Journal of Chromatography B, 951–952 (2014) 110–118

Contents lists available at ScienceDirect

Journal of Chromatography B

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

A simple quantitative method analysing amikacin, gentamicin, and vancomycin levels in human newborn plasma using ion-pair liquid chromatography/tandem mass spectrometry and its applicability to a clinical study

Yuma Bijleveld[a,*], Timo de Haan[b], Jan Toersche[a], Sona Jorjani[a], Johanna van der Lee[c], Floris Groenendaal[d], Peter Dijk[e], Arno van Heijst[f], Antonio W.D. Gavilanes[g], Rogier de Jonge[h], Koen P. Dijkman[i], Henrica van Straaten[j], Monique Rijken[k], Inge Zonnenberg[l], Filip Cools[m], Debbie Nuytemans[n], Ron Mathôt[a]

[a] Department of Pharmacy, Academic Medical Centre, Amsterdam, The Netherlands
[b] Department of Neonatology, Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands
[c] Department of Paediatric Clinical Epidemiology, Emma Children's Hospital, Academic Medical Center, University of Amsterdam, The Netherlands
[d] Department of Neonatology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands
[e] Department of Neonatology, University Medical Center Groningen, Groningen, The Netherlands
[f] Department of Neonatology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[g] Department of Neonatology, Maastricht University Medical Center, Maastricht, The Netherlands
[h] Department of Neonatology, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands
[i] Department of Neonatology, Maxima Medical Center Veldhoven, Veldhoven, The Netherlands
[j] Department of Neonatology, Isala Clinics, Zwolle, The Netherlands
[k] Department of Neonatology, Leiden University Medical Center, Leiden, The Netherlands
[l] Department of Neonatology, VU University Medical Center, Amsterdam, The Netherlands
[m] Department of Neonatology, Vrije Universiteit Brussel, Brussels, Belgium
[n] PharmaCool Study, The Netherlands

ARTICLE INFO

Article history:
Received 11 October 2013
Received in revised form 16 January 2014
Accepted 22 January 2014
Available online 30 January 2014

Keywords:
LC–MS/MS
Amikacin
Gentamicin
Vancomycin
Hypothermia
Neonates

ABSTRACT

Neuroprotective controlled therapeutic hypothermia is the standard of care for newborns suffering perinatal asphyxia. Antibiotic drugs, such as amikacin, gentamicin, and vancomycin are frequently administered during controlled hypothermia, which possibly alters their pharmacokinetic (PK) and pharmacodynamic (PD) profiles. In order to examine this effect an LC–MS/MS method for the simultaneous quantification of amikacin, the major gentamicin components (gentamicin C, C1a and C2), and vancomycin in plasma was developed. In 25 μL plasma proteins were precipitated with trichloroacetic acid (TCA) and detection of the components was achieved using ion-pair reversed phase chromatography coupled with electrospray ionization tandem mass spectrometry. The chromatographic runtime was 7.5 min per sample. Calibration standards were prepared over a range of 0.3–50 mg L^{-1} for amikacin and gentamicin and 1.0–100 mg L^{-1} for vancomycin. At LLOQ accuracy was between 103 and 120% and imprecision was less than 19%. For concentrations above LLOQ accuracy ranged from 98% to 102% and imprecision was less than 6%. Process efficiency, ionization efficiency, and recovery were acceptable. Samples and stock solutions were stable during the time periods and at the different temperatures examined. The applicability of the method was shown by analysing plasma samples from 3 neonatal patients. The developed method allows accurate and precise simultaneous quantification of amikacin, gentamicin, and vancomycin in a small volume (25 μL) of plasma.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

In 2008 controlled therapeutic hypothermia was introduced in the Netherlands as the standard of neuroprotective care for newborns suffering hypoxic ischaemic encephalopathy (HIE) following perinatal asphyxia. Recent randomized controlled trials and

* Corresponding author at: Academic Medical Centre, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands. Tel.: +31 205669111; fax: +31 20 6972291.
E-mail address: y.a.bijleveld@amc.nl (Y. Bijleveld).

1570-0232/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2014.01.035

meta-analyses demonstrated that therapeutic hypothermia significantly improves the long term neurodevelopmental outcome in these cases [1]. As part of the intensive care treatment, antibiotic drugs, such as amikacin, gentamicin, and vancomycin are frequently administered. Although the action and metabolism of these drugs have both been (extensively) investigated in neonates [2,3] evidence is now emerging demonstrating possible effects of hypothermia on the pharmacokinetics (PK) of commonly used drugs [4–8]. In order to investigate the effects of hypothermia on the PK profile of antiepileptic, sedative, analgesic, and antibiotic drugs a multicentre observational cohort study (the PharmaCool study (ZONMW grant number 11-32010-01; NTR number: NTR TC 2529)) is currently being conducted in the Netherlands [9]. As the preservation of blood volume is essential in neonatal intensive care patients the development of analytical methods requiring only small blood volumes to measure the concentration of drugs is necessary. Furthermore, although gentamicin and amikacin are clinically not co-administered, simultaneous analysis of these antibiotics is required as all samples of the Pharmacool trial are analysed centrally and the participating centres apply varying antibiotic regimens. For the PharmaCool study such a method was developed for the above mentioned antibiotic drugs and will be presented here.

