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Comparing Pharmacokinetics of Meloxicam When Administered With a Needle-Free Injection Device Versus Needle-And-Syringe in Piglets

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ABSTRACT

Meloxicam is a common analgesic for castration in pigs. While needle-free technology is effective for swine vaccination, its implementation for administering meloxicam has not been fully explored. The objective of this study was to compare the pharmacokinetics (PK) of meloxicam administered via a commercial needle-free injection device (NFID) and intramuscularly via needle-and-syringe (NS) in nursing piglets. Twenty-six nursing piglets were randomly assigned to one of two treatment groups receiving the same approved label dosage of 0.4 mg/kg of meloxicam. Plasma meloxicam concentrations were measured using liquid chromatography–tandem mass spectrometry, and PK profiles were measured using non-compartmental analysis. The results indicated C_{max}, AUC_{0-last}, $AUC_{0-\infty}$, $AUMC_{0-last}$, $AUMC_{0-\infty}$, and MRT in the NFID group were all significantly lower compared with those of the NS group ($p < 0.05$). No differences in T_{max}, $T_{1/2}$, and λ_z were found between the two groups ($p > 0.05$). The study concluded that further research is needed to determine the optimal NFID setting and the clinical efficacy when using NFID for injecting meloxicam in piglets.

1 | Introduction

During the first week of life, piglets in commercial swine production undergo several common management procedures that are collectively termed 'piglet processing'. These management procedures promote health and productivity and include teeth clipping, supplemental iron administration, and castration. Castration is a routine husbandry practice in piglet processing aimed at preventing boar-related odor in the meat. However, without anesthetics or analgesics, piglet castration causes both acute and chronic pain, resulting in physiological and behavioral changes (Hay et al. 2003; Dzikamunhenga et al. 2014). According to Canadian Code of Practice for the Care and Handling of Pigs (2014), castration and tail docking, regardless of age, must be performed with analgesics for pain control after processing. In Canada, while meloxicam, flunixin meglumine, and ketoprofen are approved nonsteroidal anti-inflammatory drugs (NSAIDs) for pigs, only meloxicam is currently approved to control pain from castration.

To administer drugs, needle-and-syringe (NS) injection is commonly used in the swine industry. Recent studies showed the puncture force gradually increases after each injection due to the loss of needle sharpness, which can lead to more painful

experiences for the pig, particularly during the last use (Owen et al. 2022). Reused needles have also been shown to transmit infectious diseases among pens and rooms after being used on infected pigs (Madapong et al. 2021; Salman et al. 2023). Needle-free injection devices (NFIDs) have become broadly applied both in human (Mitragotri 2006; Shergold et al. 2006) and in veterinary medicine (van den Drunen Littel Harke et al. 2006; Chase et al. 2008; Rey et al. 2013) for administering vaccines, drugs, proteins, growth hormones, and DNA.

The advantages of using NFIDs are not limited to improving animal welfare, reducing the risk of disease transmission, and minimizing carcass lesions; they also reduce operating costs for long-term use and risks of self-stick injuries to the workers (Ko et al. 2018; Temple et al. 2020; Imeah et al. 2020; Madapong et al. 2021; Salman et al. 2023). Moreover, the NFIDs also potentially contribute to reducing the biohazard waste of used needles disposal.

In swine, previous studies have shown that NFIDs are effective for vaccine administration, and several commercial NFIDs are currently available (Chase et al. 2008; Madapong et al. 2021; Cho et al. 2022; Renson et al. 2024). However, there are few studies on the use of NFIDs for drugs in swine. To the authors' knowledge, no studies have investigated the application of NFIDs for meloxicam administration in nursing piglets; therefore, the efficacy of meloxicam delivered via NFIDs remains unclear. Establishing a pharmacokinetic (PK) profile with drugs of interest is the first step in providing empirical evidence to support the use of NFIDs in swine husbandry practices. The objective of this study was to compare the pharmacokinetics and relative bioavailability of meloxicam administered via a commercial NFID and intramuscularly (IM) via NS in nursing piglets using the same approved label dose.

2 | Material and Methods

This project followed the guidelines of the Canadian Council on Animal Care. The Animal Utilization Protocol (AUP #5021) was reviewed and approved by the University of Guelph Animal Care Committee.

2.1 | Animals and Husbandry

Twenty-eight piglets (Landrace × Duroc × Yorkshire; 13 males, 15 females) were recruited by purposive selection from a commercial operation. Two piglets per litter (1 male and 1 female) that were clinically healthy and non-processed were selected. Gilt litters were excluded. The study was conducted in three batches from November 24, 2023 to January 10, 2024 ($N_{\text{batch 1}} = 6$, $N_{\text{batch 2}} = 10$, $N_{\text{batch 3}} = 12$).

The study timeline is outlined in Figure 1. Five days before the trial began, the piglets arrived at the research facility (Department of Animal Biosciences, University of Guelph). The mean weight and age at arrival were 3.1 kg (range: 2.6–3.5 kg) and 6.2 days (range: 5–7 days), respectively. During the acclimatization period following arrival at the research facility, two pigs from the same litter were housed together in the same pen, with two heat lamps per pen in a temperature-controlled room. After jugular catheterization, piglets were housed individually in pens side by side with slotted partitions, allowing nose-to-nose contact. To protect the jugular catheters from accidental removal, piglets were fitted with a body wrap (Vetrap, 3 M Company, MN, USA) and a second body covering made from cast sleeve material (Stockinette Tubular Protouch; BSN Medical, NC, USA).

