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THERAPEUTIC DRUG MONITORING OF LEVETIRACETAM: COMPARISON OF A NOVEL IMMUNOASSAY WITH AN HPLC METHOD

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Abstract

Background. Therapeutic drug monitoring of the anti-convulsant levetiracetam may be indicated in patients with conditions that may alter pharmacokinetic characteristics, for tailoring individual dosage regimens or to investigate patient compliance. In this study the Bio-Rad HPLC method (in-use method) and the ARK™ immunoassay method (new method) for levetiracetam monitoring in serum were compared.

Methods. Levetiracetam concentrations were determined in 63 samples using: 1) "Levetiracetam by HPLC" kit by Bio-Rad (Hercules, CA) on the Agilent 1100 HPLC system and 2) "ARK™ Levetiracetam" immunoassay by ARK Diagnostics Inc. (Fremont, CA) on the CDx90 platform by Thermo Fisher Scientific Inc.

Results. Within-laboratory imprecision and bias of the new method, evaluated over a 20 day period, were respectively 7.4% and 0.5% at 7.5 µg/mL, 4.5% and 1.9% at 30 µg/mL, 3.1% and 2.0% at 75 µg/mLl. Passign-Bablok regression analysis (X:Bio-Rad; Y:Ark) showed a non significant intercept of 0.16 (95%CI -0.55-0.72) and a slope marginally significantly different from unity of 0.95 (95%CI 0.90-0.99) which suggested minimum proportional systematic error. In agreement, Bland-Altman analysis showed minimum systematic bias of 1.0 µg/mL (95%CI 0.32-1.69) with 95% of the HPLC–Ark differences ranging from -4.3 (95%CI -5.52-(-)3.16) and 6.3 (95%CI 5.16-7.52). Our data showed that the two methods were identical both within inherent imprecision as well as analytical quality specifications (maximum allowable error 15%).

Conclusions. The new Ark™ method on the CDx platform is acceptable and may be used to measure serum levetiracetam concentrations routinely.

Keywords: levetiracetam, HPLC, method comparison, immunoassay, therapeutic drug monitoring

1. INTRODUCTION

Levetiracetam (Keppra®) is an anti-convulsant drug available as a monotherapy for epilepsy in the case of partial seizures, or as an adjunctive therapy for partial, myoclonic and tonic-clonic seizures [1].

Levetiracetam displays excellent oral absorption and bioavailability (> 95%) with a plasma half-life of 6-8 hours in healthy individuals. It is not bound to plasma proteins and is not metabolized in the liver, but it is renally excreted, with 66% of the drug present in urine unchanged, and 24% present as the primary metabolite, which is pharmacologically inactive [2]. Due to its very favourable pharmacokinetic characteristics and tolerability, clinical use of levetiracetam is simple and straightforward [2, 3]. Nevertheless, therapeutic drug monitoring of levetiracetam may be indicated in patients with conditions that may alter pharmacokinetic characteristics (elderly, children, pregnancy or renal impairment), to tailor individual dosage regimens or to investigate patient compliance [4]. In the elderly, in whom the half-life of the drug is increased by about 40 % (10 to 11 hours) due to reduced renal function, and in patients with renal impairment, in whom levetiracetam clearance is correlated with creatinine clearance, the daily maintenance dose of levetiracetam has to be adjusted and drug monitoring is strongly suggested [5]. However, tailored therapeutic ranges both in paediatric and in adult populations with reduced or impaired renal function have not been reported in the literature.

The implementation of an immunoassay for levetiracetam monitoring using automated instrumentation may present several advantages over chromatographic methods, including reduced turnaround time and simplified sample preparation, leading to potential improvements in patient care and reduced costs. Monitoring could be performed daily, particularly in critically ill patients, and laboratory workflow could be reorganized.

In this study, using both Passing-Bablok regression, Bland-Altman plot and a Medical Decision chart, the in-use and validated Bio-Rad HPLC method and the ARK^{TM} immunoassay method on the CDx platform for levetiracetam monitoring were compared.

2. MATERIALS AND METHODS

2.1. Subjects and samples

Sixty-three unselected patients were enrolled with a request for levetiracetam monitoring ordered by the family doctor or by a Hospital Division. Blood samples were collected in Becton Dickinson Vacutainer Plastic serum tubes. After centrifugation, samples were divided into two aliquots, which were analysed with both methods.

