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Short communication

Quantification of cefazolin in serum and adipose tissue by ultra high performance liquid chromatography-Tandem mass spectrometry (UHPLC–MS/MS): application to a pilot study of obese women undergoing cesarean delivery

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ABSTRACT

Higher doses of cefazolin are required in obese patients for preoperative antibiotic prophylaxis, owing to its low lipophilicity. An ultra high performance liquid chromatography-tandem mass spectrometry method was developed to quantify cefazolin in serum and adipose tissue from 6 obese patients undergoing cesarean delivery, and using stable-isotope labeled cefazolin as an internal standard. The method has a 2 μg/g lower limit of quantitation. The concentration in adipose tissue was 3.4 ± 1.6 μg/mL, which is less than half of the reported minimum inhibitory concentration of 8 μg/mL for cefazolin. Serum cefazolin concentrations were more than 30-fold higher than in adipose tissue.

1. Introduction

Antibiotic preoperative prophylaxis is the standard of care to decrease the incidence of infections following surgery. Based on pharmacokinetic data and the American College of Obstetrics and Gynecology recommendations [1], a single 1–2 g dose of cefazolin no more than 1 h prior, is the standard preoperative prophylactic antibiotic for obstetrical procedures [2,3]. This regimen decreases morbidity and mortality for elective and non-elective cesarean sections [4]. However, maternal obesity increases the risks of infectious morbidity [5–7] suggesting that the standard dosing of cefazolin is insufficient for obese patients undergoing cesarean section. Obese patients require larger doses of β-lactam antibiotics [8–10] and studies suggest that dosing should be based on actual, rather than ideal bodyweight [11]. The high water solubility of cefazolin suggests low partitioning into adipose tissue, which may explain the higher than normal doses required in obese patients. In order to determine if this is the case, new assays are needed to measure cefazolin in adipose tissue.

Several studies have attempted to determine a prophylactic dose of cefazolin in obese women undergoing cesarean delivery [3,12–16]. Most of these studies utilize high performance liquid chromatography (HPLC), or microbiological assays to measure cefazolin concentrations in the blood and adipose tissues [17–19]. None of these clinical studies utilize a method validated in human adipose tissue. Analysis in biological matrices such as adipose tissue or serum can produce variations in signal, referred to as matrix effects. We therefore validated our UHPLC–MS/MS method in serum and adipose tissue and used stable isotope labeled cefazolin as an internal standard to control for matrix effects. To the best of our knowledge, this is the first UHPLC–MS/MS method to measure cefazolin in a clinical study of obese women undergoing cesarean delivery that is validated in both serum and adipose tissue utilizing stable-isotope labeled cefazolin as the internal standard.

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2. Materials and methods

2.1. Analytical method

2.1.1. Chemicals and reagents

Cefazolin was purchased from Sigma Aldrich and the Cefazolin-$^{13}C2,^{15}N$ internal standard (IS) from Toronto Research Chemicals (C242502). Mobile phases contained analytical grade formic acid, HPLC grade acetonitrile (Fisher) and water purified from a Milli-Q system. Mobile phases were filtered through a 0.2 μm nylon filter.

2.1.2. Stock solutions

Standard stock solutions of cefazolin and IS were made up to 1 mg/mL in water and stored at −80° C until use. Working solutions of 100 μg/mL were prepared from the stock solutions in water. Standard calibrations were prepared from the 100 μg/mL working solutions by diluting with blank human serum (50 μL) for concentrations between 0.5, and 100 μg/mL of cefazolin.

2.1.3. Sample preparation

Standards and samples in serum (50 μL) or adipose tissue (0.25 g) were added to the appropriate volume of IS to reach a final concentration of 2.5 μg/mL after reconstitution. ***Serum:*** The samples and standards were treated with 1 mL acetonitrile to precipitate proteins, separated by centrifugation. The supernatant was collected and dried at 45° C in a Thermo scientific Savant SPD1010 SpeedVac concentrator and reconstituted in 100 μL of 5% aqueous acetonitrile with 0.1% formic acid for analysis. ***Adipose tissue:*** samples weighed in pre-tared glass tubes, were emulsified with 1 mL hexane, and 1 mL 100 mM ammonium acetate buffer (pH 7.5) and rotated for 16 h at 110 rpm and 37° C. The aqueous phase was separated, collected, dried and reconstituted in 100 μL of 5% aqueous acetonitrile with 0.1% formic acid.

