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Population pharmacokinetics modelling for clinical dose adjustment of carboplatin in dogs

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Abstract

Background Carboplatin is a human chemotherapeutic agent which is frequently used in dogs for the management of solid tumors. In human patient, its dosage is adjusted carefully, based on the creatinine clearance computation. In dogs however, the pharmacokinetics of carboplatin is poorly known and the dose 300 mg/m2 is based mostly on empirical data. Here, we aimed at characterizing the pharmacokinetics of carboplatin and determined the influence of several covariates, including creatinine plasma concentration and neutering status, in dogs, and used this model to predict myelotoxicity.

Results Sixteen client owned dogs were included after carboplatin administration (300 mg/m^2). For each animals, three to four plasma samples were collected and free plasma concentration of carboplatin was determined by HPLC/MS and analysed using Monolix® software with Non-linear mixed effect modelling. A mono-compartmental model best described the plasma concentration of carboplatin with log plasma creatinine concentration and sterilization status as covariates. After adjustment with the covariates, median population clearance was 3.62 [3.15 – 4.12] L/h/kg and volume of distribution was 3.93 [3.84 – 4.14] L/kg. The application of this model in 14 additional dogs demonstrates that individual drug exposure (model-predicted Area Under the Curve) predicted thrombocyte blood reduction (Pearson coefficient $r^2 = 0.73$, $p = 0.002$) better than dose after 14 days following administration of carboplatin.

Conclusion Based on our results, plasma creatinine concentration and the sterilization status are relevant explanatory covariates for the pharmacokinetics variability of carboplatin in client owned dogs. Dose adjustment based on these parameters could represent a promising strategy for minimizing thrombocyte toxicity.

Keywords Carboplatin, Renal function, Thrombocytopenia, Dogs, Modelling

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Introduction

Carboplatin is a platinum-based cytotoxic compound that was developed for the treatment of epithelial carcinoma in humans. It is also widely used in dogs for the treatment of various canine solid tumors including mammary carcinoma, osteosarcoma and pulmonary or thyroid adenocarcinoma [1–3]. Its mechanism of action is based on the formation of strong chemical bonds with DNA, RNA and proteins, ultimately inducing tumor cell death. Like other chemotherapeutic agents, platinum-based drugs are highly toxic and can cause nephrotoxicity, diarrhea and emesis. These adverse effects are more severe with older drugs like cisplatin, which is why carboplatin is now preferred over first-generation platinum-based molecules. Despite being less likely to cause renal and gastrointestinal adverse effects, carboplatin is still associated however with a strong risk of myelosuppression, characterized by thrombocytopenia and neutropenia, which generally occurs 14 days after administration in dogs [4, 5].

Traditionally, chemotherapy dosages are calculated using body surface area (BSA) as it is thought to better reflect the metabolic and elimination rate of the molecules and therefore better correlate with the pharmacokinetic profile of the drug disposition [6]. In dogs, the generally accepted dose of carboplatin is 300 mg/m^2, based on a study demonstrating that this dose produces about a 1/3 incidence of mild to moderate toxicity in 30 dogs [5]. However, only a few studies have specifically studied the pharmacokinetics of carboplatin in dogs. The use of a single dose indexed on body surface area is probably not ideal, as it has been shown to increase the risk of carboplatin-related toxicity in smaller dogs [4]. In addition, the pharmacokinetics of carboplatin have also been demonstrated to be variable between individuals in humans and cats, with the major determinant for carboplatin disposition in these species being the glomerular filtration rate (GFR) [7, 8]. Consequently, it has been proposed that carboplatin dosing in humans and cats should be computed based on physiological criteria to reach a targeted value of the Area Under the Curve (AUC) of plasma carboplatin concentrations, depending on the aimed efficacy and toxicity.

In dogs, no such dosing adjustments have been proposed to date. The goal of the present study is therefore to describe the pharmacokinetics of carboplatin in client-owned dogs by the method of non-linear mixed-effects modeling in order to determine the different clinical parameters that could be used to individualize its dosing. The first part of this work then consists of the pharmacokinetics modelling of carboplatin in the recruited dogs. The second part was dedicated to determining the statistical relationship between the AUC predicted by the model and thrombocytopenia or neutropenia at 14 days in a new cohort of dogs.

Materials and methods

Study design and animal studied

Our study was a prospective, two-center clinical trial performed in client-owned dogs presented at two French veterinary schools (National Veterinary School of Alfort and Oniris VetAgroBio) from June 2019 to January 2021. The protocol and the design of the study were approved by our local clinical research ethics committee (protocol #2019–03-03). All owners of the included dogs signed an informed consent before enrollment and received a detailed written description of the study.

Inclusion criteria were dogs presenting with neoplastic disease for which carboplatin monotherapy was indicated, either for curative purposes or for the prevention of recurrence of a previous tumor. To be included in the study, dogs had to have an estimated life expectancy greater than 3 months as evaluated by the referral clinician. Dogs with any previous administration of carboplatin at the time of inclusion were excluded from the study. During the normal hospital admission process, blood samples were taken from the animals and a complete blood count (Procyte Dx®, IDEXX, Hoofddorp, Netherland) as well as creatinine and urea blood concentrations were assessed (Catalyst One®, IDEXX, Hoofddorp, Netherland). Dogs with a neutrophil blood concentration of less than 1 500/µL and thrombocyte concentration of less than 50 000/µL at admission were excluded from the study. Animals with an admission plasma creatinine concentration higher than 14 mg/L were also excluded from the study.