The study was planned according to the guidelines of the local ethical committee in conformity to the principles of the Declaration of Helsinki.

2.2. Methods and Instruments. Principle of analysis

Levetiracetam concentrations were determined using: 1) "Levetiracetam by HPLC" kit by Bio-Rad (Hercules, CA) on the Agilent 1100 HPLC system equipped with Chemstation software Rev. B. 03.01 (in-use method) and 2) "ARK^{TM} Levetiracetam" immunoassay by ARK Diagnostics Inc. (Fremont, CA) on the CDx90 platform by Thermo Fisher Scientific Inc. (Waltham, MA), distributed in Italy by Tema Ricerca S.r.l. (Castenaso, BO) (new method).

For the in-use HPLC method, 100 µL of serum were mixed with 400 µL of working solution containing the internal standard and, after centrifugation, 50 µL of supernatant was injected onto the HPLC column (isocratic; reverse phase 150x4 mm DI; flow 0.8 mL/min; column temperature 40°C; absorbance 210 nm; run 15 min; linearity range: 0.5-200 µg/mL).

In the ARK Levetiracetam homogeneous immunoassay, the drug in the specimen competes with levetiracetam labelled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds the antibody, enzyme activity decreases. In the presence of the drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance at 340 nm. The new kit was implemented on the CDx full automated platform with random access (90 test/hour).

The assay was calibrated using 6 concentrations provided by ARK Diagnostics (0, 5, 12.5, 25.0, 50.0 and 100 µg/mL). Briefly, 2.5 µL of sample were mixed with 150 µL of the antibody solution and 75 µL of the enzyme conjugate solution (levetiracetam labelled with G6PDH) and NADH formation was measured spectrophotometrically as a rate of change in absorbance at 340 nm (linearity range: 2.0-100 µg/mL).

All assays were performed, according to manufacturers' instructions, on instruments already available in the Laboratory and routinely used by highly trained technical personnel. The levetiracetam therapeutic range we used was: 5-30 µg/mL.

2.3. Performance verification

Both methods investigated have already been validated and extensively evaluated in previous studies and found to be appropriate for levetiracetam monitoring [6] . Nonetheless, before starting the comparison method study, analytical performances declared by the Manufacturers were verified. Commercially available control samples at different levels were run during each batch analysis. Precision analysis studies, useful to define combined imprecision of the methods, were performed over a 20 day period.

2.4. Statistical Analysis

All statistical analyses and graphs were performed by SPSS statistical software v. 15.0 (SPSS Inc., Chicago, IL, USA) and MedCalc v. 9.6.2 (MedCalc Software, Mariakerke, Belgium). Methods were compared by Bland-Altman plot and Passing-Bablok regression.

To judge acceptability within inherent imprecision, a combined imprecision of the methods was evaluated as $CV = \sqrt{CV_{method1}{}^2 + CV_{method2}{}^2}$ and lines corresponding to 0 ± 1.96 times the combined inherent imprecision were added to the graph where differences between methods were plotted against levetiracetam concentration.

Acceptability of the new method based on preset analytical quality specifications was judged using maximum allowable total error (TEmax) of 15% [7] and a medical decision chart (MEDx chart), in which inaccuracy and imprecision of the new and reference methods were compared [8]. The maximum allowable total error was selected by literature, considering maximum errors reported for similar drugs. Three lines representing 3 different currently accepted criteria for Total Error (TEmax) were built connecting TEmax on the y-axis to different points on the x-axix: 1) TEmax/2=7.5% (corresponding to the total error criterion TEmax = Inaccuracy + 2 x Imprecision), 2) TEmax/3=5% (corresponding to the total error criterion TEmax = Inaccuracy + 3 x Imprecision), 3) TEmax/4=3.75% (corresponding to the total error criterion TEmax = Inaccuracy + 4 x Imprecision). An operating point representing inaccuracy-imprecision pairs at the upper limit of the concentration range (30 µg/mL) (calculated by imprecision experiments and Passing-Bablok regression equations) was plotted.

To further evaluate agreement between the automated method and HPLC, continuous levetiracetam concentrations were transformed into ordinal variables with 2 levels (normal: <30 µg/mL; elevated: >30 µg/mL). Concordance between qualitative variables was then evaluated as % of concordant items or weighted Cohen's k analysis with 95% confidence interval.