Health monitoring and feeding were performed four times daily by members of the research team. The general health appearance, body weight, body temperature, catheter site reaction, and feed intake were recorded. The piglets were trained to feed in a size-appropriate trough with milk replacer (Supp-Le-Milk; Soppe Systems, IA, USA). All pigs were administered ceftiofur hydrochloride (3 mg/kg; Excenel RTU EZ; Zoetis Canada Inc., QC, Canada) into the hamstring muscles once daily for 4 days (day 1–4, Figure 1) to prevent diarrhea associated with transport and adjustment to a new research housing environment. The authors acknowledged that using ceftiofur in this manner is prohibited in the US and other countries, while it is acceptable in Canada and some other countries. This project was a research PK trial conducted under controlled conditions with piglets not entering the human food chain.

2.2 | Jugular Vein Catheterization

After acclimatization, surgical placement of catheters in the right jugular veins of piglets was performed under general anesthesia to support repeated blood sampling. The catheter was a made with Micro-Renathane Implantation Tubing (MRE) 0.80″ (Braintree Scientific Inc., Montreal, QC, Canada) with a custom configuration for piglet specifically for this trial. The catheter was gas sterilized before use. Piglets were administered IM a

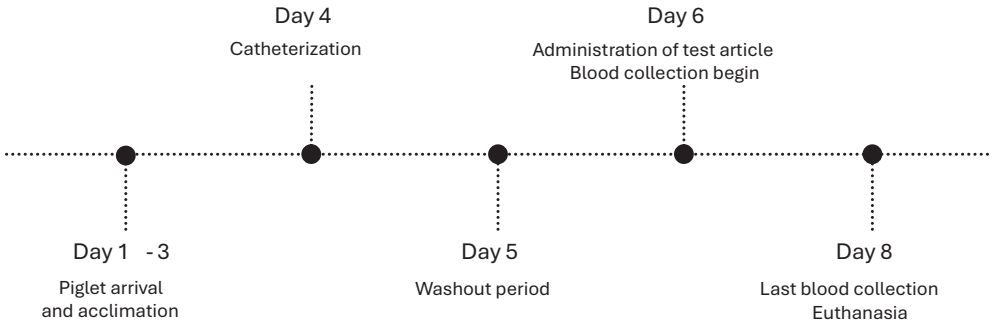

FIGURE 1 | Study timeline.

sedative (0.2 mL/kg; compounded by Ontario Veterinary College Pharmacy, University of Guelph, Guelph, ON, Canada) of ketamine (50 mg/mL; Narketan Vetoquinol N.-A. Inc., QC, Canada), xylazine (10 mg/mL; XylaMed Bimeda-MTC Animal Health Inc., ON, Canada), and butorphanol (1 mg/mL; Torphadine Dechra Regulatory BV, AD, Netherlands), and then maintained under general anesthesia with 2.5% isoflurane (AErrane Baxter Corporation, ON, Canada) via face mask. Lidocaine HCl 2% (Hikma Pharmaceuticals USA Inc., OH, USA) was administered subcutaneously (SQ) at the catheter exit site before the surgery, and buprenorphine (0.02 mg/kg; Vetergesic Multidose, Ceva Animal Health Inc., ON, Canada) was given IM upon recovery for post-operative pain management. A 2-day washout period was observed following surgery to allow for all previous medications to be eliminated. Catheters were monitored and flushed with heparinized physiological saline (10 IU/mL; compounded by Ontario Veterinary College Pharmacy) once daily during the washout period. No additional drugs were administered following the completion of the catheterization.

2.3 | Treatment Groups

2.3.1 | NFID Setting and Calibration

The NFID used in this study is commercially available (Pulse 50; Pulse NeedleFree Systems Inc., KS, USA) for veterinary medicine use only, and can administer dose volumes from 0.1 to 0.5 mL. According to the manufacturer's guidelines and the literature available at the time of the study, no specific injection pressure (pounds per square inch—psi) was recommended for administering meloxicam in piglets. As a general approach, the manufacturer recommended using a pressure of 70 psi for pigs weighing <4.54 kg, and 75 psi for those ≥ 4.54 kg up to weaning. The NFID setting was calibrated in a separate cohort of two piglets: one pig with 70 psi and another with 75 psi. Meloxicam plasma concentration (data not shown) indicated the 75-psi pressure was optimal for meloxicam administration and was selected for this pharmacokinetic study.

2.3.2 | Pharmacokinetic Study

At the time of meloxicam administration, the piglet mean weight and age were 3.4 kg (range: 3.0–4.0 kg) and 11.5 days (range: 10–13 days), respectively. The authors acknowledged that castration in piglets is recommended within the first week of life. However, the piglets' health and welfare must be ensured throughout the entire study. At the time this study was conducted, there were no reports in the literature showing that being a few days older would significantly affect the PK results when injecting meloxicam. One pig in the needle-and-syringe group was excluded from the study because the catheter was accidentally removed by the pig and could not be replaced. Piglets were randomly assigned to one of 2 treatment groups using a parallel study design: needle-free injection (NFID; 7 females, 7 males) and needle-and-syringe injection (NS; 7 females, 6 males). The approved label dose of 0.4 mg/kg of meloxicam (Metacam 20 mg/mL; Boehringer Ingelheim Animal Health Canada Inc., ON, Canada) was administered once to both treatment groups. In order to accommodate the injection volume requirements of the NFID, the stock formulation of meloxicam (20 mg/mL) was diluted 1:3 with sterile water for injection (Pfizer Canada ULC, QC, Canada) resulting in a 5 mg/mL final formulation. The NS group was administered IM by 20G $\times 1''$ needles with 1 mL syringes, while the NFID group was administered with the setting described in the calibration section. The injection site for both treatment groups was the left side of the neck to simulate on-farm practices of injecting meloxicam using NFID and needle-and-syringe.

