Study protocol and Statistical analysis plan of

Pharmacokinetic-pharmacodynamic Analysis of Sugammadex in Children

(NCT03943888)

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1) Study protocol

STUDY DESIGN AND POPULATION

This study was designed as a randomized, controlled, single-blinded, exploratory study to examine the pharmacokinetics of sugammadex and compare the pharmacodynamics of 2, 4, or 8 $mg \cdot kg^{-1}$ of sugammadex against 0.03 $mg \cdot kg^{-1}$ of neostigmine. This study was conducted at a single center. The study protocol was approved by the Institutional Review Board of the Seoul National University Hospital (1904-149-1029, approval date: 07/31/2019) and the Ministry of Food and Drug Safety of the Republic of Korea (approval no.: 32285, approval date: 07/09/2019). This study was registered at http://clinicaltrials.gov (NCT03943888, principal investigator: Hee-Soo Kim, published date: 08/13/2019). The study was conducted in accordance with the Good Clinical Practice guidelines by the International Council for Harmonization and the Declaration of Helsinki. Participants were recruited between August 2019 and February 2020.

Children aged 2−17 years, with an American Society of Anesthesiologists (ASA) physical status classification of 1 or 2, who were scheduled to undergo surgery under general anesthesia, and required early reversal of neuromuscular blockade after induction because of electrophysiology monitoring were enrolled. Written informed consent was obtained from one parent of each participant aged younger than 7 years, whereas it was obtained from both the participant and one of their parents for children aged 7−17 years. The exclusion criteria were a history of hypersensitivity to any anesthetic agents including rocuronium; the presence of underlying cardiovascular or genitourinary disease; the use of a neuromuscular blocking agent or any other drug that can influence the effect of rocuronium before surgery; a history of malignant hyperthermia; anticipation of massive hemorrhage during surgery; and one or more parent or legal guardian declining to enroll.

STUDY PROTOCOL

Upon the participant's arrival in the operating room, electrocardiogram (ECG), non-invasive blood pressure at 1-min intervals, and pulse oximetry for peripheral capillary oxygen saturation (SpO_2) were monitored. Anesthesia was induced in a

routine manner with sodium thiopental or propofol according to age. Intravenous propofol and remifentanil were continuously infused to maintain anesthesia while providing 100% oxygen via a fitting mask. Train-of-four stimulation was performed with four twitch stimulations over 2 s with an intensity of 50 mA every 15s via ToFscan® (IDMED, Marseille, France) at the participant's unilateral ulnar nerve. Responses to the train-of-four stimulations were measured by acceleromyography and automatically recorded via a program provided by the manufacturer. After the start of the recording, 1 $mg \cdot kg^{-1}$ of rocuronium was intravenously injected. Arterial catheterization was performed at one of the four extremities, and continuous monitoring of blood pressure was initiated. After confirmation of a train-of-four count of zero, the post-tetanic count, which measures the number of responses to 15 twitch stimulations at 50 Hz for 5 s, was measured at another extremity. After confirmation of zero post-tetanic count, the study drug was administered intravenously. At the end of the surgery, the train-of-four monitoring was stopped.

PHARMACOKINETIC MEASUREMENTS

Arterial blood was withdrawn nine times to measure the plasma concentrations of rocuronium and sugammadex at 2 min after rocuronium injection; immediately before study drug administration; and 2, 5, 15, 60, 120, 240, and 480 min after study drug administration. The concentration of rocuronium was measured at every time point, whereas sugammadex was excluded at the first point. In case of deviation from the scheduled time, the actual time of sampling was recorded.

MEASUREMENT OF PLASMA CONCENTRATIONS

At the previously described time points, 1 mL of arterial blood was drawn for each measurement, and the blood was immediately stored in a sodium heparin tube (BD Vacutainer® sodium heparin (N) 75 USP Units, Becton Dickinson Korea, Seoul, Korea). After centrifuging the samples at 3,000 rpm for 10 min, the supernatant was collected and stored in a sterile internal cryogenic vial (Cryotain™, SCILAB Korea, Seoul, Korea). The cryovials were stored in a freezer below -70°C until analysis.

Plasma concentrations of sugammadex and rocuronium were measured using liquid chromatography-tandem mass spectrometry. The assays were conducted in full compliance with the Good Laboratory Practice regulations. As this assay could not discriminate the sugammadex-rocuronium complex from their free forms, all plasma concentrations were considered total plasma concentrations. The internal standard was donepezil base for sugammadex and 3-acetyl rocuronium bromide for rocuronium, respectively (Toronto Research Chemicals, North York, Canada). The lower limit of quantification and upper limit of quantification were set as 0.1 and 100 $\mu g \cdot mL^{-1}$ for sugammadex and as 10 and 10,000 $ng \cdot mL^{-1}$ for rocuronium. The intra-assay coefficient of variation was no more than 14.9%, and the % bias was -12.5–2.0% for sugammadex. The intra-assay coefficient of variation was no more than 5.7%, and the % bias was -5.6–5.0% for rocuronium.

2) Control group setting and randomization method

According to a randomization table obtained from the website https://sealedenvelope.com/, the participants were allocated to one of the four groups: 2, 4, or 8 $mg \cdot kg^{-1}$ of sugammadex or a control group with 0.03 $mg \cdot kg^{-1}$ of neostigmine. According to the allocation, the study drug was prepared by a single anesthesiologist (J.H. Lee). The participants and their parents were blinded to the group allocation.