3. RESULTS

Measured levetiracetam concentrations of commercially available control samples were always found to be within the range declared by the Manufacturer for either method investigated.

In particular, within-laboratory imprecision and bias of the new method, evaluated over a 20 day period, were respectively 7.4% and 0.5% at 7.5 µg/mL, 4.5% and 1.9% at 30 µg/mL, 3.1% and 2.0% at 75 µg/mL.

Levetiracetam concentrations measured by the HPLC and the ARK methods were respectively (median, interquartile range, min-max): 18.8 µg/mL (10.3-32.2, 0.0-88.0) and 17.5 µg/mL (9.6-30.9, 1.0-81.0).

The methods were compared by Passing-Bablok regression (slope with 95%CI, intercept with 95%CI) and Bland-Altman analysis (bias with 95%CI, 95% limits of agreement). Regression analysis (X:Bio-Rad; Y:Ark) showed a non significant intercept of 0.16 (95%CI -0.55-0.72) and a slope marginally significantly different from unity of 0.95 (95%CI 0.90-0.99) which suggested minimum proportional systematic error (Figure 1).

In agreement, Bland-Altman analysis showed minimum systematic bias of 1.0 µg/mL (95%CI 0.32-1.69) with 95% of the HPLC–Ark differences ranging from -4.3 (95%CI -5.52-(-)3.16) and 6.3 (95%CI 5.16-7.52) (Figure 1).

Based on the measured imprecision of both methods, respectively 4% and 4.5% for the Bio-Rad HPLC and the Ark methods, the combined inherent CV of the methods, given single measurements, was $CV=(4^2 + 4.5^2)^{(1/2)}=6\%$, meaning that if the two methods were identical, the differences should have been symmetrically distributed around 0 µg/mL, and 95% of the differences should have been within 0 ± 1.96 times 6% = 0 ± 11.8% (at 30 µg/mL the difference between the two methods should be respectively within the interval -3.5 to 3.5). Our data showed that the Ark method was nearly identical within inherent imprecision and therefore acceptable, since up to 81% (expected proportion 95%) of the HPLC-Ark differences were within the interval 0±1,96 times the combined inherent coefficient of variation of both methods (Figure 2).

Using the slope and intercept of the Passing-Bablok regression analysis, the predicted value for a target levetiracaetam concentration of 30 µg/mL (upper limit of the range) was respectively 28.7 µg/mL (0.95x30+0.16=28.66, difference 1.34=4.5%). For an Allowable Total Error of 15%, selected from the literature, considering maximum errors reported for similar drugs, the new Ark method predicted levetiracetam values within the acceptability threshold. This fact is easily understood by observing the medical decision chart shown in Figure 2, constructed using maximum allowable total error (TEmax:15%), imprecision of the new method (4.5%) and inaccuracy as estimated by Passing-Bablok regression analysis (4.5%), which clearly showed that the Ark automated assays and the HPLC method were indeed identical within preset analytical quality specifications (TEmax) (Figure 2).

Agreement between methods was also evaluated after transformation of levetiracetam continuous concentration levels into a dichotomous qualitative variable (<30 and >=30 µg/mL). Agreement with HPLC, evaluated as concordant items (%) and by weighted Cohen's kappa analysis, was 95% and k=0.89 (95%CI 0.77-1.00). In particular, 21 out of 63 patients had levetiracetam concentrations outside the therapeutic range according to the Ark or the HPLC method; in 18 there was agreement between the two methods, while in 3 patients the difference was lower than 3 µg/mL.

4. DISCUSSION

In this study, using both Passing-Bablok regression, Bland-Altman plot and Medical Decision chart, the in-use and validated Bio-Rad HPLC method and the ARKTM immunoassay method for levetiracetam monitoring were compared. Precision (within laboratory repeatability) and bias supplied by each manufacturer were verified in order to confirm that instruments were in proper working conditions.

The Ark and HPLC method displayed comparable levetiracetam values; only minimum proportional systematic error and minimum bias were shown by Passing-Bablok regression and Bland-Altman analysis.

Moreover, our data showed that the two methods were almost identical within inherent imprecision, (Figure 2), confirming that only a minimum bias, already observed by regression, was present. Accordingly, a MEDx chart (Figure 2), built using an imprecision of 4.5% and inaccuracy of 4.5% estimated at 30 µg/mL from the regression equation of Passing-Bablok analysis, showed that the Ark method, with respect to HPLC, was identical within preset analytical quality specifications, suggesting that the two methods did not differ for an error larger that the allowable total error. Good agreement was also observed after comparing, by Cohen's k analysis, the proportion of patients above or below 30 µg/mL.