The characteristics of each animal were documented, including center of inclusion, breed, sex, neutering status, body condition score (BCS), type of cancer and date of diagnosis. Any subsequent dose of carboplatin after the first dose could be included in the analysis if occurred during the inclusion period, meaning that different administrations were analysed as different occasions for the same dog.

All animal descriptive parameters are expressed as mean ± SD.

Number of subjects

Based on previous data in dogs, cats and human, we hypothesis that carboplatin concentration pharmacokinetics would follow a one-compartment first-order absorption model [7, 8]. In an article from 2008, Ogungbenro and Aarons demonstrated that 20 to 30 subjects are required to estimate the 95% confidence interval of the parameters in the case of extravascular administration with 3 sample by individual, is between 20 and 30

subjects [9]. The target enrollment number was then 30 animals in this study.

Treatment administration

On the day of treatment, the animals were carefully weighted and we computed their Body Surface Area (BSA) using the following formula:

$$\text{BSA}\left(\text{m}^2\right) = 0.1 \times \text{Body Weight}\left(\text{kg}\right)^{2/3} \quad (1)$$

All dogs then received a slow intravenous infusion (of approximately 20 min) of carboplatin (Carboplatin 10 mg/ml, Accord HealthCare) at a dose of 300 mg/m^2 through a cephalic vein catheter. For each administration, we documented the total duration of this infusion. All animals received a single intravenous injection of maropitant (1 mg/kg, Cerenia®, Zoetis) prior the administration of carboplatin to minimize the risk of emesis during the chemotherapy.

After the administration of carboplatin, dogs were hospitalized during 24 h in accordance with French regulations for the use of chemotherapeutic agents in veterinary medicine.

Blood sample and dosage

The sample design was based on the sampling windows design with 4 pre-scheduled windows of sampling corresponding to the first hour following administration, between 1 and 2 h following administration, between 2 and 4 h and between 4 and 12 h following administration. Within each window of administration, the exact sampling time was left to the discretion of the person in charge of taking the blood sample, but the exact timing of sample was carefully noted. After sampling, the blood was immediately transferred to lithium-heparin tubes and centrifuged at 4 °C. The plasma was then stored at −80 °C before analysis.

After re-equilibration for 1 h at 37 °C, 100 µL of plasma were deposited on an ultrafiltration system (Nanosep®) and centrifuged for 1 h at 37 °C, at 2500 g. The ultrafiltrate was acidified and injected into the chromatographic system to measure the free fraction of the drug.

Free carboplatin concentrations were quantified using a validated liquid chromatography-mass spectrometry triple quadrupole method (Quantis, Thermofisher, Villebon s/Y, France) composed by a +4 °C autosampler, a binary solvent pump and a thermostatic column oven, maintained at +40 °C. Chromatographic separation was performed on a Hypersil® Gold C18 (100 mm × 2.1, 1.7 µM, Thermofisher, France).

A programmed mobile phase gradient was used at a flow rate of 0.3 mL/min at 90% of mobile phase B, a decrease to 5% of mobile phase B from 4.0 to 5.0 min, then increase to 90% of mobile phase B from 5.1 to 6 min.

Tandem-mass spectrometric detection was carried out with a TSQ Quantis® Mass spectrometer (MS) (ThermoFisher Scientific, USA) and with an electrospray ionization (ESI) for all analyses. The acquired data were processed using Trace Finder™ Clinical software version 4.1 (ThermoFisher Scientific, USA).

Optimization of the MS conditions has been performed by single direct infusion of reference standards of each analyte. MS parameters were optimized as follows: sheath gas 40 arbitrary unit (Arb); auxiliary gas 10 Arb; vaporizer temperature 250 °C, ion transfer tube temperature 200 °C, positive Ion Voltage (V): 4200 V, dwell Time 10 ms (ms). The ESI polarity was positive.

Briefly, SRM transitions used were 371.900/294.00 (quantification) and 371.900/354.917 (confirmation) for carboplatin and 398.87/305.917 for oxaliplatin, the internal standard.

Calibration standards ranged from 0.1 to 30 µg/L in water. Low, medium and high QCs were at 0.3, 2 and 25 µg/L in water to determine free plasma concentrations. Lower limits of quantification were 0.1 µg/L respectively for aqueous and plasma calibrations. Our method was validated according to international guidelines (EMA, FDA) with good precision (CV ranged from 3.1 to 8.6%) and accuracy (−4.3 to 8.8% of biais).

Population pharmacokinetics analysis

We modelled the pharmacokinetics of carboplatin in dogs using a population pharmacokinetics approach with non-linear mixed effect modelling with Monolix Software (Lixoft®, Antony, France, v.2024R1). In order to normalize the pharmacokinetics parameters computed by the model to the body weight, we divided the total dose received by each dog by its own body weight for the modelling.

A structural pharmacokinetic model was determined by fitting the free plasma concentration values of carboplatin to either a one- or two-compartment model with linear elimination and administration by infusion with no lag-time. Area Under the Curve ($AUC_{0\text{-last}}$) computation was manually added as an additional output to the Monolix model. Values below the limit of quantification of 0.1 µg/L was defined as interval censored in the analysis.

Inter-individual variability of the pharmacokinetics parameters was modelled using a lognormal distribution according to the equation:

$$\log(\theta_i) = \log(\theta_p) + \eta_i + \eta_{occ} \quad (2)$$

where θ_i represents the model-predicted pharmacokinetic parameter estimate for the i^{th} dog, θ_p represents the typical pharmacokinetic population parameter and η_i and η_{occ} are random variables representing inter-individual and inter-occasion (for dogs with several administrations) variability (IIV and IOV, respectively) and follows a normal distribution with mean zero and respective variances ω^2 and γ^2.

