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RESEARCH STUDY

Pharmacokinetics of carprofen in anaesthetised pigs: A preliminary study

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Authors' contributions

MB: sample collection, manuscript writing

MK: experiment planning, results analysis, manuscript writing

ULT: sample collection, manuscript writing

JD: analysis of the samples, manuscript writing

MČ: experiment planning and supervision, manuscript writing

AS: experiment planning and supervision, manuscript writing

Conflict of interest

The authors declare no conflict of interest.

This study has not been published previously and is not under consideration for publication elsewhere. All the authors have read the paper and agreed to the submission and have participated in the study to sufficient extent to be listed as authors.

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Pharmacokinetics of carprofen in anaesthetized pigs: a preliminary study

Abstract

Objective To investigate the pharmacokinetics of carprofen after a single intravenous (IV) dose and multiple oral doses administered to pigs undergoing electroporation of the pancreas.

Study design Prospective experimental study.

Animals A group of eight female pigs weighing 31.74 ± 2.24 kg (mean $\pm$ standard deviation).

Methods Carprofen 4 mg kg^{-1} was given IV after placement of a central venous catheter during general anaesthesia with isoflurane. Blood samples were collected 30 seconds before and 5, 10, 20, 30 and 60 minutes and 2, 4, 6, 8, 12 and 24 hours after carprofen administration. Afterwards, the same dose of carprofen was given orally, daily, for 6 consecutive days and blood collected at 36, 48, 60, 72, 96, 120, 144 and 168 hours after initial carprofen administration. Plasma was analysed using liquid chromatography with mass spectrometry. Standard pharmacokinetic parameters were calculated by compartmental analysis of plasma concentration–time curves. Data are presented as mean $\pm$ standard error.

Results The initial plasma concentration of IV carprofen was estimated at $54.57 \pm 3.92 \text{ } \mu\text{g mL}^{-1}$ and decreased to $8.26 \pm 1.07 \text{ } \mu\text{g mL}^{-1}$ 24 hours later. The plasma elimination curve showed a bi-exponential decline: a rapid distribution phase with a distribution half-life of 0.21 ± 0.03 hours and a slower elimination phase with an elimination half-life of 17.31 ± 3.78 hours. The calculated pharmacokinetic parameters were as follows: the area under the plasma concentration-time curve was $357.3 \pm 16.73 \text{ } \mu\text{g mL}^{-1} \cdot \text{hour}$, volume of distribution was $0.28 \pm 0.07 \text{ L kg}^{-1}$ and plasma clearance rate was $0.19 \pm 0.009 \text{ mL minute}^{-1} \cdot \text{kg}$. The plasma concentration of carprofen, given orally from day 2 to 7, varied from 9.03 ± 1.87 to $11.49 \pm 2.15 \text{ } \mu\text{g mL}^{-1}$.

25 **Conclusions and clinical relevance** Carprofen can be regarded as a long-acting nonsteroidal
26 anti-inflammatory drug in pigs.

27

28 **Keywords** carprofen, pharmacokinetics, pigs.

Introduction

Carprofen is a member of the arylpropionic acid class of nonsteroidal anti-inflammatory drugs (NSAID) (Ricketts et al. 1998). It is a mixture of S(+) and R(-) enantiomers, with the S(+) enantiomer being more biologically active than the R(-) enantiomer (Lees et al. 2004). The pharmacokinetics and pharmacodynamics of enantiomers are different and can vary significantly between species (Iwakawa et al. 1991; McKellar et al. 1994; Soraci et al. 1995).

