Journal of Chromatography B, 1015 (2016) 173–180

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Chiral analysis of carvedilol and its metabolites hydroxyphenyl carvedilol and O-desmethyl carvedilol in human plasma by liquid chromatography-tandem mass spectrometry: Application to a clinical pharmacokinetic study

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ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form 15 February 2016
Accepted 17 February 2016
Available online 23 February 2016

Keywords:
Carvedilol
Enantiomers
Pharmacokinetics
Metabolism
Plasma
Diabetes

ABSTRACT

Carvedilol is an antihypertensive drug, which is available in clinical practice as a racemic mixture. (S)-(−)-carvedilol is a β- and α1-adrenergic antagonist, while (R)-(+)-carvedilol only acts as an α1-adrenergic antagonist. Carvedilol is metabolized mainly by glucuronidation and, to a lesser extent, by CYP2D6 to hydroxyphenyl carvedilol (OHC) and by CYP2C9 to O-desmethyl carvedilol (DMC). This study describes the development and validation of a method for the sequential analysis of the enantiomers of carvedilol, OHC and DMC in plasma using a Chirobiotic® V chiral-phase column coupled to an LC–MS/MS system. The method was linear in the range of 0.05–100, 0.05–10 and 0.02–10 ng/mL for the carvedilol, OHC and DMC enantiomers, respectively. Application of the method to the investigation of a patient with type 2 diabetes *mellitus* treated with a single oral dose of 25 mg racemic carvedilol showed plasma accumulation of the (R)-(+)-carvedilol, (R)-(+)-DMC and (R)-(+)-OHC enantiomers. These results suggest that plasma accumulation of (R)-(+)-carvedilol cannot be explained by its oxidative metabolism.

1. Introduction

Carvedilol, a third-generation β-adrenergic antagonist, is used for the treatment of hypertension, angina pectoris, cardiac arrhythmias, and congestive heart failure [1,2]. Carvedilol is available in clinical practice as racemic mixture of the (S)-(−) and (R)-(+)-carvedilol enantiomers, which have distinct pharmacokinetic and pharmacodynamic properties. (S)-(−)-carvedilol is an ∝1- and β -adrenergic antagonist, while (R)-(+)-carvedilol only exhibits ∝1 antagonism [3]. The clearance of (S)-(−)-carvedilol (662 mL/min) is greater than that of (R)-(+)-carvedilol (605 mL/min). The oral bioavailability of (R)-(+)-carvedilol is about twice that of its antipode (31.1% for (R)-(+) and 15.1% for (S)-(−)-carvedilol) [4] and the plasma concentrations of (R)-(+)-carvedilol are approximately three times higher than those of (S)-(−)-carvedilol [5].

Carvedilol is mainly eliminated by conjugation with glucuronic acid [6,7] and by oxidation reactions to hydroxyphenyl carvedilol (OHC), (4′-hydroxyphenyl carvedilol and 5′-hydroxyphenyl carvedilol), and to O-desmethyl carvedilol (DMC) [5,8,9]. Studies using human liver microsomes have shown that (S)-(−)-carvedilol is metabolized faster than the (R)-(+)-carvedilol through oxidative reactions, although the same CYP enzymes are involved in the metabolism of both enantiomers. CYP2D6 is the main enzyme responsible for the formation of 4-OHC and 5-OHC, although CYP2E1, CYP2C9 and CYP3A4 also contribute in the production of these metabolites. CYP2C9 is responsible for the formation of DMC with additional contribution from CYP2D6, CYP1A2 and CYP2E1 [8]. In human liver microsomes, glucuronidation of (S)-(−)-carvedilol is also faster than (R)-(+)-carvedilol in the racemate, but true activities of both glucuronidations are approximately the same for the pure enantiomers. UGT1A1 and UGT2B7 are main responsible for the glucuronidation of (S)-(−) and (R)-(+)-carvedilol in human intestinal microsomes [10].

The sequential analysis of carvedilol and its metabolites 4-OHC and DMC is only described for the enantiomeric mixture in rat

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http://dx.doi.org/10.1016/j.jchromb.2016.02.028
1570-0232/

plasma using UPLC–MS/MS, with quantification limits of 0.5 ng/mL for carvedilol and of 0.05 ng/mL for 4-OHC and DMC [11]. The enantioselective analysis of carvedilol and its metabolite DMC in human plasma has only been reported by Eisenberg et al. [12] with quantification limits of 0.625 ng of each enantiomer/mL, whereas the enantioselective analysis of carvedilol and its metabolite 4-OHC has only been reported by Furlong et al. [13] with quantification limits of 0.2 and 0.02 ng of each enantiomer/mL plasma, respectively.

Therefore, the present study reports for the first time the development, validation and clinical application of a method for sequential analysis of the enantiomers of carvedilol, OHC (4-OHC + 5-OHC) and DMC in human plasma using a chiral-phase column coupled to an LC–MS/MS system. The method was applied to investigate the enantioselective carvedilol metabolism in one patient with type 2 diabetes *mellitus* who was treated with a single oral dose of racemic carvedilol.

2. Methods

2.1. Standard solutions and quality control samples

The stock solutions were prepared using carvedilol standard purchased from Sigma (purity: 99.1%; St. Louis, MO, USA), and metoprolol, DMC and 4-OHC standards purchased from Toronto Research Chemicals (purity: 98%; TRC, North York, Canada). The carvedilol stock solution was prepared at a concentration of 100 μg of each enantiomer/mL methanol (J.T. Backer, Mexico City, Mexico) and the stock solutions of OHC and DMC at a concentration of 10 μg of each enantiomer/mL methanol. Using these stock solutions, standard solutions diluted in methanol were prepared at the following concentrations: 4000, 2000, 400, 200, 40, 20, 8, 4 and 2 ng of each carvedilol enantiomer/mL; 400, 200, 40, 20, 4, 2, 0.8, 0.4 and 0.2 ng of each OHC and DMC enantiomer/mL. The standard solution of racemic metoprolol used as internal standard (IS) was prepared at a concentration of 10 μg/mL in methanol.