2.3.3 | Potency of Meloxicam Compounded With Sterile Water for Injection

In order to verify meloxicam potency in the final formulation following dilution with sterile water, a series of in vitro tests were conducted with Metacam 20 mg/mL diluted 1:1 (10 mg/mL) and 1:3 (5 mg/mL) with sterile water for injection. Samples were collected in triplicate for five consecutive days (day 0–5) from a group of two conditions: maintained in ambient light and another protected from ambient light. Collected samples were divided into equal aliquots and transferred into 1.2 mL cryogenic vials and stored at −80°C until liquid chromatography–tandem mass spectrometry (LC–MS/MS) performance.

2.4 | Blood Collection

Blood samples were collected pre-dose, and at 5, 10, 20, 30, and 45 min, and 1, 2, 4, 8, 12, 24, 36, 48, and 72 h after treatment. During each sample time point, the pre-measured 0.6 mL of dead space inside the catheter was removed and discarded before 1.5 mL of fresh whole blood was collected and placed in heparinized tubes. The catheter was flushed with heparinized physiological saline (10 IU/mL) after each blood draw. Blood samples were placed on ice immediately following collection and centrifuged at $368 \times g$ at 4°C for 20 min within approximately an hour of collection. Collected plasma was divided into equal aliquots where possible and transferred into 1.2 mL cryogenic vials and stored at −80°C until liquid chromatography–tandem mass spectrometry (LC–MS/MS) performance. After collecting the final sample, all piglets were humanely euthanized intravenously with a lethal dose of pentobarbital sodium (Euthanyl Forte; Bimeda-MTC Animal Health Inc., ON, Canada).

2.5 | Quantitation of Meloxicam in Piglet Plasma Using LC MS/MS

2.5.1 | Chemicals and Reagents

USP reference standard of meloxicam, anhydrous dimethylformamide, and ammonium formate were purchased from Sigma (St. Louis, MO, USA). Meloxicam-d3 (internal standard) was purchased from Toronto Research Chemicals (Toronto, ON, Canada). LC/MS grade of formic acid, acetonitrile, methanol, and water were purchased from Fisher Scientific Inc. (MA, USA). Stock solutions of meloxicam (1 mg/mL) and meloxicam-d3 (1 mg/mL) were prepared by dissolving standards in dimethylformamide and stored at −80°C.

2.5.2 | Sample Preparation

Meloxicam was extracted from pig plasma using simple protein precipitation. A 200 μL sample of piglet plasma was mixed with 10 μL of internal standard, followed by adding 800 μL of chilled acetonitrile acidified with 1% formic acid. The sample mixture was vortexed thoroughly and then centrifuged at 17,000 × *g* at 4°C for 10 min. The supernatant was evaporated under a gentle stream of nitrogen, and the residue from fortified samples was reconstituted with 100 μL of the mobile phase for LC–MS analysis. Calibration standards, ranging from 2 to1000 ng/mL, and quality controls (3 and 800 ng/mL) were prepared on the day of analysis by spiking working solutions in blank piglet plasma.

2.5.3 | Instruments and Conditions

A Q Exactive Focus Orbitrap mass spectrometer (ThermoFisher Scientific, Bremen, Germany) coupled with a Thermo Vanquish Flex Binary UHPLC system was used for LC MS/MS determination of plasma drug concentrations.

Meloxicam was separated using a Waters ACQUITY Premier BEH C18 Column (1.7 μm, 2.1 mm × 50 mm) with a Premier BEH C18 VanGuard FIT Cartridge (1.7 μm, 2.1 mm × 5 mm). The mobile phase consisted of ammonium formate buffer (10 mM) as phase A, and acetonitrile with 0.1% formic acid as phase B. The initial mobile phase composition was 65% A and 35% B (v/v). The flow rate was 300 μL/min with a linear gradient: 0–0.5 min, 35% B; 0.5–2.5 min, 35%–95% B; held at 95% B from 2.5–3.0 min; then changed to 35% B at 3.1 min and maintained until the end of the 4-min run. A 2 μL sample was injected onto the column, and meloxicam and its internal standard meloxicam-d3 were eluted at 2.1 min.

The Q Exactive Focus Orbitrap Mass Spectrometer was equipped with a heated electrospray (HESI) source. Meloxicam was measured in the ESI-negative mode with the Ion source parameters optimized as: Spray voltage 2.8 kV, Capillary temp 280°C, S-lens RF level 50.0, Aux gas heater temp 425°C. For MS/MS analysis, the collision energy was set at 12 eV for both meloxicam and meloxicam-d3. Data were acquired in parallel-reaction monitoring (PRM) negative ion mode, and the resulting chromatograms were extracted with a mass accuracy of 5 ppm. Deprotonated $[M\text{-}H]^-$ precursor ions were 350.0 m/z for meloxicam and 353.0 m/z for its internal standard meloxicam-d3. The product ions 286.1 m/z and 289.1 m/z were selected as quantifying ions, and 146.1 m/z and 149.1 m/z were selected as a confirming ions for meloxicam and meloxicam-d3, respectively.

2.5.4 | Method Validation

The LC MS/MS assay was validated following the FDA Bioanalytical Validation guidelines for specificity, selectivity, linearity, accuracy, intra- and inter-day precision (U.S. Food and Drug Administration 2018). Specificity and selectivity were evaluated by extracting six different piglet blank plasma samples and comparing the peak area at the retention time of meloxicam with the peak area found in the lower limit of quantitation (LLOQ; 2 ng/mL). No interfering peaks were observed in the blanks. The LLOQ was established at 2 ng/mL, representing the lowest concentration of calibration standard with a coefficient of variation (CV) ≤ 20%. Nine-point calibration curves (2–1000 ng/mL) were prepared and assayed over 15 separate days. All curves were linear and reproducible with a correlation coefficient (R^2) > 0.99 using weighted (1/×) least squares linear regression. To determine precision, triplicates of calibration standards and QCs were run on three different days. The assay demonstrated repeatability and reproducibility with both intra- and inter-day precision within ±15% CV for all standards and QCs. Accuracy was calculated as the percentage of deviation from the nominal concentrations. For all calibration standards and QCs, accuracy was within ±15%, except at the LLOQ, where it was within ±20%.