In dogs, carprofen has a bioavailability of 95% after oral administration of a 4 mg kg⁻¹, dose with a peak plasma concentration ($C_{\max}$) of $35.30 \pm 2.70 \mu\text{g mL}^{-1}$ occurring at a $T_{\max}$ (time to reach maximum concentration) of 1.25 ± 0.25 hours (McKellar et al. 1990). After intravenous (IV) administration of 0.7 mg kg⁻¹ of carprofen, the volume of distribution (V_d) at a steady state was reported to be $0.14 \pm 0.02 \text{ L kg}^{-1}$ (McKellar et al. 1990) or $0.18 \pm 0.06 \text{ L kg}^{-1}$ after approximately a 7.5 mg kg⁻¹ dose (Schmitt & Guentert 1990). The plasma elimination half-life ($t_{1/2\beta}$) varied from 8 ± 1.18 hours (McKellar et al. 1990) to 11.7 ± 3.05 hours (Schmitt & Guentert 1990). The area under the plasma concentration-time curve (AUC) at a 7.5 mg kg⁻¹ IV dose was $411.8 \pm 26.52 \mu\text{g mL}^{-1} \cdot \text{hour}$ (Schmitt & Guentert 1990) and $268.94 \mu\text{g mL}^{-1} \cdot \text{hour}$ at a 4 mg kg⁻¹ IV dose (Messenger et al. 2015). The biotransformation of carprofen in dogs takes place in the liver via glucuronidation and is followed by rapid excretion of metabolites in the faeces (70%) and urine (8–15%) (Rubio et al. 1980).

In cats, the pharmacokinetics following IV administration of 4 mg kg⁻¹ carprofen indicate a V_d of $0.14 \pm 0.05 \text{ L kg}^{-1}$, an AUC of $637 \pm 237 \mu\text{g mL}^{-1} \cdot \text{hour}$ and a $t_{1/2\beta}$ of 20.0 ± 16.6 hours (Parton et al. 2000), which is longer than that reported in dogs. In lactating cows, the V_d of carprofen administered at 0.7 mg kg⁻¹ IV was $0.091 \pm 0.003 \text{ L kg}^{-1}$ with a long $t_{1/2\beta}$ of 30.7 ± 2.3

hours (Lohuis et al. 1991). Similar pharmacokinetic parameters were observed in sheep given carprofen IV at 0.7 mg kg^{-1} , with a V_d of $0.093 \pm 0.006 \text{ L kg}^{-1}$ and a $t_{1/2\beta}$ of 26.1 ± 1.14 hours. When sheep were given a higher dose of 4 mg kg^{-1} IV, the V_d was $0.117 \pm 0.003 \text{ L kg}^{-1}$ and $t_{1/2\beta}$ was 33.7 ± 3.14 hours (Welsh et al. 1992). Compared to cows, horses given carprofen at 0.7 mg kg^{-1} IV have a larger V_d of $0.22 \pm 0.01 \text{ L kg}^{-1}$ and shorter $t_{1/2\beta}$ of 18.1 ± 1.3 hours (Lees et al. 1994). Similar results were obtained with a 3.5 mg kg^{-1} IV dose; the $t_{1/2\beta}$ was 21.9 ± 2.3 hours (McKellar et al. 1991). In horses, the AUC was $569 \pm 148 \mu\text{g mL}^{-1} \cdot \text{hour}$ after 3.5 mg kg^{-1} IV (McKellar et al. 1991) and $57.9 \pm 5.2 \mu\text{g mL}^{-1} \cdot \text{hour}$ after a 0.7 mg/kg IV dose (Lees et al. 1994).

The pig is a common research model for translational medicine due to its anatomical and physiological resemblance to humans and was therefore selected for our research on the effects of electroporation (Djokic et al. 2018; Campana et al. 2019) on the pancreas (Dežman et al. 2020). Thus far, the pharmacokinetics of carprofen in pigs have not been published, which is rather unusual given the number of experimental pigs used worldwide. Data describing the pharmacokinetics of carprofen are important not only from an animal welfare perspective but also owing to the possibility of drug-drug interactions with research results. Therefore, we carried out a prospective experimental study to investigate the pharmacokinetics of carprofen in pigs. This study was not designed solely as a classical pharmacological experiment, but in compliance with the 3Rs principle (Replacement, Reduction and Refinement) in addition to the electroporation procedure.

