Dose-dependency of pharmacokinetic/pharmacodynamic parameters after intravenous bolus doses of cisatracurium

C. Chen, N. Yamaguchi and F. Varin*

Faculté de pharmacie, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, QC, Canada H3C 3J7

*Corresponding author. E-mail: france.varin@umontreal.ca

Background. Pharmacokinetic/pharmacodynamic (PK/PD) parameters of neuromuscular blocking agents (NMBAs) are generally assumed to be dose-independent. To our knowledge, there are very few clinical reports where the PK/PD parameters of a NMBA were derived separately for each dose group during a formal dose-ranging study. The primary objective of this study was to challenge a potential dose-dependency of cisatracurium PK/PD parameters by conducting a well-controlled experimental study.

Methods. Eight dogs were anaesthetized with pentobarbital and mechanically ventilated. Two doses of cisatracurium (1.5×ED95 and 6×ED95) were administered in a randomized cross-over design after an appropriate washout period. Neuromuscular function was monitored using train-of-four (TOF) stimulation. Arterial blood was sampled continuously for the first minute after cisatracurium injection and at frequent intervals thereafter. Cisatracurium plasma concentrations were determined by high performance liquid chromatography analysis. PK/PD modelling of individual data sets was performed with NONMEM using a non-parametric approach and a descriptive sigmoid $E_{\text{max}}$ model.

Results. Cisatracurium PKs were linear over the dose range studied. Using non-parametric PK/PD analysis, mean values for plasma–effect compartment equilibration delay ($k_{\text{e0}}$) were 0.0600 vs 0.1278 min$^{-1}$ ($P<0.05$) and sensitivity (EC$_{50}$) were 323 vs 235 ng ml$^{-1}$ ($P<0.05$) for the high and low doses, respectively.

Conclusions. A dose-dependent effect on the PK/PD parameters of cisatracurium has important clinical implications as an accurate estimate of the EC$_{50}$ is desirable. PK/PD parameters derived after intubating bolus doses of cisatracurium would be more reliable.

Br J Anaesth 2008; 101: 788–97

Keywords: pharmacodynamics, cisatracurium; pharmacokinetics, cisatracurium; pharmacokinetics, dose-dependency; pharmacokinetics, early

Accepted for publication: September 19, 2008

Over the range of doses used in clinical practice, the equilibration rate constant between plasma and effect compartment concentrations ($k_{\text{e0}}$) is believed to be concentration independent for most drugs.$^{1}$ However, this may not be the case after high bolus doses of drugs that reach maximum effect within the first 2 min of i.v. administration. There are very few reports where the PK/PD parameters of neuromuscular blocking agents (NMBAs) were derived separately for each dose group during a formal dose-ranging study. In a study$^{2}$ comparing the PK parameters of three different i.v. bolus doses of cisatracurium besylate (0.075, 0.15, and 0.3 mg kg$^{-1}$ corresponding to 1.5, 3, and 6×ED95) in anaesthetized patients, we previously observed a dose-dependent effect on pharmacokinetic/pharmacodynamic (PK/PD) parameters. These changes were observed without affecting the dose proportionality of PK parameters. This dose-dependency may have resulted from methodological factors because the plasma concentration profile was not characterized properly during the first 2 min.$^{3}$ Similar changes in the dose–effect relationships had previously been reported for vecuronium in patients when using PD modelling.$^{4}$ These changes were later confirmed using a traditional PK/PD approach.$^{5}$ Finally, clinical evidence has also been provided that the dose–response relationship of an NMBA may depend on the administration rate when high bolus doses are given. Dose requirements of cisatracurium were found
significantly greater when given by high bolus (8×ED$_{95}$) than with continuous infusion during cardiac surgery.$^6$

The question whether PK/PD data obtained after very high bolus doses of NMBA should be included in a classical Sigmoid $E_{\text{max}}$ model remained to be investigated. The primary objective of this study was to challenge a potential dose-dependency of cisatracurium PK/PD parameters by conducting a well-controlled experimental study.

Methods

Study design

The study was conducted as a two-period, randomized cross-over design including a washout period. Purpose-bred dogs were naïve animals kindly donated by a pharmaceutical company. Dogs were randomly allocated to two groups. Group A first received the low dose (1.5×ED$_{95}$) whereas Group B first received the high dose (6×ED$_{95}$). To provide a maximum control on experimental conditions, four persons were responsible for drug administration, arterial blood sampling, PD monitoring, or sample processing. Their respective roles remained unchanged throughout the study.

Chemicals

A commercial preparation of cisatracurium besylate (Nimbex®, Abbott Canada Ltd, Montréal, Québec, Canada) was used. The internal standard (n-methyl laudanosine) was provided by Glaxo-Wellcome (Stevenage, UK). All solvents were of high performance liquid chromatography (HPLC) grade and purchased from Anachemia (Montréal, Québec, Canada).

