Altered cisatracurium pharmacokinetics and pharmacodynamics in patients with congenital heart defects

Zhufeng Wu, Sheng Wang, Xuemei Peng, Chunying Lu, Xiaodong Ye, Baojian Wu

Division of Pharmaceutics, College of Pharmacy, Jinan University, 601 Huangpu Avenue West, Guangzhou 510632, China (Z.W., B.W.); Department of Anesthesiology, First Affiliated Hospital of Jinan University, Guangzhou 510632, China (X.P., C.L.); and Department of Anesthesiology, Guangdong Cardiovascular Institute, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, 510080, China (S.W., X.Y.)
Running Title Page

Running Title: PK/PD characterization of cisatracurium

Address correspondence to:

Baojian Wu, Ph.D.
Division of Pharmaceutics, College of Pharmacy, Jinan University, 601 Huangpu Avenue West, Guangzhou 510632, China
E-mail: bj.wu@hotmail.com

Number of Text Page: 32
Number of Tables: 5
Number of Figures: 9
Number of References: 25
Number of Words in Abstract: 227
Number of Words in Introduction: 750
Number of Words in Discussion: 1061

Non-standard abbreviations

NMBA, nondepolarising neuromuscular blocking agents; CHD, congenital heart defects; VSD, ventricular septal defects; ASD, atrial septal defects; PK, pharmacokinetics; PD, pharmacodynamics; UPLC, ultra-performance liquid chromatography; QTOF/MS, quadrupole time-of-flight/mass spectrometry; IS, internal standard; BMI, body mass index; SD, standard deviation; AUC, the area under the curve; $k_{10}$, the elimination rate constant for central compartment; $k_{20}$, the elimination rate constant for peripheral compartment; $k_{12}$, the transfer rate constant from central to peripheral compartment; $k_{21}$, the transfer rate constant from peripheral to central compartment; $V_c$, the volume of central compartment; $V_{ss}$, the steady state volume of distribution; $T_{1/2a}$, the distribution half-life; $T_{1/2b}$, the elimination half-time; MRT, mean residence time; $k_{e0}$, the transfer rate constant of drug molecules from the central to effect compartment; AIC, Akaike information criterion.
Abstract

The neuromuscular blocking agent cisatracurium is frequently used adjunctively in anesthesia to facilitate endotracheal intubation and to provide muscle relaxation during surgery. Here we aimed to determine the pharmacokinetics/pharmacodynamics (PK/PD) of cisatracurium in patients with congenital heart defects (CHD) [i.e., ventricular septal defect (VSD) and atrial septal defect (ASD)], and to assess the effects of CHD on the PK/PD profiles of cisatracurium. A modified two-compartment model with drug clearance from both compartments was best fitted the PK data and to determine the PK parameters. The model suggested that septal defects significantly lowered the rate of cisatracurium distribution from central to peripheral compartment. The inter-compartment rate constants $k_{12}$ and $k_{21}$ were significantly reduced (35-60%, $p < 0.05$) in VSD and ASD patients as compared to control patients. Consistently, septal defects caused a marked increase (160-175%, $p < 0.001$) in the distribution half-life ($T_{1/2\alpha}$). Furthermore, significantly delayed pharmacodynamic responses to cisatracurium were observed in patients with septal defects. The onset time (i.e., the time to maximal neuromuscular block) was prolonged from 2.2 min to 5.0 min. PK/PD modeling suggested that reduced concentrations of cisatracurium in the effect compartment due to poorer distribution were the main cause of lagged pharmacodynamic responses. In conclusion, cisatracurium PK/PD were significantly altered in patients with septal defects. Our study should be of use in clinical practice for the administration of cisatracurium to CHD patients.
Introduction

Cisatracurium (marketed as Nimbex) is the R-cis, R’-cis isomer of atracurium (Kisor and Schmith, 1999). Both cisatracurium and atracurium act as nondepolarising neuromuscular blocking agents (NMBA). However, cisatracurium is about three-fold more potent as a muscle relaxant compared to atracurium (Bryson and Faulds, 1997). Further, it has less propensity to elicit histamine release, the major adverse effect of atracurium (Belmont et al., 1995). Cisatracurium has been frequently used adjunctively in anesthesia to facilitate endotracheal intubation and to provide muscle relaxation during surgery (Bryson and Faulds, 1997). The drug spontaneously degrades at physiological pH and temperature via Hofmann elimination to yield laudanosine that is subsequently metabolized to a number of conjugated metabolites (Dear et al., 1995; Welch et al., 1995; Weindlmayr-Goettel et al., 1998). Therefore, elimination of cisatracurium most likely occurs in both plasma and tissues. Although Hofmann elimination is the main mechanism for cisatracurium clearance in the body, plasma esterase-mediated hydrolysis also play a role (Kisor et al., 1996).