2.6 | Pharmacokinetics and Statistical Analysis

Meloxicam plasma concentration-time profiles were generated and PK parameters determined using noncompartmental analysis for each pig using Phoenix WinNonlin (Version 8.3; Certara USA Inc., New Jersey, USA). Relative bioavailability was calculated by Phoenix WinNonlin with natural log transformation of C_{max}, AUC_{0-last}, and $AUC_{0-\infty}$ of the NFID group by those of the NS group as a reference.

Statistical analyses were conducted with Stata 18.0 (College Station, TX: StataCorp LLC, USA), and a two-sample Student's *t*-test was utilized to compare the PK parameters between the treatment groups with 95% confidence intervals (CI). The assumptions of normality were evaluated using the Shapiro–Wilk test, and the assumption of equal variances was tested using Levene's test. If the data failed to meet both assumptions, a log or reciprocal transformation was applied, followed by selecting the one that satisfied the assumptions. The Wilcoxon rank-sum test was utilized for non-parametric covariates when transformations did not satisfy the necessary assumptions. The difference in relative bioavailability from 100% was determined using a one-sample Student's *t*-test with a 90% CI. The potency of meloxicam was analyzed using mixed linear regression comparing across days and conditions. $p < 0.05$ was considered significant for all analyses.

3 | Results

General health and physiological parameters remained within clinically acceptable ranges in both treatment groups throughout the trial: during and after treatment administration and sample collection. One male pig given meloxicam through NFID showed no detectable presence of the meloxicam in all collected blood samples when using the methods described above, which was hypothesized to be due to a technical issue during injection. Some blood samples were unable to be obtained at their nominal time, owing to catheter malfunction: two samples at the 5-min time point in the NFID group, and three samples at the 30-min, 12-h, and 36-h time points in the NS group. These animals were still used in the final PK analysis that included 26 meloxicam plasma concentration-time (C-T) profiles (13 in each treatment group).

The plasma C-T profiles of the two treatment groups were plotted on the log scale and presented in Figure 2. Pharmacokinetic parameters are reported in Table 1. C_{max}, AUC_{0-last}, $AUC_{0-\infty}$, $AUMC_{0-last}$, $AUMC_{0-\infty}$, and MRT in the NFID group were all significantly lower compared with those of the NS group. All parameter comparisons were analyzed using a standard 2-sample *t*-test, except for T_{max}, which was analyzed non-parametrically using the Wilcoxon rank-sum test. Differences of the NFID/NS ratio from 100% were detected in both C_{max} ($p < 0.001$; 90% CI: 34.75–45.24), AUC_{0-last} ($p < 0.001$; 90% CI: 23.55–39.69), and $AUC_{0-\infty}$ ($p < 0.001$; 90% CI: 23.31–39.42). The relative bioavailability of meloxicam in the NFID group was significantly lower than that of the NS group.

Results of potency testing of meloxicam diluted in sterile water showed no differences for actual versus. theoretical meloxicam levels for either dilution (data not shown). All calibration curves were linear and reproducible with the correlation coefficient (R^2) > 0.99. The intra-day and inter-day assay precision was 2.07% and 5.17%, respectively. Accuracy was within ±10% of nominal concentrations for all calibration standards and controls. There is no difference found between the two conditions among five days in linear regression models neither for 1:1 or 1:3 ratio ($p > 0.05$).

4 | Discussion

In the current study, significantly lower PK parameters and relative bioavailability of meloxicam in the NFID treatment group were observed compared with the NS group. Bioavailability is generally defined as the rate and the extent to which the active pharmaceutical ingredient (API) is absorbed from a pharmaceutical form and becomes available to the systemic circulation (Toutain and Bousquet-Mélou 2004). The rate and the extent of API availability are reflected by C_{max} and AUC, respectively. The other PK parameters including T_{max}, λ_z, $T_{1/2}$, and MRT are hybrid parameters which are influenced by the elimination of the drug (Toutain and Bousquet-Mélou 2004). Among the mentioned parameters, only MRT differed significantly ($p = 0.021$), but the difference might not be clinically significant (7.38 h in NFID group vs. 6.16 h in NS group).

At the time this study was conducted, no PK data on meloxicam administered via NFID was available. Several studies have investigated the PKs of meloxicam in pigs of similar age using needle-and-syringe and the same dosing regimen and found comparable results to the NS group in the current study (Nixon et al. 2020; Enouri et al. 2022). The decreased PK parameter values observed in the NFID group were consistent with findings from a previous study on antibiotic administration in pigs comparing NFID to NS delivery. Apley et al. (2007) conducted a study of ampicillin in pigs and found C_{max}, AUC, and bioavailability were lower when administered by NFID, although the differences were not statistically significant. The inconsistencies between our study and Apley's may be attributed to differences in the physicochemical properties of the tested drugs (final formulation, viscosity, etc.), study design (parallel vs. crossover), study sample sizes, and NFID settings (same manufacturer as the current study, but different injectors: micro-dose injector [0.1 mL—0.5 mL] vs. standard-dose injector [0.5 mL—2.5 mL]).

