The Surface Area to Volume Ratio Changes the Pharmacokinetic and Pharmacodynamic Parameters in the Subcutaneous Tissue Cage Model: As Illustrated by Carprofen in Sheep

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Introduction: Pharmacokinetic and pharmacodynamic models can be powerful tools for predicting outcomes. Many models are based on repetitive sampling of the vascular space, due to the simplicity of obtaining samples. As many drugs do not exert their effect in the vasculature, models have been developed to sample tissues outside the bloodstream. Tissue cages are hollow devices implanted subcutaneously, or elsewhere, that are filled with fluid allowing repetitive sampling to occur. The physical dimensions of the cage, namely, the diffusible surface area to volume ratio, would be expected to change the rate of drug movement into and out of tissue cages.

Methods: Seven sheep were implanted with five pairs of tissue cages, subcutaneously. Each pair of cages had a different length but a fixed diffusible surface area, so the surface area to volume ratio differed. Carrageenan was injected into half of the cages in each animal during one sampling period in a cross-over design. Samples from each cage and the bloodstream were obtained at 14-time points during two sampling periods. The concentration of carprofen was measured using LC–MS/MS and the results were modeled using nonlinear mixed-effects techniques. Prostaglandin metabolites were also measured and the change over time was analyzed using linear mixed effect modeling.

Results: The presence of carrageenan within an animal changed the systemic pharmacokinetics of carprofen. The rate of drug movement into and out of the tissue cages varied with the surface area to volume ratio. The concentration time curve for prostaglandin metabolites was also measured and the change over time was analyzed using linear mixed effect modeling.

Conclusion: The surface area volume ratio of tissue cages will influence the calculated pharmacokinetic parameters and may affect calculated pharmacodynamics, thus, it is an important factor to consider when using tissue cage data for dosing regimes.

Keywords: pharmacokinetics, pharmacodynamic, surface area to volume ratio (SAV), tissue cage model, sheep, carprofen
INTRODUCTION

Pharmacokinetic and pharmacodynamic (PKPD) models are powerful tools for predicting outcomes from pharmacological interventions when they accurately model reality. Many PKPD models are based on sampling from the vascular space as blood, plasma, and serum are easily sampled over multiple time points with reliable techniques. However, the vasculature is not the target site of action for many drugs, e.g., antibiotics and anti-inflammatories are commonly given to treat ailments outside the bloodstream. To improve the accuracy of PKPD model predictions, in vivo models have been developed to obtain and measure drug concentrations and effects in other tissues.

Ideally, these in vivo models allow individual tissues within an animal to be sampled with high frequency over a relatively short period of time. Clearly, tissue collection that requires the sacrifice of animals or produces significant damage to the tissue (e.g., muscle biopsy) is not ideal. Therefore, the tissue cage (or chamber) model, which was developed by Guyton (1) to study physiological parameters, was quickly adopted for pharmacological studies of antibiotics (2). Hollow devices (cages) are implanted subcutaneously or within tissue in a manner that allows percutaneous sampling by a needle and syringe. Cages are made permeable to drugs by perforating portions of their surfaces, thus, creating a diffusible surface area. Two to three weeks after implantation, cages are filled with a fluid that can be sampled (3, 4). This model has the advantage of producing relatively large sample volumes compared with alternative approaches that use skin windows, blistering, or wicking. Furthermore, the cages can be maintained for long periods (e.g., 22 weeks in cattle) and still produce viable samples (3).

Several variations in tissue cage shape, material, and size have been used in published studies. While silicone cylinders, as described by Bengtesson and Sidhu (3, 4), are the most common construction material and shape, the size of cages can vary significantly between studies. This variation in size, coupled with variations in the diffusible surface area, leads to variations in the surface area to volume ratio (SA/V) of the cages (Table 1). The SA/V parameter, under the Fick’s law of diffusion, is expected to be an important contributor to the pharmacokinetics that are subsequently measured. Modeling of various SA/V ratios in vitro showed a marked difference in the pharmacokinetics of the cages according to their SA/V (5). An in vivo model by Van Ettà also showed that cages of differing dimensions but the same SA/V produce the same pharmacokinetic profiles (6), consistent with the theoretical prediction under the Fick’s law.

