“Pharmacokinetics and efficacy of meloxicam administered subcutaneously and intramuscularly in sheep”

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Abstract

Objective To determine the pharmacokinetic profiles and efficacy of meloxicam when administered subcutaneously (S/C) and intramuscularly (IM) in sheep at different doses.

Procedures Ewes were injected with 0.1mL of oil of turpentine in a forelimb, followed by either a 1.0mg/kg or 2.0mg/kg dose of meloxicam administered either subcutaneously or intramuscularly (n=3). Ewes were examined at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h, with blood collected (3-4mL) at each timepoint and behavioural and physiological responses recorded. Responses measured included skin temperature, limb circumference, limb sensitivity and gait. Pharmacokinetics were analysed using HPLC analysis.

Results Oil of turpentine successfully induced inflammation in the ewes with affected limbs demonstrating higher skin temperature ($p < 0.001$), limb circumference ($p < 0.001$) and sensitivity ($p = 0.01$). Whilst significantly affected by meloxicam, minimal variations of skin temperature ($p < 0.001$), limb circumference ($p = 0.012$) and gait ($p < 0.001$) were observed between treatments. S/C 1.0mg/kg, IM 2.0mg/kg and S/C 2.0mg/kg treatments significantly reduced limb sensitivity when compared to the control at 48hrs post drug administration ($p < 0.001$). The IM treatment at 1.0mg/kg had a significantly higher plasma concentration of meloxicam than S/C at 1.0mg/kg from 0.5-4hrs post drug administration ($p < 0.001$). Both IM and SC treatments demonstrated long terminal half-lives at 12.47 and 10.24 hrs respectively.

Conclusion Meloxicam was effective at providing some analgesia post-injection of turpentine in sheep however analgesic efficacy could not be distinguished between the five treatments (IM 1.0mg/kg, S/C 1.0mg/kg, IM 2.0mg/kg, S/C 2.0mg/kg and control).

Key words

analgesia; animal welfare; meloxicam; pharmacokinetics; sheep

Abbreviations

AUC = Area under the curve

Cmax = Maximum serum concentration

$T_{1/2}$ = Half-life of the terminal portion of the curve

Tmax = Time to maximum serum concentration

COX = cyclo-oxygenase

NSAID = non-steroidal anti-inflammatory drug

Introduction

Meloxicam is a non-steroidal, anti-inflammatory drug (NSAID) currently registered for pain management in a number of species including humans, cats, dogs,[1] cattle, pigs and most recently sheep.[2] Currently meloxicam is the only registered NSAID for use in sheep,[3] with Metacam® 20 (Boehringer Ingelheim, Australia) officially registered for use in 2016.[2] Meloxicam is an enolic acid that provides analgesia and antipyretic action via the inhibition of the cyclo-oxygenase (COX) pathway.[4] The COX pathway is responsible for the biosynthesis of arachidonic acid to prostaglandins (PGE_2), which impart pain via the stimulation of peripheral sensory neurons.[5] Meloxicam selectively inhibits the COX-2 pathway in a number of species including humans,[6] primates[6] and horses,[7] resulting in the low ulcerogenic potential of the drug, alongside other favourable characteristics for animal use, including an extended elimination half-life and effective bio-absorption.[4] Meloxicam has been shown to significantly

reduce abnormal behaviours associated with pain during husbandry procedures such as castration and tail docking in sheep.[8]

The pharmacokinetics of meloxicam have been documented in numerous species including goats,[9] horses,[10] cattle[11] and sheep.[1] However, the pharmacokinetics of meloxicam in sheep have only been assessed via intravenous and oral routes of delivery with no literature currently available in regards to subcutaneous and intramuscular administration routes.[1] Metacam® 20 administration guidelines in sheep recommend a subcutaneous injection of 1.0mg/kg,[2] therefore it is surprising that a knowledge gap is present in regards to the pharmacokinetics and efficacy of subcutaneous administration in sheep at the recommended dose. With known benefits such as on-farm practicality and slower absorption resulting in the potential for longer-lasting analgesia,[12] subcutaneous administration is ideal, however with a lack of knowledge available in sheep, alternative routes of delivery, such as intramuscular, may prove to be more efficacious for providing pain relief. Whilst there is no literature currently available on pharmacokinetics of intramuscular administration of meloxicam in sheep, previous studies investigating alternative NSAID's in pigs, sheep and horses indicate greater bioavailability and maximum concentrations when utilising intramuscular routes compared to alternative routes investigated.[13-15]