3) Data analysis and statistical analysis method

NONCOMPARTMENTAL ANALYSIS

The noncompartmental pharmacokinetic parameters of sugammadex and rocuronium were calculated using a validated software, Phoenix WinNonlin (Version 8.1; Certara USA, Princeton, NJ, USA). The maximum concentration (C_{max}) and time to reach Cmax were determined from the observed values. The area under the plasma concentration-time curve (AUC) from time zero to the last measurable concentration (AUC_{last}) was calculated using linear-up log-down trapezoidal method. Partial AUCs from time zero to 15 minutes (AUC_{0-15m}) and 1 h post-dose (AUC_{0-1h}) were calculated using the same method. AUC from time zero to infinity (AUC_{inf}) was calculated as the sum of AUC_{last} and the last measureable concentration divided by

terminal elimination constant (λ_z) estimated by linear regression. Terminal-phase elimination half-life ($t_{1/2}$) was calculated as natural logarithm of 2 divided by λ_z. Clearance (CL) and terminal-phase volume of distribution (V_z) were calculated as $Dose \cdot AUC_{inf}^{-1}$ and $Dose \cdot (\lambda_z \cdot AUC_{inf})^{-1}$.

POPULATION PHARMACOKINETIC MODELING OF SUGAMMADEX

Population pharmacokinetic model for sugammadex was developed using nonlinear mixed-effect modelling software (NONMEM 7.4.4 software, ICON Development Solutions, Ellicott City, MD, USA). Data processing and diagnostics were performed using R version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria) and Perl-Speaks-NONMEM (version 4.9.0, https://uupharmacometrics.github.io/PsN/). Plasma concentrations were fitted into one/two/three-compartment models via the ADVAN 6 subroutine and first-order conditional estimation with interaction. During model building, inter-individual variabilities of parameters were assumed to be log-normally distributed and introduced as exponential. The residual error was described with a proportional model. The minimum objective function value was obtained, which was equivalent to the -2 log likelihood of the model. For an alpha-error probability of 0.05, a reduction in the objective function value by more than 3.84 was regarded as significant according to the χ^2 distribution at degrees of freedom = 1.

After determining the base pharmacokinetic models, covariates of age, sex, weight, height, and serum creatinine concentration5 were evaluated for selection. The effect of bodyweight on clearance was considered with allometric scaling as follows:

$$P_i = \theta_p \times ((BW_i)/(MeanBW_i))^{0.75} \times e^{\eta i}$$

where Pi denotes the individual value, θ_p represents the population estimates, BW_i represents the individual bodyweight, and η_i denotes the inter-individual random effect.

After development, goodness-of-fit plots comparing observations and individual predictions, observations and population predictions, conditional weighted residuals and population predictions, conditional weighted residuals, and time after dosing were sketched. For internal validation, median values and their 95% confidence intervals for each parameter were obtained from a nonparametric bootstrap analysis of 1,000 simulated datasets. Prediction-corrected visual predictive check was performed with the R package 'xpose'. The prediction-corrected visual predictive check evaluated whether the observed data were within the median and 90%

prediction interval of 1,000 simulated datasets from the final model. The models were executed, and diagnostics were performed using Pirana (version 2.9.9, https://www.pirana-software.com). During the modeling, the rocuronium-sugammadex complex was not discriminated from the free form of sugammadex or rocuronium, since they were indistinguishable during the plasma concentration measurement. Referring to previous studies, we assumed elimination constant of rocuronium-sugammadex complex as identical to that of sugammadex and built a pharmacokinetic model of sugammadex, regardless of complex formation.

PHARMACODYNAMIC MEASUREMENTS

During or after surgery, neuromuscular blockade was monitored by evaluating the response to a peripheral nerve stimulation. Train-of-four stimulation, which consists of four successive supramaximal stimuli delivered at 2Hz on the ulnar, facial, or posterior tibial nerve, is the most common means of monitoring. The count of responses to the stimuli and the ratio of the fourth twitch to the first twitch (T_4/T_1 ratio) represent the receptor occupancy by rocuronium. A T_4/T_1 ratio greater than 90% is considered sufficient for extubation. Usually, the efficacy of the reversal agents for neuromuscular blockade is measured as the time after administration of reversal agents to recovery of the T_4/T_1 ratio greater than 90%.

The count of twitches and the T_4/T_1 ratio to the train-of-four stimulations were automatically recorded until the end of the surgery and were compared according to the group. The time elapsed from the study drug administration to the attainment of a T_4/T_1 ratio $\geq$ 0.9 was the primary pharmacodynamic outcome. To bind the pharmacokinetics of sugammadex to pharmacodynamic measurements, the time to recovery of the train-of-four ratio larger than 0.9 was plotted against the Cmax and AUClast of sugammadex.

MONITORING OF SAFETY

Monitoring of the participants' ECG, mean blood pressure, pulse oximetry, and body temperature was started from the beginning of anesthesia and continued until 24 h after the end of surgery. The presence of hemodynamic instability (more than 30% change from baseline for heart rate and mean blood pressure), hypoxemia (less than 92%), hyperthermia (above 38.3°C), hypothermia (below 35.5°C), nausea, vomiting, urticaria, and any anaphylactic reactions was monitored and recorded.

STATISTICAL ANALYSIS

Data for the baseline characteristics and pharmacodynamics were tested for normality using the Kolmogorov–Smirnov test for data from the whole study population and the Shapiro–Wilk test for data from individual study groups. For nonparametric comparison of baseline characteristics of individual study groups, the Kruskal–Wallis test and a consequent Mann–Whitney U test with Bonferroni correction for post-hoc analysis were employed using the SPSS® version 22 (IBM®, Chicago, IL, USA). Differences in the pharmacokinetic (PK) parameters of rocuronium related to systemic exposure (i.e., C_{max}, AUC_{last}, and AUC_{inf}) according to the sugammadex dose groups were compared using the Kruskal–Wallis test, followed by the nonparametric post-hoc evaluation using the Dwass, Steel, Critchlow-Fligner procedure implemented in the SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).