Journal of Chromatography B, 877 (2009) 303–310

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Quantitative measurements of corticosteroids in *ex vivo* samples using on-line SPE-LC/MS/MS

Lan Gao*, William J. Chiou, Heidi S. Camp, David J. Burns, Xueheng Cheng

Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL, USA

ARTICLE INFO

Article history:
Received 26 August 2008
Received in revised form 9 December 2008
Accepted 14 December 2008
Available online 24 December 2008

Keywords:
High performance liquid chromatography (HPLC)
Solid phase extraction (SPE)
Mass spectrometry (MS)
Tandem mass spectrometry (MS/MS)
Quantitation
Corticosteroid
Steroid
Cortisol
Hydrocortisone
Cortisone
Dexamethasone
Corticosterone
Dehydrocorticosterone
Dehydrodexamethasone
Ex vivo
Endogenous
11β-Hydroxysteroid dehydrogenase (11β-HSD)

ABSTRACT

Abnormal elevation of 11β-HSD1 activities in tissues, such as fat and brain, may contribute to the development of the abdominal obesity and Alzheimer disease, and the inhibition of 11β-HSD1 might be beneficial to the management of these diseases. To assess the effects of pharmacologic inhibitors of 11β-HSD1, we developed a fast LC/MS/MS method to quantify corticosteroids in minced tissue samples in the presence of 11β-HSD substrates. The novel on-line SPE-LC/MS/MS method was developed with dual binary gradient and a throughput of 4.5 min/sample. A total of six corticosteroids (cortisol, cortisone, corticosterone, dehydrocorticosterone, dexamethasone, and dehydrodexamethasone) were studied. The lower limit of quantitation from 0.40 to 11.4 fmol and 4.5 orders magnitude of dynamic range were obtained for these six compounds. Three novel enzymatic bi-products, all isomers of cortisol, were observed in the liver or fat samples. Two of them were identified by matching the HPLC retention times and MS/MS spectra with authentic compounds. The potential interferences of these isomers and their removal are discussed.
© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Two isoforms of 11β-hydroxysteroid dehydrogenase, type 1 (11β-HSD1) and type 2 (11β-HSD2), regulate intracellular levels of glucocorticoids (GC) [1,2]. 11β-HSD1 converts inactive glucocorticoids (such as cortisone and dehydrocorticosterone) into active glucocorticoids (cortisol and corticosterone, respectively), and 11β-HSD2 catalyzes the reversed reaction [3]. 11β-HSD1, expressed in liver, adipose, brain, and many other tissues, is thought to exert its effect by increasing active GC concentrations in tissues. It has been reported that transgenic mice over-expressing 11β-HSD1 in adipose tissue leads to visceral obesity [4] and that 11β-HSD1 knockout mice showed attenuated glucocorticoid-inducible responses and resisted hyperglycemia on obesity or stress [5]. Moreover, 11β-HSD1 has been found to play a role in mammalian brain [6,7], and the administration of carbenoxlone (a known 11β-HSD inhibitor) improved cognition function in man [8]. Thus, 11β-HSD1 has been postulated to be a therapeutic target for visceral obesity and Alzheimer disease. 11β-HSD2 inactivates GC by catalyzing the dehydrogenation reaction in mineralocorticoid (MC) sensitive tissues, such as kidney and colon. The mineralocorticoid receptor (MR) has equal functional affinity for cortisol and its native ligand aldosterone, which regulates blood pressure and sodium balance in the kidney. Patients with apparent mineralocorticoid excess (AME), caused by the loss of 11β-HSD2 function, have hypertension due to kidney MR activation by cortisol. Consequently, a potential therapeutic 11β-HSD1 inhibitor must selectively inhibits 11β-HSD1 over 11β-HSD2 to minimize the side effects [2].

* Corresponding author at: Current address: Schering Plough Research Institute, 2015 Galloping Hill Road, K-15-3-3800 Kenilworth, NJ 07033, USA.
Tel.: +1 908 7403948; fax: +1 908 7403966.
E-mail address: Lan.Gao@spcorp.com (L. Gao).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.12.029

In *ex vivo* studies [9–11], minced liver, kidney, brain, and adipose tissues from animals pre-dosed with 11β-HSD1 inhibitors were incubated in the presence of excess amount of enzyme substrate. The concentrations of both substrate and product were then determined to evaluate the inhibition and selectivity of the 11β-HSD1 inhibitors.

LC/MS/MS methods have been reported for corticosteroids analysis [12–16] using both positive and negative electrospray ionization (ESI) tandem mass spectrometry (MS/MS) monitoring for *in vitro* and *in vivo* samples [15,17–20]. It is generally believed that MS/MS is specific enough to distinguish compounds of interest from the interferences from the biological samples, so chromatography with partially overlapping peaks were often used [12,16,21,22]. However, we observed that interferences from the tissue sample matrix were significant when the substrate concentration was much higher than the product and that MS/MS alone was not sufficient to filter out these interferences. In order to separate the inactive forms from the active corticosteroids in chromatography for quantitative analyses, we developed this on-line SPE LC/MS/MS method to minimize the potential interference. Flumethasone was used as an internal standard for MS/MS analysis and was monitored with multiple ion reaction monitoring (MRM) transition of 411 → 253. In addition to the detection of cortisol and cortisone, this method can also be used to detect the other two pairs of corticosteroids (dexamethasone/dehydrodexamethasone and corticosterone/dehydrocorticosterone).