Residual error variability (ε), which includes intra-individual and analytical variability, was estimated by testing different error model (combined, constant or proportional). The best model was chosen through visual inspection of the relationship between observed and predicted individual concentrations, the distribution of weighted residuals (IWRES), and normalized prediction distribution errors (NPDE) as well as, the Bayesian Information Criteria (BIC) [10].

After the final structural model was selected, the effect of different covariates were evaluated. Age, weight, and natural logarithm of the plasma creatinine at the time of administration were tested as continuous covariates. Sex, inclusion center, body condition score and neutered status were considered as categorical covariates. The relationship between pharmacokinetics parameters and covariates was described by modifying Eq. (2) as follows:

$$\log(\theta_i) = \log(\theta_p) + \beta.Cov_{\theta i} + \eta_i + \eta_{occ} \quad (3)$$

where β represents the coefficient to be determined and $Cov_{\theta i}$ represents the value of the continuous covariate, or 0 or 1 for categorical covariates.

Distribution normality of the covariate and the pharmacokinetics parameters was verified by Shapiro–Wilk test and the relationship between each covariate and pharmacokinetics parameters were tested with Pearson's test for continuous covariates or ANOVA for categorical covariate. The covariate was selected to be tested into the model based on the results of these correlation tests. The covariate was included finally in the model if its inclusion resulted in a decrease in BIC value of 2 as usually recommended [10].

Finally, in order to verify the parameters precision by an additional method than standard errors, we conducted a bootstrap analysis using the bootstrap analysis module of Monolix [10]. This analysis was conducted using 200 replications on the population parameters for a similar sample size than our study population.

Model evaluation on toxicity

We recruited additional dogs to investigate whether our model could predict carboplatin toxicity in animals that were not used for the model definition. Specifically, we assessed the potential relationship between the $AUC_{0-\infty}$ values predicted by the model (taking into accounts covariates) and the thrombocytopenia or neutropenia, which are the major limiting adverse effect with carboplatin.

For each animal that received carboplatin administration between January 2022 and June 2023 at our hospitals, we documented the precise dose of carboplatin administered, initial blood concentration of neutrophils and thrombocytes, as well as the covariate values (plasma creatinine concentration and sterilization status) used in the definition of the model. After 14 days, the animals returned to our veterinary hospital for another venous blood sampling to assess the values of thrombocytes and neutrophils blood concentration at the expected nadir in dogs [4, 5]. Dogs unable to return for follow-up blood count were excluded from this second study.

For each dog included, a simulation of free carboplatin blood concentration based on its own plasma creatinine concentration and sterilization status was performed using Simulx software (Lixoft®, Antony, France, v.2024R1) to predict its blood concentration profile and to estimate its $AUC_{0-\infty}$ value. For each dog, simulations were repeated 1000 times by Monte-Carlo method in order to compute the median $AUC_{0-\infty}$ value for each animal. This Monte-Carlo simulation allowed to take into account for each animal the uncertainty of the prediction (intra-individual variability of the PK parameters).

To evaluate our model's ability to predict the observed toxicity, we quantified the non-parametric correlations by Spearman coefficient between predicted $AUC_{0-\infty}$, dose expressed in mg/kg and dose expressed in mg/m^2 versus the percentage of platelet or neutrophil reduction between day 0 and day 14. When a significant correlation was evidenced, we performed a non-linear least square regression with a Hill model using GraphPad Prism software (v.10.2.3) according to the equation:

$$Y = \frac{E_{max}.X^n}{(EC_{50}^n + X^n)} \quad (4)$$

where the variable Y represents the fraction of platelet or neutrophil reduction (ranging between 0 and 1), X, the explanatory variable ($AUC_{0-\infty}$, Dose in mg/kg or Dose in mg/m^2), E_{max} the maximal effect (lower than 1), EC_{50} the value of the explanatory variable leading to 50% of the maximal effect and n the Hill coefficient.

Results

Population pharmacokinetic analysis of carboplatin in dogs

Study dogs

Between June 2019 and January 2021, 27 dogs were initially included in the study from the two centers (22 in

National Veterinary School of Alfort and 5 in Oniris VetAgroBio) but 11 animals were excluded due to deviations in the experimental protocol. Among the 16 dogs finally included, several received successive carboplatin administrations that were included in the analysis leading to a total of 39 carboplatin plasma concentration profiles analyzed (4 dogs received 5 administrations, 2 received 3 administrations, 3 dogs received 2 administrations, and 7 dogs received only one administration). All dogs were treated with carboplatin in the context of prevention of recurrence tumor following surgical removal of the tumor (carcinoma for 4 dogs, osteosarcoma for 3 dogs, melanoma for 3 dogs, mammary adenocarcinoma for 2 dogs, ovarian dysgerminoma for 2 dogs, one chondrosarcoma and one fibrosarcoma). The mean age of the animals at the time of the inclusion was 11.1 ± 1.72 years and the mean weight was 21.5 ± 7.8 kg. The sex repartition was 14 females and 12 males (4 neutered females and 5 neutered males). The mean dose of carboplatin was 300.4 ± 7.6 mg/m^2 equivalent to a mean dose of 10.7 ± 1.0 mg/kg.

At the time of the carboplatin administration, the mean value of plasma creatinine concentration was 7.32 ± 1.86 mg/L, the mean thrombocytes and neutrophil blood count were 415.10^3 ± 170.10^3/µL and 7671 ± 5789/µL, respectively.