Congenital heart defects (CHD) are an abnormality in the structure of the heart, resulting in a change in the normal flow of blood through the heart (Garg, 2006). CHD is one of the most common types of birth defect, affecting 8 out of every 1000 newborns (Mozaffarian et al., 2015). It is reported that more than 35000 infants in America are born with congenital heart defects every year (Mozaffarian et al., 2015). Cardiac septal defects, characterized by holes in the
 septum, are the main type of CHD (Garg, 2006). Further, there are two types of septal defects, namely, ventricular septal defects (VSD) and atrial septal defects (ASD) (McDaniel, 2001). CHD can be repaired with a high success rate via catheter procedures or surgery (Napoleone and Gargiulo, 2007; Blanche et al., 2013). However, numerous adult patients are still suffering from CHD in the developing countries because they fail to receive timely treatment in their childhood.

Cisatracurium is a commonly used muscle relaxant in anesthesia (Bryson and Faulds, 1997). It is frequently used in anesthesia during repairment surgery for CHD patients (Pinard et al., 2003; Mirinejad et al., 2007). However, the pharmacokinetics (PK) and pharmacodynamics (PD) of cisatracurium in CHD patients remain underexplored. The lack of PK/PD information in this specific population raises serious concerns about administration of cisatracurium with a regular dose (0.15 mg/kg). The objectives of the present study were to determine the PK/PD of cisatracurium in CHD patients, and to assess the effects of CHD on PK/PD profiles of cisatracurium. To this end, a clinical trial was performed with patients suffering from CHD [i.e., ventricular septal defect (VSD) and atrial septal defect (ASD)]. Cisatracurium levels in plasma samples were quantified using a UPLC-QTOF/MS method. The neuromuscular block effects were measured using the train-of-four (TOF) technique. Customized PK and integrated PK/PD models were used to analyze the data. We demonstrated for the first time that PK and PD behaviors of cisatracurium were significantly altered in CHD patients.
Materials and Methods

Materials

Cisatracurium (>98% pure) was purchased from Sigma–Aldrich. SNX-2112 (used as an internal standard) was kindly provided by Prof. Yifei Wang (Biomedicine Research and Development Center, Jinan University, Guangzhou, China). MS-grade acetonitrile was obtained from Merck (Darmstadt, Germany). H$_2$SO$_4$ (analytical grade) was purchased from Runhao biological technology Ltd (Guangzhou, China).

Subjects

The clinical trial study of cisatracurium (No.GDREC2015297H, Supplemental Figure S1) was approved by the Guangdong General Hospital, Guangdong Academy of Medical Sciences (Guangzhou, China). The clinical trial was performed in the Guangdong General Hospital (Guangzhou, China). A total of 43 patients were included in the trial (written informed consent forms were obtained from all patients). These patients were divided into three groups, namely, control, VSD, and ASD groups ($n = 15$ for control and ASD groups, $n = 12$ for VSD group). Patients in control group were diagnosed with gallstone or inguinal hernia but had intact cardiac structure and normal function. VSD group patients suffered from ventricular septal defect, whereas ASD group patients suffered from atrial septal defect. The echocardiograms for all CHD patients were available in Supplementary Materials (Supplemental Figures S2 & S3). Basic demographic data were collected for each patient (Table 1). All patients had normal renal and...
liver functions and were free of clinically significant blood, psychiatric, neurologic or neuromuscular diseases.

**Anesthesia (cisatracurium administration)**

A peripheral catheter was inserted into the median cubital vein for administration of anesthetic agents. About 1 ml blank blood was collected for pH measurement prior to anesthesia execution. Blood pH was measured using a blood gas analyzer (IRMA TruPoint, USA). Anesthesia was induced with a loading dose of 0.05 mg/kg midazolam, 1~1.5 mg/kg propofol, and 5 μg/kg fentanyl. After loss of eyelash reflex (about 3-4 minutes later), 0.15 mg/kg cisatracurium was administered by intravenous bolus injection. Anesthesia was then maintained by continuous infusion of propofol (4 mg/kg/h) and remifentanil (0.1~0.5 μg/kg/min). Body temperature of each patient before drug dosing was measured using the Primus system (Drager, Germany).

**Neuromuscular monitoring**

The neuromuscular block response to cisatracurium was assessed using the train-of-four (TOF) twitch technique. TOF measurements were performed with TOF-Watch® SX (Organon, Ireland) according to the manufacturer’s protocol. In brief, the skin over the ulnar at the wrist was gently abraded and then cleaned with an alcohol wipe. The negative electrode was placed on the wrist, in line with the smallest digit, 1-2 cm below skin crease and the positive electrode was 2-3 cm proximal to the negative electrode. The adductor pollicis muscle twitch upon nerve stimulation was monitored at a 15 s interval. Single stimuli at 1 Hz were administered for 3 min for
stabilization before switching to TOF stimulation (2 Hz every 15 s) prior to cisatracurium administration. The percentage of neuromuscular block was calculated using T1 (the first twitch) values as described (Bergeron et al., 2001). The onset time was defined as the time to reach maximum block (Bergeron et al., 2001).

**Blood sampling**

Arterial blood samples (~4 ml) were collected before (time 0) and at 1, 2, 4, 8, 12, 16, 20 min after cisatracurium administration to the patients. Blood samples were transferred to centrifuge tubes containing heparin, followed by centrifugation (4 °C) at 9,000 g for 2 min. The supernatant (plasma) was collected and immediately mixed with 100 μl H₂SO₄ (1 mM). The plasma samples were stored at -80 °C until analysis.

