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Determination of dexmedetomidine by UHPLC–MS/MS and its application to evaluate the effect of dexmedetomidine concentration on the target-controlled infusion concentration of propofol

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ABSTRACT

The combination of dexmedetomidine (DEX) and propofol (PPF) is extensively used in the field of anaesthesiology. This study aimed to develop and validate a rapid, simple and sensitive ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) method for the determination of DEX in human plasma. The method was applied to evaluate the effect of DEX concentration on the target-controlled infusion (TCI) concentration of PPF. Analytes were separated on a Waters XTERRA® MS C18 column with a mobile phase of acetonitrile-methanol-water containing 0.1% formic acid and 5 mM ammonium acetate (70:10:20, *v/v/v*) at a flow-rate of 0.3 mL/min. Mass spectrometry was performed in the positive selection reaction monitoring mode. The 201.12 → 95.12 and 515.29 → 275.68 mass transitions of DEX and IS (telmisartan), respectively, were monitored. The calibration curve of DEX was linear over the concentrations of 0.1–10 ng/mL. The intra-batch and inter-batch precisions of quality control samples were less than 10.05% and had accuracies of less than 6.25%. The newly developed method was successfully applied to quantify the DEX concentrations of plasma samples from 34 patients who were co-medicated with DEX prior to receiving anaesthesia by PPF. Results showed that comedication with DEX could reduce the requirements of PPF. Specifically, it was firstly found that the concentration of DEX is negatively correlated with the TCI concentration of PPF at the time of loss of consciousness.

1. Introduction

Dexmedetomidine (DEX, Precedex™) is an alpha-2 adrenergic receptor agonist with sedative and anaesthetic properties. In 1999, its use in the sedation and anxiolysis of adults in the intensive care unit [1] was approved by the FDA. DEX is also used in anaesthesia assistance [2] and is a highly effective premedicant for general anaesthesia owing to its sedative and sympatholytic effects in reducing the occurrence of body movement, postoperative pain and emergence agitation.

The combination of DEX and propofol (PPF) is widely used in clinical applications. PPF is one of the most commonly used intravenously administered sedative agents for the induction and maintenance of anaesthesia. Given that DEX has anaesthetic properties, it may exhibit pharmacodynamic interaction with PPF. Several studies have reported that comedication with DEX could reduce the required dosage of PPF [3–6]. However, treatment with the same DEX dosage results in considerable inter-individual differences in the extent of PPF dosage reduction and in the plasma concentration of DEX [7]. The target-controlled infusion (TCI) of PPF has been widely studied and applied in anaesthesia. Whether the reduction in the TCI concentration of PPF is dependent on the concentration of DEX has never been reported and requires elucidation.

Several chromatography methods, such as gas chromatography-mass spectrometry (GC–MS) [8], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [9–15] and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) [16,17], have been used for DEX detection. Nonetheless, these analytical methods had some limitations. For example, GC–MS analysis

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required a multistep derivatisation procedure [8]. Although LC–MS/MS and UPLC–MS/MS methods exhibited excellent lower limit of quantitation (LLOQ), these methods needed large plasma sample volumes [9,10,12], long analytical times with gradient elution [13,15] and the laborious and costly solid-phase extraction (SPE) procedure [14,17].

The objective of our study was to develop and validate a rapid, simple and highly sensitive UHPLC–MS/MS method for the quantification of DEX concentration. The effect of DEX concentration on the TCI concentration of PPF during the anaesthesia induction phase was evaluated to identify the reason for the individual variations in PPF reduction under comedication with the same dose of DEX. The results will provide guidance for the clinical application of DEX and PPF.

2. Materials and methods

2.1. Chemical and reagents

DEX standard and telmisartan (internal standard, IS) standard were purchased from Aladdin Industrial Corporation (Shanghai, China). HPLC-grade acetonitrile, methanol, ethyl acetate and dichloromethane were produced by Tedia Inc. (Beijing, China). Other reagents were of HPLC grade or analytical purity grade and met the experimental requirements. Blank human plasma from healthy people was provided by the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China).

2.2. UHPLC–MS/MS instrumentation and conditions

An UHPLC system (Thermo Fisher Scientific Inc., Boston, USA) consisting of Ultimate 3000 RSLC system was used for the chromatographic separation of DEX and IS. Sample separation was performed on a Waters XTERRA® MS C_{18} column (2.1 mm × 150 mm, 5 μm) with a column temperature of 40 °C. Acetonitrile-methanol-water containing 0.1% formic acid and 5 mM ammonium acetate (70:10:20, *v/v/v*) was used as the isocratic mobile phase and was applied with a flow rate of 0.3 mL/min. The total run time was 3.0 min.