Unlike other method comparison studies previously published on the Ark method, this study showed at least two distinctive aspects: 1) the implementation of the Ark method on the CDx analytical platform, 2) the use of advanced statistical techniques and the evaluation of acceptability. To our knowledge this is the first study in which the combination of the Ark method and CDx90 platform was evaluated and compared with a chromatographic method for levetiracetam. The CDx90 platform is a fully automated random-access system with high throughput (90 tests/hour) specially developed for therapeutic drug monitoring. The implementation of validated and verified methods on this platform may theoretically present several advantages over chromatographic methods, including reduced turnaround time, simplified sample preparation and cost reduction,

leading to the optimization of technical personnel and resource management. However, the replacement of a validated in-use assay with a new method is a thorough process which should be planned carefully to evaluate correctly both agreement and acceptability. In this study we have described two main approaches to evaluate method acceptability, taking into consideration the combined imprecision as well as the maximum allowable error suggested in the literature.

The introduction of the Ark method in our routine analytical practice significantly improved laboratory and clinical and aspects. The Ark immunoassay method required less specific training to be implemented in daily routine and reduced the workload of the HPLC system. Moreover, daily testing (before the introduction of the Ark method, the test was carried out only once per week) allowed clinicians to verify more frequently patients' compliance, closely monitor specific groups of patients (children, elderly, adults with impaired renal function) and not delay an urgent levetiracetam test (patients in the Emergency Department or Intensive Care Unit). With this regard, a revision of the laboratory data revealed that the replacement of the HPLC method with the ARK immunoassay had a favourable impact in terms of rapid achievement of the therapeutic range and therefore better outcome in patients admitted to Intensive Care Unit. With the previous HPLC method, optimal management of the dose was hindered by delayed weekly monitoring, with patients displaying levetiracetam concentrations at lower or upper reference limit, or even out of the therapeutic range, for a prolonged period of time (e.g. patient 1: 5.1 mg/l (day 1), 6.6 (day 8), 3.6 (day 15); patient 2: 3.8 mg/l (day 1), 4.8 (day 8), 3.6 (day 15); patient 3: 3.6 mg/l (day 1), 12.4 (day 8), 5.5 (day 15)). The new ARK automated method with daily monitoring allows rapid achievement of levetiracetam therapeutic range by dose adjustments (e.g. patient 1: 50.0 mg/l (day 1), 30.2 (day 6), 38.8 (day 9), 26.0 (day 10); patient 2: 46.1 mg/l (day 1), 31.2 (day 4), 26.0 (day 6), 26.4 (day 7); patient 3: 33.8 mg/l (day 1), 59.7 (day 3), 36.7 (day 6), 32.3 (day 10), 25.3 (day 17)).

In conclusion, the new Ark method on the CDx platform is acceptable and may be used routinely to measure levetiracetam concentrations. The implementation on the CDx platform allows reduced

turnaround time and simplified sample preparation, leading to potential improvements in patient care and laboratory management.

5. ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1

Passing-Bablok regression and Bland-Altman analysis of the comparison between the Ark and the HPLC method. The regression plots include the line of identity (slope=1), the regression line (solid line) with 95% confidence interval (dashed lines). The Bland-Altman plots include the reference line for no difference (at 0 µg/mL), the solid line indicating mean % difference with 95% confidence interval (dash-dotted lines) and the 95% limits of agreement of the methods (dashed lines).

Figure 2

(upper panel) A difference plot with dashed lines representing 0 ± 1.96 times the combined inherent coefficient of variation (CV) of HPLC and Ark methods (6%). The lines represent the interval within which the differences between the 2 methods should be included if the 2 methods are identical within the inherent imprecision of both methods. At 5 µg/mL the interval ranges from 0 ± 1.96 x 6% x 5 = -0.59 to +0.59; at 30 µg/mL the interval ranges from 0 ± 1.96 x 6% x 30 = -3.53 to +3.53; at 75 µg/mL the interval ranges from 0 ± 1.96 x 6% x 75 = -8.82 to +8.82.

