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Validated assay for the simultaneous determination of cortisol and budesonide in human plasma using ultra high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

An ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC/MS/MS) method was developed and validated for the quantification of cortisol and budesonide in human plasma. Charcoal stripped human plasma was used as the blank matrix during validation. Cortisol, budesonide, and dexamethasone (internal standard) were extracted from human plasma with methyl-tert-butyl ether, and the chromatographic separation of the peaks was achieved using a Waters Acquity UPLC BEH C_{18}, 1.7 μm, 2.1 mm × 50 mm column with a run time of 4.0 min. Cortisol, budesonide, and dexamethasone were monitored at the total ion current of their respective multiple reaction monitoring transition signals. The UHPLC/MS/MS system consisted of an Agilent 1290 Infinity ultra high performance liquid chromatograph coupled with an AB Sciex Qtrap® 5500 hybrid linear ion-trap triple quadrupole mass spectrometer. The method was validated for accuracy, precision, linearity, range, selectivity, lower limit of quantification (LLOQ), recovery, matrix effect, dilution integrity, and evaluation of carry-over. All validation parameters met the acceptance criteria according to regulatory guidelines. The LLOQ was 1.0 ng/mL for both compounds requiring 100 μL of sample. To our knowledge, this is the first validated LC/MS/MS method for the simultaneous quantitative analysis of cortisol and budesonide in human plasma. The method was applied successfully in a clinical investigation of the impact of nasally administered Pulmicort (budesonide) on the hypothalamic-pituitary-adrenal axis of patients with chronic rhinosinusitis.

1. Introduction

Hypothalamic-pituitary-adrenal (HPA) axis suppression is an indicator of adrenal dysfunction [1]. Primary adrenal insufficiency is associated with an autoimmune disease or an adenoma of the adrenal gland, whereas secondary adrenal insufficiency is generally linked to the long-term use of exogenous corticosteroids, such as budesonide (BUD) [2,3]. The severity of the suppression will depend on several variables, including the pharmacokinetics of the particular drug and the mode of delivery [4]. Symptoms may develop gradually and include hypoglycemia, hypotension, cardiovascular collapse, nausea, and diarrhea. Patients with a chronic inflammatory disease of the nose and paranasal sinuses known as chronic rhinosinusitis (CRS) have been successfully treated with nasally administered BUD. BUD has been demonstrated to have a better side-effect profile compared to other steroids; however, there is still a concern that if administered in sufficient amounts it could cause HPA-axis suppression [5].

Diagnosis of HPA axis suppression involves the administration of the adrenocorticotropic hormone (ACTH) adrenal stimulation test with the subsequent quantification and interpretation of plasma cortisol (CORT) levels [1]. Plasma CORT levels that are within or above the normal range of this test indicate that BUD does not impact the HPA axis. However, CORT levels that are consistently below the normal range indicate that sufficient BUD is being absorbed into the systemic circulation to cause HPA axis suppression [3].

The clinical investigation associated with the present analytical study sought to determine the impact of a novel intranasal steroid delivery device, the mucosal atomization device (MAD), (LMA North America, Inc., formerly Wolfe-Tory Inc.) on the systemic absorption of BUD. To achieve this goal, a sensitive and validated assay was required for the simultaneous quantification of CORT and BUD in human plasma.

There are several analytical methods available for the separate determination of CORT or BUD. CORT was determined in a variety of matrices using radioimmunoassay [6], liquid chromatography–mass spectrometry cubed [7], and liquid chromatography–tandem mass spectrometry (LC/MS/MS) [8–10]. Similarly, BUD was quantitated in a range of biological matrices

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using LC/MS/MS [11–13]. None of the above methods attempted the simultaneous analysis of CORT and BUD in the same sample.

Although, there have been several attempts in the literature to quantify both CORT and BUD in the same sample, those studies had their limitations. They have either used different methodologies or laboratories for these two analytes [14–16], were done in matrices other than human plasma [17,18] or have not been validated for both CORT and BUD [19].

In the present study, an ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC/MS/MS) method is presented for the simultaneous determination of CORT and BUD in human plasma using charcoal stripped human plasma as the blank matrix. The assay was validated according to regulatory guidelines [20] and applied successfully to measure the CORT and BUD plasma levels of human volunteers who received ACTH stimulation followed by BUD treatment, 1 mg, twice a day for 60 days. To our knowledge, this is the first validated LC/MS/MS assay for the simultaneous quantitation of cortisol and budesonide in human plasma.

2. Experimental

2.1. Chemicals and standards

Hydrocortisone (≥98%), budesonide (≥99%), dexamethasone (≥98%), and ammonium formate (99.995 + % metals basis) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methyl-tert-butyl ether, acetonitrile, and methanol (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NY, USA), formic acid (puriss. p.a. for mass spectroscopy) from Fluka (Steinheim, Germany), hydrochloric acid (1.0 M) from VWR (West Chester, PA, USA). Human plasma (2× charcoal stripped, K_2 EDTA-treated) was purchased from Bioreclamation, LLC (Westbury NY, USA). Ultra pure water was prepared in our laboratory using a Milli-Q Synthesis system (Millipore, Billerica, MA, USA). Cortrosyn® (cosyntropin) for injection, sterile lyophilized powder vials (containing 0.25 mg cosyntropin, 10 mg mannitol) were manufactured by Amphastar Pharmaceuticals, Inc., (Rancho Cucamonga, CA, USA), and the saline solution was manufactured by Hospira Canada (Montreal, Quebec, Canada). Pulmicort (budesonide) nebuamp capsules (0.5 mg budesonide in 2 mL saline) were manufactured by Astrazeneca (Wilmington, DE, USA).