Materials and methods

Animals

This study was approved by the National Ethics Committee and National Veterinary Administration (approval number U34401-1/2017/4, approval date 17.03.2017) and conducted in accordance with ARRIVE (Animal Research: Reporting of *in Vivo* Experiments) guidelines (Kilkenny et al. 2010). The pigs were reared according to the European Council directive for minimum standards for the protection of pigs (2008/120/EC), and all procedures complied with the relevant Slovenian and European legislation.

A total of eight female Landrace and Large White hybrid pigs (*Sus scrofa domestica*), aged 10 weeks and weighing 31.74 ± 2.24 kg were studied. Electrochemotherapy was performed using the same electrodes and technological approach as described in our previous study on electrochemotherapy of the porcine liver (Brloznik et al. 2019; Zmuc et al. 2019). The pigs were procured from an authorized swine breeder 3 to 17 days before the procedure and housed in separate indoor straw-bedded pens sized 1.25 x 4.40 m enabling visual and audible contact. They were exposed to a natural light/dark cycle and kept at a room temperature of 20 to 23 °C and relative air humidity of 50 to 65%. The pigs were fed twice daily with commercial feed for growers and had unlimited access to tap water from nipple waterers. They were continuously monitored by researchers for the first 24 hours after anaesthesia. Afterwards, they were under continuous video surveillance and clinically examined three times daily until the last day of the experiment.

Anaesthesia and interventions

Pigs were fastened for 12 hours before the induction of anaesthesia and had unlimited access to tap water. They were premedicated with ketamine (Bioketan; Vetoquinol, France) 10 mg kg^{-1} , midazolam (Midazolam Torrex; Torrex Chiesi Pharma GmbH, Austria) 0.5 mg kg^{-1} and medetomidine (Domitor; Orion Corporation, Finland) 0.02 mg kg^{-1} mixed together and given

intramuscularly (IM) approximately 5 cm behind and at the level of the ear base. Anaesthesia was induced with isoflurane (Isoflurin; Vetpharma Animal Health S.L., Spain) in oxygen using a facemask. The pigs were endotracheally intubated, and anaesthesia was maintained with isoflurane (0.8–2.5% vaporiser setting) in oxygen delivered through a circle breathing system (22 mm Flextube breathing system; Intersurgical Ltd, UK). The pigs breathed spontaneously during anaesthesia. Non-invasive blood pressure (Doppler flow monitor Model 811; Parks Medical Electronics; OR, USA), oxygen saturation (SpO_2), end-tidal carbon dioxide ($\text{FE}'\text{CO}_2$) concentration, oesophageal temperature and electrocardiography were monitored (BLT M9000 VET; Guangdong Biolight Meditech, Guangdong, China). A 22-gauge catheter (BD Venflon; Becton Dickinson Infusion Therapy AB, NJ, USA) was placed into an auricular vein, and saline solution (NaCl 0.9%; B Braun, Germany) was infused at $10 \text{ mL kg}^{-1} \text{ hour}^{-1}$ during anaesthesia.

Prior to surgical intervention, a single dose of enrofloxacin (Enroxil 10%, Krka d.d., Slovenia) 7.5 mg kg^{-1} was administered IM. The pigs were placed into dorsal recumbency, and a 16-gauge permanent central venous catheter (Intramedicut 2; Sherwood Medical, Ireland), which served for blood sampling from the external jugular vein, was surgically implanted. The subcutis of the right neck area was channelled dorsally and the catheter looped in order to provide additional length during movements of the cervical spine. The rest of the catheter was led externally to the back of the neck where it was fixed with monofilament nylon (Monosof 3-0; Covidien, Ireland) and taped with self-adhesive plaster. The catheter was flushed with saline solution and locked with trisodium citrate (Citra-Lock 30%; Dirinco B.V., the Netherlands) diluted with saline solution to a 3% solution. At each sampling, an appropriate quantity of catheter lock solution was discarded, the blood sample was collected, and the catheter was flushed with saline solution and locked again.