Experimental conditions

The experimental protocol was approved by our institutional Animal Care Committee and was in accordance with the Canadian Council on Animal Care. Veterinary care and housing facilities met Good Animal Practice standards. For the study, eight adult male beagles (7.5–10.5 kg) were singly housed and maintained under a 12 h light/dark cycle at 21 (0.9)°C and 50 (10)% relative humidity. Food was freely available up to 18 h before experiment. Water was not restricted.

General anaesthesia

Dogs were anaesthetized with an initial injection of 30 mg kg$^{-1}$ i.v. sodium pentobarbital (Somnotol®, Abbott Laboratories, Montréal, QC, Canada) administered in the cephalic vein of the left leg. Monitoring of the level of anaesthesia was based on haemodynamic parameters and neurosensory reflexes (hind feet retraction to pinching and cornea response to light touch). Measures were taken every 15 min and at blood sampling times. An adequate level of anaesthesia was maintained with supplemental i.v. doses of pentobarbital (3–5 mg kg$^{-1}$). Respiration was controlled mechanically (Model 607, Harvard Apparatus, South Natick, MA, USA) with room air delivered through a tracheal tube.

Animal preparation

After satisfactory level of anaesthesia and stability of physiological parameters were achieved, the left femoral vein was cannulated for cisatracurium and pentobarbital administration. The right femoral artery was cannulated for blood sampling. A three-way stopcock was installed on the arterial line for arterial pressure monitoring. Haematocrit was measured after administration of each dose and at the end of the experiment. Heart rate was monitored via the arterial pulse. An electromagnetic flow probe (3 mm ID, model FR-030T, Nihon Kohden, Tokyo, Japan) was installed on the left femoral artery and connected to a polygraph system (model RM-6000, Nihon Kohden) for muscle blood flow measurement. The animal was kept warm using a heated surgical table and an insulated sheet. Central body temperature was monitored by means of a rectal probe and kept constant throughout the experiment with a controller. At the end of the experiment, animals were euthanized using an overdose of pentobarbital and saturated KCl.

Neuromuscular monitoring

The medial antebrachial cutaneous nerve was stimulated supramaximally (40–70 mA) at the right forelimb through surface electrode with a train-of-four (TOF) stimulation. Impulses of 0.2 ms duration were delivered at a frequency of 2 Hz for 2 s. TOF stimulation was repeated every 12 s. The resulting force of contraction was measured using a force transducer (Grass F-10). A stabilization period where the tension of the first twitch ($T_1$) in the TOF did not change (baseline value) was allowed before injection of cisatracurium. Time zero for PD measures corresponded to 6 s before drug injection to ensure that PK samples (drawn every 6 s during the first minute) were synchronized with the beginning and middle of each 12 s TOF interval. Muscle relaxation was monitored continuously until full recovery. During the washout period, neurostimulation was stopped. A new baseline value was obtained before the second dose.

Blood sampling

After stabilization of anaesthesia and TOF response, a blank sample was drawn before the first bolus dose of cisatracurium besylate was administered. Drug administration was similar for both doses. I.V. tubing was pre-filled with the injectable drug solution and flushed completely over 2 s (~1/4 volume per 0.5 s). For the first minute, arterial
blood was let to flow freely from a cannula into 2 ml Eppendorf tubes that were changed every 6 s. The diameter of this cannula was selected such that ~1 ml of blood could be collected in a 6 s interval, yielding ~0.5 ml plasma. Thereafter, arterial blood samples (2 ml) were drawn directly from the stopcock and transferred into heparinized tubes at 1.5, 3, 5, 7, 15, 30, 45, 60, and 80 min. Additional samples were obtained for the higher dose when full recovery was not reached. After a washout period, a blood sample was drawn immediately before the injection of the second bolus (to document the residual plasma concentration, if any) and thereafter, as described above. The volume of blood obtained was replaced by saline. To minimize the in vitro degradation of cisatracurium, samples were kept in an ice water bath and centrifuged within 2 min at 4°C. Plasma was then transferred into pre-acidified tubes (30 μl of 2 M H2SO4) to obtain a final pH between 3 and 4, then frozen immediately on dry ice. Samples were stored at –70°C until analysis.