The objective of this study was to determine the pharmacokinetic profiles and efficacy of meloxicam when administered subcutaneously and intramuscularly in sheep at 1.0mg/kg and 2.0mg/kg for the alleviation of pain and inflammation in sheep.

Methods

Sheep

65 The experiment was conducted at a University of Sydney farm ("Mayfarm"), in Camden, New
66 South Wales (NSW) and was approved by the University Animal Ethics Committee. 14 Merino
67 ewes (44-68kg) were housed in a group pen (10x10m) underneath a covered shed with
68 outdoor access. Two weeks prior to the commencement of the experiment, sheep were
69 housed in the group pen in order to acclimatise to the experimental environment. Sheep had
70 their necks shaved to expose the jugular region for precise jugular venepuncture during
71 experimentation. During this time, sheep were also drenched for internal parasites with Q
72 drench (Jurox, Hunter Valley, NSW) at the dose recommended by the manufacturer. 1 week
73 prior to experimentation, sheep were habituated to experimental conditions (once daily
74 5d/wk). This included catching, tipping and restraining each individual sheep in lateral
75 recumbency and recurrent jugular venepuncture. Sheep were fed an allocation of 750g/d
76 lucerne hay cubes (MultiCube®; 18% crude protein dry matter; 9.1 MJ/kg dry matter). During
77 the 1-week washout period sheep were released onto a 3 ha paddock with ad libitum access
78 to Kikuyu pasture and water.

79 ***Drug administration***

80 A randomised crossover design with a 10-day washout period was used, which is adequate
81 for drug clearance in sheep.[1] 5 days prior to experimentation, sheep were weighed to
82 calculate drug doses of Metacam 20® (Meloxicam 20mg/mL, Boehringer Ingelheim, Australia).

83 Inflammation was induced via the injection of oil of turpentine (Sigma-Aldrich) as previously
84 utilised by Colditz et al. (2011). Oil of turpentine (0.1mL) was injected subcutaneously via an
85 18-gauge needle on the pastern midway between the fetlock and the coronet on a single
86 forelimb of sheep. The limb chosen for turpentine injection was randomised across individual

sheep, alternating between the left and right forelimb, with the unaffected limb acting as a control.

Additionally, sheep were assigned to one of five treatment groups; 1.0mg/kg meloxicam (Metacam 20®; Boehringer Ingelheim) administered intramuscularly (n=3), 1.0mg/kg administered subcutaneously (n=3), 2.0mg/kg administered intramuscularly (n=3), 2.0mg/kg administered subcutaneously (n=3) or a control group (n=2) administered no dose of meloxicam (Table 1). Treatments were randomly assigned using a random number generator. Administration was undertaken using a 6-mL syringe and therefore doses were rounded to the nearest tenth in order to ensure precise meloxicam administration.

Blood collection

Blood samples were collected for pharmacokinetic analysis of meloxicam when administered at alternative routes and doses. Sheep were restrained in an upright position and blood samples (3-4mL) were collected into lithium heparin vacutainers via jugular venepuncture at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours post drug administration. Samples were immediately centrifuged for 10 minutes at 3,500 X g. Plasma was transferred to sterile vials and frozen at -70°C until analysis. Analysis of samples took place within 35 days of collection.

Skin temperature

Skin temperature was measured using an infrared thermometer (non-contact thermometer, Jaycar Electronics) with a resolution of 0.1°C. The laser light on the thermometer was aimed at the cranial surface of both affected and unaffected carpal joints, in the region of the scaphoid and lunate bones, and held at the recommended distance (300mm). The same investigator was responsible for all skin temperature measurements to minimise inter-observer variation. Ambient temperature was also recorded for each collection time-point

(USB Temperature/humidity datalogger with LCD, Jaycar Electronics). Measurements were taken at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours post drug administration.

