

## Dose-Dependent Pharmacokinetics of Ropivacaine in Anesthetized Rabbits: Absence of Changes in Protein Binding

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### Abstract

**Objective:** The primary objectives of this study were to investigate the *in vivo* pharmacokinetics and *in vitro* plasma protein binding of ropivacaine at the concentration range observed after intravenous administration of different doses in anesthetized rabbits.

**Study design:** Prospective experimental study

**Animals:** Thirteen New Zealand White rabbits

**Methods:** Rabbits were anesthetized with isoflurane. Different doses (0.62 to 6.52 mg/kg) of 0.5 % plain ropivacaine hydrochloride were administered and arterial blood sampled at frequent intervals up to 10 hours. Ropivacaine plasma concentrations were determined by high performance liquid chromatography. Pharmacokinetic analysis of individual data sets was performed using non-compartmental approach to calculate area under the plasma concentration–time curve from time zero to infinity ( $AUC_{0-\infty}$ ). *In vivo* pharmacokinetics linearity was verified using analyses of variance for between group comparisons of the effect of dose on the  $AUC_{0-\infty}$  normalized by the dose. A population pharmacokinetic model was developed to describe ropivacaine disposition after intravenous administration in the linear range of dose in anesthetized rabbits. Determination of protein binding was performed, under controlled temperature and pH conditions, in plasma samples spiked extemporaneously with <sup>3</sup>H-ropivacaine and unlabeled ropivacaine (2.5 and 3500 ng/ml) using an ultrafiltration method.

**Results:** At intravenous doses higher than 3.67 mg/kg, dose-dependent changes in ropivacaine systemic clearance were observed. In contrast, protein binding was found to be concentration independent in the tested range.

**Conclusions and clinical relevance:** In anesthetized rabbits, saturation of ropivacaine plasma protein binding is not the mechanism responsible for the pharmacokinetic nonlinearity of ropivacaine observed at intravenous doses higher than 3.67 mg/kg. Disposition parameters obtained in our study will enable accurate characterization of ropivacaine pharmacokinetics after a peripheral nerve block.

**Keywords:** Ropivacaine; Protein Binding; Linearity; Pharmacokinetics; Intravenous; Rabbits

**List of abbreviations:**  $\omega_i^2$ : The variance of  $\eta_{ij}$ ;  $\eta_{ij}$ : Proportional difference between  $P_{ij}$  and  $\theta_j$ ;  $\theta_j$ : The j-th typical parameter value of the population; AAG:  $\alpha$ 1-acid glycoprotein; ANOVA: Analyses of variance;  $AUC_{0-\infty}$ : Area under the plasma concentration–time curve from time zero to infinity;  $AUC_{0-t}$ : Area under the plasma concentration–time curve from time zero to the time corresponding to the last measurable concentration; Cl: Total body clearance;  $Cl_i$ : Distributional clearance between compartment 1 (central) and compartment 2 (peripheral); dpm: Disintegrations per minute; ELS: Extended least squares; FOCE: First order conditional estimation; fu: Unbound fraction; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC: High performance liquid chromatography; IS: Internal standard;  $K_{10}$ : First-order rate constant of elimination from the central compartment,  $K_{12}$ : First-order rate constants associated with drug transfer from compartment 1 to compartment 2;  $K_{21}$ : First-order rate constants associated with drug transfer from compartment 2 to compartment 1;  $P_{ij}$ : The j-th parameter value for individual i; PK: Pharmacokinetics; PNB: Peripheral nerve block; UV: Ultraviolet detection;  $V_2$ : Apparent volume of the peripheral compartment;  $V_c$ : Apparent volume of the central compartment;  $\alpha$ : Type I error

### Introduction

Peripheral nerve block (PNB) is widely used to facilitate and accelerate recovery following major orthopedic surgery [1]. The introduction of long-acting local anesthetic with a safer profile as well as better equipment for nerve blockade resulted in the increased use of such techniques for postoperative analgesia [2,3].

Despite the high number of rabbits undergoing surgical procedures, anaesthesia and analgesia protocols for rabbits are still limited

compared with those for cats and dogs [4,5]. Currently, it appears that veterinarians treating rabbits (including those kept in laboratories) are unfamiliar and unsure of available drugs, dosage ranges and drug effects [6,7]. Pain can be prevented and reduced in many ways, and in pet rabbit medicine, multimodal analgesia is advised [8,9]. Common local anaesthetic drugs used in rabbits are EMLA® cream (a eutectic mixture of 2.5% prilocaine and 2.5% lidocaine) as well as lidocaine and bupivacaine injectable solutions [9].