Mass spectrometry was performed with a TSQ Quantum Access Max API mass spectrometer (Thermo Fisher Scientific Inc, Boston, USA). DEX and IS were analysed under positive ion mode through selection reaction monitoring (SRM) with an electrospray ionisation ion source (ESI). The most abundant fragment ions of DEX and IS were explored, and *m/z* transitions from precursor ions to product ions were 201.12 → 95.12 and 515.29 → 275.68 for DEX and IS, respectively. The mass fragmentation pattern of DEX and IS are shown in Suppl. Fig. 1. The following parameters were applied as source-dependent parameters: Spray voltage: 3500 V. Vaporiser temperature: 300 °C. Sheath gas pressure: 35 Psi. Ion sweep gas pressure: 15 Psi. Aux gas pressure: 10 Psi. Capillary temperature: 350 °C. Tube lens offset: 124 V. Collision gas pressure: 1.5 mTorr. Collision energy (CE) was 20 eV for DEX and 45 eV for IS.

2.3. Preparation of calibrators and quality control samples

Stock solutions were dissolved in methanol-water (50:50, *v/v*) and the concentration of DEX and IS were 100 and 40 μg/mL, respectively. DEX working solutions with concentrations of 1, 2, 10, 25, 50, 80 and 100 ng/mL were prepared by diluting the DEX stock solution with a mixture of methanol-water (50:50, *v/v*). IS working solution (2.5 ng/mL) was prepared from the corresponding stock solution in the same manner.

The calibration standard solution and quality control (QC) samples were prepared by spiking 90 μL of human blank plasma with 10 μL of standard working solution. The DEX calibration curve points included 0.1, 0.2, 1, 2.5, 5, 8 and 10 ng/mL. QC samples with concentrations of 0.2, 1 and 8 ng/mL were prepared through the methods mentioned above. The stock solutions, calibrators and QC samples were all stored at 4 °C.

2.4. Sample preparation

Firstly, 100 μL of human plasma sample and 10 μL of IS were mixed in a centrifuge tube. Then the mixture was mixed through 30 s of vortexing. Afterwards, 0.5 mL of extraction solvent (ethyl acetate-dichloromethane, 4:1, *v/v*) was added to the mixture and then vortexed for 3 min. After 3 min of deposition at 25 °C, the mixture was centrifuged at 15,000 rpm for 10 min at 4 °C. Then, 350 μL of the organic upper phase was transferred to another clean tube and evaporated to dryness at 25 °C by using a vacuum drying concentrator. The residue was redissolved in 100 μL of the mobile phase solution and vortexed for 2 min, followed by centrifuged at 15,000 rpm at 4 °C for 5 min. Finally, the upper phase was transferred into an autosampler vial and 10 μL of the reconstituent was injected into the UHPLC–MS/MS system.

2.5. Method validation

The method validation included specificity, linearity and lower limit of quantification (LLOQ), intra- and inter-day precision and accuracy, extraction recovery, matrix effect and stability according to the validation of bioanalytical methods of China Food and Drug Administration (CFDA) guideline [18].

2.6. Method application

The method was used to quantify DEX concentrations in patients who were scheduled for thyroid surgery or breast surgery under total intravenous anaesthesia. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University.

Thirty-four patients participated in the present research. The physical statuses of the patients were classified as ASA I–II on the basis of the guidelines of the American Society of Anaesthesiologists. Without receiving any other medication prior to the procedure, the patients underwent 10 min of premedication with 0.50 μg/kg DEX before the induction of anaesthesia with PPF. In accordance with Marsh's pharmacokinetic models [19], a TCI pump of PPF was used for anaesthetic induction and maintenance. Anaesthetic induction was initiated with a PPF target plasma concentration (Cp) of 4.0 μg/mL. The Cp of PPF was adjusted at any time until the patient fell asleep. Cp was adjusted on the basis of the Nacortrend stage, which was maintained between D2 and E0. Blood samples were collected at the time of loss of consciousness (LOC, Nacortrend value at D2 stage). The TCI Cp and TCI effect site concentration (Ce) of PPF at the time of LOC were documented. Statistical analyses were conducted with SPSS 22.0 (SPSS Inc., Chicago, USA). The statistical description of non-normally distributed data was shown as the median and interquartile range. Spearman rank correlation was applied to assess the relationship between the plasma concentration of DEX and the TCI concentration of PPF.

3. Results and discussions

3.1. Method development

3.1.1. HPLC–MS/MS optimisation

In this study, a rapid, simple and sensitive UHPLC–MS/MS method for DEX quantification was developed and validated using the more straightforward isocratic elution. Several analytical methods, including GC–MS and LC–MS, for DEX quantification have been