9120/

mass spectrometry (LC-MS/MS), allowing the measurements of multiple of exogenous small compounds in a drop of blood [10]. Breakthroughs with prelaboratory preparation of microliter whole blood samples to dried plasma and the design of micro-devices for collection of a defined sample volume, makes it likely that DBS will become an attractive sampling technique for quantitative analysis [11–14].

Drugs vary in their distribution between the cell fraction and plasma in whole blood, described as the specific blood-to-plasma ratio (b/p-ratio) or red blood cell/plasma ratio (RBC/p-ratio) of the drug [6,9,15–17]. Apart from the RBC/p-ratio, which can be concentration dependent, the patients individual HCT should in theory also affect the relation between DBS and plasma concentrations. In order to compare DBS concentrations with traditional plasma concentrations, a conversion algorithm may be required to calculate an estimated plasma value based on the DBS concentration. Different approaches, with experimental data of paired plasma and DBS concentrations, corrected for sample HCT [18] or not corrected for HCT [19,20] have been used to calculate a ratio which is then used as a conversion factor. Theoretical approaches constructing algorithms considering RBC/p ratios and individual patient HCT levels have also been presented [21].

VPA is mainly distributed in plasma and yields lower concentrations in whole blood, e.g. using DBS [20–22]. In CBZ and LTG, the distribution between whole blood and plasma is more equal since the RBC/p ratio is close to one and direct comparisons have been proposed for these drugs [21,23–26]. Clinical validations are needed to introduce DBS as an alternative matrix in routine TDM [27]. We present an approach on how TDM decisions can be made with DBS concentrations.

In this study on children with epilepsy and concomitant neurological diagnoses, we evaluated capillary DBS concentrations of three commonly used AEDs with concentrations of simultaneously collected capillary plasma. DBS concentrations were analyzed using a recently developed liquid chromatography tandem mass spectrometry (LC-MS/MS) method while plasma concentrations from capillary blood were analyzed with the routine laboratory methods. We also investigated if conversion factors were needed to calculate estimated plasma concentrations from DBS concentrations for the use in clinical practice TDM.

2. Material & method

2.1. Patients

This study was approved by the local research ethics committee (Regionala etikprövningsnämnden EPN at Karolinska Institutet, Stockholm, 2012/2146-3) and the work conducted in accordance with the Declaration of Helsinki. Patients and/or their guardians approved participation in the study by informed consent prior to blood sampling. Inclusion criteria for the study population were children and adolescents aged 2 to 18 years and treated for epilepsy with CBZ, LTG or VPA as a single or combined drug therapy at Department of Neuropediatrics, Karolinska University Hospital, Huddinge.

2.2. Sample collection

Samples were collected by pediatric nurses at the neuropediatric clinic from April 2013 to March 2014. DBS samples were collected at the same occasion as routine TDM samples for plasma and did not require extra visits to the clinic or additional fingerpricks. All samples reflected trough drug levels. Time for sampling, reported prescriptions of antiepileptic drug and last intake of drug were recorded. Fingerprick blood (three to five drops) was collected on filterpaper (Whatman 903 protein saver card, GE Healthcare, Westborough, MA) using a Microtainer Lansett, 1.8 or 2.0 (Becton, Dickinson and Company, Franklin Lakes, NJ). Guidelines from CLSI, Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens, was followed [28]. Hands were always warm before sampling and first drop of blood was wiped away. From the same fingerprick, approximately 300–500 μL of capillary blood was collected in a Li-heparin capillary tube for routine plasma analysis. An additional 300–500 μL was collected in a capillary tube for HCT measurement (K2 EDTA Microvette, both tubes from Sarstedts, Nümbrecht, Germany).

HCT was analyzed on a SYSMEX XE-5000 (Sysmex, Kobe, Japan), Department of Clinical Chemistry, Karolinska University Hospital, Huddinge. Whatman cards were left drying at room temperature for at least 3 h and then stored in zip-lock bags (Joka 11-68, VWR, Radnor, PA) kept at 4 °C with desiccant packages (Millipore, Darmstadt, Germany) until analysis. On arrival in the laboratory DBS samples were visually inspected and blood spots with a diameter of <7 mm, corresponding to an approximate volume of <15 μL were excluded since they were not within validation ranges [29].

2.3. Analytical methods

CBZ, LTG and VPA concentrations in DBS samples were measured using a recently developed LC-MS/MS method [29]. The method could be applied in the hematocrit range 0.30–0.60 in CBZ and LTG, VPA 0.35–0.60 and for volumes between 15 and 50 μL.

In the analysis of plasma concentrations, LTG and CBZ were analyzed by immunochemical methods, QMS LTG and CEDIA CBZ II on an Indiko Plus analyzer (all from Thermo Scientific, Waltham, MA). The methods were performed according to the manufacturer's protocols and kit-inserts. An accredited in-house LC/MS method was used for routine VPA analysis [22].

2.4. Statistics

Capillary DBS concentrations and plasma concentrations were compared using Passing and Bablok regression analysis. No constant bias between the methods was defined as when the 95% confidence interval (CI) of the intercept of the regression line included zero. In analogy, no proportional bias was defined as when the 95% confidence interval for the slope of the regression line included one. Bland-Altman plots were used to identify outliers or tendencies. Criteria for cross validation from European Medicines Agency (EMA) guidelines on bioanalytical method validation (≥67% of the samples should have a difference within ±20% of the mean) [30], were applied on unconverted DBS concentrations. Drugs that needed conversion were evaluated with these criteria a second time, after being converted to estimated plasma concentrations.

All calculations, analyses and figures were made using Microsoft Excel 2013 and Addinsoft *XL*STAT, 2016.

2.5. Conversion of DBS concentrations to estimated plasma concentrations

Two different approaches were used to calculate estimated plasma concentrations based on DBS concentrations. The first approach was to use the average ratio between measured plasma concentrations and DBS concentrations and multiply the DBS concentration with this ratio to achieve an estimated C_{plasma}. This simple *ratio-approach* has been used in earlier TDM comparison studies [19,20].

The second was partly a *theoretical approach* taking into account RBC/plasma ratio (= K in the equation below) and patient HCT and then converting it based on linear regression (21). The following equation was used to create a theoretical plasma concentration.

$$\textit{Theoretical } C_{plasma} = C_{DBS}/[1-\mathrm{HCT}(1-\mathrm{K})] \quad (1)$$

C_{DBS} is the drug concentration in DBS, HCT is the individual hematocrit value and K is the specific drug RBC concentration/plasma concentration ratio derived from in-house in vitro tests, see S3. In calculations, the individual HCT was also replaced with the mean HCT of the patient group for evaluation of individual HCT measurement necessity.

Please cite this article as: C. Linder, et al., Comparison between dried blood spot and plasma sampling for therapeutic drug monitoring of antiepileptic drugs in children with ep..., Clin Biochem (2016), http://dx.doi.org/10.1016/j.clinbiochem.2016.12.008