Clinica Chimica Acta 441 (2015) 63–70

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact

Matthijs Oyaert [a,*], Nele Peersman [a], Davy Kieffer [a,b], Kathleen Deiteren [c], Anne Smits [d,e], Karel Allegaert [d,e], Isabel Spriet [f,g], Johan Van Eldere [a,b], Jan Verhaegen [a,b], Pieter Vermeersch [a,h], Steven Pauwels [a,h]

[a] University Hospitals Leuven, Department of Laboratory Medicine, B-3000 Leuven, Belgium
[b] KU Leuven — University of Leuven, Department of Microbiology and Immunology, B-3000 Leuven, Belgium
[c] Antwerp University Hospital, Department of Laboratory Medicine, B-2650 Edegem, Belgium
[d] KU Leuven — University of Leuven, Department of Development and Regeneration, B-3000 Leuven, Belgium
[e] University Hospitals Leuven, Neonatal Intensive Care Unit, B-3000 Leuven, Belgium
[f] KU Leuven — University of Leuven, Department of Clinical Pharmacology and Pharmacotherapy, B-3000 Leuven, Belgium
[g] University Hospitals Leuven, Pharmacy Department, B-3000 Leuven, Belgium
[h] KU Leuven — University of Leuven, Department of Cardiovascular Sciences, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form 8 December 2014
Accepted 10 December 2014
Available online 16 December 2014

Keywords:
LC–MS/MS
Vancomycin
Tandem mass spectrometry
Therapeutic drug monitoring

ABSTRACT

Background: Accurate quantification of vancomycin in plasma is important for adequate dose-adjustment. As literature suggests between-method differences, our first objective was to develop a novel liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for total vancomycin in human plasma and to compare frequently used immunoassays with this method. Secondly, we investigated the clinical impact of between-method quantification differences.

Methods: For LC–MS/MS, lithium heparin plasma was extracted by adding a precipitation reagent containing the internal standard (vancomycin-des-leucine). Analysis was performed on an Acquity TQD mass spectrometer equipped with an Acquity UPLC 2795 separations module. Our method was analytically validated and compared with four frequently used immunoassays from four different manufacturers. Vancomycin concentrations were clinically classified as toxic, therapeutic and sub-therapeutic. Clinical discordance was calculated using LC–MS/MS as a reference.

Results: A novel LC–MS/MS method using protein precipitation as sole pretreatment and an analysis time of 5.0 min was developed. The assay had a total imprecision of 2.6–8.5%, a limit of quantification of 0.3 mg/L and an accuracy ranging from 101.4 to 111.2%. Using LC–MS/MS as reference, three immunoassays showed a mean proportional difference within 10% and one showed a substantial mean proportional difference of >20%. Clinical discordant interpretation of the obtained concentrations ranged from 6.1 to 22.2%.

Conclusions: We developed a novel LC–MS/MS method for rapid analysis of total vancomycin concentrations in human plasma. Correlation of the method with immunoassays showed a mean proportional difference >20% for one of the assays, causing discordant clinical interpretation in more than 1 out of 5 samples.

1. Introduction

Vancomycin is a glycopeptide antibiotic with strong bactericidal activity against gram-positive bacteria. These do not only include methicillin-resistant *Staphylococci*, but also penicillin resistant organisms, such as *Streptococci* and *Corynebacteria* [1]. Large inter- and intra-patient variability, combined with a correlation between low plasma concentrations and therapeutic failure on the one hand, and high plasma concentrations and toxicity on the other hand, makes the molecule an excellent candidate for therapeutic drug monitoring (TDM). In addition, the potential rise in minimum inhibitory concentrations of vancomycin target organisms makes it increasingly important to adjust its dosage in order to ensure adequate concentrations [2,3]. In clinical practice, therapeutic intervals, target levels and dose-adjustment schemes in function of administration mode and sampling time are used.