**HPLC analysis**

Plasma concentrations of cisatracurium were determined using a HPLC coupled to a fluorescence detector set at 280 nm (excitation) and 320 nm (emission). Bond-Elut® phenyl solid-phase extraction cartridges (Varian, Harbor City, CA, USA) were used for extraction of cisatracurium from plasma. The method published by Bryant and colleagues for urine samples was slightly modified and validated for plasma in our laboratory. Cisatracurium was separated from its major metabolites on a Spherisorb SCX column (150×4.6 nm ID, 5 μm; Phenomenex, Torrance, CA, USA), using a stepwise gradient (Thermo Separation Products, Riviera Beach, FL, USA). The mobile phase changed from a first phase (14 mM Na2SO4 in 0.5 mM H2SO4:acetonitrile:H2O 40:60:6) during 5 min to a second phase (70 mM Na2SO4 in 0.5 mM H2SO4:acetonitrile 40:60) during 6 min. The solvent flow rate was 2 ml min⁻¹ and the column maintained at 50°C. This assay proved to be sensitive (lower limit of quantification, 6.5 ng ml⁻¹), precise (mean coefficient of variation, 11%), and linear up to 2500 ng ml⁻¹ (r²: 0.995). Samples obtained during the first minute were diluted with control plasma before extraction. Dilution algorithms were established during preliminary experiments. When samples still exceeded the upper limit (generally between 30 and 90 s), samples were further diluted and re-assayed. This procedure has been previously validated. When running diluted samples, a diluted quality control sample was extracted at the same time. The mean coefficient of variation for precision was 3.3% with an overall bias of 5.4%.

**Pharmacodynamic analysis**

Only the first twitch response (T₁) to the TOF was considered. The degree of neuromuscular block was expressed as the percentage of twitch height depression relative to the T₁ baseline value. The first times where recovery reached final T₁ value and full recovery (T₁=T₄) were recorded. Onset time was defined as the time required for reaching maximum block after drug injection. Clinical duration was the time comprised between injection and recovery of twitch height to 25% of its baseline value. Recovery index, or the time elapsed between 25% and 75% twitch height recovery, was also measured.

### Dose linearity of pharmacokinetics

Linearity in PK of the complete data sets was first verified using standard non-compartmental methods (WinNonLin Pro, Pharsight Corp, version 5.2). During the first minute, the time assigned to each sample was the midpoint of the 6 s sampling interval. The terminal elimination rate constant, kₑₜ, was determined by log linear regression. Dose-normalized area under the curves (AUCs) of the plasma concentration vs time curve for the first minute (AUC₀₋₁) and for time zero to infinity (AUC₀₋∞) were calculated using trapezoidal integration. The maximum plasma concentration (Cₘₐₓ) and time to reach Cₘₐₓ (Tₘₐₓ) occurring during the first minute were read directly from the experimental data.

### Non-parametric PK/PD analysis

All individual PK/PD analyses were performed using NONMEM VI (GloboMax LLC, Hanover, MD, USA). We used a non-parametric approach 5,8 where concentrations between the preceding and the subsequent measured values are interpolated as a straight line when the concentrations are increasing and as a simple log-linear function when the concentrations are decreasing. The convolution of this with kₑ₀ e⁻ᵏₑ₀ t calculates the effect-site concentration–time profile. The effect site was then assumed to be linked to the plasma by a compartment of trivial volume with a first-order equilibrium constant (kₑ₀). The sigmoid Eₘₐₓ model was used to correlate the effect with the effect compartment concentration, thus computing the effect compartment concentration at 50% of maximal effect (EC₅₀) and the slope factor (γ). Eₘₐₓ values fixed to 100% or estimated were tested. Values for dose-normalized ECₘₐₓ (ECₘₐₓ/D) and Tₑ_ECₘₐₓ were also estimated.

For the initial analysis, we used a two-stage approach in which the PK/PD values for each animal and each dose are determined independently. To determine whether the PK/PD parameters of cisatracurium varied as a function of dose, data from both doses were analysed simultaneously. Two types of analyses were performed for each individual: (i) values for kₑ₀, EC₅₀, and γ were assumed to be identical for both doses and (ii) values for kₑ₀, EC₅₀, and γ were permitted to vary between doses. Goodness of fit was indicated by an improvement in the NONMEM objective function and by visual inspection of the fit of predicted vs observed values.
Statistical analysis

On the basis of our previous study, a sample size of 8 was estimated to provide 80% power to detect a 25% difference in the EC50 for the low and high dose with the expected standard deviation of ±27 ng ml⁻¹ at an alpha significance level of 0.05 (SigmaStat, version 3.1; Systat Software, Inc., Point Richmond, CA, USA).

Physiological values, PD, PK, and PK/PD parameters obtained in each animal after the high and low doses were compared using a paired t-test with resampling technique using Insightful software version 8 (Seattle, WA, USA). NONMEM objective function (−2 log likelihood values) from the analyses in which kdo, EC50, and γ were permitted to vary between doses were also analysed using a paired sample t-test with resampling technique to determine whether this dose-related effect was systematic.

