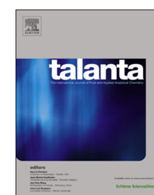
Talanta 149 (2016) 142–148

Contents lists available at ScienceDirect

Talanta

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



Quantitative determination of the enantiomers of methadone in human plasma and saliva by chiral column chromatography coupled with mass spectrometric detection

Rani George [a,*], Michael Lobb [b], Alison Haywood [a,c], Sohil Khan [a,c], Janet Hardy [c,d], Phillip Good [c,d,e], Stefanie Hennig [f], Ross Norris [a,c,g,h]

[a] School of Pharmacy, Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia
[b] Mater Pathology Services, South Brisbane, QLD, Australia
[c] Mater Research Institute-The University of Queensland, Brisbane, QLD, Australia
[d] Department of Palliative and Supportive Care, Mater Health Services, Brisbane, QLD, Australia
[e] Palliative Care, St. Vincent's Private Hospital, Brisbane, QLD, Australia
[f] Pharmacy Australia Centre of Excellence, The University of Queensland, Brisbane, QLD, Australia
[g] SydPath, St Vincent's Hospital, Sydney, NSW, Australia
[h] St Vincent's Clinical School, University of New South Wales, Australia

ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form
17 November 2015
Accepted 17 November 2015
Available online 19 November 2015

Keywords:
Methadone
Enantiomers
HPLC–MS/MS
Plasma
Saliva
Adsorption

ABSTRACT

Methadone is a potent lipophilic synthetic opioid that is effective in the treatment of cancer pain and perceived benefit in difficult pain control scenarios (especially in cases of neuropathic pain). The use of methadone in clinical practice is challenging however, due to the narrow therapeutic window and large inter- and intra-individual variability in therapeutic response. Quantitation of the enantiomers d- and l-methadone (d- and l-MTD) in plasma and saliva provides a basis for studying its pharmacokinetics in patients with cancer and for monitoring efficacy, toxicity and side-effects. This assay involves quantitation of the enantiomers of methadone using their respective deuterated internal standards, in plasma and saliva matrices with no impact of ion suppression in either matrix. The analytical recoveries of d- and l-MTD from the saliva collection devices (Salivette®) are optimised in this novel method with an accurate and simple extraction method employing dichloromethane. Optimal enantioselective separations were achieved using an α1-acid glycoprotein chiral stationary phase and triple quadrupole tandem mass spectrometer. Linearity was demonstrated over 0.05–1000 μg/L for both enantiomers in plasma and in saliva with correlation coefficients greater than 0.998. The lower limit of quantitation (LLOQ) was determined to be 0.1 μg/L in plasma and saliva for d- and l-MTD. Accuracy of the method ranges from 100% to 106% even at the LLOQ and total precision, expressed as the coefficient of variation, was between 0.2% and 4.4% for both analytes in both matrices. A simple one step extraction procedure resulted in recoveries greater than 95% for both analytes, at concentrations as low as 0.5 μg/L, from the Salivette®. The validated method was applied successfully in 14 paired plasma and saliva samples obtained from adult patients with cancer pain receiving methadone.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Moderate to severe pain in cancer is common and affects 70–80% of patients with advanced malignancy. Methadone (6-(dimethylamino)-4, 4-diphenylheptan-3-one) is a synthetic μ opioid receptor agonist that is effective in treating cancer pain [1]. It is administered as a racemic mixture of two enantiomers with distinct actions and elimination profiles. The (R)- or l-methadone enantiomer is more potent than the (S)- or d-isomer by a factor of eight to 50 and is believed to be almost entirely responsible for the analgesic properties [2]. Clinical studies have indicated that methadone metabolism and disposition is enantioselective [3,4]. This

Abbreviations: ANOVA, analysis of variance; CDB, cotton dental bud; Conc, concentration; CV, coefficient of variation; d,l-MTD, racemic methadone; d,l-MTD, D3-deuterated racemic methadone; ESI, electrospray ionisation; HPLC, MS/MS–liquid chromatography coupled with tandem mass spectrometry; IS, internal standard; LLOQ, lower limit of quantitation; MMT, methadone maintenance treatment; MRM, multiple reaction monitoring; MTD, methadone; PCI, post-column infusion; PK, pharmacokinetics; QC, quality control

* Correspondence to: School of Pharmacy, Gold Coast campus, Griffith University, QLD 4222, Australia.
E-mail address: ranijee21@gmail.com (R. George).

http://dx.doi.org/10.1016/j.talanta.2015.11.044
0039-9140/© 2015 Elsevier B.V. All rights reserved.

suggests that the d- and l-enantiomers of methadone have different pharmacokinetic (PK) profiles. Studies on the CYP involvement in stereo-selective metabolism of methadone suggest that CYP2B6 is the main determinant of enantioselective metabolism [5,6]. The free fraction of l-methadone is greater, yet has a slower clearance rate relative to d-methadone [3]. It is, therefore, useful to quantify both enantiomers individually when investigating the PK of methadone [7].

In addition to cancer pain, methadone is also used in the management of other forms of acute and chronic pain scenarios including neuropathic pain and in opioid replacement therapy. The doses used in methadone maintenance treatment (MMT) are generally much higher than those used for pain management. A narrow therapeutic window between pain control and toxicity as well as large inter-individual variation in the pharmacokinetics and pharmacodynamics of methadone challenge the management of severe pain with this drug [8,9]. If opioid dose individualisation, based on PK modelling from saliva or plasma concentrations, without the need for dose titration was feasible, it would represent a major breakthrough in the use of methadone in cancer pain. This would be a significant step forward in improving pain control in cancer patients on a worldwide basis, as methadone is one of the least expensive opioids currently available. In addition, personalised therapy would facilitate effective methadone dosing and pain control without compromising the patient's safety. Moreover, a convenient assay could facilitate effective and safe individualised conversion schedules for methadone.