After placement of the central venous catheter, carprofen (Rycarfa 50 mg mL⁻¹ solution for injection; Krka d.d., Slovenia) 4 mg kg⁻¹ was administered IV through the catheter placed in the auricular vein, and a fentanyl transdermal patch (Duragesic; Janssen Pharmaceutica, Belgium) 75 µg hour⁻¹ was applied to the lateral aspect of the thorax and left *in situ* until the end of the experiment on day 8 or until the patch fell off. Carprofen (Rycarfa Flavour 100 mg kg⁻¹ and 20 mg kg⁻¹ tablets with meat aroma; Krka d.d., Slovenia) 4 mg kg⁻¹ was given orally once daily for 6 days from day 2 to day 7. The tablets were crushed, mixed with homemade jam and spoon fed to the pigs.

Computed tomography (CT)-guided electroporation of the pancreas was performed in four pigs, and surgical exposure of the pancreas for electroporation through a median laparotomy was performed in the remaining four pigs. In the latter group, the edges of the incision were infiltrated before the end of the surgery with L-bupivacaine (Chirocaine; Abb Vie, Italy), at a dose not exceeding 2 mg kg⁻¹. Additional post-operative analgesia was provided to all pigs using morphine (Morphini Chl.; Alkaloid, FYROM) 0.2 mg kg⁻¹ IM, the dose to be repeated every 4 hours if required. Pigs had free access to water and food postoperatively. On day 8, they were re-anaesthetized using the same protocol as previously administered, and a CT scan of the abdomen was performed. Afterwards, they were euthanized with T61 euthanasia solution (Intervet; the Netherlands) 3 mL10 kg⁻¹ IV. After confirmation of cardiac arrest with auscultation of the heart, a median laparotomy was performed, and the pancreas explanted for the purpose of the primary study.

Blood sampling

For each sequence, blood samples were collected into heparinized tubes (Vacurette; Greiner Bio-One GmbH, Austria), the first 30 seconds before carprofen administration (time point 0) and at 5,

10, 20, 30 and 60 minutes and 2, 4, 6, 8, 12 and 24 hours thereafter. After the first 24 hours, carprofen was given orally once per day, after morning blood collection. On day 2 and 3, blood was collected every 12 hours (time points 36, 48, 60 and 72 hours) and on day 4 to 7 only in the morning (time points 96, 120, 144 and 168 hours). Blood samples were centrifuged within 10 minutes after collection at $1500 \times g$ for 15 minutes at 20 °C. Plasma samples were separated into aliquots and stored at -20 °C until analysis.

Laboratory analysis

Carprofen concentrations were determined using liquid chromatography (Waters Alliance 2695; Waters, MA, USA) with mass spectrometry (Quattro Micro API; Micromass, Waters, MA, USA). Acetonitrile 0.4 mL was added to a centrifuge tube containing 0.20 mL of plasma and vortexed. The samples were centrifuged at $3400 \times g$ for 10 minutes. Supernatants were transferred to a new centrifuge tube. The solvent was evaporated to dryness. The residues were dissolved in 0.1 mL acetonitrile/methanol (V/V=1/1) and vortexed. Those solutions were transferred to the injection vial. An extracted matrix matched calibration curve was processed parallel with samples using a fortified sample of blank plasma.

The mobile phase consists of A: 0.1% formic acid and B: 0.1% formic acid in acetonitrile at a flow rate of 0.25 mL minute⁻¹. The mobile phase started at 50% A with a linear gradient to 20% A at 10 minutes, followed by a linear gradient to 90% A from 10.1 to 11 minutes with a total run time of 15 minutes. Separation was achieved with a 2.0 x 150.0 mm, 3 µm column (Gemini C18; Phenomenex Corp., CA, USA) maintained at 40 °C.