Ropivacaine is a long-acting amide-type local anesthetic. In comparison with bupivacaine, ropivacaine is equally effective for postoperative analgesia after a peripheral nerve block and, owing to its markedly lower lipid solubility, presents a lower cardiovascular and central nervous system toxicity in healthy volunteers [10,11]. However, recommended dose of ropivacaine is mostly based on avoiding systemic toxicity rather than achieving a targeted duration of action for a specific PNB. The first step in dose optimization is to establish a concentration-time relationship for each drug-PNB combination.

The major challenge during the development of a pharmacokinetic (PK) model for local anesthetics is the complexity in estimating the delayed and slow absorption rate into the bloodstream as they exhibit flip-flop kinetics after neural blockade [12]. Flip-flop kinetics implies that the rate of drug absorption from the site of administration into the systemic circulation is slower than drug elimination. When systemic absorption becomes a limiting factor, the terminal half-life reflects the rate and the extent of absorption and not the elimination process [13]. Numerical deconvolution of plasma concentration-time curve is used to determine the rate of drug absorption to the systemic circulation after extravascular administration; it requires that disposition parameters be obtained after intravenous drug administration [14]. Therefore, characterizing the PK of ropivacaine after intravenous injection is a prerequisite before attempting to study the PK after peripheral nerve injection [15].

Several pharmacokinetic studies with ropivacaine have been conducted on animals after intravenous administration of single doses, namely on dogs (3 mg/kg), sheep (2 mg/kg) and rhesus monkeys (1 mg/kg) [16-19]. To our knowledge, these studies were all performed under the assumption of a linear PK, most probably because previous reports in humans documented that ropivacaine PK was linear up to total plasma concentrations of 2000 ng/ml [20].

In humans, ropivacaine is eliminated predominantly by hepatic metabolism with an intermediate to low extraction ratio [21]. Therefore, total body clearance would depend mostly on both the unbound fraction in plasma and hepatic enzyme activity [22]. The high extent of protein binding (94%) in humans suggests that any change in protein binding, mostly  $\alpha$ 1-acid glycoprotein (AAG), will have a significant effect on the pharmacokinetics and pharmacodynamics of ropivacaine [23]. Saturable plasma protein binding of ropivacaine would not be surprising in rabbits, since AAG concentration is known to be one third of that in human plasma (12 - 16 mg/dl and 30 - 40 mg/dl, respectively) [24]. A species-related difference in drug binding is generally recognized and warrants investigation. The aims of this study were to investigate the *in vivo* pharmacokinetics and *in vitro* plasma protein binding of ropivacaine at the concentration range observed after intravenous administration of different doses in anesthetized rabbits.

## Materials and Methods

### Drugs and chemicals

An injectable preparation of 0.5% plain ropivacaine hydrochloride (Naropin®; AstraZeneca, ON, Canada) was used. Ropivacaine pure analytical standard (Molecular weight: 274.4 mg base) was kindly provided by AstraZeneca (Montreal, QC, Canada). Tetracaine (internal standard, IS) was obtained from Sigma-Aldrich (Milwaukee, WI). <sup>3</sup>H-ropivacaine 1 mCi/ml, specific activity 2.8 Ci/mmol, (Moravek Biochemicals, Brea, CA, USA) was used for protein binding assay. All solvents were of HPLC grade and purchased from Anachemia (Montréal, QC, Canada). All other chemicals were from Fisher Scientific (Montreal, QC, Canada).

### Animals

The experimental protocol was approved by our institutional Animal Care Committee and the study conducted in accordance with the Canadian Council on Animal Care. Veterinary care and housing facilities met Good Animal Practice standards. For the study, thirteen male New Zealand white rabbits (Charles-River, St-Constant, QC, Canada) with a mean weight of 3.41 kg (range: 2.48-4.8 kg) were singly housed and maintained under a 12-h light/dark cycle at 21.8 °C and 43.3 % relative humidity for one week before the experiment. There was no restriction of food and water.

### Analytical method

Plasma concentrations of ropivacaine were determined using high performance liquid chromatography with ultraviolet detection (HPLC-UV) method similar to that described by Gaudreault, *et al* [25]. The method was adapted to quantify ropivacaine in a smaller volume (0.35 ml) of rabbit plasma; a plasma volume of 0.7 ml was necessary to detect ropivacaine in the last two samples for rabbits receiving the lowest dose of 0.62 mg/kg. To avoid degradation of our internal standard (IS) tetracaine, ice-cold acetonitrile was used to precipitate butyrylcholinesterases instead of enzymatic inactivation by ethiothiophate. Calibration curves were generated by weighted regression ( $1/x^2$ ) of the analyte/IS peak height ratio versus ropivacaine nominal concentration.