PK model evaluation

The evolution of carboplatin free plasma concentration *versus* time was best described by a one-compartment model with linear elimination and characterized by the volume of distribution (V) and clearance (Cl). The residual variability (ε) was described by a combined error model according to the equation:

$$Obs = C + \sqrt{a^2 + (b * C)^2} * \varepsilon \quad (5)$$

where Obs represents the observed concentration, C is the predicted concentration and a and b are component of the error model.

As illustrated by Fig. 1A, the examination of the individual prediction *vs* observation suggests that most of the observations fell into the prediction interval centered on the identity line, suggesting a good description of the data by this model. The Normalized Prediction Distribution Error (NPDE) plots presented in supplemental material (Supplemental Fig. 1) also show the NPDE distribution centered on zero.

As illustrated by Fig. 1B, the conditional distributions of the random effects, η_V and η_{Cl}, were centered on zero and a Shapiro–Wilk test suggested a normal distribution ($p = 0.46$ and $p = 0.50$ for η_V and η_{Cl}, respectively) with low shrinkage values (11.1% and −4.7% for V and CL, respectively).

Covariate analysis

No correlation between covariates was evidenced. As illustrated in Fig. 2, log transformed plasma creatinine concentration at the day of carboplatin administration inversely correlated with individual plasma clearance values ($p < 0.001$). Additionally, we also found that sterilization status was a covariate associated with clearance, as neutered animals had a significantly lower clearance

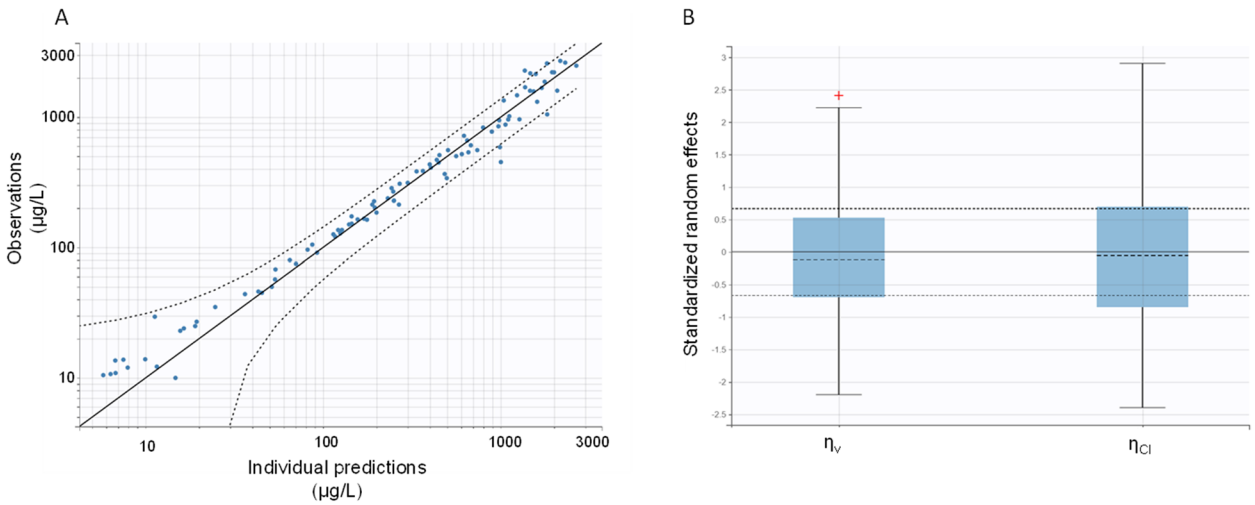

Fig. 1 **A** Individual predictions *vs.* observations expressed in a log10-log10 scale. Blue dots represents the individual observations. Black line represents the identity line and dotted black line the 90% prediction interval. **B** Distribution of the standardized random effect η_V and η_{Cl} for Volume of distribution and Clearance respectively

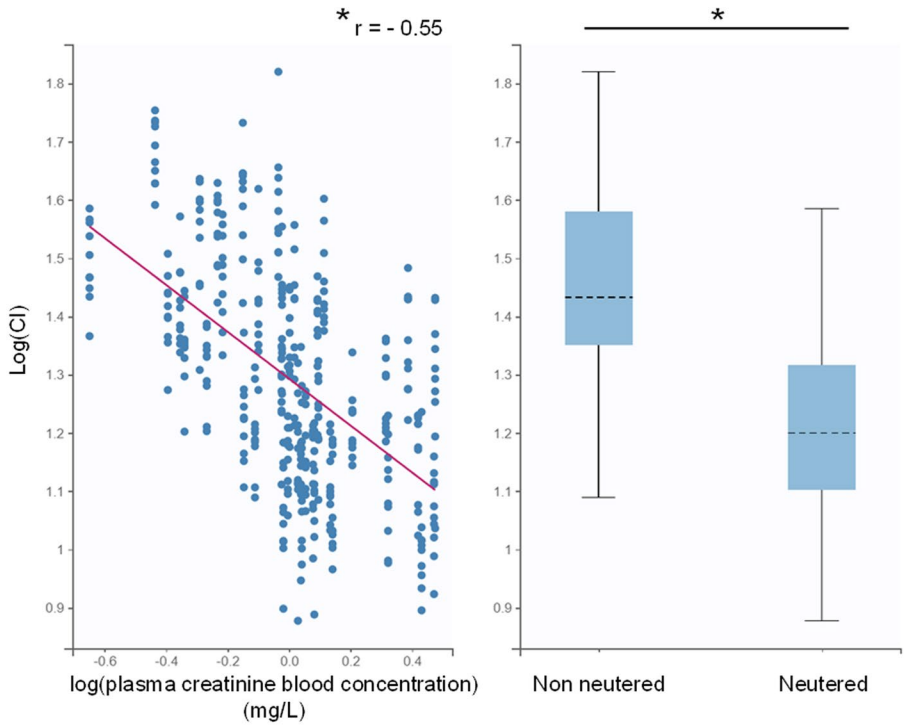

Fig. 2 Correlations between creatinine blood concentration or sterilization status of the animal and clearance. Both relationships were statistically significant according to Pearson correlation test (creatinine blood concentration) or ANOVA (sterilization status). r, Pearson correlation coefficient; Cl, Clearance (L/h/kg); *, $p < 0.005$