Most new bioanalytical methods for drug assays utilise high performance liquid chromatography coupled with mass spectrometry (HPLC–MS/MS) which permits simultaneous analysis of multiple, non-volatile, polar and/or high molecular weight compounds in various biological matrices even with low sample volume. The use of saliva in therapeutic drug monitoring has increased over the last four decades for anticonvulsants, analgesics and opioids [10–12]. Strong correlations between plasma and saliva concentrations for analgesics such as paracetamol [10] and hydromorphone [13] have been described. Other opioids including codeine [14], diamorphine [11], methadone (for methadone maintenance treatment) [12], morphine [15], dihydrocodeine [16], oxycodone [17], and fentanyl [18], have also been analysed to investigate saliva/plasma (*S*/*P*) ratios of drug concentration. Saliva sampling is non-invasive and painless and does not require specially trained personnel. In studies involving patients with advanced malignant disease, this method of sample collection, avoids reluctance on the part of health professionals to subject their patients to venesection. Though Shiran et al. [10] reported insignificant correlation for saliva and plasma concentrations of methadone among a MMT patient population, Wolff et al. [19] reported a positive correlation ($r=0.81$) between paired saliva and plasma methadone concentrations in 21 patients receiving MMT. The enantiomeric ratio of methadone in saliva has been found to have good correlation with the d/l ratio in serum [20]. Methadone's lipophilicity could contribute to passive diffusion into saliva and better reflect either free or total plasma concentration. Despite the advantage of saliva sampling, there are challenges involved with sample analysis, including low sample volumes (associated with xerostomia in cancer patients) and adsorption of methadone to sample collection devices. The nonspecific adsorption of methadone to the cotton dental bud (CDB) has resulted in recoveries of $\leq 70\%$ [21,22]. To date, there have been no studies addressing this problem. For quantitative purposes, accurate determination of concentration is essential [23]. In a recent study, involving quantitation of fentanyl in saliva samples, adsorption to Salivette® was addressed by an extraction procedure involving multiple steps of extraction [18].

Several hyphenated analytical methods have been proposed for the analysis of methadone enantiomers in matrices including plasma, serum, saliva, whole blood, liver microsomes and urine [24–34]. All of these methods were developed to assay methadone enantiomers in plasma for methadone maintenance patients or toxicological studies. HPLC-MS/MS methods to quantitate methadone enantiomers in cancer patients especially in both matrices (plasma and saliva) samples are lacking. The aim of this study was therefore to develop and validate a simple, simultaneous HPLC–MS/MS method to quantify the d- and l- enantiomers of methadone in plasma and saliva to study the pharmacokinetics of methadone in cancer patients. Absolute chromatographic separation was achieved by isocratic elution and an extended run time assured repeatable results and ruggedness of the assay. Multiple reaction monitoring (MRM) mode was used in mass spectrometer which added specificity to the assay as compared to previous methods, without any loss in sensitivity (lower limit of quantitation (LLOQ)=0.1 μg/L). Deuterated internal standards for each analyte were used to correct for any loss in sensitivity to ion suppression. However, the evaluation of ion suppression in both the matrices with multiple samples confirmed no interference throughout the entire chromatographic run. This assay method utilised protein precipitation for plasma samples, and a simple, direct and validated extraction of the analytes from the saliva collection device (Salivette®), overcoming the adsorption of methadone to the Salivette®, including the cotton dental bud (CDB) with recoveries approaching 100% even with very low sample volumes.

2. Materials and methods

2.1. Chemicals and reagents

Racemic methadone (d,l-MTD) and deuterated racemic methadone (d,l-MTD-D3) were obtained from Cerilliant®, Round Rock, Texas, USA. The pure enantiomers, d- and l-MTD, were obtained from Ultrafine Chemicals (Manchester, UK) by custom synthesis. HPLC grade acetonitrile, dichloromethane and isopropyl alcohol were obtained from Merck, New Jersey, USA. Ammonium acetate and Ammonium hydroxide were obtained from Scharlau Chemie, Spain and Sigma Aldrich Cheme, Germany. 18 MΩ water was obtained from a Labmate water purification system (Aquacure, Brisbane, Australia). Salivettes® were obtained from Sarstedt, Nümbrecht, Germany. The blank plasma samples were obtained from the Pathology Services, Mater Health Services, Brisbane and the blank saliva samples were obtained from volunteer staff within the laboratory.

2.2. HPLC–MS/MS instrumentation and conditions

Samples were analysed using a Shimadzu HPLC system (DGU-20A3 Degasser; LC-20 AD Liquid Chromatography; CBM-20A Communication Module; SIL-20AC Autosampler) (Nakagyo-Ku, Koyoto, Japan) coupled to an API 3200 tandem mass spectrometer (AB SCIEX, Mount Waverly, Victoria, Australia). Analyst software version 1.4.2 from AB SCIEX was used for data acquisition and analysis. The electrospray ion source (ESI) was operated in positive ion mode, employing MRM with optimised declustering potential, entrance potential, collision energy and collision cell exit potential as reported in Table 1. Nitrogen was used as the source gas. Curtain gas pressure was maintained at 30 psi, collision gas at 5 psi, ion source gas 1 (GS-1) and ion source gas 2 (GS-2) were maintained at 60 and 70 psi respectively. Ion spray voltage was maintained at 5500 V and drying gas temperature at 750 °C. For chromatographic separation of the enantiomers, a chiral α-acid glycoprotein (AGP), 5 μ, 100 × 4.0 mm ID column (ChromTech, Hagersten,

Download English Version:

https://daneshyari.com/en/article/1241815

Download Persian Version:

https://daneshyari.com/article/1241815

Daneshyari.com