Current recommendations, however, do not take into account that routine plasma vancomycin quantification by commercial immunoassays can show substantial between-method differences [4–6]. Next to standardization issues, immunoassays can also lack specificity. For

Abbreviations: LC–MS/MS, liquid chromatography–tandem mass spectrometry; TDM, therapeutic drug monitoring; IS, internal standard; QC, quality control; MRM, multiple reaction monitoring; S/N, signal to noise ratio; CV, coefficient of variation; ME, matrix effect; DR, detection range; FPIA, fluoro polarized immuno assay; PETINIA, particle enhanced turbidimetric inhibition immunoassay; LOQ, limit of quantification; LOA, limit of agreement.

* Corresponding author at: University Hospitals Leuven, Laboratory Medicine, Herestraat 49, B-3000 Leuven, Belgium. Tel.: +32 16 34 70 00; fax: +32 16 34 79 31.
E-mail address: matthijsoyaert@telenet.be (M. Oyaert).

http://dx.doi.org/10.1016/j.cca.2014.12.012
0009-8981/

example, cross-reacting substances such as vancomycin degradation products have been described to interfere with some immunoassays [7]. Also, several structurally related compounds are formed during the production process and can be present in the isolated substance. A study of Diana et al. investigated the impurities present in a commercial vancomycin sample and found 15 different impurities, together composing 16.6% of the sample [7]. The *clinical impact* of these issues was recently suggested in a paper by Zhao et al., in which the predictive performances of different neonatal pharmacokinetic models for vancomycin administration were compared [8]. They found different predictive performances between different analytical methods for serum vancomycin concentrations, thereby highlighting that dosage individualization of vancomycin in neonates should not only consider patients' characteristics like body weight, but also the methods used to measure vancomycin [9]. Moreover, it remains often difficult to track the analytical details of the methods used to measure vancomycin in determining therapeutic intervals and target values [10], thereby shedding doubt on the applicability of the guidelines in specific hospital settings. Lastly, current guidelines use the total concentration of vancomycin (free and bound) for dosage adjustment [11], even though it is known that, as for most antibiotics [12], it is probably the free concentration that is critical for diffusion into infected areas [13,14]. Whether reported protein-binding percentages for vancomycin are stable and predictable when only looking at total vancomycin concentrations is part of an ongoing discussion. The same holds true for the added value of measuring free concentrations.

To tackle the above-mentioned limitations, a number of methods using mass spectrometry (LC–MS/MS) for the quantification of plasma total vancomycin concentration have been described. These methods, however, rely on internal standard (IS) compounds that are structurally not related to the target analyte (teicoplanin, atenolol, kanamycin-B) [15–18], use a labor-intensive sample preparation [5,16], or have very long runtimes [5].

It is known that the IS has a crucial role in compensating for sample specific matrix effects (MEs) in LC–MS/MS assays. As most studies relied on other, structurally and hence physically and chemically unrelated compounds as IS, it is not surprising that significantly different percentages of ME between vancomycin and IS (up to 50% difference [18]) have been described in published methods, shedding serious doubt on the quantification accuracy of clinical samples presenting with varying matrices. A recently published method tried to cope with this problem by synthesizing a homemade vancomycin derivative [5]. Although this method was intended as a reference method, the use of a homemade vancomycin derivative as IS is time-consuming and offers no workable solution for other groups trying to easily measure vancomyin with mass spectrometry. Moreover, this method has an analysis time of up to 20 min per sample [5].

The first aim of our study was to develop a novel LC–MS/MS method for measuring total vancomycin concentrations with acceptable runtimes and using an adequate IS. To chart between-method differences, we compared four frequently used immunoassays with our method. In addition, we investigated the clinical impact of the observed differences.

2. Materials and methods

2.1. Chemicals and solutions

Vancomycin HCl was purchased from Toronto Research Chemicals (Toronto, Canada). Vancomycin-des-leucine formiate was purchased from Alsachim (Strasbourg, France; formulation on request) and acetonitrile (LC–MS grade) from BioSolve (Valkenswaard, The Netherlands). HPLC-grade water was generated using a Milli-Q-water-purification system (Millipore, Molsheim, France). Pooled blank lithium heparin (Becton Dickinson, Franklin Lakes, USA) blood samples were collected from a healthy volunteer.