2.2. BUD administration to human volunteers

Human volunteers with CRS were recruited from the St. Paul's Hospital Sinus Center in Vancouver, British Columbia, Canada, for a clinical safety and efficacy trial of BUD delivered via MAD. Ethics approval was obtained from the University of British Columbia Providence Health Care Research Ethics Board prior to the start of the study. Patient consent was obtained two months prior to starting the trial in order to ensure that patients currently on BUD treatment could undergo a four week 'washout period' consistent with previously published data on the pharmacokinetics and pharmacodynamics of BUD [16,21]. Patients were provided with a bailout dose of BUD in the event they required immediate therapy for an acute sinusitis flare-up during the washout period. Patients were seen three times over the 60-day trial. Prior to the start of BUD therapy, patients' plasma CORT was measured at the first visit in order to establish a baseline plasma CORT level. The ACTH stimulation test was then administered to rule out pre-existing adrenocortical insufficiency in the patient. The ACTH stimulation test is the standard to test for suspicion of adrenocortical insufficiency [1]. An intramuscular injection of cosyntropin 0.25 mg (synthetic ACTH) reconstituted in 1 mL of saline was administered followed by a post-stimulation blood sampling at 60 min [22]. A post-stimulation level of plasma CORT, which was above a threshold of 18 mg/dL or twice the baseline amount, was expected in healthy patients [23]. At the end of the first visit, patients were randomly divided into two equal groups, i.e., the experimental group (BUD via MAD) and control group (BUD via impregnated nasal saline irrigation). For the duration of the study, patients were administered 1 mg of BUD twice daily (2 mg/day) for 60 days. The ACTH stimulation test was administered at days 30 and 60. Blood samples were collected in a consistent manner at each visit, immediately processed for plasma, and stored at −80 °C until the analysis by UHPLC/MS/MS.

2.3. Instrumentation and experimental conditions

The UHPLC/MS/MS system consisted of an Agilent 1290 Infinity Binary Pump, a 1290 Infinity Sampler, a 1290 Infinity Thermostat, and a 1290 Infinity Thermostatted Column Compartment (Agilent, Mississauga, Ontario, Canada) connected to an AB Sciex QTrap® 5500 hybrid linear ion-trap triple quadrupole mass spectrometer equipped with a Turbo Spray source (AB Sciex, Concord, Ontario, Canada). The mass spectrometer was operated in positive ionization mode and data were acquired using the Analyst 1.5.2. software on a Microsoft Windows XP Professional operating platform.

Chromatographic separation was achieved using a Waters Acquity UPLC BEH C_{18}, 1.7 μm, 2.1 mm × 50 mm column maintained at 30 °C, and the autosampler tray temperature was maintained at 10 °C. Solvent A was water with ammonium formate (AF) (2.5 mM), solvent B was methanol with AF (2.5 mM). The mobile phase initial conditions were solvent A (40%) and solvent B (60%), which was ramped to solvent A (5%) by 2.0 min, held until 3.0 min and followed by an equilibration with solvent A (40%) and solvent B (60%) for 1 min. The flow rate was 0.2 mL/min, injection volume was 15 μL with a total run time of 4.0 min.

Mass spectrometric conditions were as follows: curtain gas 30 units, collision gas (CAD) high, ionspray 5500 V, temperature 450 °C, ion source gas 1, 40 units, ion source gas 2, 60 units. Nitrogen gas was used for curtain gas, collision gas, ion source gas 2 (vaporizing gas), and zero air was used for ion source gas 1 (nebulizing gas). Entrance potential 10 units, resolution Q1 low, resolution Q3 unit, and dwell time was 100 ms for all the compounds.

CORT and BUD were quantitated using the total ion current (TIC) of the multiple reaction monitoring (MRM) transitions as follows. For CORT (declustering potential DP, 80, collision cell exit potential CXP, 12), m/z 363.0 → 327.3 (collision energy CE, 23), m/z 363.0 → 267.0 (CE, 26), m/z 363.0 → 121.2 (CE, 34), for BUD (DP, 70, CXP, 18), m/z 431.0 → 323.2 (CE, 19), m/z 431.0 → 173.1 (CE, 38), m/z 431.0 → 147.1 (CE, 47). Dexamethasone (DEX) was monitored (DP, 70, CXP, 14) using the TIC of MRMs of m/z 393.0 → 373.4 (CE, 13), m/z 393.0 → 355.1 (CE, 17), and m/z 393.0 → 337.3 (CE, 19). To protect the mass spectrometer from contamination from the samples and to reduce the solvent load in the source, the mobile phase flow was diverted to the waste before 1.2 min and after 3.4 min during the chromatographic run.

2.4. Preparation of stock solutions

Two separate master stock solutions of CORT (100 μg/mL), and BUD (100 μg/mL) were prepared in methanol. The CORT and BUD master stock solutions were combined in equal parts into a mixed working stock solution of CORT and BUD (50 μg/mL, each). The mixed working stock solution was further diluted with methanol to yield a series of diluted working stock solutions. The series of diluted working stock solutions were used to prepare the calibration standards as described in Section 2.5. A 100 μg/mL DEX internal standard (IS) solution was also prepared in methanol and