After the addition of the internal standard (SNX-2112), plasma samples (200 μl) were deproteinized using acetonitrile (800 μl). The resulting mixture was vortexed for 3 min, followed by centrifugation at 15,000 g for 15 min. The supernatant was collected and dried using Eppendorf Concentrator Plus (Hamburg, Germany). The residue was reconstituted in a solution of water/acetonitrile (50:50, v/v; 200 μl) and centrifuged at 15,000 g for 15 min (4 °C). A 5 μl aliquot of the supernatant was injected into the UPLC-QTOF/MS system for drug quantification.

**Quantification of cisatracurium by UPLC-QTOF/MS Analysis**

Quantification of cisatracurium was performed using the UPLC-QTOF/MS system equipped with ACQUITY UPLC and Xevo G2 QTOF mass spectrometry (Waters). Instrument configuration and
parameter settings have been described in our previous publication (Liu et al., 2014). In brief, chromatographic separation was performed on a BEH column (2.1 × 50 mm, 1.7 μm; Waters). A gradient elution was applied using formic acid (0.1%) in water (mobile phase A) versus acetonitrile (mobile phase B) at a flow rate of 0.45 ml/min. The gradient elution program was 5% B at 0–1 min, 5% to 85% B at 1–3 min, 85% B at 3–3.5 min, and 85% to 5% B at 3.5–4 min. Quantitation was performed based on the full scan analysis and extracted ion chromatograms using MassLynx version 4.1 as described (Liu et al., 2014).

**Pharmacokinetic (PK) modeling**

**Model selection**

Two conventional PK models (i.e., one-compartment and two-compartment models) and a modified two-compartment PK model (Figure 1A) were used to describe the pharmacokinetic data of cisatracurium. In conventional models, drug is cleared from the central compartment only (Figure 1A). By contrast, the modified model assumed that drug clearance occurred in both central and peripheral compartments (Figure 1A). Elimination of cisatracurium from both compartments was highly possible because Hofmann degradation (the primary clearance route of the drug) was organ-independent. It was noted that several studies had indicated that the pharmacokinetic data of cisatracurium were well described by this modified PK model (Kisor et al., 1996; Schmith et al., 1997; Bergeron et al., 2001).

The mean plasma concentrations (at different time points) for three groups of patients were
obtained by averaging the data of all individuals in each group. Each of the three PK models was fitted to the data of mean plasma concentrations versus time. Model construction and data fitting were performed using MATLAB (The Mathworks Inc., Natick, MA, USA). Goodness of fit was assessed by Akaike information criterion (AIC) and dialogistic plots. The model with the smallest AIC value was regarded as the best model.

**Estimation of PK parameters**

The best PK model was fitted to individual PK data to derive PK parameters for each subject. The mean value of each parameter for each study group was obtained by averaging the values derived from the individuals. This method of parameter derivation allowed for adequate assessment of the inter-subject variability of PK parameters. Model building and parameter estimation were performed using MATLAB (The Mathworks Inc., Natick, MA, USA).

**Pharmacokinetic/pharmacodynamic (PK/PD) modeling**

**Model selection**

Three PK/PD models [i.e., model 1 (indirect link E\(_{\text{max}}\) model), model 2 (indirect link sigmoid E\(_{\text{max}}\) model), and model 3 (direct link sigmoid E\(_{\text{max}}\) model)] were established by linking an effect compartment to the best PK model (Figure 1B & Table 4). The effect compartment represented the site of cisatracurium action. In the indirect link models, the rate constant k\(_{\text{e0}}\) described the transfer of drug molecules from the central compartment to the effect compartment. In the sigmoidal E\(_{\text{max}}\) models, the slope factor (γ) was a parameter describing steepness of the
response curve. In all models, $E_{\text{max}}$ was the maximal effect. EC$_{50}$ was the drug concentration required to produce half of the maximal effect.

The mean neuromuscular block effects (at different time points) for three groups of patients were obtained by averaging the data of all individuals in each group. Each of three PK/PD models was fitted to the data of mean neuromuscular block effects versus time. PD parameters were derived following a two-step fitting procedure. First, PK parameters were obtained by fitting the PK model alone to the average PK data. Second, PD parameters were estimated by fitting the PK/PD link model to the PD data. In the second step, PK parameters in the PK/PD model were fixed at those corresponding ones derived from the first step. Model construction and data fitting were performed using MATLAB (The Mathworks Inc., Natick, MA, USA). Goodness of fit was assessed by AIC value and dialogistic plots. The model with the smallest AIC value was regarded as the best model.

**Estimation of PD parameters**

The best PK/PD model was fitted to individual PK/PD data to derive PD parameters for each subject. PD parameters were obtained following a two-step fitting procedure as described above. The only difference was that the individual data were used here. The mean value of each PD parameter for each study group was obtained by averaging the values derived from the individuals. Again, this method of parameter derivation allowed for adequate assessment of the inter-subject variability of modelled parameters. Model building and parameter estimation were
performed using MATLAB (The Mathworks Inc., Natick, MA, USA).