### *In Vivo* experiments

Rabbits were anesthetized with 2 to 3% isoflurane administered via a mask until optimal conditions for orotracheal intubation were achieved; anesthesia was maintained with 1.5 to 2.5% isoflurane. Monitoring of the level of anesthesia was based on hemodynamic

parameters as well as corneal reflex. The left femoral artery was cannulated for blood sampling. A three-way stopcock was installed on the arterial line for femoral artery blood pressure and heart rate monitoring. The femoral vein of the same leg was cannulated for ropivacaine administration and fluid therapy. During the whole experiment, body temperature was monitored by means of a rectal probe and kept at 39 °C. After reaching a stable level of anesthesia and before starting ropivacaine infusion, a blank blood sample (5 ml) was collected into heparinized tube for *in vitro* protein binding. Different dosing regimens and duration of infusion of ropivacaine hydrochloride (0.62, 1.20, 3.67 and 6.52 mg/kg over 10, 10, 30 and 45 min, respectively) were administered to anesthetized rabbits (n: 4, 3, 3 and 3, respectively). Up to 17 arterial blood samples (1.8 ml) were drawn during and post-administration of ropivacaine. Samples were collected before drug administration (blank plasma), near the middle and at the end of the infusion. The arterial blood sampling times were 1, 2, 4, 8, 15, 30 min, 1, 2, 3, 4, 6, 8, 9.5 hours post-administration. Samples were collected into heparinized tubes, kept in an ice-water bath, centrifuged within 30 min at 4 °C and stored at -70 °C until HPLC analysis.

### PK Linearity Evaluation

Rabbits were administered four different regimens of ropivacaine hydrochloride. Total plasma concentrations versus time data sets were analyzed individually using standard non-compartmental analysis with Phoenix® WinNonlin® version 7.0 (Certara L.P., Princeton, NJ, USA). The apparent terminal elimination rate constant,  $k_{10}$ , was obtained by log-linear regression of the plasma concentration–time curve (using three or more data points). The area under the plasma concentration–time curve from time zero to the time corresponding to the last measurable concentration ( $AUC_{0-t}$ ) or up to infinity ( $AUC_{0-inf} = AUC_{0-t} + C_{last}/k_{10}$ ) were calculated via the linear trapezoidal method.

Linearity in PK was verified using analyses of variance (ANOVA) for between group comparisons of the effect of dose on the  $AUC_{0-inf}$  normalized by the dose ( $AUC_{0-inf}/Dose$ ). When significant difference was found, Holm-Sidak test was used for between groups pairwise comparisons. The threshold for statistical significance ( $\alpha$ ) was set at  $P < 0.05$ . SigmaPlot® statistical software for Windows® (version 11.0, build 11.2.0.5) was used.

### Population PK Analysis

Following PK linearity evaluation, only the 10 rabbits in groups that showed linear PK were used for population PK analysis (0.62, 1.20 and 3.67 mg/kg). Population PK analysis was conducted using Phoenix® NLME® version 7.0 (Certara L.P., Princeton, NJ, USA). The first order conditional estimation - extended least squares method (FOCE-ELS) was used throughout the analysis. SigmaPlot® for Windows® (version 11.0, build 11.2.0.5) was used for graphic display of data. Models were evaluated on the basis of change in the objective function value ( $-2 \times \log$ -likelihood), assessment of diagnostic plots, and precision of parameter estimation.

Ropivacaine PK after intravenous administration was best described by a two-compartment model with zero-order input rate and first-order elimination rate from the central compartment. The following parameters were fitted: distributional clearance between compartment 1 (central) and compartment 2 (peripheral) ( $Cl_d$ ), total body clearance (Cl), apparent volume of the central compartment ( $V_c$ ) and apparent volume of the peripheral compartment ( $V_2$ ). The following exit-site dependent PK parameters were also calculated from the above estimated parameters: first-order rate constant of elimination from the central compartment ( $K_{10} = Cl/V_c$ ), first-order rate constants associated with drug transfer from compartment 1 to compartment 2 ( $K_{12} = Cl_d/V_c$ ) and from compartment 2 to compartment 1 ( $K_{21} = Cl_d/V_2$ ).

Between-subject variability of the PK parameters was characterized using the exponential error model (Equation 1):

$$P_{ij} = \theta_j \cdot \exp(\eta_{ij})$$

Where  $P_{ij}$  is the  $j$ -th parameter value for individual  $i$ ,  $\theta_j$  is the  $j$ -th typical parameter value of the population, and  $\eta_{ij}$  is the proportional difference between  $P_{ij}$  and  $\theta_j$ , assumed to be normally distributed with a mean of 0 and a variance of  $\omega_j^2$ . The difference between predicted and observed values (residual error) was evaluated using a proportional error model. To assess model stability, a non-parametric bootstrap analysis was conducted with 1000 replicates, the lower and upper limits for 95% confidence interval were calculated.