2.2. Chromatographic analysis

The ACQUITY UPLC® H-Class chromatographic system coupled to a Xevo TQ-S® triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with Zspray® electrospray ionization (ESI) was used for analysis. The devices were controlled with the MassLynx 4.1 program (Waters Corp.).

The carvedilol, OHC and DMC enantiomers were separated on an Astec Chirobiotic® V column (0.46 × 25 cm, 5-μm particle size; Supelco, Bellefonte, PA, USA) maintained at a temperature of 24 °C. The mobile phase consisted of 99% of mixture A and 1% of mixture B and was eluted at a flow rate of 0.8 mL/min. Mixture A consisted of 60% ethanol (purity: 99.9%; Panreac, Barcelona, Spain) and 40% methanol (purity: 99.95%; J.T. Backer, Mexico City, Mexico) plus 1.8 mL/L glacial acetic acid (purity: 99.7%; Synth, Diadema, Brazil) and 0.2 mL/L diethylamine (purity: 100%; J.T. Baker, Phillipsburg, NJ, USA). Mixture B consisted of purified water plus 1.8 mL/L acetic acid and 0.2 mL/L diethylamine. The water was purified using the Synergy® UV system (Millipore, Molsheim, France).

The operating conditions of the Xevo TQ-S® system were optimized in the multiple reaction-monitoring mode by direct infusion of the standard solutions of carvedilol, OHC, DMC and metoprolol (IS) into the mobile phase. The capillary voltage was set at 3.30 kV and the source and desolvation temperatures at 150 °C and 550 °C, respectively. The collision energy was 15, 20, 22 and 25 V for metoprolol, DMC, OHC and carvedilol, respectively. The cone voltages were 50 and 30 V for metoprolol and DMC, respectively, and 35 V for OHC and carvedilol. The desolvation and cone gas (nitrogen) flow rates were 1000 and 150 L/h, respectively, and the flow rate of the collision gas (argon) was maintained at 0.19 mL/min. Protonated carvedilol, OHC, DMC and metoprolol and their respective product ions were monitored at transitions of m/z 407 > 100, 423 > 222, 393 > 210 and 268 > 116, respectively.

Table 1
Quality controls concentrations of carvedilol enantiomers and their metabolites.

Sample	carvedilol	OHC	DMC
	concentration (ng/mL)		
LLOQC	0.05	0.05	0.02
LQC	0.08	0.08	0.04
MQC	4	0.4	0.4
HQC	80	8	8
DQC[a]	400	40	40

LLOQC, LQC, MQC, HQC e DQC are the quality controls of Lower limit of quantification, low, medium and high concentrations and dilution.

[a] DQC: the samples were diluted with blank plasma in the proportion 1/5 immediately before the sample preparation. OHC: hydroxyphenylcarvedilol. DMC: O-desmethylcarvedilol. The samples were prepared as described in Section 2.3 sample preparation and 70 μL were injected.

2.3. Sample preparation

Plasma aliquots (1 mL) were added to 25 μL of the IS solution and 5 mL di-isopropyl ether (purity: 98.5%; Sigma-Aldrich, St. Louis, MO, USA). The samples were shaken for 50 min in a mechanical horizontal shaker at 220 ± 10 cycles/min (MA 139/CFT, Marconi, Piracicaba, Brazil) and centrifuged at 1800 *g* for 10 min at 5 °C in a refrigerated centrifuge (Himac CF 8DL, Hitachi, Tokyo, Japan). Volumes of 4 mL of the organic phase were separated and evaporated to dryness in a vacuum evaporator (Christ RVC 2–25 CD and Christ CT 04–50 SR, Osterode am Harz, Germany). The residues were resuspended in 100 μL of mixture A and 70 μL was injected into a Chirobiotic® V column. The processed samples were kept in the automatic injector at 12 °C.

2.4. Determination of elution order

The standard solutions in methanol at concentrations of 2, 4 and 3 μg/mL, respectively for (R)-(+)-carvedilol, (R)-(+)-DMC and (R)-(+)-OHC were prepared with standards purchased from Toronto Research Chemicals (purity: 98%; TRC, North York, Canada). Aliquots (25 μL) of the standard solutions were evaporated to dryness and submitted to chromatographic analysis. The retention times were compared to those obtained by analyzing the racemic standards in methanol.

2.5. Validation of the method

The analytical method was validated according to the recommendations of the US-FDA guidance for industry [14] for the validation of analytical methods and of the guidelines of the European Medicines Agency for the validation of bioanalytical methods [15].

The calibration curves were prepared in triplicate using 1 mL aliquots of blank plasma spiked with 25 μL of each standard solution of carvedilol and its metabolites, including a blank sample and a zero sample. The calibration curves were constructed in the range of 0.05–100 ng of each carvedilol enantiomer, 0.05–10 ng of each OHC enantiomer, and 0.02–10 ng of each DMC enantiomer per mL plasma.

Quality control samples were prepared from the stock solutions in blank plasma, corresponding to the lower limit of quantification (LLOQC), low (LQC), medium (MQC) and high (HQC) concentrations, and dilution quality control (DQC). The concentrations are shown