An analysis of variance (ANOVA) test was also carried out to exclude the presence of sequence or period effect for PD and PK/PD parameters (R 2.6.2 Software, the R Foundation for Statistical Computing). The factors included in the ANOVA were sequence, period (first vs second administration), treatment (high or low dose), and subjects nested within sequence. Therefore, results are presented as individual data (n=8) and expressed as mean values (sd) in the summary tables. The threshold for statistical significance (α) was set at 0.05.

Results

Physiological parameters are reported in Table 1. Arterial pressure, heart rate, and hind limb vascular resistance were stable throughout the experiment (baseline and onset time data presented only). Haematocrit was similar during the first and second period [38% (4%) vs 38% (4%), respectively].

The experimental time flow for each randomization sequence is summarized in Table 2. For the baseline value, a stabilization period of ~5 min was allowed before injection of cisatracurium. Times to recovery to final T1 value and T1=T4 did not differ markedly. Recovery was assumed complete after a stable period of T1=T4 of ~5 min.

Return to 100% of baseline value was observed in four (low dose) and three dogs (high dose). When the final T1 response did not return to the baseline value, all recovery parameters based on the twitch height were adjusted to the final T1 value (normalization). After the low dose, normalization ranged from 95% to 107% (n=4) whereas it ranged from 89% to 111% (n=5) for the high dose. A systematic bias was ruled out statistically.

All eight dogs reached 100% neuromuscular block after both doses (Table 3). After the high dose, 100% block lasted for 44–59 min (mean=52 min). Onset time after the higher dose was decreased by approximately two-fold compared with the lower dose. After the high dose, 4–8 measures of muscle twitch (mean: 6.6) that were not the 0 and 100% values were observed during onset of block. Clinical duration and recovery times were increased by more than two-fold after the high dose. No statistical difference was observed for the recovery index.

For illustration purposes, mean dose-normalized cisatracurium plasma concentrations vs time observed for each dose group were adjusted to a 0.1 mg kg⁻¹ dose and found to be superimposable (Fig. 1). Individual dose-normalized

| Table 1 | Physiological parameters during baseline and at onset time in pentobarbital-anæsthetized dogs after sequential administration of two doses of cisatracurium. Dogs were randomized for sequence (n=8). Values represent mean (sd) |
| Baseline | | Onset time | |
| 0.15 mg kg⁻¹ | 0.6 mg kg⁻¹ | P-value | 0.15 mg kg⁻¹ | 0.6 mg kg⁻¹ | P-value |
| Heart rate (beats min⁻¹) | 129 (45) | 138 (39) | 0.494 | 134 (45) | 144 (35) | 0.388 |
| Systolic arterial pressure (mm Hg) | 129 (22) | 131 (32) | 0.872 | 127 (24) | 143 (31) | 0.164 |
| Diastolic arterial pressure (mm Hg) | 89 (18) | 98 (24) | 0.262 | 93 (21) | 94 (27) | 0.880 |
| Muscle blood flow (ml min⁻¹ kg⁻¹) | 7.4 (3.4) | 6.8 (3.9) | 0.690 | 8.0 (3.6) | 7.1 (4.4) | 0.564 |
| Hind limb vascular resistance (mm Hg min kg ml⁻¹) | 17.7 (11.0) | 21.8 (12.9) | 0.332 | 16.7 (10.4) | 23.0 (14.8) | 0.092 |
| Body temperature (°C) | 37.5 (0.6) | 37.2 (0.6) | 0.690 | 37.5 (0.6) | 37.2 (0.6) | 0.440 |

| Table 2 | Study time flow. Dogs were randomized sequence (n=4). Values represent mean (sd). During the washout period, neurostimulation was stopped |
| Duration (min) | Sequence 1 | | Sequence 2 | | |
| | Dose 1 | Dose 2 | Dose 1 | Dose 2 |
| Baseline stabilization | 10.3 (4.4) | 7.3 (2.3) | 8.5 (2.5) | 8.5 (2.5) |
| Bolus administration | 0.033 | 0.033 | 0.033 | 0.033 |
| Recovery time to final T1 value | 28.0 (5.3) | 63.5 (10.9) | 77.0 (12.2) | 35.7 (6.7) |
| Recovery time to T1=T4 | 33.2 (5.2) | 70.6 (10.8) | 90.1 (18.7) | 40.6 (4.9) |
| Stable period of T1=T4 | 7.7 (4.0) | 5.5 (0.9) | 5.5 (3.5) | 4.3 (2.3) |
| Washout period | 124.5 (5.8) | 92.1 (6.1) | | |
plasma concentrations observed during the first minute after the low and high dose are presented in Figure 2A and B, respectively. Before dose administration, cisatracurium plasma concentrations were always below the lower limit of quantification, thus confirming an adequate washout period. Cisatracurium peak concentrations occurred between 12 and 30 s dosing in dogs.

