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High performance liquid chromatography–tandem mass spectrometric assay of dexmedetomidine in plasma, urine and amniotic fluid samples for pregnant ewe model

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ABSTRACT

Dexmedetomidine (DEX; Precedex®), approved by the Food and Drug Administration (FDA) in 1999 as a sedative for use in the intensive care unit, is a potent and highly selective α_2-adrenoceptor agonist with significant sedative, analgesic and anxiolytic effects. However, the research of DEX use during pregnancy is limited and the impact of DEX on the fetal development is unclear. This article describes a high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay suitable for various biomatrices of plasma, urine and amniotic fluid, as a prerequisite for pharmacokinetic characterization of DEX in the pregnant ewe model. DEX and testosterone (internal standard; IS) were extracted from 200 μL of plasma, urine or amniotic fluid with ethyl acetate. The HPLC resolution was achieved on an Agilent ZORBAX SB-CN column with a gradient elution at a flow rate of 0.5 mL/min using a mobile phase of 5–100% of acetonitrile with 0.5% formic acid (mobile phase B) in water (mobile phase A). The detection was performed by a triple quadrupole tandem mass spectrometer with positive electrospray ionization. The precursor/product transitions (m/z) in the positive ion mode $[M+H]^+$ were m/z 201.5 → 95.4 for DEX and m/z 289.2 → 109.1 for IS. The method was validated in the concentration range of 25 (lower limit of quantification; LLOQ)–5000 pg/mL for both maternal and fetal plasma, and of 50 (LLOQ)–5000 pg/mL for urine and amniotic fluid, respectively. The intra- and inter-day precision and accuracy were within ±9%. The overall recoveries of DEX were 82.9–87.2%, 85.7–88.4%, 86.2–89.7% and 83.7–88.1% for maternal plasma, urine, fetal plasma and amniotic fluid, respectively. The percentage matrix factors in different biomatrices were less than 120%. Stability studies demonstrated that DEX was stable after three freeze/thaw cycles, in the autosampler tray at 20 °C for 24 h and during the 3 h sample preparation at room temperature. The validated HPLC–MS/MS method has been successfully employed for pharmacokinetic evaluation of DEX in pregnant ewes and fetuses.

1. Introduction

Dexmedetomidine (DEX; Precedex®, Fig. 1), approved by FDA in 1999 as a sedative for use in the intensive care unit, is a potent and highly selective α_2-adrenoceptor agonist with significant sedative, analgesic and anxiolytic effects. Unlike traditional sedatives, DEX offers hemodynamic stability during surgical stimulation and does not cause respiratory depression. Moreover, DEX has emerged as an effective therapeutic agent in a wide range of anesthetic management during the past years, offering benefits in the perioperative use due to its favorable pharmacodynamics properties [1,2]. The mechanism of DEX action mediated by signaling pathways other than the α_2-adrenoceptor has been reported to play a role in neuroprotection [3,4]. Animal studies have also suggested that DEX could provide neuroprotective effects on anesthetic-induced neurotoxicity in neonatal rats [5,6]. Therefore, DEX may be

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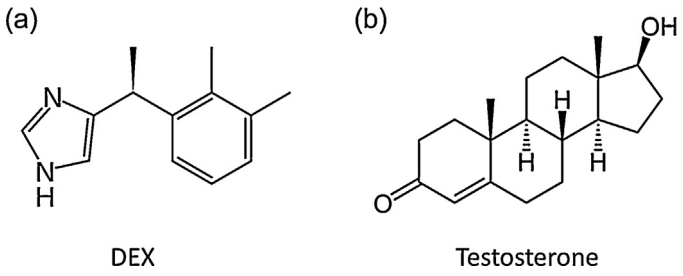

Fig. 1. Chemical structures of (a) DEX and (b) testosterone (internal standard).

desirable for pregnant mothers who undergo anesthesia, by exhibiting hemodynamic stability, requiring decreased dose of anesthetics and offering an added benefit of neuroprotection for fetuses. However, the impact of the use of DEX which may be associated with hypotension and bradycardia, on fetal development during pregnancy has not yet been fully understood; this greatly limits its use in pregnant women. Therefore, the maternal–fetal pharmacokinetic data of DEX during pregnancy is essential, but unavailable for the pregnant women (a search was performed in PubMed and Web of Science).

One of the major challenges to acquisition of maternal–fetal information in humans is the ethical issues in performing experiments on fetuses. Alternatively, a variety of animal models have been developed for pharmacokinetics studies in pregnancy. While no animal model truly recapitulates human pregnancy, the pregnant ewe model has been used extensively to delineate the maternal–fetal interactions. This is in part due to the suitability of its fetus to mimic human fetal response, and the distinguished merit of the relatively large size of the fetus that permits the implantation of catheters in both maternal and fetal blood vessels for repeated sampling from both maternal and fetal sides of the placenta [7–10]. The pregnant ewe model enables the investigation of the maternal–fetal pharmacokinetics of DEX for fetal safety study that involves drug exposures in both mothers and fetuses. Therefore, analytical methods for the quantification of DEX in both pregnant ewes and fetuses are the prerequisite for pharmacokinetic study.