In addition to the variation in physical characteristics, inflammation may be induced using carrageenan within one or more cages when studying anti-inflammatory drugs. This is designed to allow comparison between inflamed spaces and non-inflamed spaces within an individual animal (12, 19, 25, 26). It is unknown if inducing inflammation within one or more cages in an animal changes the observations in either the blood or in the non-inflamed cages compared to similar observations with no inflammation.

The tissue cage model continues to be used for pharmacological research (27, 28) without standardization or comprehensive verification. Notably, the dosing interval for robenacoxib of 24 h is based on tissue cage modeling in cats (10).

This study aimed to evaluate the effects of SA/V and inflammation on the PK and PKPD results from tissue cage models, using the well-characterized NSAID drug carprofen. We predicted that as the volume of the cage increases relative to the surface area, changes in the pharmacokinetics will result in changes in the predicted pharmacodynamic parameters. Second, we predicted that the presence of carrageenan induced inflammation within the individual animal would change the pharmacokinetics of the non-inflamed cages.

MATERIALS AND METHODS

In this experiment, we simultaneously sampled blood and tissue cage fluid from implanted cages with varying SA/V and with and without induced inflammation, in a cross-over design.

Tissue cages were prepared in the manner of Sidhu et al. (4) utilizing 17 mm outer diameter silicone laboratory tubing. In total, five sizes were prepared; 3, 6, 10, 14, and 18 cm in length. Each end was sealed with silicone putty and 24 holes were created in each using a 4 mm biopsy punch. Once the putty had set, the
cages were packaged in sets of five and sterilized by ethylene oxide. The calculated surface area, volume and SA/V ratio are displayed in Table 2.

In total, seven merino wethers, approximately 18 months old and ranging from 39 to 59 kg, were enrolled (University of Melbourne Animal Ethics approval 1814590). Each wether was determined to be healthy by veterinary clinical examination and routine hematological and biochemical testing prior to enrolment. All the sheep were housed in a corrugated iron shed on slatted floors with water supplied ad libitum. Pellets (Sheep & Cattle Rumevite, Townsville, QLD, Australia) and lucerne chaff were provided daily.

To prepare the animals, under general anesthesia, five hollow silicone cylinders were implanted subcutaneously on each side of the neck of each wether to form ten tissue cages as described previously (29). The cages were inserted in size order, with the shortest cage being most cranially positioned. A cross-over two-phase pharmacokinetic study was conducted at 3 and 7 weeks after implantation of the tissue cages. An indwelling over-the-needle intravenous catheter was placed in a cephalic or jugular vein (18 ga Jelco Optiva, Smiths Medical Macquarie Park, NSW) and an injection port was attached and flushed with heparinised saline between each use. Ventilation was provided by passive air movement through doors and windows, and experiments were conducted between April 2019 and August 2019 in Werribee, Victoria, Australia (29).

Table 2 | Dimensions of the subcutaneously implanted tissue cages.

| Length (cm) | Diffusible surface area (cm²) | Volume (mL) | Surface area to volume ratio (cm⁻¹) |
|-------------|------------------------------|-------------|-----------------------------------|
| 3           | 3.0159                       | 5.3014      | 0.5669                            |
| 6           | 3.0159                       | 10.6029     | 0.2844                            |
| 10          | 3.0159                       | 17.6715     | 0.1707                            |
| 14          | 3.0159                       | 24.7400     | 0.1219                            |
| 18          | 3.0159                       | 31.8086     | 0.0948                            |

To inject the catheter or by direct venipuncture. Each set of 11 samples was divided into 1.5 ml microcentrifuge tubes while blood samples were divided between lithium heparin tubes and serum tubes containing indomethacin (C₁₉H₁₈CINO₄, CAS: 53-86-1) to prevent ex vivo formation of eicosanoids (30). All the samples were kept at 4°C until centrifugation and decanting of plasma, serum, and the liquid fraction of tissue cage fluid into 1.5 ml microcentrifuge tubes and then stored at −80°C until analysis.