Limb circumference

Limb circumference was measured, to the nearest millimetre, around the proximal aspect of the carpus by use of anatomic reference points, on both the affected and control limb using a measuring tape. The same investigator was responsible for all limb circumference measurements to minimise inter-observer variation. Measurements were taken at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours post drug administration.

Limb sensitivity

Sensitivity of the affected and unaffected forelimb was measured with a calibrated hand-held pressure algometer (Wagner Pain Test FPIX Digital Algometer, Wagner Instruments, Riverside, CT, USA) which has a maximum pressure of 10kg/f. The device consisted of a $1cm^2$ blunt rubber tip and was applied, with increasing pressure at a perpendicular angle to the target site, midway between the fetlock and the coronet, on both the affected and unaffected limb of the animal. The force required for withdrawal of the limb was recorded as the mechanical nociceptive threshold (MNT) to the nearest 0.5kg/f. Sheep that were unresponsive to the applied force were recorded at the maximum threshold of 10kg/f. The hand-held device was returned to zero after each pressure test. The MNT was recorded in the second phase of experimentation by a single investigator to minimise inter-observer variation. Measurements were taken at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours post drug administration.

Gait score

Sheep were assessed for gait using a numerical rating scale (NRS) based on behavioural characteristics of lameness (Table 2). Scoring took place once sheep were released back into the group pen after final measurements were taken for physiological and behavioural

indicators of pain. The observer stood within the group pen at a distance of 1-2 metres from the flock, observing voluntary movement in individual sheep for 1-3 minutes in order to obtain a lameness score. If individual sheep were out of sight, the observer entered the flight zone in order to stimulate movement within the flock and allow view of the desired individual. Measurements took place at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours post-injection by a single observer to minimise inter-observer variation.

Serum meloxicam concentration measurement

The concentration of meloxicam in serum was measured using high-pressure liquid chromatography (HPLC) analysis. The methodology was previously developed and validated for the University of Sydney HPLC laboratory in which analysis took place. [16] The HPLC system was comprised of a Shimadzu CBM-20A module (Kyoto, Japan) equipped with a LC-20AT delivery unit with DGU-20As degassing solvent delivery unit and SIL-20AC auto injector.

A reversed phase C^{18} column (Synergi™ 4µm MAX-RP 80A, 150 x 4.6mm, Phenomenex, Lane Cove, NSW) was used for separation. The isocratic mobile phase comprised of 50mM potassium phosphate buffer (pH 2.15) and acetonitrile (55:45, v/v). The mobile phase was run at a flow rate of 1 mL min^{-1} with an oven temperature of 30°C. The solution was monitored at a wavelength of 355nm via an SPD-M20A diode array detector (Kyoto, Japan) and Shimadzu class VP data system (software version 7.4) (Kyoto, Japan). The HPLC method was validated prior to the analysis of samples.

Meloxicam extraction was undertaken by the addition of 400µL of acetonitrile, containing the internal standard (IS) piroxicam, to 200µL of the serum sample (2:1 ratio). A vortex was used for 5 seconds to mix the samples and centrifugation at 14,0000 g resulted in a precipitate. 100 µL of supernatant was pipetted to an injection vial, with the HPLC injection volume set to

20µL. Meloxicam plasma concentration within the samples was defined by obtaining standard curves via the analysis of blank plasma samples which were spiked with meloxicam. Meloxicam was observed at 8.5 minutes and the IS at 5.5 minutes (Figure 1.)