### Protein Binding Assay

Determination of protein binding was performed in plasma samples using slight modifications of the ultrafiltration methods described by Cameron, *et al.* and Koivisto, *et al.* [26,27]  $^3H$ -ropivacaine 1 mCi/ml was diluted with HPLC grade water to achieve a concentration of 100 ng/ml.

On the day of ultrafiltration experiment, blank plasma samples were unfrozen and transferred into glass tubes placed under low agitation into a water bath incubator with temperature controlled by an automatic feedback thermometer (Thermistor, Oakton, Singapore) [28]. A pH meter electrode was put into each plasma sample for continuous monitoring. This system provided accurate pH and temperature recordings before and during the incubation period. The pH of plasma was maintained to the mean value reported in rabbits under anesthesia (mean: 7.381) by the periodic addition of 1 to 5  $\mu$ L of HEPES buffer. Similarly, water bath temperature was adjusted to the mean *in vivo* temperature recorded during the experiments for each rabbit. Time zero for incubation corresponded to the addition of ropivacaine. A fixed amount (2.5 ng) of  $^3H$ -ropivacaine was spiked extemporaneously to plasma containing unlabeled ropivacaine to obtain final concentrations of 2.5, 10, 50, 100, 1000

and 3500 ng/ml of total ropivacaine. After 5 min equilibrium, two samples of 50  $\mu$ l were transferred to measure radioactivity in whole plasma while a 750  $\mu$ l sample was applied into a microseparation device having a cut-off of 30 000 MW (Centrifree, Amicon Micropartition System, Beverly, MA). The microseparation device was centrifuged (Evolution RC Superspeed Centrifuge, Sorvall-Kendro, Newtown, CT, USA) for 5 min at 1076 x g and 39 °C. Duplicate aliquots of ultrafiltrate were transferred to measure radioactivity of unbound ropivacaine. As previously reported by Arvidsson, *et al.* non-specific adsorption of ropivacaine to the microseparation devices was observed and determined to be 10% by an initial aqueous washing of the ultrafiltration device [29].

### Radioactivity Assay

Radioactive samples of whole plasma and ultrafiltrate were mixed with scintillation liquid (Ultima Gold, Perkin-Elmer, MA, USA), and total radioactivity in each sample was determined using a liquid scintillation counter. Unbound fraction ( $f_u$ ) of ropivacaine in plasma was calculated by the ultrafiltrate/plasma disintegrations per minute (dpm) ratio, after correction for background radioactivity and non-specific adsorption of ropivacaine to the microseparation devices.

### Protein Binding Linearity Evaluation

ANOVA was used to exclude the presence of any intra-assay concentration-dependent effect in protein binding; when the normality test failed, Kruskal–Wallis one-way ANOVA was applied. The threshold for statistical significance ( $\alpha$ ) was set at  $P < 0.05$ . SigmaPlot® statistical software for Windows® (version 11.0, build 11.2.0.5) was used.

### Results

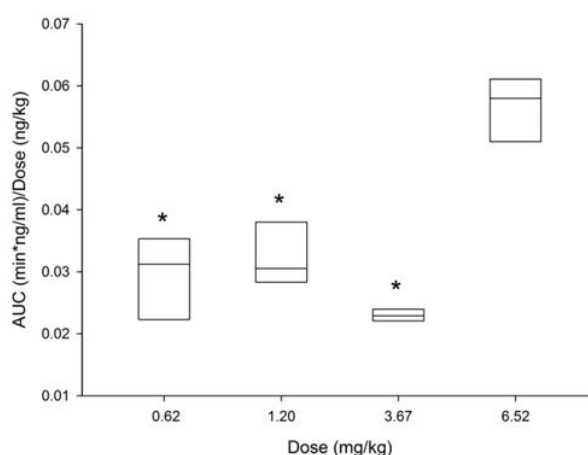
Rabbits were maintained under anesthesia during approximately 11 h. Heart rate was considered stable throughout the experiment (mean:  $245 \pm 25$  bpm; range: 118–298). Inconsistent decreases in arterial blood pressure were observed toward the end of the experiment (8 hours post infusion) in five rabbits. A more pronounced decrease in arterial blood pressure was observed in two rabbits at the end of the infusion but values returned spontaneously to nearly baseline 30 to 40 min afterwards. No correlation was found between these changes and the dose given. Throughout the study, each rabbit's body temperature was maintained to its baseline value which averaged  $39.0 \pm 0.4$  °C (range: 37.6–40.1 °C). Accordingly, during *in vitro* protein binding studies, water-bath temperature was adjusted to its mean *in vivo* temperature (mean:  $38.6 \pm 0.78$  °C; range: 36.7–39.2). The stability of pH was well documented throughout *in vitro* studies (mean:  $7.38 \pm 0.02$ ; range: 7.35–7.42).