**Statistical Analysis**

Data are presented as mean ± standard deviation (SD). The unpaired Student's t-test was used to analyze the significant differences in PK or PD parameters between control and CHD groups. The level of significance was set at $p < 0.05$ (*) or $p < 0.01$ (**) or $p < 0.001$ (***).
Results

Study subjects

A total of 43 patients, divided into one control and two CHD groups (i.e., VSD and ASD groups), were included in current clinical trial (Table 1). These patients were randomized to maximize the similarity in demographic features (e.g., age, sex, race and BMI) between control and CHD groups (Table 1). The main clearance pathway for cisatracurium was Hoffman degradation that was pH and temperature-dependent (Kisor et al., 1996). Hence, it was necessary to measure the blood pH and body temperature for all patients. The result showed that there were no significant differences in pH or body temperature values between any two of the three groups (Table 1).

Quantification of plasma cisatracurium by UPLC-QTOF/MS

Concentrations of cisatracurium in plasma were quantified using UPLC-QTOF/MS with a 4-min elution gradient. Cisatracurium and SNX-2112 (used as an internal standard) were eluted at 2.61 and 2.88 min, respectively (Figure 2). We selected SNX-2112 as an internal standard because this compound was rather stable and showed a high response in the UPLC-QTOF/MS system. The analytical method were rigorously validated with respect to linearity (50–5000 ng/ml), precision (RSD <10%), and accuracy (within 90–110%). The limit of quantification was estimated at 12.5 ng/ml.
Altered cisatracurium pharmacokinetics in patients with cardiac septal defects

The plasma concentrations of cisatracurium versus time curves were determined for all individuals (Figure 3A-C). All pharmacokinetic curves clearly showed two distinct phases, namely, the distribution and elimination phases. Mean plasma concentrations versus time curves for control and CHD groups were plotted and compared (Figure 4). It was found that mean plasma concentrations of cisatracurium at early time points (≤ 4 min) were significantly higher (p < 0.01) in CHD group than in control group patients (Figure 4). The results suggested that cardiac septal defects were associated with an elevation in the plasma level of cisatracurium in the distribution phase. This was accounted for by altered drug distribution in CHD patients (see next paragraph).

A modified two-compartment model (Figure 1) was best fitted to the pharmacokinetic data according to the AIC value (Table 2). Best fitting of this custom model to the data was also justified by the diagnostic plots (Supplemental Figures S4 & S5). The predicted values from the model were closest to the observed ones (Supplemental Figure S4). Also, the model generated smallest and randomly distributed residuals (Supplemental Figure S5). The mean value of each parameter for each study group was obtained by averaging the values derived from the individuals (Table 3). Cardiac septal defects led to obvious alterations in drug transfer between central and peripheral compartments (Table 3). The rate constants $k_{12}$ and $k_{21}$ were significantly reduced (35-60%, $p < 0.05$) in the VSD and ASD groups as compared to the control group (Table 3). Consistently, septal defects caused significant increases (160-175%, $p < 0.001$) in the
distribution half-life value ($T_{1/2\alpha}$). Taken together, the modeling results indicated that ASD and VSD defects delayed distribution of cisatracurium to peripheral compartment, leading to higher levels of the drug in the central compartment (or plasma) in the distribution phase. By contrast, none of other parameters [including the volume of central compartment ($V_c$), steady state volume of distribution ($V_{ss}$), elimination rate constant ($K_{10}$), elimination half-time ($T_{1/2\beta}$) and mean residence time (MRT)] were changed by cardiac septal defects (Table 3).

**Altered cisatracurium pharmacodynamics in patients with cardiac septal defects**

The profiles of neuromuscular block effect versus time were determined for all individuals (Figure 5). By using the individual data, the mean neuromuscular block effects versus time curves for three study groups were plotted and compared (Figure 6). It was clear that cardiac septal defects delayed the pharmacodynamic effects of cisatracurium by visual inspection (Figure 6). Furthermore, the pharmacodynamic data was best described by the PK/PD model 2 consisting of the modified PK model and an indirect link sigmoidal $E_{max}$ model (Table 4; Figures 1B & 6). Best fitting of this integrated model to the data was also justified by the diagnostic plots (Supplemental Figures S6 & S7). The predicted PD values from the model were closest to the observed ones, generating smallest residuals (Supplemental Figures S6 & S7). Further, the residuals were randomly distributed, suggestive of model adequacy (Supplemental Figure S7).

The estimated pharmacodynamic parameters were summarized (Table 5). The mean $k_{e0}$ value was 30.8-38.5% smaller ($p < 0.001$) in patients with cardiac septal defects than in control
patients, suggestive of slower distribution of drug molecules to the effect compartment from blood in septal defect patients. The onset time (i.e., the time to maximal neuromuscular block effect) was significantly prolonged to 5.0 min (p < 0.001) in septal patients (Table 5). By contrast, septal defects did not cause changes in any of the other parameters (i.e., $E_{\text{max}}$, $EC_{50}$ or $\gamma$) (Table 5), indicating that drug potency and binding affinity to the target remained unchanged.