Non-compartmental PK parameters are listed in Table 4. Mean terminal elimination half-lives (T_{1/2}B) for the 0.15 and 0.6 mg kg^{-1} dose were 17.6 and 17.8 min, respectively. No dose-dependency in any PK parameters was observed.

Mean dose-normalized cisatracurium effect compartment concentrations at each dose level are shown in Figure 3. At the time of the second dose, predicted effect compartment concentrations of cisatracurium were negligible. The hysteresis curves observed between plasma concentration and effect after a high and low dose in a representative dog are presented in Figure 4A. Using non-parametric PK/PD analysis, the corresponding sigmoid E_{max} curves derived from the observed effect and predicted effect compartment concentration are presented in Figure 4B.

Table 3 PD parameters in pentobarbital-anaesthetized dogs after sequential administration of two doses of cisatracurium. Onset, time to maximum neuromuscular block; Max block, magnitude of maximum neuromuscular block; Duration, time to 25% Recovery of twitch height; Recovery index, time from 25% to 75% recovery of twitch height. Dogs were randomized for sequence (n=8). Values represent mean (sd). *P<0.05

<table>
<thead>
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<th>0.15 mg kg^{-1}</th>
<th>0.6 mg kg^{-1}</th>
<th>P-value</th>
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<tr>
<td>Onset (min)</td>
<td>2.14 (0.48)</td>
<td>0.98 (0.17)</td>
<td>0.012*</td>
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<td>Max block (%)</td>
<td>100 (0)</td>
<td>100 (0)</td>
<td>1.00</td>
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<tr>
<td>Duration (min)</td>
<td>21.1 (3.0)</td>
<td>58.0 (8.9)</td>
<td>0.002*</td>
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<tr>
<td>Recovery times (min)</td>
<td></td>
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</tr>
<tr>
<td>25%</td>
<td>21.1 (3.0)</td>
<td>58.0 (8.9)</td>
<td>0.012*</td>
</tr>
<tr>
<td>50%</td>
<td>24.0 (4.0)</td>
<td>60.7 (9.3)</td>
<td>0.012*</td>
</tr>
<tr>
<td>75%</td>
<td>27.0 (4.8)</td>
<td>64.5 (10.0)</td>
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<tr>
<td>Recovery index (min)</td>
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<td>0.436</td>
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</table>

Table 4 PK parameters in pentobarbital-anaesthetized dogs after sequential administration of two doses of cisatracurium. C_{max}/D, dose-normalized maximum plasma concentration; T_{max}, time to reach C_{max}; AUC_{0–∞}/D, dose-normalized area under the curve from 0 to ∞; AUC_{0–1}/D, dose-normalized area under the curve from 0 to 1 min; k_{el}, elimination rate constant. Parameters were normalized for a 0.1 mg kg^{-1} dose. Dogs were randomized for sequence (n=8). Values represent mean (sd) *P<0.05

<table>
<thead>
<tr>
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<th>0.15 mg kg^{-1}</th>
<th>0.6 mg kg^{-1}</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>C_{max}/D (ng ml^{-1})</td>
<td>4678 (1806)</td>
<td>4485 (767)</td>
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<tr>
<td>T_{max} (min)</td>
<td>0.26 (0.06)</td>
<td>0.25 (0.11)</td>
<td>0.406</td>
</tr>
<tr>
<td>AUC_{0–∞}/D (ng min ml^{-1})</td>
<td>9593 (1258)</td>
<td>10 383 (1655)</td>
<td>0.294</td>
</tr>
<tr>
<td>AUC_{0–1}/D (ng min ml^{-1})</td>
<td>1448 (296)</td>
<td>1483 (249)</td>
<td>0.772</td>
</tr>
<tr>
<td>k_{el} (min^{-1})</td>
<td>0.0402 (0.0042)</td>
<td>0.0409 (0.0096)</td>
<td>1.000</td>
</tr>
</tbody>
</table>
With the final model, $k_{e0}$ values were typically two-fold lower after the high dose compared with the low-dose values. A typical example of the collapsed hysteresis curve is presented in Figure 5. Sigmoid $E_{\text{max}}$ curves were shifted to the right resulting in a 38% higher predicted EC50 for the high dose (Table 6 and Fig. 4B). Predicted $E_{\text{max}}$ was used because the objective function was slightly better than when $E_{\text{max}}$ was fixed to 100%. Only minor differences (<1%) were observed for the PK/PD parameters between the two approaches. Individual data for $k_{e0}$ and EC50 at each dose level are represented in Figure 6. The results of the ANOVA showed no evidence of a statistically significant sequence or period effects for PK/PD parameters $k_{e0}$, EC50, $T_{E_{\text{max}}}$ and $E_{\text{max}}/D$ (power >80%).