2.1.4. UHPLC–MS/MS method

The Shimadzu Nexera UHPLC system coupled to a Shimadzu MS-8040 triple quadrupole mass spectrometer was operated using electrospray and atmospheric pressure chemical ionization (DUIS) in positive ion mode with multiple reaction monitoring (MRM). Nitrogen drying and nebulizing gas flow rates were 15 and 2 L/min, respectively. The desolvation line and heating block temperatures were 250° C and 400° C, respectively. Samples were injected in 3 μL volumes on a Restek C18 1.9 μm reverse phase 50 × 2.1 mm column at 45° C using mobile phase A (water in 0.1% formic acid v/v) and mobile phase B (acetonitrile in 0.1% formic acid v/v) with a flow rate of 400 μL/min. 5% B is held for 1 min, stepped to 85% B and held for 3 min, stepped to 95% B for 0.5 min then stepped down to 5% B and held for 3.5 min. MRM events were optimized using the LabSolutions LCMS software and the parameters displayed in Table 1.

2.1.5. Method validation

Inter-day and intra-day variations were measured to validate the method in human serum and adipose tissue. Standard curves were prepared in either 50 μL blank human serum or 0.25 g blank human adipose tissue by emulsification with cefazolin and IS working solutions as per sample preparation in section 2.1.3. Inter-day standard curves were prepared and analyzed each day for 6 consecutive days between 0.5 and 100 μg/mL cefazolin (2.5 μg/mL IS) in serum and between 0.2 and 40 μg/g (1 μg/g IS) in adipose tissue. Intra-day variability was evaluated by 6 repeated injections at every calibrated level. Measurements were expressed by the peak area ratio of cefazolin/IS. Calculating the RSD% and bias from the theoretical concentration of each level determined the lower limit of quantitation (LLOQ) over the inter-day analysis (Table 2). The threshold LLOQ was the lowest level at which the average calculated concentration from the inter-day analysis that had a bias no greater than ±20%. The bias was calculated as $(C_{average} - C_{theoretical})/C_{theoretical}$, where $C_{average}$ is the calculated average concentration of cefazolin for a standard level over the inter-day analysis (n = 6) and $C_{theoretical}$ is the theoretical concentration of that level. Quality control samples at 12.5, 25 and 37.5 μg/mL (2.5 μg/mL IS) in serum and 8, 12, 16 μg/g (1 μg/g IS) in adipose were included everyday throughout the validation.

2.2. Pilot clinical study patients and methods

Obese patients in labor (body mass index ≥ 35 or body weight > 80 kg) were asked to participate in this pilot study (Protocol Reference B2010:063). Enrollment occurred once it had been decided that the patient required a Cesarean delivery. Patients enrolled received 1 dose of cefazolin for preoperative antibiotic prophylaxis (Table 3). Exclusion criteria included: allergy to cefazolin or penicillin, chorioamnionitis, multiple gestation, and antibiotic use up to 1 week prior to labor (however, patients receiving group B strep prophylaxis in labor were not excluded).

Patient characteristics recorded included age, weight, height, gravity, parity, gestational age, presence of diabetes or hypertension, duration of operation, type of surgical incision, number of previous cesarean deliveries, length of ruptured membranes and cervical dilation at the time of cesarean delivery, presence of antenatal Group B *strepococcus*, presence of internal fetal monitoring, and postoperative recovery room temperature.

3. Results and discussion

3.1. Method development and validation

The gold standard in assay development is stable isotope labeled internal standards as there is no chemical difference between the standards and IS (Fig. 1). The IS is added to account for any mechanical loss during the sample preparation, since the isotopically labeled IS shares the same chemical properties as the analyte and behaves exactly the same as the analyte during sample preparation (especially the extraction procedure). This type of internal standard method cannot be used in HPLC-UV assays as the analyte and IS cannot be resolved by chromatography and must be differentiated by mass (Fig. 2). Currently, the few validated LC–MS/MS assays for cefazolin in biological matrices use internal standards such as cloxacillin [20], odansetron-D3 [21], and others [22] but none have used stable-isotope labeled cefazolin and been validated in human adipose tissue. Since cephalosporin antibiotics are highly bound to plasma proteins [23], the difference in affinity to plasma proteins between IS and standard is a major cause of inter-assay variability. Moreover, the high water solubility of cefazolin suggests limited partitioning into adipose tissue. The differences in protein affinity, water solubility and ionization would interfere with reliable quantification when using a non-isotopically labeled internal standard. Therefore, stable-isotope cefazolin was chosen as an internal standard to account for all of these variables.

3.1.1. Linearity and low limit of quantification

The LLOQ for cefazolin was found to be 5 μg/mL in serum and 2 μg/g adipose tissue. This was the lowest level that met the criteria of bias no greater than 20%. Previous studies have reported lower LLOQ values for cefazolin, [22] however, for clinically relevant concentrations of cefazolin our LLOQ values were sufficient. Linearity was achieved with concentrations between 5 and 50 μg/mL in serum and 2 and 20 μg/g in adipose tissue (Fig. 2) with no point within this range deviating more than 20% of the expected concentration (Table 2). Any samples above the linear range of the curves