Currently, no method has been reported for the determination of DEX in pregnant ewes (a search was performed in PubMed and Web of Science) [11,12]. Analytical methods have been published to quantify DEX concentration in human plasma [13–18], among which sensitive LC–MS/MS methods with LLOQ of 5 pg/mL have been developed and validated [16–18]. However, these methods may not be suitable for the determination of DEX in the ewe, because the performance of LC–MS/MS methods can significantly vary among species due to the matrix effects of different biofluids [19–24]. Analytical bias between species due to different phospholipid profiles among human, rodent and non-rodent species has been reported [24]. Matrix components present in biological samples can suppress or enhance the response of the analyte of interest, which may affect the assay sensitivity and/or accuracy.

The purpose of our study was to develop a sensitive, specific, accurate and reliable HPLC–MS/MS assay that are suitable for the determination of DEX in maternal–fetal unit of pregnant ewes, specifically in maternal plasma and urine, as well as fetal plasma and amniotic fluid. The method has been successfully employed to the pharmacokinetic evaluation of DEX in the pregnant ewe model.

2. Experimental

2.1. Chemical and materials

Dexmedetomidine HCl was purchased from Fisher Scientific (Pittsburgh, PA, USA) and testosterone (internal standard; IS) from Indofine Chemical Co., Inc. (Hillsborough, NJ, USA). Ewe samples were supplied by Texas Children's Hospital (Houston, TX, USA) and stored at −80 °C prior to the assay quantification. HPLC-grade methanol, acetonitrile, and ethyl acetate were purchased from EMD Chemicals USA, and formic acid (~98%) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Chromatographic conditions

Chromatographic analysis was performed using an Agilent 1200 series HPLC system (Foster City, CA, USA). DEX and IS were resolved on Agilent ZORBAX SB-CN column (5.0 μm, 150 mm × 2.1 mm I.D.). The mobile phases consisted of water (mobile phase A) and 0.5% formic acid in acetonitrile (mobile phase B). A gradient elution was started with 5% of mobile phase B, maintaining for 1 min and with a linear increment to 100% of mobile phase B from 1 min to 3.5 min. The elution was kept constant at 100% of mobile phase B for 1.5 min, and then decreased to 5% of mobile phase B in 0.5 min. This composition was maintained at 5% of mobile phase B for 2.5 min until the end of run (8.0 min). The flow rate was delivered at 0.5 mL/min, and the injection volume was 50 μL.

2.3. Mass spectrometry conditions

The column effluent was monitored using an HPLC–MS/MS of 3200 QTRAP®, which is a hybrid triple quadrupole linear ion trap equipped with a TurboIonSpray ion source. Pure nitrogen was generated by a Parker Balston Source 5000 Tri Gas Generator. The IonSpray heater was maintained at 500 °C with the curtain gas, nebulizer gas and heater gas set at 10, 20 and 60 psi, respectively. IonSpray needle voltage was set at 5500 V, and the collision activate dissociation (CAD) gas was set to medium.

Optimal multiple reaction monitoring (MRM) was used to detect transition ions from a specific precursor ion to product ion $[M+H]^+$, m/z 201.5 → 95.4 for DEX and m/z 289.2 → 109.1 for IS, respectively. The collision energy was set at 22 and 34 eV for DEX and IS, respectively. Other compound parameters were determined using the QTRAP instrument and version 1.5 of the Analyst® Software. Finally, the positive ion electrospray MS/MS product ion spectra of DEX and IS are established (Fig. 2). The formic acid of 0.1%, 0.3% and 0.5% in mobile phase B were tested, and 0.5% was selected as it resulted in the most sharp and symmetrical peak shape, and no significant suppression issue in positive ion mode.

2.4. Preparation of calibration standards and quality control (QC) samples

Stock solutions of DEX and IS were prepared at concentrations of 1.0 mg/mL in methanol and 100 μM in acetonitrile, respectively. Stock solutions were stored at −20 °C until use for the preparation of working solutions. A series of DEX standard working solutions were freshly prepared by adding appropriate volumes of DEX stock solution (1.0 mg/mL) with 30% aqueous acetonitrile to obtain the DEX concentrations of 0.25, 0.50, 1, 2.5, 5, 10, 25 and 50 ng/mL, respectively. These standard working solutions of DEX (20 μL) were spiked to blank ewe samples (180 μL) to yield calibration standards of 25, 50, 100, 250, 500, 1000, 2500 and 5000 pg/mL in plasma, and 50, 100, 250, 500, 1000, 2500 and 5000 pg/mL in urine or amniotic fluids, respectively. Three levels of QC samples of 50, 500 and 2500 pg/mL for plasma and 100, 500 and 2500 pg/mL for urine or amniotic fluid, were prepared in the same manner.

2.5. Preparation of plasma, urine and amniotic fluid samples

Standards and QC samples were extracted by liquid–liquid extraction. An aliquot (200 μL) of plasma, urine or amniotic fluid