Cisatracurium concentrations in the effect compartment were simulated based on the PK/PD model 2 (Figure 7). The delayed onset of cisatracurium effect was associated with reduced concentrations of cisatracurium in CHD groups due to poorer distribution (Figure 7). This suggested that the differences in drug distribution may explain the distinct pharmacodynamic effects in CHD patients. Dose-dependent pharmacodynamic effects in VSD and ASD patients were also simulated (Figure 8). It was predicted that an escalation of dose from 1.5 to 2.0 mg/kg would result in an equal pharmacodynamic effect in septal defect patients as compared to control patients (Figure 8).
Discussion

In this study, we for the first time determined the pharmacokinetics and pharmacodynamics of cisatracurium in patients with two common types of CHD (i.e., VSD and ASD patients). The results suggested that septal defects significantly altered the kinetics and extent of drug distribution from central to peripheral compartment, resulting in a higher drug level in central compartment (plasma) during the distribution phase (Figure 4). Furthermore, significantly delayed pharmacodynamic responses to cisatracurium were observed in patients with septal defects (Figure 6). PK/PD modeling suggested that reduced concentrations of cisatracurium in the effect compartment due to poorer distribution may be the main cause of the lag in pharmacodynamic response (Figure 7). Therefore, our study provided a strong PK/PD basis for rational dosing of cisatracurium in patients with septal defects to achieve a desired pharmacodynamic response.

A modified two-compartment model (Figure 1A) with elimination from both central and peripheral compartments was best fitted to the pharmacokinetic data of cisatracurium herein. The pharmacokinetic data of cisatracurium were also well described by the same model in previous studies (Schmith et al., 1997; Bergeron et al., 2001). This model was highly relevant to cisatracurium because Hofmann elimination as the main elimination route of the drug was organ-independent (Fisher et al., 1986). The conventional two-compartment models with elimination from the central compartment alone would result in an underestimation of $V_{ss}$ value.
and an inadequate description of the concentration versus time curve (Kisor et al., 1996). The derived $K_{10}$ values (0.08-0.10 min$^{-1}$) were larger than the $K_{20}$ value (0.03-0.04 min$^{-1}$). This was in line with the fact that in addition to Hofmann elimination, the drug was also cleared via other pathways such as the renal excretion (Kisor et al., 1996). Nevertheless, the custom model was an empirical model due to the lack of a mechanistic justification using the peripheral compartment data.

The pharmacokinetics and pharmacodynamics of cisatracurium were best described by an integrated PK/PD model (i.e., PK/PD model 2) with an effect compartment linked to the central compartment (Figure 1B). The same model had been successfully used to analyze the pharmacokinetics and pharmacodynamics of cisatracurium in patients with other types of diseases (e.g., end-stage liver disease and severe sepsis) (De Wolf et al., 1996; Liu et al., 2012). Our study and previous ones highlighted that the PD effects of cisatracurium were directly related to the drug concentrations in the site of action (Mellinghoff and Diefenbach, 1997). Hence, the first-order rate constant $K_{e0}$, characterizing the transfer of drug from the central to the effect compartment, were an key determinant to the onset time of cisatracurium (Minto and Schnider, 2008). Due to a decrease in $K_{e0}$ value, the onset time of cisatracurium was significantly prolonged in VSD and ASD patients as compared to control patients (Figure 6).

It was a novel finding that cardiac septal defects, the most common types of congenital heart diseases, led to significant alterations in both pharmacokinetics and pharmacodynamics of
cisatracurium. The PK/PD model suggested that altered (delayed) pharmacodynamic effects may be due to reduced drug exposure in the effect compartment caused by poorer distribution. The exact reasons remained unknown as to why cisatracurium distribution from central/blood to peripheral/effect compartment was altered in patients with septal defects. Nevertheless, septal defects allow blood shunting between the atria and ventricles, resulting in marked changes in hemodynamics (Penny and Vick, 2011; Geva et al., 2014). Hemodynamic changes are known to be associated with pulmonary hypertension, tricuspid valve regurgitation and mitral valve regurgitation in septal defect patients (Penny and Vick, 2011; Geva et al., 2014). Therefore, it was reasonable to speculate that the hemodynamic changes in patients with septal defects underlay poorer drug distribution of cisatracurium to the tissues from blood.

Pharmacokinetic analyses showed that the elimination rate constants $K_{10}$ and $K_{20}$ remained unchanged in CHD as compared to control patients (Table 3). Since the main elimination pathway for cisatracurium is Hoffman degradation (Kisor et al., 1996; Schmith et al., 1997; Bergeron et al., 2001), the unchanged $K_{10}$ and $K_{20}$ may imply that degradation of cisatracurium by Hoffman reaction was not altered in CHD patients. This was supported by the fact that the pH and body temperature (two critical factors determining the rate of cisatracurium degradation) of CHD patients were identical to those of control patients (Table 1). Since it was unlikely that CHD caused a difference in the rate of metabolite production, there was a very low possibility that metabolite production would impact the cisatracurium pharmacodynamics in CHD patients.
Determination of pharmacokinetics and pharmacodynamics of cisatracurium in subpopulations assumed great importance in formulation of population-specific dosage regimen to achieve optimized therapy. Accurate dosing of cisatracurium contributed to improvement of patient safety during anesthesia (Merry et al., 2009). Failing to perform endotracheal intubation at the peak of the muscle-relaxing effect (i.e., at the onset time point) will compromise the hemodynamic stability of the patients and cause a disturbance in surgery operating, thereby increasing the rate of anesthetic accidents. In clinical practice, anesthetists usually perform intubation 2 minutes after intravenous injection of 0.15 mg/kg cisatracurium (Bryson and Faulds, 1997). However, such treatment would cause tissue damages in patients with septal defects due to a lag in the onset of neuromuscular block. It was suggested that intubation should be performed ~5.0 minutes after cisatracurium administration. Alternatively, the dose should be escalated to about 2 mg/kg to achieve a regular pharmacodynamic effect (Figure 8).