The standard curve used for determining meloxicam concentration through use of sheep plasma was linear from 0.049 to 25µg/mL. The lowest limit of quantification (LLOQ) was determined using the formula LLOQ = 10 x σ/S whereby σ refers to the standard deviation (SD) and S refers to the slope of the calibration curves[16]. The LLOQ was defined as 0.096 µg/mL with the acceptance threshold defined as precision less than 15% and accuracy within ± 20% of the nominal concentration across analyses as recommended by the International Guidelines for Bioanalytical Method Validation.[17] Intra-day accuracy as determined by the formulae: [(estimated value/nominal value) × 100][16] was 102 ± 2.96%. Intra-day precision as calculated using the formula: coefficient of variation (CV) x [(SD/mean value) x 100][16] was 0.97 ± 0.87%. Intra-day accuracy and precision were determined through triplicates for meloxicam concentrations of 0.2, 2.0, and 20.0 µg/mL across 3 consecutive days.

Pharmacokinetic analysis

As a result of time constraints only S/C and IM 1.0mg/kg treatments were analysed using HPLC. The PK parameters calculated were: area under plasma concentration vs. time curves AUC0-t; where t refers to the final data time-point, maximum concentration of meloxicam in plasma (Cmax), time taken to reach maximum concentration in plasma (Tmax) and terminal half-life ($t^{1/2}$). AUC0-t was calculated using the linear trapezoidal rule for approximation of the definite integral. Cmax was calculated by obtaining $\bar{x}$ of the maximum concentrations of the samples (n=3). Tmax was calculated by obtaining the $\bar{x}$ of the time taken to reach maximum

concentration for the samples where $\bar{x}$ is the mean. The formula used for t1/2 = 0.693/kel where kel is the elimination rate constant.

Statistical analysis

Skin temperature, limb circumference and limb sensitivity were analysed in GenStat (VSN International Ltd, 14th Edition 2011) with a restricted maximum likelihood linear mixed model (REML). The model fitted the effects of treatment, timepoint and limb with a random effect of sheep ID. Ambient temperature was statistically significant against skin temperature and was therefore included in the model for skin temperature as a random effect. Limb sensitivity data was converted to binomials; 10kg/f = 1 and <10kg/f = 0 for statistical analysis. This was undertaken as transformation did not assist in normalising the dataset. Gait scores were subjected to ordinal logistic regression (OLR) in ASReml® 3.0 statistical software (VSN International, Hemel Hempstead UK). The fixed effects of the model were treatment x timepoint and the random effect of the model was sheep ID. Data from the OLR analysis are presented as a cumulative odds ratios with the statistical probabilities of sheep having gait scores of *Y* = 0, 1, 2, or 3. Pharmacokinetic values were analysed in GenStat version 14.0 (VSN International Ltd, Hemel Hempstead, UK) with a restricted maximum likelihood linear mixed model (REML) as mentioned above for skin temperature, limb circumference and limb sensitivity. Pair-wise comparison was undertaken in Excel (Microsoft Excel® 2016 MSO) for any significant treatment x timepoint interactions to compare differences across timepoints and treatments, using least significant differences (LSD). LSD was calculated using the formula: 1.96 x average predicted standard error of differences (SED).

Data plotted in figures are predicted means ± standard error of the mean (SEM), with the exception of limb sensitivity and gait score which are reported as probabilities. P <0.05 was considered statistically significant.

Results

Animals

The mean ± SD body weight of the sheep during the study phase was 56.8 ± 6.69kg. No adverse effects were observed following intramuscular or subcutaneous administration of meloxicam.

Skin temperature

There was a significant treatment x timepoint interaction (p <0.001) and effect of limb on skin temperature (p <0.001). Ambient temperature had a significant effect on skin temperature (p <0.001). Mean temperature of the affected limb (23.9˚C) was greater than control limbs (22.4˚C). Skin temperature of affected limbs slightly increased over time with a marked drop in temperature observed at 6 hours post-administration, followed by a steady, continual incline until 48 hours (Table 3). There was no significant difference between treatments until 12 hours after administration where treatments: S/C 1.0mg/kg and IM 2.0mg/kg were significantly less than the control (Table 3). At 24 hours, S/C 1.0mg/kg resulted in a significantly lower skin temperature than all treatments except S/C 2.0mg/kg.