value compared to non-neutered ($p < 0.0001$). The model without covariate had an absolute BIC value of 1193.61 and the addition of the sterilization status or log plasma creatinine concentration to this model reduced the BIC value to 1164,82 (−28.79) or to 1179.18 (−23.43), respectively. The addition of both covariates of clearance resulted in a new BIC of 1160.32, corresponding to a reduction of 33.29 points of BIC as compared to model without any covariate. The use of non-transformed creatinine plasma concentration did not improve the likelihood of the model as much as log-transformed values. No other covariates were identified in our study. Interestingly, despite the fact that carboplatin dose was linearly computed based on the BSA and reported based on body weight as input of the model, weight was not significantly correlated with any of the pharmacokinetics parameters ($p = 0.32$ and $p = 0.86$ for V and Cl, respectively) and did not improve the quality of the model.

The effects of plasma creatinine concentration and sterilization status were added to the model and their respective coefficients were computed. The values of each covariate's coefficient are presented in Table 1. It is important to note that, based on these coefficients, a variation of a creatinine blood concentration within the normal range interval, *e.g.* from 5 mg/L to 15 mg/L, will reduce carboplatin clearance of about 30% according to our model. Similarly, in our study, animals neutered at the time of carboplatin administration had a clearance reduction of around 25%.

Parameters estimates

The final model parameters are presented in Table 1. Precision of the parameter's estimates were satisfactory as RSE was lower than 25% for both V and Cl. After the adjustment on the different covariate, the median value of clearance and volume of distribution for the population were 3.62L/h/kg and 3.93L/kg, respectively. The first estimation of V with a model including inter-individual variability, *i.e.* between dogs (IIV), and inter-occasion variability, *i.e.* between different administrations for a given dog (IOV), was very imprecise with a RSE higher than 100%. To improve this estimation, IIV and IOV standard deviations were fixed to 0.1 for volume of distribution during the modelling process, as previously described [11].

As illustrated by Fig. 3, the final good performance of our model was assessed by the Visual Predictive Check of the model and individual fits. This was also evidenced by a good correlation between typical values of the population model estimates and the bootstrap estimates as

Table 1 Estimated population pharmacokinetics parameters for carboplatin disposition after intravenous administration in dogs associated with their relative standard error (RSE %)

Pharmacokinetics parameter	Symbol	Model estimate		Bootstrap analysis		
		Typical value	Relative Standard Error (%)	Median	[95% CI]	Bias (%)
Volume of distribution (L/kg)	V	4.05	6.04	4.02	[3.65 – 4.52]	0.3
Clearance (L/h/kg)	Cl	6.9	24.7	6.82	[3.94 – 10.35]	−1.1
Covariates						
Coefficient for the effect of creatinine plasma concentration (log-transformed) on clearance	$\beta_{creatinine}$	−0.25	50.3	−0.24	[−0.45 – 0.07]	−10.9
Coefficient for the effect of sterilization status on clearance	$\beta_{neutered}$	−0.22	34.2	−0.22	[−0.44—−0.07]	7.9
Random effects						
IIV of V	ω_v	0.1[a]	-	-	-	-
IIV of Cl	ω_{cl}	0.074	53.1	0.044	[0.004 – 0.16]	−22.4
IOV of V	γ_V	0.1[a]	-	-	-	-
IOV of Cl	γ_{Cl}	0.11	28.3	0.11	[0.02 – 0.15]	−4.1
Residual error model						
Additive constant of the combined error model	a	12.28	17.2	11.72	[7.3 – 19.9]	−0.002
Proportional constant of the combined error model	b	0.23	12.2	0.22	[0.17 – 0.25]	−5.72

IIV and IOV: inter-individual and inter-occasion variability represented as standard deviation of the random effect

[a] IIV and IOV have been fixed at 0.1 for Volume of distribution as data were too sparse for correct estimation

demonstrated by the low bias values of the bootstrap analysis. This demonstrates the good ability of the model to describe the observed variability, as most observed values fell into the prediction interval.

Toxicity prediction

Study dogs

In a second set of experiments, we included 17 additional dogs receiving carboplatin for establishing whether our model could predict medullary toxicity (*i.e.* thrombocytopenia and neutropenia at 14 days after carboplatin administration). Among these animals, 3 were excluded due to loss of follow-up, leaving 14 dogs finally included in the analysis. The mean age of the animals at the time of the inclusion was 9.34 ± 2.91 year and the mean weight was 22.43 ± 11.28 kg. The sex repartition was 5 females and 9 males (2 neutered females and all males were intact). The mean administered dose was 306.1 ± 22.6