**Analytical Methods**

**Reagents**

Deionized water was purified using a MilliQ system to 18 MΩ (Millipore North Ryde NSW). Chromatography grade acetonitrile (ACN) and formic acid were sourced from Merck Australia (North Ryde, NSW). Carprofen analytical standard (C₁₃H₁₂CINO₂, CAS: 53716-49-7) and meclofenamic acid (MFA) analytical standard (C₁₄H₁₁Cl₂NO₂, CAS: 644-62-2) were obtained from the Sigma–Aldrich Australia (North Ryde, NSW). High throughput 96 well protein precipitation and phospholipid removal plates (Ostro, Waters Australia Rydalmere, NSW) and 1 ml polystyrene round bottom 96 well plates were obtained from Waters Australia (Rydalmere, NSW).

**Instrumentation**

The Shimadzu LCMS 8050 system included an autosampler, solvent pumps, a column oven chamber, and a triple quadrupole mass spectrometer (Shimadzu Australia, Rydalmere NSW). Analytes were separated during the LC phase using a C18 Poroshell 120 SB 2.1 × 50 mm 2.7 µm Column (Agilent Technologies Mugrave VIC) with a matching guard column.

**Sample Preparation**

Following thawing at room temperature from −80°C, 100 µl of sample was added to the pass-through plate, 390 µl of internal standard working solution (MFA), and 110 µl ACN were added and aspirated several times to mix. The samples were drawn through the plate into the wells of a 96-well round bottom plate by −15 psi negative pressure for 5 min. This plate was capped and placed in the autosampler, which was maintained at 4°C. Calibration standards were included on every plate.

**LCMS Method**

The mobile phases consisted of 0.1% v/v aqueous formic acid (aqueous) and 100% ACN (organic), and needle wash was 100% ACN. A total flow rate of 0.35 ml/min was maintained throughout the analysis with a linear gradient from 5.0% organic to 95% organic over 4.0 min. The conditions were returned to the starting conditions over another 1 min and held at this point for a further 3 min to allow re-equilibration. The injection volume was 5 µl.

The analytical and guard columns were maintained at 50°C. Nebulizing gas flow was 1.5 L/min, heating gas was 12 L/min, and drying gas was 8 L/min of nitrogen. The interface and DL temperatures were 300°C with the heating block held at 400°C. The collision gas was argon, with detection in the third quadrupole in multiple reaction monitoring (MRM) mode. Positive ionization was utilized for the internal standard MFA with the precursor ion set at 297.10 m/z, and product ions at...
Validation of the method was carried out with intra-assay variability <10% CV and inter-assay variability was <16% for concentrations between 50 and 0.125 ng/ml, inter-assay variability for 0.0625, 0.0313, and 0.0156 ng/ml was 20.7, 38.2, and 51.9% CV, respectively. The signal-to-noise ratio at 0.0156 ng/ml was >10, therefore, the limit of detection was deemed to be below this level. A lower limit of quantification is not reported, as recently recommended (31).

RESULTS
Pharmacokinetic Model
A 2-compartment model was found to be a reasonable fit for the plasma concentrations. This model was created in the absence of the tissue cage data and described the plasma pharmacokinetics of carprofen.

A third compartment was added to the plasma model to represent the carprofen concentration in the tissue cages. The rates of influx and efflux (k13) are first order and are driven by the central compartment concentrations without altering the central compartment concentrations. The changes in the central compartment have already been accounted for in the “stand-alone” 2 compartment plasma model. This approach was taken because only a negligible proportion of carprofen drug would enter the tissue cages: this is similar to the approach taken by Sheiner et al. (39). A schematic depiction is displayed in Figure 1.