Sensitive and precise quantitation of aminoglycosides and vancomycin in biological matrices is challenging due to their high polarity and lack of any notable chromophores or fluorophores. Furthermore, gentamicin consists of the gentamicin C-complex (gentamicin C1, C1a, C2, C2a, and C2b) and minor co-produced polar gentamicin components. The components of the gentamicin C-complex differ by methylation and stereochemistry on the 6′-C position [10]. The chemical structures are shown in Fig. 1. Although gentamicin C2a and C2b have been considered as minor components, they have been shown to take up a substantial proportion of the gentamicin C-complex [11–13] and should therefore be taken into account. The content of the components in gentamicin sulphate can vary and are defined by the United States Pharmacopeia; the content of gentamicin C_1 should be between 25% and 50%, the content of gentamicin C1a between 10% and 35%, and the sum of the contents of gentamicin C2a and gentamicin C2 between 25% and 55%.

Commercially available automated immunoassays (e.g. enzyme multiplied immunoassay technique (Enzyme Multiplied Immunoassay Technique (EMIT) and Fluorescence Polarization Immunoassay (FPIA)) are widely used to quantify drugs in plasma or serum for therapeutic drug monitoring [14,15]. Although immunoassays allow easy and rapid analysis and offer adequate accuracy and precision for TDM analysis they are less suitable for pharmacokinetic studies in which high specificity, sensitivity, accuracy, and precision are required over a wide concentration range. Furthermore, analysing metabolites and different compounds (such as the components of the gentamicin complex) individually is important as their PK/PD properties can differ. Chromatographic methods do meet the above mentioned requirements for pharmacokinetic studies. Several HPLC methods have been reported for measuring gentamicin [12,16–18], amikacin [19–23], and vancomycin [24–31] in different matrices. In order to chromatographically separate the different gentamicin components by HPLC, these methods require long chromatographic runtimes. Also, because of the mentioned absence of any notable chromo- or fluorophores, sample preparation mainly consisted of time consuming extraction and derivatization steps using relatively high volumes of sample (0.5–1 mL). Specifically in the neonatal patient population small samples sizes are preferably used. Liquid chromatography in combination with tandem mass spectrometry (LC–MS/MS) can be used to determine these drugs in only small amounts of sample with high precision, sensitivity, and selectivity without the need for extraction and derivatization steps. Several LC–MS/MS methods have been reported for the analysis of gentamicin, amikacin, or vancomycin in animal tissue [32–38], animal plasma or serum [35,38–40], in hospital waste water [41], in human serum or urine [38,42,43], and in pharmaceutical preparations [44], however, these methods use relatively large sample volumes or use complicated extraction procedures. Furthermore, they did not include the analysis of vancomycin in human blood samples. Also, to the best of the author's knowledge, no validated LC–MS/MS method for the simultaneous quantification of amikacin, gentamicin, and vancomycin in human plasma or serum have been reported.

The aim of the present study was to develop and validate a sensitive method for the quantification of the components of the gentamicin C-complex, amikacin, and vancomycin with LC–MS/MS using simple protein precipitation and ion pair chromatography with perfluoropentanoic acid. The performance of the developed LC–MS/MS method was compared to Fluorescence Polarization Immunoassay (FPIA) for gentamicin and vancomycin by analysing clinical samples from adult patients. Finally, the applicability of the method is shown by presenting the data of 3 patients participating in the PharmaCool study.

2. Materials and methods

2.1. Reagents and chemicals

The reference standards amikacin, gentamicin sulphate (C1 26.4%, C1a 24.5%, sum C2 and C2a 42.5%), kanamycin B (internal standard), and vancomycin, all USP/PhEur quality, were obtained from Sigma–Aldrich (Steinheim, Germany). The reagents perfluoropentanoic acid, trichloroacetic acid, formic acid, and ammonium acetate, all of at least pro analysis quality, were also obtained from Sigma–Aldrich. Acetonitril, used for HPLC, was obtained in HPLC supra gradient quality from Biosolve (Lexington, MA, USA). Water was purified and deionized using an ELGA purelab Optron Q (Veolia Water; Saint Maurice, France). Drug free, non sterile, K_2 EDTA human pooled plasma, used for preparation of the calibration and QC samples, was obtained from Equitech-Bio (Kerrville, TX, USA).

2.2. Instrumentation

The LC–MS setup comprised a Thermo Scientific Surveyor LC (Waltham, MA, USA) system coupled to a Maylab Mistraswitch column oven (Spark Holland, Netherlands, Emmen), and a Thermo Scientific TSQ Quantum Access MS system with an ESI source. The Xcalibur 2.0.7 SP1 (Thermo Scientific) software package was used for controlling the LC–MS system and for data processing. The immunoassays were performed using the FPIA Gentamicin and Vancomycin II assays for the AxSYM Immunoassay Analyzer (Abbott Laboratories, Chicago, IL, USA).

2.3. LC–MS/MS conditions

For chromatographic separation a ternary gradient was applied using A: purified H_2O, B: acetonitril 100%, and C: perfluoropentanoic acid (200 mM)/ammonium acetate (130 mM) in purified H_2O. As LC column a Thermo Scientific Hypurity Aquastar, 100 mm in length and with a 2.1 mm internal diameter and 5 μm particle size was used. The chromatographic gradient profile: C was set at 5% throughout the run; at the start of the gradient A was set at 90% and B at 5%. A 5 min linear gradient was applied to 95% B and 0% A. The gradient was brought back to the starting conditions in the next 0.1 min and re-equilibrated for 2.4 min giving a total chromatographic run time of 7.5 min. The flow rate was 0.400 mL min^{-1}. To minimize carry-over effects, the LC injection system was rinsed with 20% formic acid in water after

Download English Version:

https://daneshyari.com/en/article/7617925

Download Persian Version:

https://daneshyari.com/article/7617925

Daneshyari.com