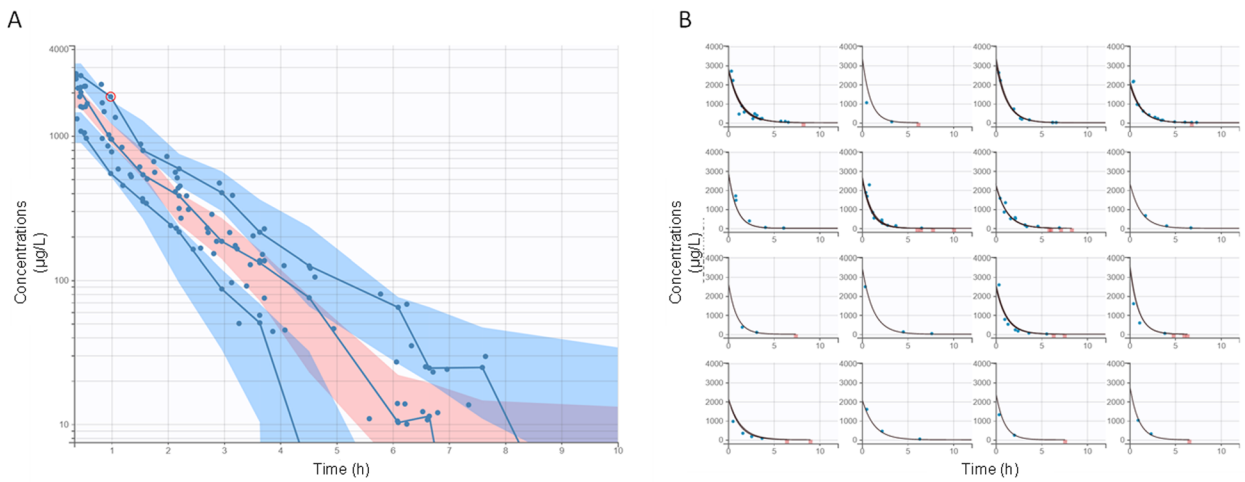

Fig. 3 **A** Visual Predictive Check (VPC) of the carboplatin concentration (log10 scale) *vs.* time. The individual values are shown as blue dots. The observed and predicted 10th and 90th percentiles of the interval prediction or empirical percentiles are shown by the blue area or blue line, respectively. Outliers are shown by red circle. **B** Individual predictions of carboplatin plasma concentration in dogs from the final selected model. Plots of individual observed (blue dot) and individual predicted (black line) concentration time course. Each black line represent the prediction of one occasion. Censored data (below Limit of Quantification) are shown as pink squares

mg/m^2 corresponding to a dose of 11.5 ± 2.4 mg/kg. The mean creatinine blood concentration was 10.9 ± 3.6 mg/L at the time of carboplatin administration. As illustrated by Fig. 4, dogs exhibited a significant decrease in thrombocytes and neutrophil blood count between the day of treatment and the 14th day after the treatment. The mean reduction observed was 66 ± 16% and 65 ± 25% for thrombocytes and neutrophils, respectively.

Computation of AUC

In each animal included in this second study, we computed the $AUC_{0-\infty}$ value for carboplatin using Simulx® software, based on the previously developed model, along with the values of creatinine plasma concentration as well as the neutering status. The predicted $AUC_{0-\infty}$ median values were 3342 [3121 – 4017] mg.h/L in these animals.

Modelling toxicity

We tested the correlation between the predicted AUC $_{0-\infty}$, the dose in mg/kg and the dose in mg/m^2, with the observed hematological toxicity. No significant correlations were found regarding the neutrophil reduction, however, the percentage reduction in thrombocytes correlates with both the $AUC_{0-\infty}$ and the dose in mg/kg ($p < 0.0001$and $p = 0.0019$, respectively). For both relationships, an E_{max} model with Hill coefficient was fitted and the coefficient of determination r^2 was reported. The values of the E_{max} model for the two relationships were are presented in Table 2. Although the dose in mg/kg showed a good fit with E_{max} model ($r^2 = 0.57$), the use of $AUC_{0-\infty}$, *i.e.* weighted by the explicative covariates of the model, improved the fitting ($r^2 = 0.73$) as illustrated by Fig. 5.

Discussion

Carboplatin free plasma concentration profiles following IV administration were modelled in client-owned dogs using a one-compartment population pharmacokinetics model. We documented a median clearance of 3.62 L/kg/h (3.25 L/kg/h and 4.26 L/kg/h in sterilized and non-sterilized animals, respectively) and a median volume of distribution of 3.93L/kg for the total population after weighting by the explicative covariates, which corresponds to 36.2 L/h/m^2 and 39.3 L/m^2, respectively. These values are close to previous clinical reports in dogs with mammary carcinoma and receiving 300 mg/m^2 carboplatin where clearance was 34.3 [13.8 – 85.3] L/h/m^2 and volume of distribution at steady state was 34 [20.1 – 57.5] L/m^2 [12]. In our model, exposure to carboplatin was affected by the plasma creatinine concentration value at administration as well as the sterilization status of the dogs.