Table 3 shows the final model parameters generated and the relative standard error of those estimates. Of note is the relatively large change in k21 when carrageenan stimulates inflammation within the sheep, although this has a high degree of uncertainty. While the change in k31 for the right hand side has a low p-value (p = 0.0016), the magnitude of change is very small and the estimate is not precise (RSE 163%). Carrageenan administration was randomized between the left and right hand sides. Inflammation induced by carrageenan in the cage decreases the rate of drug movement both into and out of the cage, as shown by the change in k31 and k13. Cage size has a moderate effect on the rate of drug movement into and out of the cages, with a 25–30% change in the rate constants for each cm change in the cage length (p < 0.001). The half life for drug removal from the tissue cage is estimated to be 1.75 (1.08–3.36) h for a 3 cm cage to 15.4 (6.48–43.0) h for an 18 cm cage. The half-life for drugs entering the cage is estimated to be 6.29 (3.14–11.9) h for a 3 cm cage and 8.95 (3.03–29.6) h for an 18 cm cage. Table 5 displays the maxima, minima and median values of these rate constants.

The time to reach the maximum concentration of carprofen (Tmax) and the maximum concentration reached (Cmax) were extracted from the raw data for each cage in each period (Figures 2, 3). The median and range for these parameters by cage size are shown in Table 4. Tmax for the 3 cm cages had a median of 8 h, while the 14 cm cages had the longest time to maximum concentration with a 48 h median. The median Cmax observed was 43.10–21.57 μg/ml for the 3 to 18 cm cages, respectively.

Statistical and Data Analysis Methods
Carprofen concentration data were modeled in Monolix (Lixoft, Antony France) utilizing a custom-built, population pharmacokinetic model which was fitted to the intravenous plasma data first then expanded to include the tissue cage data. Covariate data for sheep, period, cage side, carrageenan in cage, and carrageenan in sheep were included in the data set as discrete variables. Cage size (cm) was included as a continuous covariate variable.

In total, two thousand iterations were run to achieve convergence, with automatic stopping disabled. Diagnostic plots of the Markov chain Monte Carlo chains were visually assessed for evidence that convergence had been achieved.

ELISA
Duplicate samples were processed using commercial ELISA kits (Cayman Chemical; Prostaglandin E Metabolite ELISA Kit Item No. 514531 and Thromboxane B2 ELISA Kit Item No. 501020). Thromboxane concentrations were measured in serum samples as indicated in the instructions, without sample purification. A prostaglandin metabolite was measured in a selection of tissue cage fluid samples. Because native prostaglandin-EP2 is unstable and rapidly metabolized in vivo, with an extensive first pass effect through the lungs, measurement of the metabolite produces a more reliable measurement of the PGE2 generated (30). The commercial method was modified by exchanging an ACN precipitation in place of ethyl acetate extraction. Samples were evaporated to dryness in a centrifugal evaporator (Environmental Speedvac Savant, USA) on a medium setting (42°C–C) for approximately 1.5 h before being resuspended in the ELISA buffer and derivatised with the supplied carbonate buffer overnight to produce a single stable compound for analysis. The derivatised samples were used directly in the ELISA without acidification.

The intra-assay precision reported in the PGEM kit manual was 8.1–24.7% CV, and the inter-assay precision was 7.2–123% CV. The reported interference from non-PGE molecules was 8.1–23.7% CV, and the inter-assay precision was 7.2–123% CV. The reported interference is 9.9–12.9% CV, with interference from non-thromboxane molecules of 0.8% or less (33). Further validation was not performed because of the cost and material constraints.

279.10 and 244.05 m/z having collision energies (CE) of −13.0 and −25.0 eV, respectively. Negative ionization was employed for carprofen with the precursor ion set at 272.10 m/z, and product ions of 228.20 and 226.10 m/z having CE of 14.0 and 13.0 eV, respectively. The signal-to-noise ratio at 0.0156 ng/ml was 20.7, 43.2, and 51.9% CV, respectively. The signal-to-noise ratio at 0.0625, 0.0313, and 0.0156 ng/ml was 20.7, 38.2, and 51.9% CV, respectively. The signal-to-noise ratio at 0.0156 ng/ml was >10, therefore, the limit of detection was deemed to be below this level. A lower limit of quantification is not reported, as recently recommended (32).