In summary, we have for the first time established an integrated PK/PD model for cisatracurium in patients with cardiac septal defects. The effects of septal defects on cisatracurium PK and PD were fully assessed. PK modeling suggested that septal defects significantly lowered the rate of cisatracurium distribution from central to peripheral compartment, resulting in a higher drug level in central compartment (plasma) during the distribution phase. Furthermore, significantly delayed pharmacodynamic responses to cisatracurium were observed in patients with septal defects. PK/PD modeling suggested that reduced concentrations of cisatracurium in the effect compartment due to poorer distribution may be the main cause of the
lag in pharmacodynamic response. Our study should be of use in clinical practice for the administration of cisatracurium to CHD patients.
Authorship Contributions

Participated in research design: Z Wu, Wang, Peng and B Wu

Conducted experiments: Z Wu, Wang, Lu, Ye and Peng

Contributed new reagents or analytic tools: Wang, and Peng

Performed data analysis: Z Wu, Wang, Peng and B Wu

Wrote or contributed to the writing of the manuscript: Z Wu, Wang, Peng and B Wu
References


Footnotes

This work was supported by the Young Scientist Special Projects in biotechnological pharmaceutical field of 863 Program [Grant 2015AA020916]; the Fundamental Research Funds for the Central Universities [Grant 11615463]; and the National Natural Science Foundation of China [Grant 81573488].

ZW, SW and XP contributed equally to this work.
Legends for Figures

**Figure 1**  Schematic diagram of the PK (A) as well as the PK/PD (B) models for data analyses in this study. $k_{10}$, the elimination rate constant for central compartment; $k_{20}$, the elimination rate constant for peripheral compartment; $k_{12}$, the transfer rate constant from central to peripheral compartment; $k_{21}$, the transfer rate constant from peripheral to central compartment.

**Figure 2**  Representative extracted ion chromatograms of cisatracurium and SNX-2112 (the internal standard) from UPLC–QTOF/MS analysis. IS, internal standard. Panel A: Extracted ion chromatogram of cisatracurium. Panel B: Extracted ion chromatogram of SNX-2112.

**Figure 3**  Plasma concentration versus time curves of cisatracurium in various types of patients after intravenous administration of cisatracurium (0.15 mg/kg). Panel A: Individual plasma concentration versus time curves for 15 control patients. Panel B: Individual plasma concentration versus time curves for 12 VSD patients. Panel C: Individual plasma concentration versus time curves for 15 ASD patients.

**Figure 4**  Comparisons of mean plasma concentration versus time curves between control and CHD groups. Panel A: A comparison of mean plasma concentration versus time curves between control group and VSD groups. Panel B: A comparison of mean plasma concentration versus time curves between control group and ASD groups. Solid lines were predicted data from the modified two-compartment PK model (Figure 1A). Statistically significant differences in plasma concentrations between control and disease groups at specific time points were indicated by asterisks (**$P < 0.01$; ***$P < 0.001$).

**Figure 5**  The neuromuscular block effect versus time curves of cisatracurium in various types of patients after intravenous administration of cisatracurium (0.15 mg/kg). Panel A: A comparison of individual neuromuscular block versus time curves between control group and VSD groups. Panel B: A comparison of individual neuromuscular block versus time curves between control group and ASD groups.

**Figure 6**  Comparisons of mean PD effect versus time curves between control group and CHD groups. Panel A: A comparison of mean PD effect versus time curves between control and VSD groups. Panel B: A comparison of mean PD effect versus time curves between control and ASD groups. Solid lines were predicted data from the integrated PK/PD model 2 (Figure 1B).

**Figure 7**  Comparisons of the drug concentrations in effect compartment ($C_e$) derived from the integrated PK/PD model 2. Panel A: Comparisons of the $C_e$ between control and VSD groups. Panel B: Comparisons of the $C_e$ between control and ASD groups.
**Figure 8** Comparison of the predicted PD effect versus time curves derived from PK/PD model 2 (Figure 1B). Panel A: A comparison of the predicted PD effect versus time curves between control (at intravenous dose of 0.15mg/kg) and VSD groups (at intravenous dose of 0.15mg/kg, 0.2mg/kg and 0.25 mg/kg). Panel B: A comparison of the predicted PD effect versus time curves between control (at intravenous dose of 0.15mg/kg) and ASD groups (at intravenous dose of 0.15mg/kg, 0.2mg/kg and 0.25 mg/kg).
### Table 1