The lower plasma meloxicam concentrations, relative bioavailability, C_{max}, and AUC values for the NFID group compared to the NS group suggest reduced meloxicam absorption from the injection site into the systemic circulation. The NFID used in this study is a commercial gas-powered jet injector that propels molecules of interest at high velocity through a small orifice using compressed air to penetrate the skin. Most NFIDs can deliver medication intradermally, subcutaneously, or intramuscularly by adjusting the exit force generated at the orifice and depending on the physicochemical characteristics of the drug formulation of interest (Aguiar et al. 2001; Mitragotri 2006). However, penetration and dispersion can vary based on injection pressure, drug

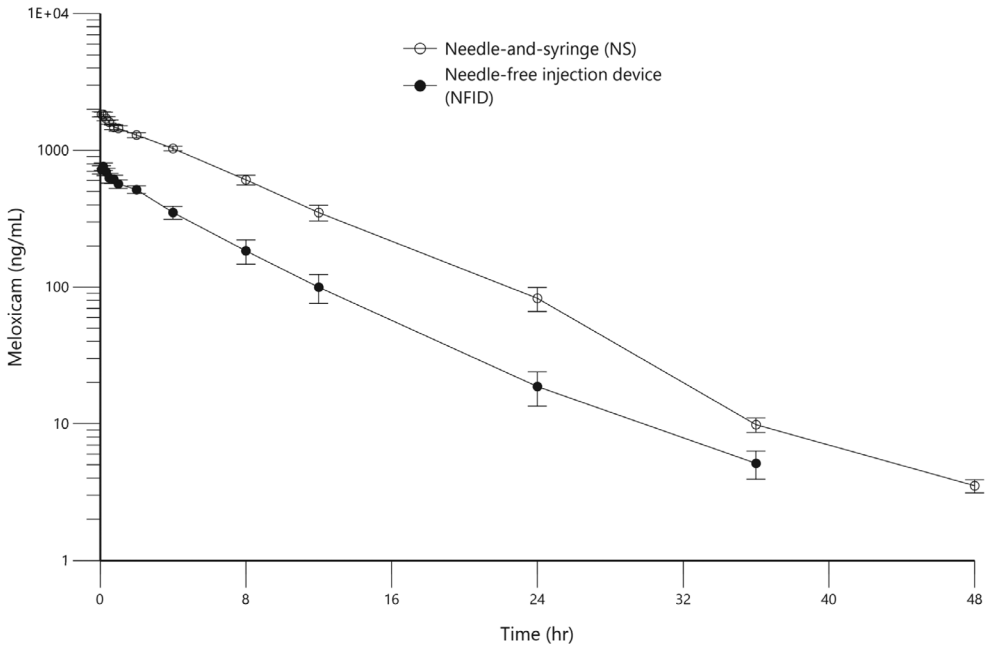

FIGURE 2 | Plasma drug concentration (± standard error) versus time curve for meloxicam administered by NS and NFID to piglets.

TABLE 1 | Pharmacokinetic parameters following noncompartmental analysis for meloxicam administered using needle-and-syringe (NS, $n = 13$) and needle-free injection (NFID, $n = 13$) in piglets.

Parameter	Units	Treatment	Average	90% conf. interval		p[a]
				LL	UL	
C_{max}	μg/mL	NS	1.90[c]	1.77	2.02	**< 0.001**
		NFID	0.77[c]	0.68	0.85	
AUC_{0-last}	h x μg/mL	NS	12.62[d]	11.09	14.37	**< 0.001**
		NFID	3.83[d]	3.01	4.87	
$AUC_{0-\infty}$	h x μg/mL	NS	12.67[d]	11.13	14.42	**< 0.001**
		NFID	3.87[d]	3.05	4.92	
$AUC_{\%extrap}$	%	NS	0.32[c]	R: 0.11	0.66	**0.003**
		NFID	1.17[c]	R: 0.22	4.18	
$AUMC_{0-last}$	h x h x μg/mL	NS	89.96[d]	72.48	111.65	**< 0.001**
		NFID	21.16[d]	14.57	30.72	
$AUMC_{0-\infty}$	h x h x μg/mL	NS	92.13[d]	74.46	114.00	**< 0.001**
		NFID	23.10[d]	16.19	32.96	
T_{max}	h	NS	0.17[e]	R: 0.08	0.33	0.858[b]
		NFID	0.17[e]	R: 0.08	0.17	
λ_z	1/h	NS	0.12[c]	0.11	0.14	0.155
		NFID	0.14[c]	0.12	0.16	
$T_{1/2}$	h	NS	5.64[f]	5.10	6.31	0.155
		NFID	4.83[f]	4.20	5.68	
$MRT_{0-\infty}$	h	NS	7.38[c]	6.75	8.01	**0.021**
		NFID	6.16[c]	5.37	6.95	

Note: Bold indicates significant differences and reports exact p-values.
Abbreviations: AUC, area under the curve; AUMC, area under the moment curve; C_{max}, maximum concentration; LL, lower limit; MRT, mean residence time; R, range; $T_{1/2}$, elimination half-life; T_{max}, maximum time; UL, upper limit; λ_z, elimination rate constant.
[a]Standard two-sample Student's t-test.
[b]Wilcoxon rank-sum test.
[c]Arithmetic mean.
[d]Geometric mean.
[e]Median.
[f]Harmonic mean.

viscosity, and differences in skin elasticity at various pig growth stages (Schramm-Baxter and Mitragotri 2004; Mitragotri 2006; Chase et al. 2008; Mohizin and Kim 2018). In this study, the penetration depth and pattern of meloxicam dispersion at the injection site were not evaluated following NFID injection.