The individual and population predicted values were plotted with the raw data and were visually inspected for goodness-of-fit.

Pharmacodynamic data were analyzed in RStudio (34, 35). Initially, plots were explored for relationships between variables. Non-linear mixed effect models were created using the NLME package (36) and evaluated for goodness-of-fit visually by assessing quantile-quantile (Q-Q) plots, Akaike's Information Criterion (AIC) and coefficient of determination (R²) values [MuMin package (37, 38)].
### TABLE 3 | Coefficients for the model parameters estimated by Monolix.

| Fixed effects                         | Units     | Maximum likelihood estimate | Relative standard error (%) | Confidence Interval 2.5% | Confidence Interval 97.5% | p-value |
|---------------------------------------|-----------|-----------------------------|-----------------------------|--------------------------|---------------------------|---------|
|                                       |           |                             |                             |                          |                           |         |
| **POPPULATION PARAMETERS ESTIMATION** |           |                             |                             |                          |                           |         |
| Central volume                        | L/kg      | 0.0924                      | 6.42                        | 0.0846                   | 0.101                     |         |
| k12                                   | h⁻¹       | 0.121                       | 7.91                        | 0.0904                   | 0.160                     |         |
| Covariate for k12 for Carrageenan not in sheep | h⁻¹ | 0*                           |                             |                          |                           |         |
| Covariate for k12 for Carrageenan in sheep | h⁻¹ | 0.00114                      | 9410                        | −0.324                   | 0.327                     | 0.992   |
| k21                                   | h⁻¹       | 0.200                        | 7.15                        | 0.157                    | 0.253                     |         |
| Covariate for k21 for Carrageenan not in sheep | h⁻¹ | 0*                           |                             |                          |                           |         |
| Covariate for k21 for Carrageenan in sheep | h⁻¹ | 0.336                        | 35.7                        | −0.0536                  | 0.726                     | 0.00507 |
| Clearance                             | L/h.kg    | 0.00235                     | 5.69                        | 0.00192                  | 0.00288                   |         |
| Covariate for k31 for Cage Side (Left) | h⁻¹ | 0*                           |                             |                          |                           |         |
| Covariate for k31 for Cage Side (Right) | h⁻¹  | −0.0701                      | 163                         | −0.0863                  | −0.0539                   | 0.0016  |
| Covariate for k31 per cm Cage size    | h⁻¹       | −0.147                       | 8.14                        | −0.309                   | 0.0153                    | <2.2e−16|
| Covariate for k31 for Carrageenan not in cage | h⁻¹ | 0*                           |                             |                          |                           |         |
| Covariate for k31 for Carrageenan in cage | h⁻¹ | −0.104                       | 130                         | −0.301                   | −0.0995                   | 0.0331  |
| k13                                   | h⁻¹       | 0.124                        | 11.5                        | 0.120                    | 0.134                     |         |
| Covariate for k13 for Cage Side (Left) | h⁻¹ | 0*                           |                             |                          |                           |         |
| Covariate for k13 for Cage Side (Right) | h⁻¹  | −0.024                       | 377                         | −0.122                   | 0.0743                    | 0.0641  |
| Covariate for k13 per cm Cage size    | h⁻¹       | −0.0378                      | 23.8                        | −0.0473                  | −0.0283                   | 7.53e−13|
| Covariate for k13 for Carrageenan not in cage | h⁻¹ | 0*                           |                             |                          |                           |         |
| Covariate for k13 for Carrageenan in cage | h⁻¹ | −0.184                       | 58.3                        | −0.300                   | −0.0883                   | 0.0045  |
| **STANDARD DEVIATION OF THE RANDOM EFFECTS** |           |                             |                             |                          |                           |         |
| Volume                                | L/kg      | 0.188                        | 16.2                        |                          |                           |         |
| Clearance                             | L/h.kg    | 0.448                        | 15.8                        |                          |                           |         |
| k31                                   | h⁻¹       | 0.522                        | 10.8                        |                          |                           |         |
| k13                                   | h⁻¹       | 0.482                        | 7.43                        |                          |                           |         |
| Error Model Parameters                |           |                             |                             |                          |                           |         |
| b1 (Plasma)                           |           | 0.136                        | 3.62                        |                          |                           |         |
| b2 (Tissue Cage)                      |           | 0.468                        | 2.36                        |                          |                           |         |