(lower panel) Medical decision chart (MEDx chart) to evaluate acceptability based on preset analytical quality specifications, using a maximum allowable total error (TEmax) of 15%. The 3 dashed lines represent 3 different currently accepted criteria for Total Error (TEmax). Lines connect TEmax on the y-axis to different points on the x-axix, respectively from right to left: 1) TEmax/2=7.5% (corresponding to the total error criterion TEmax = Inaccuracy + 2 x Imprecision), 2) TEmax/3=5% (corresponding to the total error criterion TEmax = Inaccuracy + 3 x Imprecision), 3) TEmax/4=3.75% (corresponding to the total error criterion TEmax = Inaccuracy + 4 x Imprecision). The lines divide the chart into zones corresponding, from the origin to the right, to excellent, good, marginal, poor performances. The symbol represents the operating point given by

inaccuracy-imprecision pairs (calculated by imprecision experiments and Passing-Bablok regression equations) at the concentration of 30 µg/mL.

Figure 1

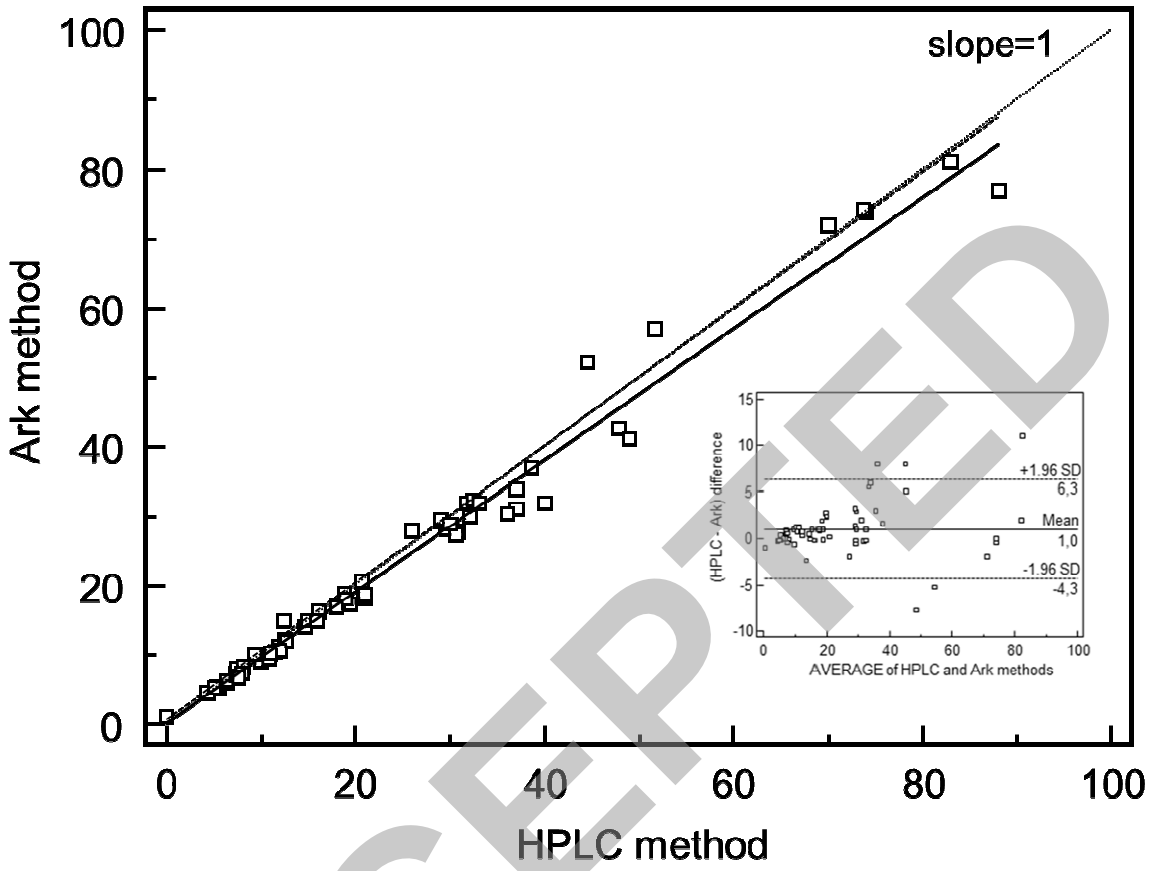

Figure 2