Journal of Chromatography A, 1439 (2016) 137–143

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Quantitation of repaglinide and metabolites in mouse whole-body thin tissue sections using droplet-based liquid microjunction surface sampling-high-performance liquid chromatography-electrospray ionization tandem mass spectrometry☆

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ARTICLE INFO

Article history:
Received 19 July 2015
Received in revised form 7 October 2015
Accepted 25 October 2015
Available online 3 November 2015

Keywords:
Liquid microjunction
Droplet-based liquid extraction
Quantitation
Autosampler
Spatial distribution
Repaglinide

ABSTRACT

Herein, quantitation aspects of a fully automated autosampler/HPLC-MS/MS system applied for unattended droplet-based surface sampling of repaglinide dosed thin tissue sections with subsequent HPLC separation and mass spectrometric analysis of parent drug and various drug metabolites were studied. Major organs (brain, lung, liver, kidney and muscle) from whole-body thin tissue sections and corresponding organ homogenates prepared from repaglinide dosed mice were sampled by surface sampling and by bulk extraction, respectively, and analyzed by HPLC-MS/MS. A semi-quantitative agreement between data obtained by surface sampling and that by employing organ homogenate extraction was observed. Drug concentrations obtained by the two methods followed the same patterns for post-dose time points (0.25, 0.5, 1 and 2 h). Drug amounts determined in the specific tissues was typically higher when analyzing extracts from the organ homogenates. In addition, relative comparison of the levels of individual metabolites between the two analytical methods also revealed good semi-quantitative agreement.

1. Introduction

Tissue distribution studies in animals are an indispensable part of the drug discovery and development pipeline [1]. The information these studies provide is important for the understanding and prediction of pharmacological target engagement, toxicological target tissue exposure and drug disposition.

A number of different technologies are currently used to measure drug and metabolite distributions directly from tissues. For example, quantitative whole-body autoradiography (QWBA) [2,3] and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [4–6] are used to analyze drug dosed thin tissue sections. High performance liquid chromatography-mass spectrometry (HPLC-MS) is used to measure these species in whole organ tissue homogenates [1] or in punched thin tissue sections [7]. However, each of these approaches has analytical shortcomings. QWBA requires the use of an often expensive radiolabeled drug and it is unable to distinguish between parent drug and its metabolites. MALDI-MS analysis requires coating the sample with a chemical matrix prior to MS analysis and it is not well-suited to detect fragile molecules like phase II metabolites [5]. In addition, MALDI lacks the capability to transfer the ablated material for post-sampling processing such as an HPLC separation. Post-sampling processing is advantageous in dealing with complex sample matrices, multiple analytes, and/or for separation and identification of isomeric compounds not distinguishable by MS methods alone. Such is the case when employing HPLC-MS analysis with the above mentioned punched samples or whole organ tissue homogenates. The use of HPLC-MS also enables absolute analyte quantitation. However, HPLC-MS analysis of punched samples and tissue homogenates typically requires extensive sample handling, extraction and cleanup steps. While an unattended workflow might be employed for this multi-step process using conventional

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http://dx.doi.org/10.1016/j.chroma.2015.10.093
0021-9673/

laboratory preparative schemes and robots, simplifying and speeding up the analytical workflow is desirable.

Liquid extraction-based surface sampling probes [8] have been increasingly applied with tandem MS (MS/MS) for sampling and detecting drugs [9] and phase I, [10–12] and phase II metabolites [13–20] directly from animal thin tissue sections. The rise in interest in this spatially resolved surface sampling/analysis methodology is due, in part, to the enhanced sensitivity compared to other ambient surface sampling techniques [21,22], and to the integration with HPLC-MS/MS systems [16–20,23,24]. Comparative relative quantitation of drug and metabolites distributions determined by the liquid extraction-based surface sampling probes, by QWBA and HPLC-MS [17], and QWBA and radio thin-layer chromatography [18] analyses have shown good agreement.

Repaglinide is an insulin secretagogue used in the treatment of type 2 diabetes. In humans, repaglinide is rapidly absorbed and cleared with a time to maximum serum concentration of 0.5 h (oral solution) and a terminal half life of 0.53 h. A dicarboxylic acid metabolite, M2, is a major metabolite in humans. Other metabolites include hydroxylated metabolite M4 and an acyl glucuronide metabolite M7. It is of our interest to study the tissue distribution of repaglinide in a time scale that is consistent with its rapid absorption and elimination [25,26]. In this paper, we quantitate repaglinide and its metabolites in organs of whole-body mice thin tissue sections using a fully automated droplet-based liquid microjunction surface sampling probe (LMJSSP)-HPLC-MS/MS system [16–20,27] and compare those results with quantitation using HPLC-MS/MS analysis of bulk extracts from organ homogenates. Absolute drug concentrations obtained by the two methods differed but showed good relative agreement with concentrations determined generally higher with the organ homogenate extracts. Reasons for differences observed in concentration values and strategies to correct for them are discussed in detail.