Demographic characteristics of patients in the clinical trial

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 15)</th>
<th>VSD (n = 12)</th>
<th>ASD (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M/F</td>
<td>7/8</td>
<td>7/5</td>
<td>9/6</td>
</tr>
<tr>
<td>Race</td>
<td>Chinese</td>
<td>Chinese</td>
<td>Chinese</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.7 ± 9.73</td>
<td>31.2 ± 8.73</td>
<td>36.5 ± 13.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.6 ± 6.78</td>
<td>52.8 ± 5.61</td>
<td>53.8 ± 7.82</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.7 ± 0.88</td>
<td>20.6 ± 1.57</td>
<td>20.5 ± 2.36</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.2 ± 0.28</td>
<td>36.2 ± 0.29</td>
<td>36.2 ± 0.28</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.39 ± 0.02</td>
<td>7.37 ± 0.03</td>
<td>7.38 ± 0.02</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
Table 2

Pharmacokinetic parameters and statistics derived from fitting of each of three pharmacokinetic models (Figure 1A) to the average data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>VSD</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>One-compartment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V$ (ml/kg)</td>
<td>84.2 ± 8.3</td>
<td>67.3 ± 6.9</td>
<td>61.7 ± 7.11</td>
</tr>
<tr>
<td>$K_{10}$ (1/min)</td>
<td>0.10 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>AIC</td>
<td>86.6</td>
<td>87.1</td>
<td>88.2</td>
</tr>
<tr>
<td><strong>Two-compartment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_c$ (ml/kg)</td>
<td>57.2 ± 6.23</td>
<td>54.7 ± 4.98</td>
<td>52.8 ± 5.92</td>
</tr>
<tr>
<td>$K_{10}$ (1/min)</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>$K_{12}$ (1/min)</td>
<td>0.25 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>$K_{21}$ (1/min)</td>
<td>0.25 ± 0.03</td>
<td>0.17 ± 0.02</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>AIC</td>
<td>61.2</td>
<td>59.7</td>
<td>62.6</td>
</tr>
<tr>
<td><strong>A modified model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_c$ (ml/kg)</td>
<td>50.1 ± 2.18</td>
<td>48.9 ± 2.17</td>
<td>47.2 ± 2.11</td>
</tr>
<tr>
<td>$K_{10}$ (1/min)</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>$K_{20}$ (1/min)</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>$K_{12}$ (1/min)</td>
<td>0.23 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>$K_{21}$ (1/min)</td>
<td>0.24 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>AIC</td>
<td>49.6</td>
<td>48.3</td>
<td>50.1</td>
</tr>
</tbody>
</table>

Data presented as estimated value ± SD.

*Two-compartment model with drug elimination only from central compartment.

bTwo-compartment model with drug elimination from both central and peripheral compartment.
Table 3

Pharmacokinetic parameters estimated from fitting the modified PK model (Figure 1A) to individual data. The mean value of each parameter for each study group was obtained by averaging the values derived from the individuals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>VSD</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vc (ml/kg)</td>
<td>42.2 ± 6.72</td>
<td>50.5 ± 11.1</td>
<td>41.3 ± 6.56</td>
</tr>
<tr>
<td>K10 (1/min)</td>
<td>0.10 ± 0.05</td>
<td>0.08 ± 0.002</td>
<td>0.09 ± 0.003</td>
</tr>
<tr>
<td>K12 (1/min)</td>
<td>0.37 ± 0.17</td>
<td>0.14 ± 0.04**</td>
<td>0.16 ± 0.04**</td>
</tr>
<tr>
<td>K21 (1/min)</td>
<td>0.23 ± 0.08</td>
<td>0.15 ± 0.06*</td>
<td>0.14 ± 0.06*</td>
</tr>
<tr>
<td>K20 (1/min)</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>T1/2α (min)</td>
<td>1.31 ± 0.05</td>
<td>2.29 ± 0.08***</td>
<td>2.08 ± 0.05***</td>
</tr>
<tr>
<td>T1/2β (min)</td>
<td>19.3 ± 6.78</td>
<td>21.2 ± 7.08</td>
<td>21.0 ± 7.42</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>6.86 ± 1.45</td>
<td>6.72 ± 1.34</td>
<td>6.51 ± 1.18</td>
</tr>
<tr>
<td>Vss (ml/kg)</td>
<td>142 ± 37.9</td>
<td>142 ± 29.9</td>
<td>129 ± 29.2</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>23.3 ± 8.76</td>
<td>24.6 ± 9.45</td>
<td>24.7 ± 8.91</td>
</tr>
<tr>
<td>AUC (min*μg/ml)</td>
<td>23.8 ± 5.18</td>
<td>28.6 ± 6.57*</td>
<td>30.5 ± 6.65**</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD. *P < 0.05 compared with control group; **P < 0.01 compared with control group; ***P < 0.001 compared with control group.
### Table 4

Pharmacodynamic parameters and statistics derived from fitting of each of three PK/PD models (Figure 1B) to the average data