k12 is the rate constant for drug movement between the central compartment and the peripheral compartment, and k21 is the constant for drug movement from the peripheral compartment to the central compartment. k13 and k31 are the population rate constants for drug movement into and out of the tissue cages, respectively. These constants are modified by the covariates; the presence of carrageenan in the individual animal, with the reference being no carrageenan present for systemic pharmacokinetics. The tissue cage constants are modified by the cage length (size) in a continuous manner, i.e., k31 decreases by −0.147 for each centimetre of cage length. The presence of carrageenan in an individual cage and the side of the neck the cage is on also modifies the rate constants. * The reference values are for the left hand side and there is no carrageenan in the cage.

Tables 6, 7 show the difference in estimated marginal means between the Tmax and Cmax for each cage size.

**Pharmacodynamic Results**
A total of 279 PGEM results were available for analysis and, of these, 74 results were from cages without carrageenan. This imbalance was intentional due to the expectation that non-inflamed cages would have PGEM concentrations below the level of detection, all samples analyzed were above the LOD. All the samples were taken from animals that received carprofen, therefore, the expected PGEM concentration without carprofen is not known and the 50% inhibitory concentration (IC50) cannot be calculated. Overall, the PGEM concentrations increased from time zero to 72 h after carprofen administration with a high degree of variability.
The prostaglandin E2 metabolite results were modeled with a linear mixed effects model. PGEM was log transformed, and cage size was analyzed as a discrete covariate with 5 levels. Individual sheep was included as a random variable. The concentration of carprofen in the cage was not included in the final model as it is collinear with time.

The final model was
\[
\log_{10}(\text{PGEM}) \sim (\beta_0, y_{\text{sheep}}) + \beta_1 \cdot \text{Time} + \beta_2 \cdot \text{Cage Size} + \beta_3 \cdot \text{Time} \cdot \text{Cage Size} + N(0, \sigma)
\]

where the logarithm of PGEM concentration is predicted by time, cage size, and their first order interaction with individual sheep is included as random effects. \(\beta_0\) is the estimated population intercept and \(y_{\text{sheep}}\) is the variance in intercept for the individual subject. \(\sigma\) is the SD of the unexplained variability.

The model explains some of the variation seen with the marginal and conditional \(R^2\) of 0.27 and 0.42, respectively.

The coefficients of the fixed effects and their interactions are displayed in Table 8. Time is a significant predictor of PGEM concentration in this model. Only the 14 cm level of the cage size covariate differed significantly from the 3 cm cage reference. A significant interaction occurs \((p = 0.0029)\) between the 6 cm cage and time in our dataset.

The fitted linear model (Figure 4) shows a rapid increase in PGEM concentrations in the 6 cm cages compared with the other cage sizes, with a predicted concentration of 0.75–1 logarithm (2 vs. 2.75) higher than the other cages. There is significant variability in the results within timepoints, with the 3 cm cage having approximately 1.5 logarithms spread at the 72 h timepoint.
FIGURE 1 | Schematic diagram of the pharmacokinetic model, plasma kinetics are modeled with a 2 compartment model with intravenous (instantaneous) administration into the central compartment (1). Clearance is from the central compartment. k12 and k21 are the rate constants (h\(^{-1}\)) for the drug movement from the central compartment (1) to the peripheral compartment (2) and from the peripheral compartment to the central compartment, respectively. The kinetics of the tissue cage compartment are driven by the central compartment concentration with no change in the central compartment concentration. The drug’s movement into and out of the cage is modeled by the rate constants k13 and k31, respectively. The volume of the tissue cage compartment is specified by the size of the tissue cage (Table 1).