Even when the penetration depth reached IM, it remains unclear whether the majority of the meloxicam delivered in the NFID group accumulated in the dermis, SC, or IM. Drug absorption and bioavailability can vary among different routes of administration due to factors such as blood flow, lipophilicity, molecule size, and other physiochemical properties of the API. To the authors' knowledge, no literature is available comparing the PK profiles of meloxicam in pigs when given SC and IM. In sheep, the SC administration of meloxicam resulted in a significantly lower $AUC_{0-\infty}$ value compared to IM when administering the same dose of 1 mg/kg (Woodland et al. 2019). Thus, the lower AUC in the NFID group in our study could be explained by differences in the ratio of meloxicam accumulated in each tissue layer between the two treatment groups.

Additionally, it is possible that a certain proportion of the meloxicam dose in the NFID group was trapped in the dermis or SC and could not be absorbed into systemic circulation. Miranda-Muñoz et al. (2024) conducted two trials using meloxicam applied by a microneedle patch (2.5 mg/kg) on 1-week-old nursing pigs' ears compared to oral delivery (0.5 mg/kg). At 24 h post-dosing, the systemic blood concentration of meloxicam achieved in the patch group was significantly lower, approximately 4% of that in the oral group. At the same time, meloxicam from the patch was successfully diffused into the ear tissues. The authors concluded that meloxicam in the patch remained largely trapped within the tissue and was poorly absorbed into plasma. Furthermore, the physiochemical properties of meloxicam

may not support absorption from the dermis to plasma. Chen and Gao (2016) reported that meloxicam has poor solubility in water, moderate lipophilicity, and a high melting point, which can strongly affect the delivery of the drug through the skin to the systemic circulation. To the authors' knowledge, no research in the literature has examined meloxicam concentrations at the injection site and in plasma following NFID administration to determine the amount absorbed into systemic circulation versus the portion retained at the injection site.

The lower bioavailability of meloxicam in the NFID group could also be due, in part, to potential loss of the drug during the NFID delivery procedure. Theoretically, being propelled from a nozzle at high velocity could compromise the structural integrity of the API. Another possibility is the physical loss of drug splashing out at the injection site surface during drug delivery. Previous studies have documented a "wetness" on the injection surface following NFID vaccine administration due to residual vaccine loss (Jones et al. 2005; Chase et al. 2008). Finally, the consistency of drug volume injected by the NFID during the reloading phase with each use could vary. Trimzi and Ham (2021) conducted a trial measuring the drug volume per injection, over 10 repeated injections, using different NFID devices but the same air-powered technology. The authors concluded that the drug delivery efficiency averaged 96.7% for 0.2 mL per injection and 97.8% for 0.5 mL per injection.

The reduced bioavailability of meloxicam and plasma drug levels in the NFID group could result in reduced therapeutic efficacy. However, NSAID efficacy is primarily determined by the concentration of drug at the site of inflammation (i.e., target site), and that NSAID plasma concentrations are not well correlated to the NSAID concentration at the site of inflammation (Lees, Giraudel, et al. 2004; Lees, Landoni, et al. 2004; Brune and Furst 2007; Messenger et al. 2016). Enouri et al. (2022) documented a significantly lower relative bioavailability, $AUC_{0-\infty}$, and C_{max} when meloxicam compounded with iron dextran (M + D) was compared to meloxicam alone (M) in non-processed piglets. Using the same compounded meloxicam formulation on a similar age group of piglets, Reynolds et al. (2020) reported no significant differences in chute navigation times and cortisol levels one hour post-castration between the M + ID and M treatment groups. It was concluded in Reynolds's study that efficacy was not different in the two groups despite showing lower plasma meloxicam levels in the M + ID group compared to the M group in Enouri's study.

In order to reduce the development of significant diarrhea, all piglets were administered ceftiofur hydrochloride daily for 4 days. It is important to note that the 48-h washout period observed would not be sufficient to eliminate all the ceftiofur administered prior to the administration of meloxicam. Ceftiofur hydrochloride has an elimination half-life of approximately 20 h in pigs (Excenel RTU EZ; Zoetis Canada Inc., QC, Canada). At the time, other antimicrobial choices were deemed not to be as effective as ceftiofur. Based on the following, we do not believe there is concern for a significant drug–drug interaction. A review of the literature and ceftiofur and meloxicam drug labels revealed no mention of drug interactions. All piglets received ceftiofur versus one treatment group and not the other. Our results of the NS treatment group are similar to previous work with meloxicam in piglets (Nixon et al. 2020; Enouri et al. 2022).

In summary, this study offers a comparison of the PK of meloxicam administered by NFID compared to NS, providing a preliminary assessment of NFID's for meloxicam administration in piglets. The PK profiles in this study reveal significantly reduced relative bioavailability of meloxicam administered via NFID compared to NS. Since there is no recommended standard NFID setting for each medication category, including meloxicam, further research is needed to determine the optimal injection setting for each drug of interest, and regarding NSAIDs, efficacy as a clinical response when using NFID for injecting meloxicam in piglets. Additionally, the assessment of the penetration depth achieved by meloxicam when administered by NFID will provide possible insight explaining differences in PKs compared to NS administration.