Quantification and qualification were performed using two ion transitions with mass/charge ratios (m/z) 274 > 193 and 274 > 228.

The analytical range of the carprofen assay was from 5 to 100 $\mu\text{g mL}^{-1}$. The relative measurement uncertainty (at confidence of 95%) at the concentration level of 20 $\mu\text{g mL}^{-1}$ was 3%.

Pharmacokinetic and data analysis

Individual and pooled carprofen plasma concentration data derived from eight independent measurements were analysed using GraphPad Prism (GraphPad Prism software, CA, USA). The following pharmacokinetic parameters were calculated:

C_0 (concentration of carprofen in the blood at time 0) value was extrapolated from the semilogarithm of concentration *versus* time plot for each individual and pooled data set. The area under the plasma concentration-time curve (AUC) using the trapezoid method from time 0 to 24 hours ($\text{AUC}_{0 \rightarrow t}$) and plasma clearance (CL) were calculated.

$$\text{CL} = \text{dose (mg)} / \text{AUC}_{0 \rightarrow t} (\mu\text{g mL}^{-1} \cdot \text{hour}) \quad (1)$$

The elimination rate constant (k_{el}) was determined from the slope of the concentration *versus* time curve, and plasma elimination half-time was calculated using

$$t_{1/2} = 0.693 / k_{el} \quad (2)$$

and the volume of distribution (V_d) was calculated using

$$V_d = [t_{1/2} (\text{minute}) \times \text{CL} (\text{mL minute}^{-1})] / 0.693 \quad (3)$$

The area under the curve (AUC) was calculated as $AUC_{(0-t)}$ from time 0 to the last quantifiable data point using a linear trapezoidal method.

Data are presented as mean $\pm$ standard error of the mean (SEM), except for the weight of the pigs and duration of anaesthesia, which are presented as mean $\pm$ standard deviation of the mean (SD). The difference in duration of anaesthesia between the CT-guided and the open abdomen model (Student's t-test) was considered significant at a value of $p < 0.05$.

Results

A total of eight pigs were enrolled and completed the study. The physiological variables measured during anaesthesia were within normal limits in all pigs (heart rate 80–110 beats minute^{-1} , respiratory rate 20–40 breaths minute^{-1} , systolic arterial blood pressure 80–140 mmHg, SpO_2 above 95%, $\text{PE}'\text{CO}_2$ 35–45 mmHg) for almost the entire duration of anaesthesia. The lowest body temperature recorded during anaesthesia was 35.0 °C. A hydroxyethyl starch 130/0.4 (Voluven 6%; Fresenius Kabi, Germany) was administered at 5 mL kg^{-1} hour^{-1} over 30 minutes in one pig until systolic blood pressure increased to a value > 80 mmHg. Assisted ventilation was periodically employed in three pigs when $\text{PE}'\text{CO}_2$ exceeded 50 mmHg (6.67 kPa). No difference ($p = 0.34$) in the duration of anaesthesia was observed between CT-guided (202.5 ± 37.75 minutes) and open abdomen model (228.75 ± 33.26 minutes) of electroporation. All pigs recovered uneventfully from anaesthesia. Only one dose of morphine was given postoperatively to all pigs because they showed no signs of pain or distress, behaved normally, and had normal food and water consumption by the end of that day.