DISCUSSION

The plasma pharmacokinetic parameters are similar to those reported by Welsh et al. (40). The terminal plasma half-life reported by Welsh was 33.7 h for 4 mg/kg and 26.1 h for 0.7 mg/kg, the terminal plasma half-life estimated in this study is 27.2 h. The volume of distribution reported by Welsh was 117.3 and 92.7 ml/kg with our model point estimate for the population being 92.4 ml/kg. A small degree of enterohepatic recirculation was suspected at 4 mg/kg by Welsh based on visual inspection of...
the raw data plots and this was also seen in our data, although it was not included in the PK model.

As previously reported, there is some evidence for carrageenan-induced inflammation slowing the movement of drugs into and out of the tissue cage, thus, prolonging their effect. This effect was highly uncertain, as evidenced by the high relative standard errors. The change in plasma kinetics when carrageenan is present in the individual animal is of note. The rate constant of drug return to the central compartment ($k_{21}$) more than doubled at a population level when carrageenan was present in the sheep, with a large residual uncertainty in this estimate. This change in plasma kinetics in the presence of local inflammation is an important point as previous models have included carrageenan in all sampling periods and compared the pharmacokinetics and pharmacodynamics between inflamed and non-inflamed cages in the same individual (21, 22). The values from the non-inflamed cages in these studies may not accurately reflect PK in the true absence of inflammation, as our findings showed changes in the systemic pharmacokinetics in cases with tissue cage localized inflammation. It is important to note that the cumulative amount of carrageenan introduced in this model is higher than in other published models. All five cages on one side had carrageenan introduced, as opposed to most other studies where carrageenan is introduced into only one cage within the animal per sampling period. The model presented in this article could be criticized as all cages received 1 ml of carrageenan regardless of cage volume. This may lead to unequal degrees of inflammation between the cages as the smaller cages would have a higher concentration of carrageenan. The degree of inflammation induced was not measured.

We detected a clear negative effect on the rate of drug movement into and out of peripherally implanted tissue cages based on the cage length. The resulting concentration time curves are visually different, with key parameters $T_{\text{max}}$ and $C_{\text{max}}$ varying with cage size. $T_{\text{max}}$ occurred later in larger cages, with a 6-fold change between the earliest median $T_{\text{max}}$ and the longest. $C_{\text{max}}$ was lower in the larger cages, with the median $C_{\text{max}}$ in the 18 cm approximately half of the $C_{\text{max}}$ observed in the 3 cm cages. This result is expected, as the Fick’s law predicts that the diffusion is proportional to the concentration gradient and the surface area, i.e., the amount of drug entering or exiting a cage is limited by the diffusible surface area (the total area of the fenestrations). If we assume that the drug is equally dispersed within the cage, then the concentration is a function of the surface area to volume ratio of the cage. Bengtsson et al. (41) describe an equation based on Fick’s law to model tissue cage concentrations. It includes the free concentration of drug in the cage and serum at any given time, the surface area to volume ratio of the cage, and a constant for the permeability of the tissue between the blood vessels and the cage. They also assume that the amount of drug in the tissue is so small compared to the serum that it will not affect the serum concentrations, as is the case for the PK model we describe in this article. In this study, we measured total carprofen with the implicit assumption that the unbound proportion would remain constant. Other studies have examined the protein concentrations of tissue cage fluid during sampling periods and found them to be relatively stable (3) which supports our assumption.

Carprofen is a racemic drug with a single chiral center, pharmacokinetic differences between the two enantiomers have been shown in sheep, horses, and dogs but no evidence of in vivo chiral conversion was found (7, 19, 42). The length of the tissue cage was used as a surrogate measure for SA/V in the pharmacokinetic model as the model failed to converge with
FIGURE 4 | Scatterplot of the logarithmically transformed PGEM concentrations over time, separated by cage size. The blue line represents the concentrations predicted by the mixed effect model, with 95% CIs in gray.