The fact that log-transformed plasma creatinine concentration was a covariate that correlates with clearance in our model was expected, as carboplatin is mostly eliminated by kidney, and GFR results have been found to be correlated with carboplatin pharmacokinetics in cats and humans [7, 8]. In humans, the carboplatin dosage is usually adjusted based on the GFR computed from plasma creatinine concentration with the Cockcroft-Gault formula [8]. In cats, a study from 2009 demonstrated that carboplatin AUC and toxicity increase when GFR decreases [7]. In dogs, the impact of GFR on carboplatin

Table 2 Estimated E_{max} model parameters for the least square regression of the relationship between pharmacokinetics parameters and the observed toxicity

Correlation	$AUC_{0-\infty}$ (mg.h/L) *vs* Thrombocyte reduction (%)	Dose (mg/kg) *vs* Thrombocyte reduction (%)
Parameter	**Estimate [95%CI]**	**Estimate [95%CI]**
E_{max}	0.99 [0.76 – 1]	0.76 [0.68 – 1]
E_{50}	2679 [2221 – 2886]	9.0 [7.64 – 9.32]
Hill coefficient	3.35 [2.14 – 7.44]	17.80 [3.47 – 47.14]
r^2	0.73	0.57

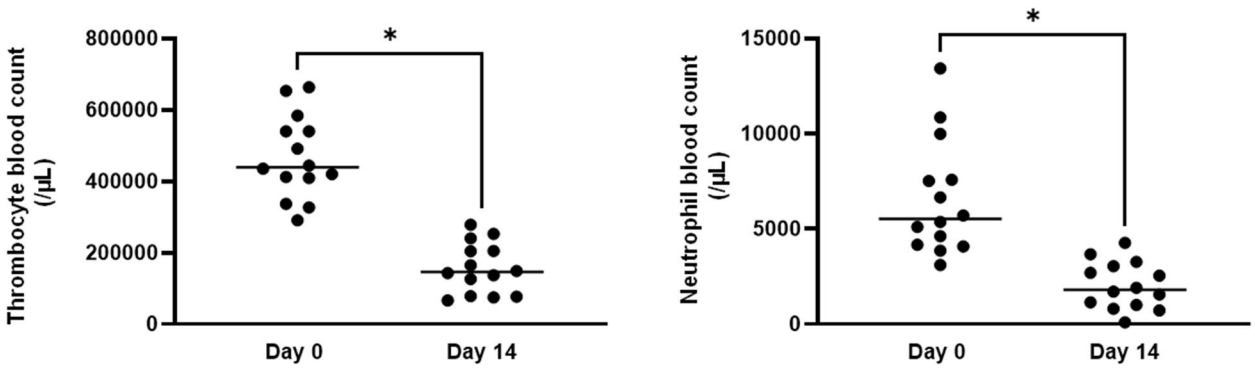

Fig. 4 Thrombocyte and neutrophil blood count between day 0 and 14 days after carboplatin administration. *$p < 0.05$ between day 0 and day 14

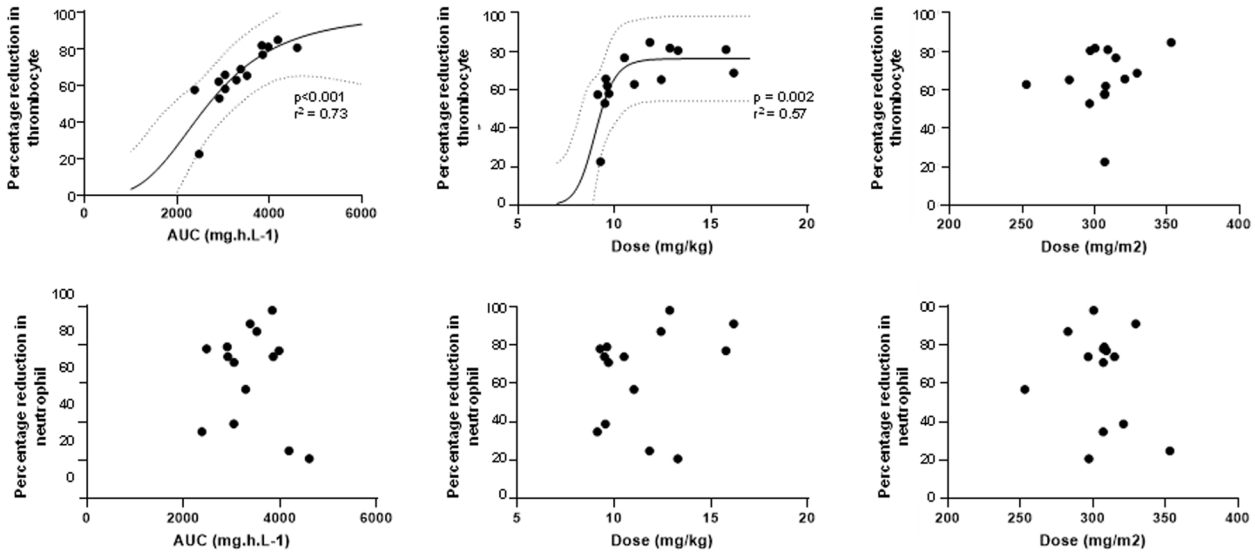

Fig. 5 Relationship between the different pharmacokinetics variable ($AUC_{0-\infty}$, dose in mg/kg or dose in mg/m^2) and thrombocyte or neutrophil blood count reduction between the first day and 14 days. Each black line represent relationship predicted by the E_{max} model and dotted line represents the 90% prediction band when a significant relationship was evidenced

elimination has never been directly evidenced, but it has been shown that after carboplatin administration, 70% of the platinum is excreted in the urine, which suggests that kidneys are the main route of carboplatin elimination, as in other species [13]. In our model, we used the natural logarithmic transformation of plasma creatinine concentration instead of absolute plasma creatinine concentration because clinical studies in dogs demonstrated that log plasma creatinine concentration correlates linearly with GFR [14]. More surprisingly, we also demonstrated a good improvement of the model when adding the sterilization status as an explanatory covariate for clearance. This result is intriguing because no effect of sterilization status on the GFR is expected. For example, a study from 2021 demonstrated that sterilization status was not a relevant covariate to explain the variability of iohexol pharmacokinetics in dogs [15]. In our study, despite not being statistically significant, we observed a trend toward an older age associated with sterilization, with a median age of 9.98 and 11.31 years in the intact and neutered animals, respectively. Similarly, even if this association in not statistically significant, there is also a trend toward a higher plasma creatinine concentration in neutered animals (mean 6.2 *vs* 7.3 mg/L in intact and neutered animals, respectively). Even if sterilization status could have an influence on hormonal status, which could alter the disposition of carboplatin in dogs, it is more probable that in our case, sterilization status presents a weak statistical association with other parameters (age, plasma creatinine concentration or other factors not evaluated here) that could have biased these results. Due to the small number of animals, other investigations are required in order to conclude on the effect of sterilization status.