All pigs were initially given a single IV injection of carprofen and were included in the whole pharmacokinetics analysis set. There were no major protocol deviations. After IV administration of 4 mg kg^{-1} of carprofen, the initial plasma concentration (C_0) of injected carprofen was calculated to $54.57 \pm 3.92 \text{ } \mu\text{g mL}^{-1}$. The plasma concentration of carprofen decreased to $8.26 \pm 1.07 \text{ } \mu\text{g mL}^{-1}$ 24 hours later (Fig. 1). The elimination curve showed a bi-exponential decline; the steep, with the rapidly declining phase immediately after the IV administration of carprofen representing the distribution of carprofen from the plasma to well perfused organs, whereas the shallower straight line represents the elimination rate of the compound from the systemic blood flow. The distribution half-life ($t_{1/2\alpha}$) was 0.21 ± 0.03 hours and $t_{1/2\beta}$ was 17.31 ± 3.78 hours. The calculated pharmacokinetic parameters were as follows: AUC was $357.3 \pm 16.73 \text{ } \mu\text{g mL}^{-1} \cdot \text{hour}$, V_d was $0.28 \pm 0.07 \text{ L kg}^{-1}$ and plasma CL was $0.19 \pm 0.009 \text{ mL minute}^{-1} \cdot \text{kg}$.

The plasma concentration of carprofen, administered orally 4 mg kg^{-1} once daily from day 2 to 7, varied from 9.03 ± 1.87 to $11.49 \pm 2.15 \text{ } \mu\text{g mL}^{-1}$ (Fig. 2).

Discussion

This prospective experimental study reports the pharmacokinetics of carprofen after a single 4 mg kg^{-1} IV dose followed by oral doses given once a day for 6 consecutive days in eight pigs. Carprofen plasma concentration after IV dose decreased bi-exponentially. The high SEM of pooled carprofen concentration data determined 30 minutes after the injection (Fig. 1) reflects the overlap of the distribution with the elimination phases of carprofen, while at 60 minutes elimination predominated and the SEM stabilized. A rapid distribution phase with a $t_{1/2\alpha}$ of 0.21 ± 0.03 hours and slower elimination phase with a $t_{1/2\beta}$ of 17.31 ± 3.78 hours was similar to that

described in horses, where $t_{1/2\alpha}$ was 0.625 ± 0.05 hours and $t_{1/2\beta}$ was 18.1 ± 1.3 hours (Lees et al. 1994). The IV dosing regimens were different, at 4 mg kg^{-1} in pigs and 0.7 mg kg^{-1} in horses, but the elimination kinetics were similar. McKellar et al. (1991) reported an even longer $t_{1/2\beta}$ of 21.9 ± 2.3 hours following the IV administration of 3.5 mg kg^{-1} carprofen to horses; the latter dose being closer to that we used in this study. A similar $t_{1/2\beta}$ (20.0 ± 16.6 hours) was also reported for cats, with the dose and route of administration being the same as that reported in our study (Parton et al. 2000). In other animal species either much longer or much shorter $t_{1/2\beta}$ s have been reported. A longer $t_{1/2\beta}$ of 30.7 ± 2.3 hours was reported in healthy cows given 0.7 mg kg^{-1} IV carprofen, and $t_{1/2\beta}$ increased to 43.0 ± 2.3 hours in cows with mastitis (Lohuis et al. 1991). The slower elimination rate in diseased cows was explained by peripheral vasoconstriction and the redistribution of blood flow during the onset of the febrile state (Blatteis et al. 1988). In sheep, the $t_{1/2\beta}$ was 33.7 ± 3.14 hours (4 mg kg^{-1} IV) and 26.1 ± 1.14 hours (0.7 mg kg^{-1} IV) (Welsh et al. 1992).

Typically, a faster elimination rate was reported in dogs, from $t_{1/2\beta}$ of 8.0 ± 1.18 hours (0.7 mg kg^{-1} IV) (McKellar et al. 1990) to 11.7 ± 3.05 hours (7.5 mg kg^{-1} IV) (Schmitt & Guentert 1990). Welsh et al. (1992) suggested that differences in the degree of the enterohepatic circulation of carprofen between animal species might account for the wide margin of $t_{1/2\beta}$ values seen.