TABLE 8 | Table of coefficients for the fixed effects and interactions for the PGEM mixed effect model.

| Term               | Estimate | p-value | Confidence interval 2.5% | Confidence interval 97.5% |
|--------------------|----------|---------|--------------------------|--------------------------|
| (Intercept)        | 1.55000  | <0.001  | 1.37000                  | 1.72000                  |
| Time               | 0.00731  | <0.001  | 0.00317                  | 0.01150                  |
| CageSizeFac6       | 0.01580  | 0.839   | −0.13700                 | 0.169000                 |
| CageSizeFac10      | 0.06640  | 0.395   | −0.08710                 | 0.220000                 |
| CageSizeFac14      | 0.16900  | 0.0385  | 0.00899                  | 0.329000                 |
| CageSizeFac18      | 0.06440  | 0.423   | −0.09360                 | 0.223000                 |
| Time:CageSizeFac6  | 0.00800  | 0.00295 | 0.00275                  | 0.013200                 |
| Time:CageSizeFac10 | −0.00160 | 0.551   | −0.00687                 | 0.003670                 |
| Time:CageSizeFac14 | −0.00416 | 0.161   | −0.01000                 | 0.001670                 |
| Time:CageSizeFac18 | −0.00501 | 0.0693  | −0.01040                 | 0.000398                 |

The 95% CI and p-value is displayed for each covariate.

SA/V or logarithmically transformed SA/V. However, the work by Van Etta (5, 6) demonstrated that SA/V was the parameter of interest, not the physical size of the tissue cage. Nevertheless, because the diffusible surface area was held constant between cages of different lengths in this study, the length and SA/V are directly related and colinearity occurs, so these effects under this design have poor identifiability. Ideally, the model would include variation in both surface area and volume.

It appeared from our data that carprofen suppressed the inflammation in the early stages of the experiment, as expected. This is in agreement with Cheng (21) with substantial suppression of PGE by carprofen for 32 h. While our model did not explicitly include the concentration of carprofen as an explanatory variable, carprofen concentration was highly correlated with time by design, so it would add no meaningful information.

The primary aim of this study was to evaluate the effect of SA/V and inflammation on carprofen’s PK and PKPD results estimated using a tissue cage model, where we had hypothesized that as the volume of the cage increases relative to the surface area, changes would occur to the estimated pharmacokinetic and pharmacodynamic parameters. Our results showed that different SA/V ratios changed the observed PK and PKPD of carprofen. It is now clear that the SA/V ratio of subcutaneously implanted tissue cages markedly affects derived pharmacokinetic parameters, with the highest median Cmax double the lowest median observation and the longest median Tmax five times greater than the shortest median observation. Our findings give weak evidence that dependent pharmacodynamic parameters may also be influenced by the SA/V ratio.

Understanding the relationship between the SA/V ratio and observed PK may allow results from other studies where the SA/V is known to be extrapolated and compared in a meta-analysis (43). If a target body tissue or compartment, such

Frontiers in Veterinary Science | www.frontiersin.org 9 July 2022 | Volume 9 | Article 905797
as a joint space, had a known SA/V ratio and permeability constant, then, dosage regimens could be accurately simulated to provide target concentrations of drug at the site of interest. It is this linking of tissue cage data to real biological spaces that would allow the true potential of tissue cage models to be utilized.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The animal study was reviewed and approved by Faculty of Ethics State.

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**AUTHOR CONTRIBUTIONS**

RM, TW, and AW contributed to the planning, design, execution, and analysis of the study. All authors contributed to the article and approved the submitted version.

**FUNDING**

RM was supported by the Australian Government Research Training Program Scholarship during this work.

**ACKNOWLEDGMENTS**

The authors would thank Jessica Leung, Ibrahim Marsoomi, Jamie Wearn, Dianne Rees, Avril Lim, Annie Kicinski, Babak Jalilian, Sebastien Bauquier, and Thierry Bels for their assistance with this work.
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