### Validation Results

This assay proved to be sensitive with a lower limit of quantification of 3.91 and 1.9 ng/ml (when 0.35 and 0.7 ml of rabbit plasma were used, respectively), precise (mean coefficient of variation, 4.8%), accurate (bias, 1.5%), and linear up to 4000 ng/ml ( $r^2$ : 0.996).

### PK Linearity Data Analysis

The results of ANOVA showed significant dose-dependency of dose normalized  $AUC_{0-\text{inf}}$ . The source of the dose-dependency between the groups was further investigated using Holm-Sidak test. Significant dose-dependency was found between each of the 3 lower doses and the 6.52 mg/kg dose. Individual  $p$  values were the following: 0.62 mg/kg ( $p < 0.001$ ), 1.20 mg/kg ( $p = 0.001$ ), and 3.67 mg/kg ( $p < 0.001$ ). No significant dose-dependency was observed between doses of 0.62, 1.20, and 3.67 mg/kg (Figure 1).

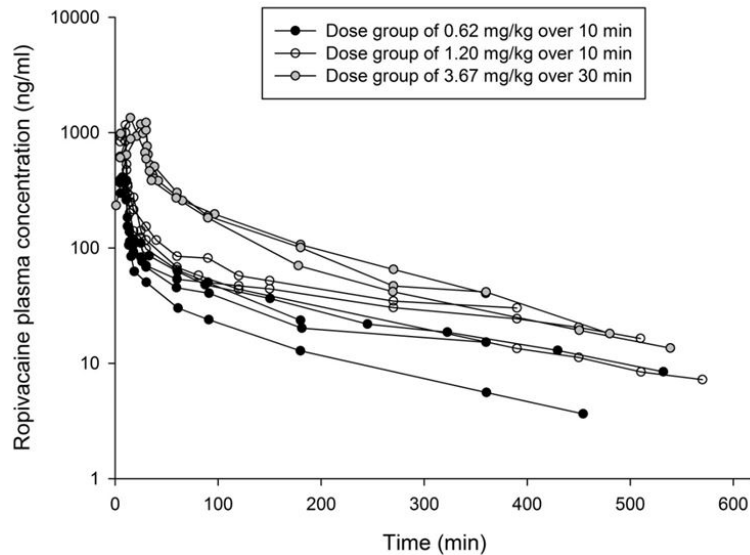


(\* statistically significant  $p < 0.05$  compared with 6.52 mg/kg group)