Limb circumference

There was a significant treatment x timepoint interaction (p = 0.012) and effect of limb on limb circumference (p <0.001). Mean circumference of the affected limb (14.16mm) was greater than control limbs (13.74mm). Limb circumference of affected limbs increased slightly over time with an observed drop in limb circumference at 1 hour and 12 hours post drug

224 administration (Table 3). The S/C 2.0mg/kg treatment resulted in a significantly lower limb
225 circumference than IM 2.0mg/kg at 0.5 hours after administration with no other significant
226 differences observed between treatments until 10-12 hours post administration where the
227 IM 1.0mg/kg treatment had a significantly lower circumference than the control treatment.

228 ***Limb sensitivity***

229 There was a significant treatment x timepoint interaction ($p < 0.001$) and effect of limb on limb
230 sensitivity ($p = 0.01$). The maximum limb sensitivity threshold (10kg) was achieved by 65.3%
231 of affected limbs compared to 76.4% of control limbs, indicating a greater limb sensitivity
232 associated with affected limbs. Limb sensitivity increased over time, with all treatments
233 reaching the maximum threshold at 0 hours compared to zero treatments at 24-48 hours
234 post-drug administration (Table 3). Significant differences between treatments over time
235 were observed with all treatments demonstrating reduced limb sensitivity when compared
236 to the control at 6 hours post drug administration. At 48 hours, treatments: S/C 1.0mg/kg, IM
237 2.0mg/kg and S/C 2.0mg/kg had significantly reduced limb sensitivity when compared to the
238 control.

239 ***Gait score***

240 There was a significant treatment x timepoint interaction for gait score ($p < 0.001$). Lameness
241 was most apparent from 0.5 to 2 hours post drug administration with a decrease apparent
242 over the 48 hours (Figure 2). A lameness score of 1, 2 or 3 was most probable between 0.5
243 and 8 hours, with 1-hour post-administration associated with the highest probability of a
244 lameness score of 3. At 48 hours post drug administration, the S/C 2.0mg/kg treatment was
245 the only one observed to return gait of all sheep to a baseline score of 0. Treatments: S/C
246 1.0mg/kg (94.6%) and IM 2.0mg/kg (82.2%) also demonstrated a high likelihood of achieving
247 a lameness score of 0, with the IM 1.0mg/kg treatment (33.8%) less likely to achieve this score

than the control treatment (52.9%). Whilst there was a trend for a significant treatment effect, pair-wise comparison revealed no differences between treatments were significant.

Pharmacokinetic analysis

The mean serum concentrations versus time for meloxicam administered IM at 1.0mg/kg or S/C at 1.0mg/kg were graphically displayed (n=3) (Figure 3). The mean Cmax for meloxicam administered IM 1.0mg/kg (n=3) was 10.62 ± 1.56 µg/mL and was achieved at a mean Tmax of 1.17 ± 0.76 h. The mean Cmax for meloxicam administered S/C 1.0mg/kg was 7.23 ± 0.75 µg/mL and was achieved at a mean Tmax of 4.67 ± 1.15 h. Due to time constraints calculations of pharmacokinetic parameters of IM 1.0mg/kg and S/C 1.0mg/kg were calculated from 6 sheep (Table 4). There was a significant treatment x timepoint interaction ($p < 0.001$) for the meloxicam concentrations within plasma. The IM 1.0mg/kg treatment had a significantly higher meloxicam plasma concentration when compared to S/C 1.0mg/kg at timepoints: 0.5, 1, 2 and 4 hours post drug-administration. There was no significant difference between the two treatments at any other timepoints.

Discussion

The present study was the first conducted to assess the pharmacokinetics and efficacy of meloxicam when administered intramuscularly and subcutaneously in sheep. Since pharmacokinetic data for NSAID's cannot be extrapolated between species,[1] these two routes of administration and alternative doses to current recommendations were investigated in order to determine whether more efficacious pain management is available. Analysis of study results indicated that meloxicam was partially efficacious at providing analgesia in sheep injected with oil of turpentine.