Author Contributions

Minh Man Pham: animal selection and care, anesthesia assistance, data acquisition, data curation, PK analysis, statistics analysis, data interpretation, data visualization, manuscript drafting, manuscript revision. **Terri L. O'Sullivan:** conceptualization, funding acquisition, methodology, project administration, animal selection and care, catheterization surgery, data acquisition, data interpretation, manuscript drafting, manuscript revision. **Maria del Rocio Amezcua:** animal selection and care, surgery assistance, data acquisition, manuscript revision. **Saad Enouri:** animal care, surgery assistance, data acquisition, data curation, manuscript revision. **Yu Gu:** LC/MS–MS analysis, data curation, manuscript revision. **Zvonimir Poljak:** data interpretation, manuscript revision. **Jennifer M. Reinhart:** PK analysis validation, data interpretation, manuscript revision. **Ron Johnson:** conceptualization, methodology, animal care, anesthesia assistance, data acquisition, PK analysis validation, data interpretation, manuscript drafting, manuscript revision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Aguiar, J. C., R. C. Hedstrom, W. O. Rogers, et al. 2001. "Enhancement of the Immune Response in Rabbits to a Malaria DNA Vaccine by Immunization With a Needle-Free Jet Device." *Vaccine* 20, no. 1–2: 275–280. https://doi.org/10.1016/S0264-410X(01)00273-0.

Apley, M. D., J. F. Coetzee, P. M. Imerman, L. A. Karriker, and R. Gehring. 2007. "Ampicillin Pharmacokinetics in Swine Following Needle-Free, Intramuscular, and Intravenous Administration." *Journal of Veterinary Pharmacology and Therapeutics* 30, no. 5: 5. https://doi.org/10.1111/j.1365-2885.2007.00887.x.

Brune, K., and D. E. Furst. 2007. "Combining Enzyme Specificity and Tissue Selectivity of Cyclooxygenase Inhibitors: Towards Better

Tolerability?" *Rheumatology* 46, no. 6: 911–919. https://doi.org/10.1093/rheumatology/kem070.

Chase, C., S. Daniels, R. Garcia, F. Milward, and T. Nation. 2008. "Needle-Free Injection Technology in Swine: Progress Toward Vaccine Efficacy and Pork Quality." *Journal of Swine Health and Production* 18, no. 5: 254–261. https://doi.org/10.54846/jshap/555.

Chen, J., and Y. Gao. 2016. "Strategies for Meloxicam Delivery to and Across the Skin: A Review." *Drug Delivery* 23, no. 8: 8. https://doi.org/10.3109/10717544.2016.1157839.

Cho, H., Y. Ahn, T. Oh, J. Suh, and C. Chae. 2022. "Non-Inferiority Field Study Comparing the Administrations by Conventional Needle-Syringe and Needle-Free Injectors of a Trivalent Vaccine Containing Porcine Circovirus Types 2a/2b and *Mycoplasma hyopneumoniae*." *Vaccine* 10, no. 3: 358. https://doi.org/10.3390/vaccines10030358.

Code of Practice for the Care and Handling of Pigs. 2014. "National Farm Animal Care Counci." http://www.nfacc.ca/pdfs/codes/pig_code_of_practice.pdf.

Dzikamunhenga, R. S., R. Anthony, J. Coetzee, et al. 2014. "Pain Management in the Neonatal Piglet During Routine Management Procedures. Part 1: A Systematic Review of Randomized and Non-Randomized Intervention Studies." *Animal Health Research Reviews* 15, no. 1: 14–38. https://doi.org/10.1017/S1466252314000061.

Enouri, S. S., T. L. O'Sullivan, S. Ramkissoon, R. M. Friendship, Y. Gu, and R. J. Johnson. 2022. "Pharmacokinetics of Combined Administration of Iron Dextran With Meloxicam or Flunixin Meglumine in Piglets." *Canadian Veterinary Journal = La Revue Veterinaire Canadienne* 63, no. 7: 727–734.

Hay, M., A. Vulin, S. Génin, P. Sales, and A. Prunier. 2003. "Assessment of Pain Induced by Castration in Piglets: Behavioral and Physiological Responses Over the Subsequent 5 Days." *Applied Animal Behaviour Science* 82, no. 3: 3. https://doi.org/10.1016/S0168-1591(03)00059-5.

Imeah, B., E. Penz, M. Rana, C. Trask, and For the Needle-less Injector Study Team. 2020. "Economic Analysis of New Workplace Technology Including Productivity and Injury: The Case of Needle-Less Injection in Swine." *PLoS One* 15, no. 6: e0233599. https://doi.org/10.1371/journal.pone.0233599.

Jones, G., V. Rapp-Gabrielson, and R. Wilke. 2005. "Intradermal Vaccination for *Mycoplasma hyopneumoniae*." *Journal of Swine Health and Production* 13, no. 1: 19–27.

Ko, E. Y., J. Cho, J. H. Cho, et al. 2018. "Reduction in Lesion Incidence in Pork Carcass Using Transdermal Needle-Free Injection of Foot-and-Mouth Disease Vaccine." *Korean Journal for Food Science of Animal Resources* 38, no. 6: 6. https://doi.org/10.5851/kosfa.2018.e46.

Lees, P., J. Giraudel, M. F. Landoni, and P. L. Toutain. 2004. "PK–PD Integration and PK–PD Modelling of Nonsteroidal Anti-Inflammatory Drugs: Principles and Applications in Veterinary Pharmacology." *Journal of Veterinary Pharmacology and Therapeutics* 27, no. 6: 6. https://doi.org/10.1111/j.1365-2885.2004.00618.x.

Lees, P., M. F. Landoni, J. Giraudel, and P. L. Toutain. 2004. "Pharmacodynamics and Pharmacokinetics of Nonsteroidal Anti-Inflammatory Drugs in Species of Veterinary Interest." *Journal of Veterinary Pharmacology and Therapeutics* 27, no. 6: 6. https://doi.org/10.1111/j.1365-2885.2004.00617.x.

Madapong, A., K. Saeng-chuto, A. Tantituvanont, and D. Nilubol. 2021. "Safety of PRRSV-2 MLV Vaccines Administrated via the Intramuscular or Intradermal Route and Evaluation of PRRSV Transmission Upon Needle-Free and Needle Delivery." *Scientific Reports* 11, no. 1: 1. https://doi.org/10.1038/s41598-021-02444-3.