Compared to other animal species, the V_d of carprofen in pigs in our study ($0.28 \pm 0.07 \text{ L kg}^{-1}$) was among the highest values reported. Nevertheless, the calculated V_d indicates that carprofen is distributed in plasma and extracellular fluid. Ruminants have the smallest V_d , cows (Lohuis et al. 1991) and sheep (Welsh et al. 1992) given carprofen had a V_d of 0.09 L kg^{-1} . At the dose used in our pigs (4 mg kg^{-1}), the V_d in sheep was 0.117 L kg^{-1} (Welsh et al. 1992). The V_d in cats given 4 mg kg^{-1} was 0.14 L kg^{-1} (Parton et al. 2000) and in dogs given 0.7 or 7.5 mg kg^{-1} , it

ranged between 0.14 and 0.18 L kg⁻¹ (McKellar et al. 1990; Schmitt & Guentert 1990). Similar to pigs in our study, horses (0.7 to 3.5 mg kg⁻¹) have a large V_d of 0.22 to 0.23 L kg⁻¹ (Lees et al. 1994; McKellar et al. 1991). The V_d in different animal species, including the pig, is small mainly because of negligible distribution of carprofen from the central into the peripheral compartment. Carprofen also shows a high degree (99%) of protein binding in plasma, a feature typical of lipophilic drugs, which means they are retained within the central compartment (DrugBank.ca).

The calculated AUC in our study was 357.3 ± 16.73 µg mL⁻¹ · hour. In horses given two different doses of carprofen, 3.5 mg kg⁻¹ and 0.7 mg kg⁻¹ IV, AUC values were 569 ± 148 µg mL⁻¹ · hour (McKellar et al. 1991) and 57.9 ± 5.2 µg mL⁻¹ · hour (Lees et al. 1994), respectively. These results indicate that plasma levels are directly proportional to the dose rate. In cats given the same dose of carprofen as that used in our study (4 mg kg⁻¹ IV) (Parton et al. 2000), the AUC (637 ± 237 µg mL⁻¹ · hour) was higher than the values we report here. Conversely, the AUC value (268.94 µg mL⁻¹ · hour) reported for dogs at a 4 mg kg⁻¹ IV dose (Messenger et al. 2015) was much lower than that in cats and in pigs. In cows, at a 0.7 mg kg⁻¹ IV dose, there was a difference in AUC between healthy cows (294.3 ± 12.9 µg mL⁻¹ · hour) and cows with *E. coli* endotoxin induced mastitis (507.4 ± 52.1 µg mL⁻¹ · hour) (Lohuis et al. 1991). Plasma levels of drugs may be altered during disease. A slower systemic clearance rate of carprofen during the febrile condition (Blatteis et al. 1988) should be considered when prescribing the drug to animals, which are pyrexia.

The plasma clearance (CL_p) in pigs in our study was 0.19 ± 0.009 mL minute⁻¹ · kg. It was higher than that reported in sheep (0.042 ± 0.002 mL minute⁻¹ · kg; Welsh et al. 1992), cows (0.04 ± 0.003 mL minute⁻¹ · kg; Lohuis et al. 1991) and cats (0.1 ± 0.03 mL minute⁻¹ · kg; Taylor et al. 1996) but lower than that reported in dogs (0.28 ± 0.05 mL minute⁻¹ · kg; Schmitt & Guentert,

1990). The CLp value most similar to that determined in our study in pigs was in horses (0.2 mL minute⁻¹ · kg; Lees et al 1994). The low plasma clearance indicates low extraction of carprofen by the liver, and some enterohepatic circulation of carprofen has also been described (Rubio et al. 1980).