Sheep demonstrated an immediate hyper-acute response to the turpentine injection by demonstrating restricted weight bearing on the affected limb as well as lying and pawing behaviour. Agitation was evident with the affected limb being held off the ground or frequently raised, up until 12 hours post-administration, with all affected limbs demonstrating a significantly higher skin temperature, limb circumference and limb sensitivity when compared to the control limb. In several sheep, irritation persisted up until 48 hours, slightly longer than the 24 hours previously observed in literature.[18] This method has been previously used to assess the efficacy of oral administration of NSAID's including flunixin and carprofen in sheep.[19]

Skin temperature and limb circumference were selected as measurement points as both are indicators of inflammation and presumably would emphasize the anti-inflammatory action of meloxicam on oedema and vasodilation.[10,18] Both were significantly affected by meloxicam administration, however proved ineffective at evaluating the efficacy of alternative treatments against the control, with zero treatment variations observed until 12 hours after drug administration. Similar observations were identified in previous studies where skin temperature and limb circumference did not significantly differ between NSAID treated ewes and those only administered turpentine, with weak to no effects observed.[10,18,19]

As ambient temperature had a large effect on skin temperature, wool present on the forelimb during measurements may have limited accurate measurement of indicators in this study as meloxicam has previously been observed to affect local skin temperature in horses at various doses.[10] Skin temperature increases observed from 24-48 hours in this study were likely related to a decline in meloxicam concentration in the blood as skin temperature of

turpentine affected sheep remained elevated when compared to the the control for up to 72 hours in a previous study.[18]

Studies utilising limb circumference as an assessment of the analgesic efficacy of meloxicam vary. Alternatively, meloxicam has been found to return limb circumference to that of control limbs 4 hours earlier than sheep receiving only turpentine, yet also demonstrate no effect in other studies.[10,18] This study found a slight increase in limb circumference over time, similar to literature assessing efficacy of alternative NSAID's, however only the IM 1.0mg/kg treatment differed significantly from the turpentine-only sheep at 12-24 hours post administration.[19] This may be related to the large limb circumference measurements at baseline which were approximately 2-3mm larger when compared to Colditz et al. (2011) resulting in a less marked increase over time and difficulty in assessing efficacy of alternative meloxicam treatments.

Limb sensitivity has previously been effective in assessing the anti-nociceptive action of meloxicam in sheep.[18] Technical issues with the algometer meant that data on limb sensitivity was limited to only one phase of this study and therefore data was difficult to analyse. Data was therefore converted to a binomial score whereby any sheep achieving a threshold of 10kg/f were assigned a score of 1 and those unable to achieve this threshold, assigned a score of 0. Limb sensitivity was observed to increase over time, with meloxicam treatments playing a significant role in limb desensitisation. This correlates with additional pharmacokinetic studies indicating meloxicam administered intravenously at 1.0mg/kg can alleviate pain-related signs of induced lameness.[1] At 6 hours post-administration, limbs were significantly less sensitive in all meloxicam treated groups when compared to the control. From 8-10 hours, all treatments were significantly similar, however meloxicam treatments: S/C

315 1.0mg/kg, IM 2.0mg/kg and S/C 2.0mg/kg were again associated with decreased limb
316 sensitivity when compared to the control treatment at 12 hours. This agrees with current
317 literature where limb sensitivity was increased in sheep receiving no meloxicam for pain relief,
318 confirming the anti-nociceptive effects of meloxicam previously observed.[18]

319 The anti-nociceptive action of meloxicam however has been disputed as analgesia was
320 ineffective in mulesed Merino lambs administered meloxicam subcutaneously at 0.5mg/kg.[20]
321 Paull et al. (2008) assessed gait using an ethogram, however studies utilising alternative
322 methods for assessing gait, such as the numerical scoring used in this study, achieved similar
323 results. It was observed that in the administration of alternative NSAID's including flunixin
324 and carprofen, analgesic efficacy could not be determined,[19] indicating that methodology
325 likely does not play a role in these findings. Comparisons between various studies indicate
326 there may be a breed difference associated with pain as studies using Merino's have had
327 limited success when using gait as a measure of efficacy. However, the study by Colditz et al.
328 (2011) utilising Merino x Romney ewes proved somewhat successful indicating treatment
329 with meloxicam was associated with a lower lameness score when compared to the control
330 at 8 and 24 hours post-administration. This notion of pain disparity has been previously
331 recognised indicating that pain responses greatly vary depending on species, sex, age, body
332 size and even between individual animals as it is a multifaceted experience affected by a
333 variety of physical and non-physical factors such as previous experience and actual tissue
334 damage.[19,21,22]