Messenger, K. M., J. A. Wofford, and M. G. Papich. 2016. "Carprofen Pharmacokinetics in Plasma and in Control and Inflamed Canine Tissue Fluid Using *In Vivo* Ultrafiltration." *Journal of Veterinary Pharmacology and Therapeutics* 39, no. 1: 32–39. https://doi.org/10.1111/jvp.12233.

Miranda-Muñoz, K., K. Midkiff, A. Woessner, et al. 2024. "A Multicomponent Microneedle Patch for the Delivery of Meloxicam for Veterinary Applications." *ACS Nano* 18, no. 37: 37. https://doi.org/10.1021/acsnano.4c08072.

Mitragotri, S. 2006. "Current Status and Future Prospects of Needle-Free Liquid Jet Injectors." *Nature Reviews Drug Discovery* 5, no. 7: 543–548. https://doi.org/10.1038/nrd2076.

Mohizin, A., and J. K. Kim. 2018. "Current Engineering and Clinical Aspects of Needle-Free Injectors: A Review." *Journal of Mechanical Science and Technology* 32, no. 12: 12. https://doi.org/10.1007/s12206-018-1121-9.

Nixon, E., G. W. Almond, R. E. Baynes, and K. M. Messenger. 2020. "Comparative Plasma and Interstitial Fluid Pharmacokinetics of Meloxicam, Flunixin, and Ketoprofen in Neonatal Piglets." *Frontiers in Veterinary Science* 7: 82. https://doi.org/10.3389/fvets.2020.00082.

Owen, K., N. Blackie, and T. J. Gibson. 2022. "The Effect of Needle Reuse on Piglet Skin Puncture Force." *Veterinary Sciences* 9, no. 2: 2. https://doi.org/10.3390/vetsci9020090.

Renson, P., S. Mahé, M. Andraud, et al. 2024. "Effect of Vaccination Route (Intradermal vs. Intramuscular) Against Porcine Reproductive and Respiratory Syndrome Using a Modified Live Vaccine on Systemic and Mucosal Immune Response and Virus Transmission in Pigs." *BMC Veterinary Research* 20, no. 1: 5. https://doi.org/10.1186/s12917-023-03853-4.

Rey, M. R., M. Undi, J. C. Rodriguez-Lecompte, et al. 2013. "A Study of the Effectiveness of a Needle-Free Injection Device Compared With a Needle and Syringe Used to Vaccinate Calves Against Bovine Viral Diarrhea and Infectious Bovine Rhinotracheitis Viruses." *Veterinary Journal* 198, no. 1: 235–238. https://doi.org/10.1016/j.tvjl.2013.06.019.

Reynolds, K., R. Johnson, J. Brown, R. Friendship, and T. L. O'Sullivan. 2020. "Assessing Pain Control Efficacy of Meloxicam and Ketoprofen When Compounded With Iron Dextran in Nursing Piglets Using A Navigation Chute." *Animals* 10, no. 7: 1237. https://doi.org/10.3390/ani10071237.

Salman, M., H. Lin, R. Suntisukwattana, et al. 2023. "Intradermal Needle-Free Injection Prevents African Swine Fever Transmission, While Intramuscular Needle Injection Does Not." *Scientific Reports* 13, no. 1: 4600. https://doi.org/10.1038/s41598-023-31199-2.

Schramm-Baxter, J., and S. Mitragotri. 2004. "Needle-Free Jet Injections: Dependence of Jet Penetration and Dispersion in the Skin on Jet Power." *Journal of Controlled Release* 97, no. 3: 3. https://doi.org/10.1016/j.jconrel.2004.04.006.

Shergold, O. A., N. A. Fleck, and T. S. King. 2006. "The Penetration of a Soft Solid by a Liquid Jet, With Application to the Administration of a Needle-Free Injection." *Journal of Biomechanics* 39, no. 14: 14. https://doi.org/10.1016/j.jbiomech.2005.08.028.

Temple, D., M. Jiménez, D. Escribano, G. Martín-Valls, I. Díaz, and X. Manteca. 2020. "Welfare Benefits of Intradermal Vaccination of Piglets." *Animals* 10, no. 10: 1898. https://doi.org/10.3390/ani10101898.

Toutain, P. L., and A. Bousquet-Mélou. 2004. "Bioavailability and Its Assessment." *Journal of Veterinary Pharmacology and Therapeutics* 27, no. 6: 455–466. https://doi.org/10.1111/j.1365-2885.2004.00604.x.

Trimzi, M. A., and Y.-B. Ham. 2021. "A Needle-Free Jet Injection System for Controlled Release and Repeated Biopharmaceutical Delivery." *Pharmaceutics* 13, no. 11: 1770. https://doi.org/10.3390/pharmaceutics13111770.

U.S. Food and Drug Administration. 2018. *Bioanalytical Method Validation Guidance for Industry*. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf.

van den Drunen Littel Harke, S., S. Babiuk, and L. A. Babiuk. 2006. "Needle-Free Delivery of Veterinary DNA Vaccines." In *DNA Vaccines*, edited by B. L. Janet, S. L. Hong, and S. W. Mark, vol. 127, 91–106. Humana Press. https://doi.org/10.1385/1-59745-168-1:91.

Woodland, A. N., D. van der Saag, B. Kimble, P. J. White, M. Govendir, and S. Lomax. 2019. "Plasma Pharmacokinetic Profile and Efficacy of Meloxicam Administered Subcutaneously and Intramuscularly to Sheep." *PLoS One* 14, no. 4: e0215842. https://doi.org/10.1371/journal.pone.0215842.