### Discussion

Using a non-parametric PK/PD approach and a descriptive sigmoid $E_{\text{max}}$ model, a dose-dependent effect on the PK/PD parameters of cisatracurium was observed in anaesthetized dogs. Mean EC50 values increased and mean $k_{e0}$ values decreased by almost 50% after the high bolus dose. Several factors have to be considered in the interpretation of this dose-dependency.

When conducting the animal study, experimental conditions were rigorously controlled to avoid any systematic bias. As both low and high bolus doses were given in each dog, any period, sequence effect in the cross-over design, or both was statistically ruled out before pooling data. At each dose level, there were no significant changes in muscle blood flow or hind limb vascular resistance during onset time when compared with baseline values, excluding changes in regional haemodynamics as a potential bias. Instead of giving a continuous infusion, small doses of pentobarbital were given when indicated by corneal reflex. This approach was preferred because, once catheters were installed, there were no manipulations of the animal.

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**Fig 3** Mean dose-normalized cisatracurium effect compartment concentrations vs time after a dose of 0.15 mg kg$^{-1}$ (black triangles) and 0.6 mg kg$^{-1}$ (white circles). For illustration purpose, concentrations were normalized for a 0.1 mg kg$^{-1}$ dose.

**Fig 4** Hysteresis curves (A) and sigmoid $E_{\text{max}}$ curves (B) derived for dog #1.

**Table 5** NONMEM objective function values ($-2 \log \text{ likelihood}$) obtained during stepwise exploration of dose impact on PK/PD parameters. $k_{e0}$, effect compartment equilibration rate constant; EC50, effect compartment concentration at 50% of maximal observed effect; $\gamma$, slope factor. Obj, NONMEM objective function value. Symbols: $\approx$, one estimation for both doses; $\Delta$, separate estimations for both doses. Values represent mean (SD) ($n=8$). *$P<0.05$ for model discrimination.
These small doses did not produce any noticeable effect on physiological parameters.

It is well recognized that the blood sampling schedule during the first minute after a bolus of a neuromuscular blocking agent may have a significant impact on the estimation of its PK and, in turn, PK/PD parameters. Plasma concentrations were therefore measured frequently during the first minute after each administration of cisatracurium to our dogs. We have first confirmed that the PK parameters of cisatracurium obtained for the complete and first minute data sets were both linear before proceeding to the PK/PD analysis. Therefore, the dose-dependent effects are not likely to result from differences in the central PK.

Given the four-fold difference in dose, a higher span of plasma concentrations were observed at a given degree of block during onset of action (ascending limb) while the concentrations associated with a given effect did not vary greatly during recovery (descending limb) of the hysteresis curves (Fig. 4A). This difference did not have a major impact on the efficiency of the link model since plasma concentrations from both the onset and the recovery limbs were adequately collapsed, and that, independently of the dose given (Fig. 5). Many data points were either 0% or 100% after the higher dose, rendering the collapsing of the hysteresis loop mostly dependent on the ascending and descending limbs. This potential modelling artifact cannot be excluded; however, it is unclear to what extent it may contribute to the rightward shift of the effect vs effect compartment concentration curve after the higher bolus dose.

**Table 6** PK/PD parameters in pentobarbital-anaesthetized dogs after sequential administration of two doses of cisatracurium. EC$_{50}$, effect compartment concentration at 50% of maximal observed effect; $T_{EC_{max}}$, time required to reach $EC_{max}$; $EC_{max}/D$, dose-normalized maximum concentration in effect compartment; $E_{max}$, maximum block; $k_{eq}$, effect compartment equilibration rate constant; $\gamma$, slope factor. Parameters were normalized for a 0.1 mg kg$^{-1}$ dose. Dogs were randomized for sequence ($n=8$). Values represent mean (s.d.). *P $< 0.05$

<table>
<thead>
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<th>0.15 mg kg$^{-1}$</th>
<th>0.6 mg kg$^{-1}$</th>
<th>P-value</th>
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<tbody>
<tr>
<td>EC$_{50}$ (ng ml$^{-1}$)</td>
<td>235 (35)</td>
<td>323 (57)</td>
<td>0.006*</td>
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<tr>
<td>$T_{EC_{max}}$ (min)</td>
<td>6.13 (0.79)</td>
<td>10.69 (1.39)</td>
<td>0.006*</td>
</tr>
<tr>
<td>$EC_{max}/D$ (ng ml$^{-1}$)</td>
<td>325 (30)</td>
<td>226 (43)</td>
<td>0.016*</td>
</tr>
<tr>
<td>$E_{max}$ (%)</td>
<td>102 (1)</td>
<td>102 (1)</td>
<td>1.000</td>
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<tr>
<td>$k_{eq}$ (min$^{-1}$)</td>
<td>0.1278 (0.0224)</td>
<td>0.0600 (0.0072)</td>
<td>0.002*</td>
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<td>$\gamma$</td>
<td>7.70 (5.44)</td>
<td>7.70 (5.44)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