<table>
<thead>
<tr>
<th>Model</th>
<th>Control</th>
<th>VSD</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(E_{\text{max}})</td>
<td>(999 \pm 898)</td>
<td>(989 \pm 986)</td>
</tr>
<tr>
<td>Model 1 (Indirect link (E_{\text{max}}) model)</td>
<td>(2.45 \pm 32.1)</td>
<td>(5.75 \pm 19.8)</td>
<td>(5.57 \pm 15.5)</td>
</tr>
<tr>
<td></td>
<td>(K_{\text{e0}}) (1/min)</td>
<td>(0.14 \pm 0.05)</td>
<td>(0.08 \pm 0.02)</td>
</tr>
<tr>
<td></td>
<td>AIC</td>
<td>(219)</td>
<td>(227)</td>
</tr>
<tr>
<td>Model 2 (Indirect link sigmoid (E_{\text{max}}) model)</td>
<td>(102 \pm 2.16)</td>
<td>(103 \pm 2.78)</td>
<td>(104 \pm 3.12)</td>
</tr>
<tr>
<td></td>
<td>(0.22 \pm 0.03)</td>
<td>(0.24 \pm 0.04)</td>
<td>(0.27 \pm 0.05)</td>
</tr>
<tr>
<td></td>
<td>(5.18 \pm 0.78)</td>
<td>(6.12 \pm 0.46)</td>
<td>(6.56 \pm 0.78)</td>
</tr>
<tr>
<td></td>
<td>(0.12 \pm 0.02)</td>
<td>(0.07 \pm 0.01)</td>
<td>(0.08 \pm 0.01)</td>
</tr>
<tr>
<td></td>
<td>AIC</td>
<td>(112)</td>
<td>(92.4)</td>
</tr>
<tr>
<td>Model 3 (Direct link sigmoid (E_{\text{max}}) model)</td>
<td>(34.4 \pm 878)</td>
<td>(41.3 \pm 978)</td>
<td>(39.9 \pm 867)</td>
</tr>
<tr>
<td></td>
<td>(1.21 \pm 17.8)</td>
<td>(2.34 \pm 32.8)</td>
<td>(2.21 \pm 12.4)</td>
</tr>
<tr>
<td></td>
<td>(0.0006 \pm 788)</td>
<td>(0.0003 \pm 841)</td>
<td>(0.0005 \pm 745)</td>
</tr>
<tr>
<td></td>
<td>AIC</td>
<td>(274)</td>
<td>(298)</td>
</tr>
</tbody>
</table>

Data presented as estimated value ± SD.
Table 5

Pharmacodynamic parameters estimated from fitting the PK/PD model 2 (Figure 1B) to individual data. The mean value of each parameter for each study group was obtained by averaging the values derived from the individuals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>VSD</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC_{50} (μg/ml)</td>
<td>0.20 ± 0.04</td>
<td>0.21 ± 0.04</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>K_{e0} (1/min)</td>
<td>0.13 ± 0.02</td>
<td>0.09 ± 0.02**</td>
<td>0.08 ± 0.01***</td>
</tr>
<tr>
<td>\gamma</td>
<td>7.18 ± 2.02</td>
<td>6.51 ± 1.82</td>
<td>6.28 ± 1.61</td>
</tr>
<tr>
<td>E_{max}</td>
<td>102 ± 2.17</td>
<td>101 ± 1.82</td>
<td>103 ± 2.51</td>
</tr>
<tr>
<td>Onset time* (min)</td>
<td>2.16 ± 0.27</td>
<td>4.87 ± 0.51***</td>
<td>4.95 ± 0.78***</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD. *P < 0.05, compared with control group; **P < 0.01, compared with control group; ***P < 0.001, compared with control group.

*Onset time was experimentally measured.
Figure 2

A

100
%

0

2.61

1: MS ES+
464.32 0.0200 Da
7.46e4

B

100
%

0

2.88

1: MS ES+
465.24 0.0200 Da
8.78e4

IS
Figure 3

A

Control

Plasma concentration, µg/ml

Time, min

B

VSD

Plasma concentration, µg/ml

Time, min

C

ASD

Plasma concentration, µg/ml

Time, min

Figure 4

A

Plasma concentration, $\mu$g/ml

- Control
- VSD

Time, min

B

Plasma concentration, $\mu$g/ml

- Control
- ASD

Time, min
Figure 5

A

Neuromuscular block (%)

Time, min

Control (n = 15)
VSD (n = 12)

B

Neuromuscular block (%)

Time, min

Control (n = 15)
ASD (n = 15)
Figure 6

A

Neuromuscular block (%)

Control
VSD

Time, min

0

B

Neuromuscular block (%)

Control
ASD

Time, min

0
Figure 7

A  
Effect Compartment Concentration, ng/ml  
0  4  8  12  16  20  
Time, min  
Control  
VSD

B  
Effect Compartment Concentration, ng/ml  
0  4  8  12  16  20  
Time, min  
Control  
ASD
Figure 8

A

B

Neuromuscular block (%)

Time, min

Neuromuscular block (%)

Time, min

Control
VSD
VSD_2mg/kg
VSD_2.5mg/kg

Control
ASD
ASD_2mg/kg
ASD_2.5mg/kg