A stock solution of vancomyin in water at 4.0 mg/mL was prepared. Ten calibration standards at vancomycin concentrations of 0.6, 1.3, 2.5, 5.0, 10.0, 20.0, 30.0, 50.0, 75.0 and 100.0 mg/L were prepared by appropriate addition of stock solution to the blank plasma pool. An IS working solution of 5.0 mg/L vancomycin des-leucine in Milli-Q was used. In each routine analysis, four levels of quality control (QC) (3.0, 15.0, 30.0 and 75.0 mg/L) were analyzed. These were prepared by the appropriate addition of another (independently prepared and weighed) stock of vancomycin (4.0 mg/mL in water) to blank pooled plasma. QCs, calibration standards and IS working solution were stored at −20 °C until use.

2.2. Sample preparation and LC–MS/MS conditions

Lithium heparin blood samples were centrifuged for 10 min at 1912 *g*. 40 μL plasma was immediately vortexed with 40 μL IS working solution and 160 μL acetonitrile in glass tubes. After centrifugation (10 min at 16,100 *g*), 5 μL of supernatant was injected (auto-sampler) into the chromatographic system. Chromatographic separation was carried out on an Acquity UPLC separations module (Waters Ltd, Watford, UK). As analytical column, an Acquity UPLC BEH HILIC (100 mm × 2.1 mm; 1.7 μm, Waters Ltd, Watford, UK), maintained at 50 °C, was used with a Phenomenex C-18 guard column (100 mm × 4 mm, Torrance, CA, USA) as pre-column.

The mobile phase was a mixture of acetonitrile (buffer A) and water (buffer B) both containing 0.1% formic acid. A linear gradient starting from 95% buffer A descending to 40% buffer A at 2.50 min was applied. At 2.60 min, buffer A was set at 99% and kept till 4.00 min. From 4.00 to 5.00 min 95% buffer A was used to re-equilibrate for the next injection. The flow rate was set at 0.45 mL/min, the total runtime was 5.0 min. Mass spectrometric analysis was performed using a tandem mass spectrometer (Acquity TQD detector, Waters Ltd, Watford, UK) equipped with an electrospray ionization source operating in the electrospray-positive mode. The source and desolvation temperature were set at 150 °C and 500 °C, respectively. Nitrogen was used as desolvation gas and was set at a flow rate of 750 L/h. Capillary voltage was set at 3.5 kV, cone voltage at 20 V and collision energy at 20 eV. Vancomycin was detected by multiple reaction monitoring (MRM) with a dwell time of 0.085 s. The following MRM transitions were monitored: m/z 725.2 → 144.0 and 726.1 → 144.0 for vancomycin, and 662.1 → 144.0 for vancomycin des-leucine. Vancomycin was quantified by means of calibration to each run, using a weighted least square ($1/X^2$) regression in MassLynx software (Waters Ltd, Watford, UK) of the 10 calibration standards. For vancomycin the 2 MRMs were summed.

2.3. Analytical validation

Method imprecision was evaluated by analysis of four QC concentrations and three concentrations of patient samples on ten consecutive days [19]. A total imprecision of <15% was acceptable [20].

The limit of quantification (LOQ) was defined as the lowest analyte concentration with a signal to noise ratio (S/N) of more than 10, a coefficient of variation (CV) and accuracy within ±20% [21].

Linearity was evaluated by comparing if higher order equations give significantly better fits using Microsoft Excel Analyse-it software. To determine the amount of carry-over, we analyzed in the sequence HHHBBB, where H is the highest calibration standard and B is a blank. The percentage of carry-over was calculated with the formula 100 × (B1 − B3) / (H3 − B3) [19].

Accuracy was calculated from the QC samples (n = 4) in ten different runs as the percentage deviation from the theoretically added vancomycin concentration. An accuracy of <15% was accepted [20]. To the best of our knowledge, no reference plasma exists for vancomycin.

Freeze and thaw, short-term, and long-term stability of plasma samples were determined at three concentration levels. Freeze and thaw stability was tested by comparing freshly prepared samples to samples