These small doses did not produce any noticeable effect on physiological parameters. It is well recognized that the blood sampling schedule during the first minute after a bolus of a neuromuscular blocking agent may have a significant impact on the estimation of its PK and, in turn, PK/PD parameters. Plasma concentrations were therefore measured frequently during the first minute after each administration of cisatracurium to our dogs. We have first confirmed that the PK parameters of cisatracurium obtained for the complete and first minute data sets were both linear before proceeding to the PK/PD analysis. Therefore, the dose-dependent effects are not likely to result from differences in the central PK.

Given the four-fold difference in dose, a higher span of plasma concentrations were observed at a given degree of block during onset of action (ascending limb) while the concentrations associated with a given effect did not vary greatly during recovery (descending limb) of the hysteresis curves (Fig. 4A). This difference did not have a major impact on the efficiency of the link model since plasma concentrations from both the onset and the recovery limbs were adequately collapsed, and that, independently of the dose given (Fig. 5). Many data points were either 0% or 100% after the higher dose, rendering the collapsing of the hysteresis loop mostly dependent on the ascending and descending limbs. This potential modelling artifact cannot be excluded; however, it is unclear to what extent it may contribute to the rightward shift of the effect vs effect compartment concentration curve after the higher bolus dose.
Onset time ($T_\text{Emax}$) is dose-related when NMBAs are given at doses producing complete block. Conversely, time to peak effect compartment concentration ($T_{EC_{\text{Emax}}}$) coincides with onset time only when the pharmacological effect is submaximal and had therefore to be derived mathematically for the present study. Although $T_{EC_{\text{Emax}}}$ is generally assumed to be dose-independent, it was significantly delayed after the higher dose in our dogs. Thus, a shorter onset time was associated with a longer $T_{EC_{\text{Emax}}}$. This apparent discrepancy can be explained by the fact that, for a given drug, calculated $T_{EC_{\text{Emax}}}$ depends solely on the plasma–effect compartment equilibration rate ($k_e$). It is noteworthy that a shorter onset is not always associated with a faster $k_e$ as this proved to be the case for succinylcholine. Since the rate-limiting factors for $k_e$ and onset times are different, the net effect may differ.

The equilibration rate $k_e$ lumps together access to the receptor and any other post-receptor event that might contribute to time delays in response. As previously mentioned, the equilibration rate $k_e$ is believed to be concentration independent for most drugs at doses generally used in clinical practice, and is thus presented as a constant value. However, this appeared not to be the case in our study where a significant decrease in $k_e$ was observed in the higher bolus dose group. According to our stepwise exploration, there was a statistically significant improvement in the objective function, when $k_e$ values were allowed to change in the two dose groups. The first determinant single parameter for adequate fitting of the PK/PD model was $k_e$, whereas changes in both the EC$_{50}$ and the $k_e$ came second. At high input rates, plasma concentrations increase more rapidly than do effect-site concentrations. As a result, circulation delays and effect site/plasma equilibration times become determinant.

In view of its high molecular weight, low lipid solubility, and ionic nature, the transcapillary transfer of cisatracurium is expected to be mostly restricted to pores. In the human forearm, the intercellular pore radius is ~16 nm. In addition, muscle capillaries present numerous membranous vessels that, after transient fusion, would create transendothelial channels or ‘small pores’. It is unknown whether these pore sizes are different in canines, but capillary permeability in the muscle tissue was found similar across mammalian species. In addition, microperoxidase (molecular weight of 1900, and molecular span of 2 nm) was found to diffuse readily through the small pore of muscle capillary in rats. Since the molecular span of many NMBAs is ~2 nm between both quaternary amines, transcapillary exchange should not be a rate-limiting step for cisatracurium. Muscle interstitial concentrations of rocuronium measured by microdialysis under steady-state conditions were found equivalent to those predicted for the effect compartment. Although cisatracurium is a larger molecule, it appears unlikely that restricted access to muscle interstitial space is responsible for the dose-dependency.