Another important finding is that body weight is not a statistically significant covariate in our model, despite the fact that in our study, carboplatin dosage administered to the dogs was based on the BSA. The use of the total BSA for the computation of dosage in chemotherapy is based on the old empirical assumption that metabolic rate and thus, clearance of anti-neoplastic drugs, correlates with the body surface [16]. However, this is now under debate, especially for drugs like melphalan, doxorubicin or mitoxantrone [17–19]. With these chemotherapeutic molecules, it has indeed been shown that smaller dogs experienced a higher risk of adverse effects, probably due to relative overdosing as compared to much larger animals. For carboplatin, it has been demonstrated similarly that dogs with smaller body weight (< 10 kg) experienced a significantly higher incidence of adverse effects (neutropenia) than larger dogs when carboplatin dosage was adjusted to the BSA [4]. This was also correlated with another study showing that small body weight was correlated with lower neutrophil blood counts at nadir following carboplatin administration [20]. In our study, the mean body weight of our study dogs was 21.5 ± 7.8 kg, and only 4 dogs had a body weight lower than 10kg, which did not allowed to evidence differences in plasma carboplatin concentration. However, we did not find evidence of a correlation between the dose expressed in meter square and thrombocyte toxicity, whereas dose in mg/kg was a good predictor of thrombocytopenia. All

together, these findings reinforce the hypothesis that carboplatin dosage should be based on body weight rather than BSA.

Finally, we demonstrated that both the dose in mg/kg and the predicted AUC positively correlated with the percentage of thrombocyte reduction after 14 days following the administration, following an E_{max} model with Hill coefficient. We evidenced a better prediction when using the predicted AUC as compared to the dose (Pearson coefficient $r^2 = 0.73$ and 0.57 for AUC and dose, respectively). Because the dose and the $AUC_{0\text{-}\infty}$ are proportionally related to clearance, this suggest that the adjustment of the clearance value by log plasma creatinine concentration and sterilization status improves the ability of the model to predict toxicity on thrombocytes as compared to using a median population value of clearance. The fact that the toxicity model has been constructed in additional dogs using a model previously developed also suggests an external validity of our model. We also evidenced a steeper (higher Hill coefficient value) E_{max} model with the dose as compared to the AUC, showing that the model-predicted AUC is a more discriminating approach than dose, which could be used in clinical setting for dose adjustments. In our study, we were not able to find any correlation between the predicted AUC or dose and neutrophil count reduction. One of the hypothesis to explain this absence of correlation could be related to the variability of the nadir of the neutrophil blood count. A study from 2020 demonstrates that even if the nadir mostly occurs at 2 weeks, the neutropenic event can be delayed up to 3 weeks following carboplatin administration, whereas the thrombocytes nadir occurs mostly within 2 weeks after administration [4]. Similarly, a 2023 study demonstrated that dogs could experience delayed neutropenic events after carboplatin administration [21]. Additionally, and contrary to thrombocytes, neutrophil blood counts can also be influenced by pre-existing or intercurrent infections that would have been undetected by clinical staff. All of these factors could contribute to the absence of clear prediction of neutropenia in our model.

Our study presents several limits. The first limit is related to the absence of documentation of the efficacy of carboplatin in the treated animals. In our studied animals, all animals received carboplatin administration for the prevention of recurrence of soft tumors following surgical resection. In this context, and due to the high variability of the tumor type, efficacy was not specifically addressed. Another limitation is related to the absence of measurement of GFR. Even if the use of plasma creatinine concentration was used as an indirect marker of kidney function, the use of GFR as covariate would have certainly provide a better prediction by the model.

Conclusion

We successfully modelled the pharmacokinetics of the free concentration of carboplatin in client-owned dogs. We demonstrated that both plasma creatinine concentration and sterilization status are relevant covariates for explaining clearance variability of carboplatin between dogs. We also used this model to predict the thrombocyte reduction after 14 days following carboplatin administration with a good accuracy, which paves the way for individualized dosing strategy based on plasma creatinine concentration and sterilization status in dogs.

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None.

Authors' contributions
B.J.: inclusion of the animals, conceptualization and methodology, supervision. M.S.: data curation and analysis. U.M.: inclusion of the animals, statistical analysis. R.A.: inclusion of the animals, I.C.: inclusion of the animals, F.A.A.: data analysis, P.L.: data analysis, H.A.: data curation and supervision, K.M.: visualization, writing the original draft, data curation and analysis, supervision, formal analysis and funding. All authors reviewed and edited the manuscript.

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Data availability
Data are available upon request to the corresponding author.

Declarations

Ethics approval and consent to participate
The protocol and the design of the study were approved by our local clinical research ethics committee (protocol #2019–03-03). All owners of the included dogs signed an informed consent before enrollment and received a detailed written description of the study.

Consent for publication
All participants consents to publications of the data.

Competing interests
The authors declare no competing interests.

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