Three isomers of cortisol were found when cortisone was incubated with the minced liver or epididymal fat tissues. These three isomers were separated by HPLC completely from cortisol. Two of the isomers identified were 4-pregnene-11α,17α,21-triol-3,20-dione and 5β-pregnane-17,21-diol-3,11,20-trione. The inability to separate these isomers in the samples could result in a quantitation error up to 8.5 times higher for cortisol detection. The LC/MS/MS method we are reporting here allows the accurate determinations of the corticosteroid concentrations of the biological samples.

2. Experimental

2.1. Materials

Cortisol, cortisone, flumethasone, dexamethasone, dehydrodexamethasone, corticosterone, dehydrocorticosterone and potassium phosphate buffer (K_2HPO_4, 1 M, pH 7.4) were purchased from Sigma (St. Louis, MO, USA). Formic acid was obtained from Sigma–Aldrich (Milwaukee, WI, USA). Acetonitrile and methanol were purchased from EMD Chemicals (Gibbstown, NJ, USA). 4-Pregnene-11α,17α,21-triol-3,20-dione and 5β-pregnane-17,21-diol-3,11,20-trione were purchased from Steraloids, Inc (Newport, RI, USA). Water was treated with Millipore purification system (Billerica, MA, USA).

2.2. Preparation of standard samples

Standards of all corticosteroids were prepared as 1.0 mM stocks in 50% acetonitrile and stored at −80 °C. The working stock was prepared by diluting the 1.0 mM stock in acetonitrile:water (1:1; v:v) containing 50 mM K_2HPO_4 (pH 7.4) to reach a final corticosteroid concentration of 10 μM. The working stocks of corticosteroids were then added together and diluted in the same solvent to prepare a working standard mixture containing 1.0 μM of each corticosteroid. The working standards were stored at 4 °C and used within three months after preparation. No decomposition or signal decrease of the standards was observed during the storage period. The working standards were serially diluted in 50% acetonitrile containing 50 mM K_2HPO_4 (pH 7.4) before LC/MS/MS analyses for the construction of corticosteroid standard curves. Flumethasone was used as the internal reference for the normalization of MS signals. Right before the LC/MS/MS analysis, an equal volume of 50 nM flumethasone in 50% acetonitrile was added to all samples including the diluted standard mixtures.

2.3. Ex Vivo assay

A known *in vitro* inhibitor of 11β-HSD1 (compound A) was dissolved in 1% Tween 80 in 0.2% hydroxypropyl methylcellulose, and administered to mice ($N = 4$) as a single oral dose at 30 mg/kg. At 1, 2, 7, and 16 h post-dose, fresh tissues including liver, epididymal fat pad (EFP), brain, and kidney were removed, and immediately immersed in ice-cold PBS buffer. Whole brain and kidney, or portions (∼0.3 mg) of liver and EFP, were weighed and minced into 2–3 mm pieces in ice-cold PBS (volume:tissue = 5 ml:1 g) in a 12-well tissue culture plate. The substrate (cortisone for 11-β HSD1, cortisol for 11-β HSD2), prepared in 50% ethanol at 1 mM, was added to the minced tissue suspensions to a final concentration of 10 μM. The plate was then incubated for the indicated periods of time at 37 °C in a tissue culture incubator. The incubation time was within the linear activity range of the mouse tissue 11-β HSD for each tissue type (20 min for liver, 30 min for kidney, 2 h for EFP, and 3 h for brain). At the end of the incubation, 150 μL of the media were transferred to microcentrifuge tubes and spun at 10,000 × g for 2 min at 4 °C. 100 μL of the clear supernatant was then mixed with same volume of acetonitrile and spun again to precipitate proteins. 50 μL of the clear supernatant was mixed with 50 μL of 50 nM internal standard (flumethasone) before LC/MS/MS analysis. The corticosteroid concentrations in the samples were determined by triplicate injections of 20 μL samples using standard calibration curves. The percent inhibition of 11-β HSD activities was calculated relative to a vehicle-treated control group. Corticosterone and dehydrocorticosterone used as native 11-β HSD substrates for the *ex vivo* studies involving mice; however, the level of endogenous corticosteroids need to be pre-determined for background correction. Dexamethasone and dehydrodexamethasone are stable artificial HSD substrates, and they were used for the measurement of 11-β HSD activity in *ex vivo* studies where the interferences by endogenous corticosteroids are prominent.

2.4. Chromatography

A Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MD, USA) consisting of one SIL-HTc autosampler and four LC-10Advp pumps was used for sample analysis. The HPLC system was running in a dual binary gradient mode [23]. An Alltima C18 guard cartridge (2.1 mm × 7.5 mm, 5 μm) from Alltech Associates, Inc. (Deerfield, IL, USA) was used as on-line solid phase extraction column (SPE) for cleaning up the samples prior to the injection of analytes to the analytical column. An YMC ODS-AQ column (2.0 mm × 100 mm, 3 μm) purchased from Waters (Milford, MA. USA) was heated and kept at 55 °C to assist the analyte separations.

A column switching strategy, designed to increase the throughput by performing the sample cleaning and HPLC analysis simultaneously, was developed and achieved an average analysis time of 4.5 min/sample. Solvent A and B, consisting of 0.1% formic acid in water and 0.1% formic acid in MeOH, respectively, formed the primary binary mobile phases (Analytical Binary) for eluting analytes from both SPE cartridge and analytical column. Solvent C and D, consisting of 0.1% formic acid in 5% MeOH and 0.1% formic acid in 90% acetonitrile, respectively, formed the secondary binary mobile phases (SPE Binary) for loading samples onto the SPE cartridge and cleaning the autosampler and SPE cartridge between sample injections. A 6-port switch valve controlled by the HPLC system was used to direct the flow path (Fig. 1). The valve was at position A

Download English Version:

https://daneshyari.com/en/article/1217553

Download Persian Version:

https://daneshyari.com/article/1217553

Daneshyari.com