In our opinion, the mechanisms underlying the non-linearity of the predicted effect compartment concentration of cisatracurium after a high bolus dose would most probably occur within the synaptic cleft itself. Although no anatomical barriers prevents diffusion of drugs from muscle interstitial space into the synaptic cleft, there is a limited space (30–50 nm) available for diffusion of NMBAs within the synaptic cleft itself. After a high bolus dose, a transient increase in the unbound concentration of cisatracurium may occur as nicotinic receptors and non-specific sites become suddenly occupied, thus reducing the concentration gradient of unbound cisatracurium between the interstitial fluid and the synaptic cleft. Owing to the very high density of nicotinic receptors, a slower diffusion of NMBAs into this restricted space may ensue. The nerve terminal would also represent a physical barrier to the diffusion of NMBAs out of the cleft, thus enhancing the repetitive binding to acetylcholine receptors. This concept of ‘buffered diffusion’ was recently tested and simulations predicted that time to peak effect would decrease when submaximal bolus doses of NMBAs are increased. However, our findings suggest that this may not apply for high bolus doses. All these mechanisms related to ‘steric hindrance’ may explain why the effect compartment concentration associated with a given effect is larger after the higher dose of cisatracurium (Fig. 4n).

If steric hindrance is an issue, this effect should disappear during the recovery phase when the rapid changes in concentrations have stopped. Recovery is more akin to a steady-state situation. It would have been interesting to compare these results with post-tetanic count. It is a well-established method of evaluating neuromuscular recovery during intense block and constitutes an excellent means to distinguish between intense and deep blocks, which may have allowed some better quantification. Experimental confirmation of these hypotheses was beyond the scope of this study but deserves further investigation.

Dose-dependent changes in the sensitivity of the neuromuscular junction were also observed in our dogs after the higher bolus dose of cisatracurium. Molecular span, but also factors such as the flexibility of the molecule and its conformation in the biophase at the moment of interaction with the acetylcholine receptors, would impact on the onset time and duration of action of NMBAs. The size of the active cation has once been suggested as a limiting factor influencing the rate of onset of NMBAs. Thus, physicochemical interactions as a potential mechanism should not be overlooked. Indeed, a good correlation was shown between EC$_{50}$ values obtained in anaesthetized patients and molecular weight for a series of NMBAs. This finding is in agreement with the hypothesis that a restricted diffusion within the synaptic cleft may alter the potency of NMBAs.

As mentioned previously, there are very few reports where the PK/PD parameters of a NMA were derived separately for each dose group during a formal dose-ranging
A parametric population approach was applied to data gathered from 241 patients having received up to an $8 \times \text{ED}_{95}$ bolus dose of cisatracurium during eight prospectively designed phase I–III studies. Their mean estimates for $k_e$ and $\text{EC}_{50}$ values are almost identical to those reported in the study by Bergeron and colleagues when compared with the mean value of the three doses. However, the interpatient coefficients of variability in the population analysis were higher (61% and 52%, respectively) than those reported by Bergeron and colleagues for each dose group (both <20%). As dose impact was not tested (considered as a covariate) in NONMEM population analysis, the possibility of a dose-related change in the PK/PD parameters was not examined.

Our findings clearly indicate that PK/PD data obtained after i.v. bolus doses that are beyond those used in clinical practice should be tested for linearity of PK/PD estimates before including them in the general model. For illustration purposes, using PK/PD parameters obtained after an intubating dose of cisatracurium to predict the effect after a high bolus dose would yield faster onset and recovery times than were actually observed (Fig. 7). Alternately, if PK/PD parameters obtained for the high dose of cisatracurium were used to simulate the effect after an intubating dose, the predicted peak effect would be highly underestimated (Fig. 8) and this would eventually lead to overdosing. These results have important clinical implication as an accurate estimate of the $\text{EC}_{50}$ is desirable for target-controlled infusion systems. In our opinion, PK/PD parameters derived after an intubating dose of cisatracurium would be more reliable.

In conclusion, after 1.5 and $6 \times \text{ED}_{95}$ bolus doses of cisatracurium, the effect compartment concentration at 50% block ($\text{EC}_{50}$) increased and the effect compartment equilibration rate constant ($k_{e0}$) decreased at the higher dose when using a descriptive sigmoid $E_{\text{max}}$ model. As this study was meant to be descriptive rather than mechanistic, it is not possible to delineate whether the PK/PD dose-dependency is a real phenomenon or a modelling artifact. Only hypotheses regarding the factors responsible for the dose-related changes in PK/PD parameters can be formulated and further confirmatory studies are required. However, these results indicate that dose impact should be tested when data gathered during dose-ranging studies are pooled to derive population PK/PD parameters. This is particularly the case after i.v. bolus doses of drugs with a rapid onset of action.

Acknowledgements

We would like to thank Johanne Couture and Sanae Yamaguchi for their technical support.

Funding

This research program was supported by the Canadian Institutes of Health Research (grant MA-10274).

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