The plasma concentration of orally administered carprofen between days 2 to 7 in our study varied from 9.03 ± 1.87 to 11.49 ± 2.15 µg mL⁻¹. In horses, a much lower carprofen plasma concentration of ≥ 1.5 µg mL⁻¹ was sufficient to produce an analgesic effect (Schatzmann et al. 1990). Plasma concentrations of 8.43 µg mL⁻¹ at 2 hours postoperatively that decreased to 6.29 µg mL⁻¹ at 5 hours postoperatively were reported to provide an analgesic effect in ovariohysterectomized dogs given carprofen 4 mg kg⁻¹ subcutaneously (Lascelles et al. 1998). Plasma levels of carprofen administered pre- or postoperatively were not related to the degree of analgesia. Even though plasma concentrations of carprofen in the early postoperative period were approximately two times higher when given postoperatively, the preoperative administration of carprofen had a greater analgesic effect. Lascelles et al. (1998) suggested that, in addition to the plasma concentration of carprofen, the timing is also crucial for control of postoperative pain. For this reason, carprofen was administered to pigs in our study before the electroporation procedure, *i.e.*, immediately after the placement of the central venous catheter. The presence of pain in pigs was assessed only subjectively, and no validated pain scale was used. Other analgesic drugs such as opioids were used as part of the anaesthetic and analgesic protocol, and therefore it was not possible to determine the effect of carprofen alone on the severity of postoperative pain in our pigs. However, all pigs recovered uneventfully after the surgery. They showed no signs of pain or distress, behaved normally, and had normal food and water consumption until the end of the experiment.

Our study has several limitations. First, the study was not designed solely as a pharmacokinetics study, but as an addition to the electroporation of the pancreas and the number of pigs enrolled was pre-determined by the primary study. Due to the requirements of the primary study, the pigs were given other drugs, although we specifically selected drugs which were not enzyme inhibitors. The only exception being enrofloxacin, which is a CYP 1A1 inhibitor but does not interfere with carprofen biotransformation. Fentanyl may decrease the excretion rate of carprofen which could result in a higher serum level of carprofen (DrugBank.ca). The expected fentanyl plasma concentrations (ng mL^{-1}) are lower than those of carprofen ($\mu\text{g mL}^{-1}$), so according to the Law of Mass Action the interaction is less likely to occur. Wide variations in plasma concentration of transdermal fentanyl have been reported in individual pigs (Harvey-Clark et al. 2000; Wilkinson et al. 2001; Lujan et al. 2017), and thus it is hard to draw any conclusion about the possible pharmacokinetic interaction between fentanyl and carprofen. Second, we cannot be certain that the pharmacokinetic data would be the same if carprofen had been administered to the pigs in the absence of anaesthetic drugs. Drugs such as medetomidine and isoflurane may influence the distribution of carprofen due to their effects on vascular resistance and cardiac output (Lawrence et al. 1996, Lundeen et al. 1983). Third, the pigs were given the first oral dose of carprofen 24 hours after IV administration, and we could not obtain the perfect IV elimination curve expanding over four half-lives periods of carprofen. Finally, we investigated the pharmacokinetics of a racemic mixture because we did not have an appropriate analytical method for the determination of carprofen enantiomers at our disposal.

In conclusion, carprofen can be regarded as a long-acting NSAID in pigs from a pharmacokinetic point of view. Further studies on the analgesic effects of carprofen in pigs using validated pain scales are warranted.

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Figure legends

Figure 1 Concentration-*versus*-time profile of a single 4 mg kg⁻¹ intravenous dose of carprofen administered to eight Landrace and Large White hybrid pigs. Pigs were premedicated with ketamine, midazolam and medetomidine given intramuscularly and anaesthesia induced and maintained with isoflurane in oxygen. Data are shown as mean and error bars represent standard error of the mean.

Figure 2 Concentration-*versus*-time profile of a single 4 mg kg⁻¹ intravenous (IV) dose of carprofen followed by a 4 mg kg⁻¹ oral dose given once daily for 6 days to eight Landrace and Large White hybrid pigs. The first oral dose was administered 24 hours after the IV dose. Pigs were premedicated with ketamine, midazolam and medetomidine given intramuscularly and anaesthesia induced and maintained with isoflurane in oxygen. Data are shown as mean and error bars represent standard error of the mean.