2. Experimental

2.1. Reagents and materials

Repaglinide (cpd 1) and Cremophor® RH 40 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Repaglinide-d5 (cpd 1a) was purchased from Toronto Research Chemicals (North York, Ontario, Canada). Blank BALB/c mouse plasma was purchased from BioreclamationIVT (Westbury, NY, USA). All organic solvents and water were of HPLC grade or better and purchased from Sigma Aldrich.

2.2. Sample preparation

2.2.1. Mouse dosing

All animals used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals [28]. Before the study initiation, the study protocol was approved by the Institutional Animal Care and Use Committee. Male BALB/c mice ($n = 4$, 18–25 g body weight) were purchased from Hilltop Laboratories (Scottdale, PA, USA). Animals were fasted overnight (at least 12 h) prior to, and throughout the study following dose administration. Each mouse received a single oral dose of repaglinide at 1 mg/kg as a solution in 25/75 (w/w) Cremophor® RH 40/water. One mouse per time point was euthanized by deeply anesthetizing at 0.25, 0.5, 1 and 2 h post-dose. All carcasses were frozen at −70 °C dry ice/ethanol bath and stored at −80 °C until required for embedding.

2.2.2. Preparation of whole-body thin tissue sections for surface sampling analysis

An individual mouse carcass in a sagittal position was embedded in a chilled 2% carboxymethylcellulose matrix and mounted on a microtome stage maintained in a dry ice/ethanol bath at −70 °C. Whole-body sagittal tissue sections of 50 μm thickness were collected on 845 Scotch® tape (3M, St. Paul, MN, USA) using a cryomicrotome (model Leica CM3600, Leica Microsystems Inc., Bannockburn, IL, USA) at −20 °C. Sections at various levels were collected to include the major organs investigated. The sections were dried in the cryomicrotome at −20 °C for at least 48 h before analysis.

2.2.3. Preparation of liver homogenate thin sections for surface sampling calibration curve

Liver was homogenized in water (1-to-1 ratio (w/w)) using an Omni TH homogenizer (OMNI International, Waterbury, CT, USA) with final repaglinide concentration ranging from 50 nM to 5 μM in the homogenate. (Liver tissue was used in the preparation of calibration standards since its density was similar to the majority of tissues in the whole-body section). Holes of 2.5 mm in diameter were drilled in a frozen tofu block (ca. $100 \times 50 \times 12.5\ mm^3$ in size, purchased locally) then the liver homogenate standards were added into the holes while the tofu block was on dry ice. For this reason, no significant loss of drug to the tofu block was expected during preparation of the tissue section standards. After the liver homogenates completely froze, the tofu block was embedded in 2% carboxymethylcellulose matrix at −20 °C and sections of 50 μm thickness were collected on tape and dried in the cryomicrotome at −20 °C for at least 48 h before the surface sampling analysis. These samples were used to quantify repaglinide in mouse whole-body thin tissue section samples measured by the surface sampling system.

2.2.4. Preparation of samples from bulk extraction of organ homogenates

Major organs (brain, lung, liver, kidney and muscle) were excised from each frozen carcass after preparation of whole-body tissue sections and were stored at −20 °C until required for homogenization. Each organ was homogenized in water with 1-to-1 ratio (w/w). Organ homogenates 50 μL in volume were mixed with 50 μL of mouse blank plasma and 200 μL of acetonitrile (ACN) containing 20 nM of repaglinide-d5 internal standard (IS, cpd 1a). The resulting samples were mixed well and shaken for 2 min with a mechanical shaker extensively, then centrifuged for 10 min at 4000 rpm. Two hundred and forty microliter of supernatants were mixed with 80 μL of water and transferred into a 96-well plate.

2.2.5. Preparation of samples for bulk extraction calibration curve

Hundred microliter of mouse blank plasma samples with repaglinide concentrations ranging from 0.3 nM to 10 μM were mixed with 200 μL aliquots of ACN containing 20 nM of IS. The samples were mixed well, shaken for 2 min with a mechanical shaker extensively, then centrifuged for 10 min at 4000 rpm. Two hundred and forty microliter of supernatants were mixed with 80 μL of water and transferred into a 96-well plate. These samples were used to quantify repaglinide in bulk extraction analysis of organ homogenate samples.

2.3. Sample analysis

2.3.1. Whole-body thin tissue section analysis by droplet-LMJSSP-HPLC-MS/MS

Whole-body and tofu-embedded liver homogenate thin sections were analyzed using droplet-based LMJSSP-HPLC-MS/MS. Briefly, the tissue section was attached to a 3 in. × 4 in. glass slide using double-sided tape, and then the glass slide was mounted in a microtiter plate size “universal” sample holder [15] (Advion BioSciences, Inc., Ithaca, NY, USA). The optical image of the sample was acquired using a scanner (ScanMaker 3630, Microtek Lab,