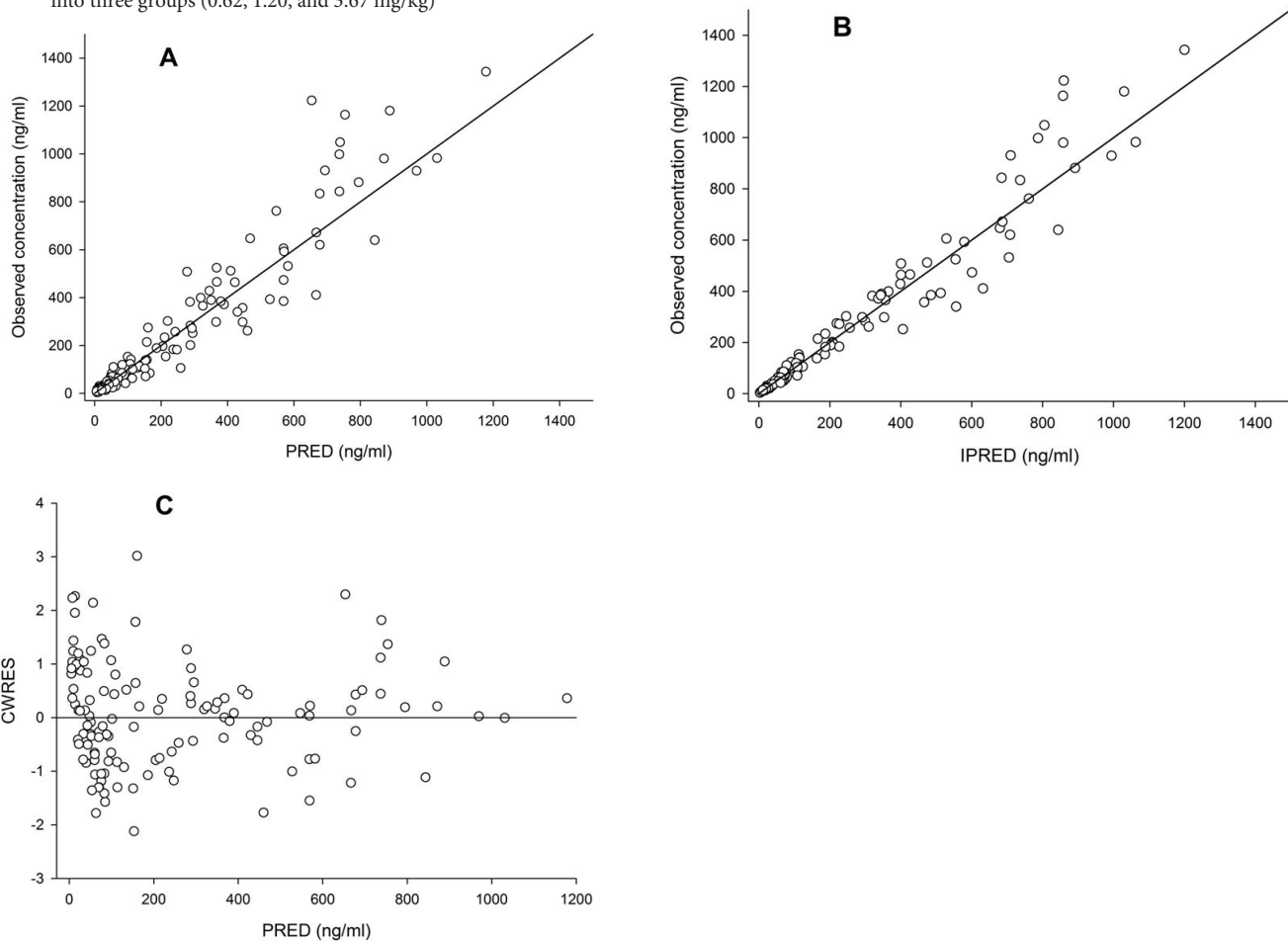
**Figure 1:** Dose-dependent dose-normalized AUC of ropivacaine in anesthetized rabbits after intravenous infusion. For illustration purposes, doses were categorized into four groups (0.62, 1.20, 3.67 and 6.52 mg/kg)

### Population PK Model

Population PK model was developed for ten rabbits in three dosing groups that showed no dose dependent PK (0.62, 1.20 and 3.67 mg/kg). For these rabbits, individual plasma concentration-time curves after intravenous infusion of different ropivacaine regimens are presented in Figure 2. Each concentration-time profile shows a biexponential decline with rapid distribution and slower elimination phases. Parameter estimates for the population PK model are presented in Table 1. Diagnostic plots revealed that the model prediction was consistent with the observed data and no systematic bias was detected (Figure 3).



**Figure 2:** Individual ropivacaine plasma concentration-time curves after intravenous infusion. For illustration purposes, doses were categorized into three groups (0.62, 1.20, and 3.67 mg/kg)



**Figure 3:** Diagnostic plots for the final population pharmacokinetic model (A) Plots of observed ropivacaine plasma concentrations against predicted (PRED); (B) Plots of observed ropivacaine plasma concentrations against individual prediction (IPRED); (C) Plots of conditional weighted residuals (CWRES) against PRED plasma concentrations by the model.

Parameter (unit)	Population estimate	Bootstrap		
		RSE(%) <sup>a</sup>	Median <sup>a</sup>	95% CI <sup>a</sup>
<b>Model-derived</b>				
V <sub>c</sub> (ml/kg)	399.6	7.98	405.6	346.5 - 465.5
Cl (ml/min.kg)	34.5	11.5	34.4	29.8 - 41.4
Cl <sub>d</sub> (ml/min.kg)	83.8	13.6	84.7	65.0 - 109.0
V <sub>2</sub> (ml/kg)	4269.0	9.51	4280	3615 - 5167
<b>Calculated</b>				
K <sub>12</sub> (1/min) <sup>b</sup>	0.210	13.4	0.210	0.159 - 0.263
K <sub>21</sub> (1/min) <sup>b</sup>	0.0196	12.2	0.0196	0.016 - 0.025
K <sub>10</sub> (1/min) <sup>b</sup>	0.086	11.3	0.085	0.074 - 0.104
<b>Between-subject variability (CV%)<sup>c</sup></b>				
ηV <sub>c</sub>	-	-	-	-
ηCl	21.3	70.7	21.1	7.66 - 33.3
ηCl <sub>d</sub>	23.1	77.1	22.5	0.289 - 36.9
ηV <sub>2</sub>	21.3	56.7	21.5	3.70 - 34.4
<b>Residual variability (CV%)</b>				
Proportional	21.8	9.09	21.5	17.9 - 24.4

V<sub>c</sub> = apparent volume of the central compartment; Cl = total body clearance; Cl<sub>d</sub> = distributional clearance between central and peripheral compartment; V<sub>2</sub> = apparent volume of the peripheral compartment; K<sub>12</sub> = first-order rate constant associated with drug transfer from compartment 1 to compartment 2; K<sub>21</sub> = first-order rate constant associated with drug transfer from compartment 2 to compartment 1; K<sub>10</sub> = first-order rate constant of elimination from the central compartment; η = the proportional difference between individual and typical parameter value of the population.

<sup>a</sup> RSE (%): relative standard error in percentage, Median and 95% CI (The lower and upper limits for 95% confidence interval) were calculated using the bootstrap (N = 1000).

<sup>b</sup> Secondary parameters calculated as: K<sub>12</sub>=Cl<sub>d</sub>/V<sub>c</sub>, K<sub>21</sub>=Cl<sub>d</sub>/V<sub>2</sub> and K<sub>10</sub>=Cl/V<sub>c</sub>

<sup>c</sup> Between-subject variability, calculated as (variance)<sup>1/2</sup>\*100%

**Table 1:** Population Pharmacokinetic Model Parameters for Ropivacaine after intravenous infusion in anesthetized rabbits

## Protein Binding Data Analysis

*In vitro* plasma protein binding experiments were performed by ultrafiltration on four different days over a concentration range of 2.5–3500 ng/mL of ropivacaine. The results of ANOVA showed no significant difference in ropivacaine percent bound to plasma proteins ( $P = 0.695$ ), excluding any concentration-dependent effect (Table 2). The overall mean percentage of protein binding of ropivacaine in rabbit plasma was 38.4%, with acceptable intra- and inter-assay variabilities represented by a maximum coefficient of variation of 5.4 and 16.6%, respectively.

% binding at different plasma concentrations (ng/ml)							Intra-assay		
Assay No.	2.5	10	50	100	1000	3500	Mean (%)	S.D.	C.V.(%)
1	39.5	44.3	-	43.7	41.4	-	42.2	2.2	5.2
2	34.4	34.5	30.3	31.8	31.0	31.9	32.3	1.8	5.4
3	42.5	42.7	39.2	43.0	42.2	39.7	41.6	1.7	4.0
4	39.5	40.7	42.2	39.0	37.5	37.1	39.3	1.9	4.9
<b>Inter-assay</b>									
Mean (%)	39.0	40.5	37.2	39.4	38.0	36.2	38.4	1.6	4.1
S.D.	3.4	4.3	6.2	5.5	5.1	4.0			
C.V.(%)	8.6	10.6	16.6	13.9	13.5	11.0			

\* Unlabeled ropivacaine and trace amount of <sup>3</sup>H-ropivacaine were spiked extemporaneously in plasma samples.

**Table 2:** *In vitro* protein binding of <sup>3</sup>H-ropivacaine\* in rabbit blank plasma with intra-assay and inter-assay precision

## Discussion

Our results show that ropivacaine PK is linear *in vivo* in the dose range of 0.62 to 3.67 mg/kg. Despite a relatively low degree of plasma protein binding, no variations are observed for *in vitro* concentrations up to 3500 ng/ml. Thus, the observed increase in

dose-normalized AUC at the 6.52 mg/kg dose suggests a capacity-limited clearance. Accurate and reliable population parameters adequately describe ropivacaine disposition after intravenous administration in the linear range of dose in anesthetized rabbits.

Different causes for nonlinearity at nontherapeutic concentrations are plausible such as a decrease in plasma protein binding, saturation of transporters or metabolizing enzymes and changes in elimination organ blood flow (mainly the liver and kidneys). Since ropivacaine is eliminated predominantly by hepatic metabolism with an intermediate to low extraction ratio, a decrease in hepatic blood flow is less likely to impact total body clearance than changes in the aforementioned mechanisms [21]. This is why dose-dependency due to saturation of plasma protein binding was first investigated *in vitro* at a pharmacologically relevant range of concentration.

Ropivacaine exhibits a high affinity low capacity association with AAG and a low affinity high capacity association with albumin in human plasma [30]. Despite the low concentration of AAG reported in rabbits, no concentration dependency of ropivacaine plasma protein binding was found in the wide range of concentration tested. Plausible explanations are first that AAG has more binding sites/affinity for ropivacaine in rabbits compared to humans or, secondly, that albumin has a higher binding capacity in rabbit.

The current study demonstrated that only 38.4% of ropivacaine is bound to plasma proteins in rabbit which, in our knowledge, is the lowest *in vitro* value reported for other species, e.g. 99% in dogs, 57% in ewes and 94% in humans [31,32,23]. This low extent of protein binding in rabbits suggests that any change in protein binding will have a minor or even no effect on the pharmacokinetics or pharmacodynamics of ropivacaine.

High plasma concentrations of ropivacaine had to be reached *in vivo* to verify PK linearity. A previous pharmacodynamic study on the toxic effects of local anesthetics on the cardiovascular and respiratory systems of 5 rabbits showed that ropivacaine administered as intravenous bolus doses of 1.372 and 2.744 mg/kg significantly decreased heart rate, while systolic and diastolic blood pressures decreased significantly only after the higher dose [33]. In our study, such changes happened in one of the three rabbits that received the highest dose. One reason that might explain this apparent lack of cardiovascular toxicity in most of our rabbits is that, in our experiment, ropivacaine at the highest dose was given over 45 min; this lower rate of administration would have less impact on baroreceptor reflex activation.

We developed a population PK model to characterize ropivacaine disposition after intravenous administration in anesthetized rabbits. Using data from all dose groups, a two compartment model with Michaelis–Menten elimination was first tried but did not improve the fit or offer any advantage over a simple two compartment model with linear elimination where only the rabbits in the three lower dosing groups were included.

To our knowledge, only one study reports the PK of ropivacaine after an intravenous bolus administration of 0.4 mg/kg ropivacaine in three male Japanese white rabbits [34]. In their conditions, ropivacaine plasma concentration-time profiles were better described by a one compartment model with a mean terminal half-life of 0.54 hour. In our conditions, the corresponding value was 2.31 hours. This discrepancy can be explained by a shorter duration of sampling (4 hours vs 10 hours in our study) and lack of analytical assay sensitivity.

PK data on ropivacaine in other animal species are quite scarce and results quite variable. In our rabbits, mean total body clearance was 34.5 ml/min.kg, which is quite compatible with the results observed in dogs and sheeps (41.1 and 29.6 ml/min.kg, respectively) but approximately 3 times higher than the value observed in rhesus monkeys.

In rabbits, ropivacaine volume of distribution at steady state based on total plasma concentrations averaged 4.7 l/kg, which is quite higher than values reported in other animal species (mean range: 1.1 to 2.2 l/kg). Based on our PK model,  $K_{21}$  is more than ten times slower than  $K_{12}$ , suggesting a retention and high affinity to peripheral tissues represented by the second compartment, which can explain the high value of  $V_2$ . Finally, elimination half-life is quite longer than in other species e.g. 0.4 hours in dogs and 1.3 hours in sheeps but close to the elimination half-life of 2.1 hours in rhesus monkeys [16,19]. Thus, the longer terminal half-life observed in rabbits would mostly result from a larger volume of distribution.

Based on total plasma concentrations in humans, ropivacaine mean total body clearance is 5.3 ml/min.kg, more than six times lower than the value observed in our rabbits [23]. Likewise, mean volume of distribution at steady state ranged between 0.48 and 0.55 l/kg, which is eight to nine times lower than the value observed in our rabbits. In contrast, based on unbound fraction in plasma, total body clearance in rabbits is quite similar to that observed in humans, representing 52% of the value in humans. Moreover, the volume of distribution based on unbound plasma concentration ( $V_u$ ) in rabbits (7.63 l/kg) is quite comparable to that reported in volunteers (mean: 9.8-11.0 l/kg) [23,35]. The apparent discrepancy in total body clearance and volume of distribution between human and rabbit is thus abolished when parameters are corrected by the free fraction of ropivacaine in plasma. These findings reinforce the necessity of determining plasma unbound fraction and of using PK parameters based on unbound concentrations when comparing different species.

Our findings, based on the *in vitro* plasma protein binding linearity, suggest that the dose-dependency of ropivacaine disposition observed at the highest dose in rabbit most probably results from saturation of hepatic enzymes or transporters. The population PK

model developed for dosing groups that showed no dose dependent PK indicate a slow release from the peripheral compartment that, in turn, would be mostly responsible for the high volume of distribution observed for ropivacaine after intravenous infusion.

## Conclusions and clinical relevance

In anesthetized rabbits, saturation of ropivacaine plasma protein binding is not the mechanism responsible for the pharmacokinetic nonlinearity of ropivacaine observed at intravenous doses higher than 3.67 mg/kg. Disposition parameters obtained in our study will enable accurate characterization of ropivacaine pharmacokinetic after a peripheral nerve block.

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