335 Whilst trends were observed in gait score, this study found no significant differences between
336 treatments, similar to previous studies studying Merino's. Lameness has previously been
337 observed to peak at 24 hours post-administration of turpentine,[18] however this study

observed the opposite, with all treatments demonstrating a high likelihood towards a low lameness score when compared to the control . Pharmacokinetic analysis in this study assists in identifying the cause of the lameness peak at 24 hours previously observed in literature. The observed peak correlates to a marked decrease in meloxicam plasma concentrations between 12 to 24 hours after administration of turpentine.

The pharmacokinetic data obtained in this study indicate that intramuscular administration at 1.0mg/kg results in higher meloxicam plasma concentration over the first 4 hours post-administration, has a longer elimination half-life and achieves pain relief more rapidly than subcutaneous at 1.0mg/kg. Time constraints limited this study and the additional treatments: IM 2.0mg/kg, S/C 2.0mg/kg and the control were not analysed. When compared to previous studies, meloxicam plasma concentrations were higher after subcutaneous (7.23 µg/mL) and intramuscular (10.62 µg/mL) administration at 1.0mg/kg than oral (1.72 µg/mL) administration at 0.99mg/kg and intravenous administration (4.25 µg/mL) at 0.5mg/kg in sheep.[1,4]

Whilst intravenous doses in sheep have only been administered at 0.5mg/kg, literature indicates that meloxicam action is independent of dose and thus comparability is possible.[1,23] Two studies have previously investigated the pharmacokinetics of intravenous administration of meloxicam at 0.5kg/mg, both varying in terms of the elimination half-life achieved. Shukla et al. (2007) reported an elimination half-life of 10.85 hours, whereas Stock et al. (2013) reported a far more superior half-life of 14.0 hours, which exceeds that of both intramuscular (12.47h) and subcutaneous administration (10.24h) achieved in this study at half the dose. This disparity may once again be a result of breed and sex difference with one study examining Dorset cross males/females and the other crossbreed female sheep.[1,4] Comparison with

previous literature indicates that both subcutaneous and intramuscular administration of meloxicam are more effective than alternative methods and could be recommended for use in sheep. However, further research of pharmacokinetics in sheep may need to account for potential breed and sex differences observed across various studies.

Whilst improved pharmacokinetic parameters were observed for intramuscular administration when compared to subcutaneous at 1.0mg/kg, plasma concentration of meloxicam was only significantly higher in intramuscular administration over the first 4 hours of inflammation. This may suggest that whilst an extended half-life and increased maximum plasma concentrations were observed, the two treatments are equally as efficacious, with intramuscular more effective at providing short-term pain relief only. Therefore, the risks and challenges associated with on-farm administration and pain management associated with intramuscular administration of NSAID's should be considered. Intramuscular administration leads to an increase in serum creatine kinase (CK) activity resulting in potential myonecrosis and muscle damage in livestock, thus requiring precise technique for injection and on-label use in regards to dosage requirements on-farm. [24-26] This may prove difficult in on-farm settings where large numbers of sheep require pain management for husbandry procedures such as tail docking and castration. Further studies are required to ensure the safety of intramuscular administration of meloxicam and to determine any toxicity risks associated with double doses at 2.0mg/kg.

Conclusion

This study demonstrates that meloxicam is effective at providing some analgesia after injection of turpentine into the limb in sheep. Whilst some knowledge of analgesic efficacy according to dosage and administration route over time were obtained, this study could not

significantly distinguish analgesic efficacy of meloxicam between treatments in sheep. Further research is required before alternative routes and doses of meloxicam should be registered for use in sheep.

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Figures

Table 1. Randomised crossover design experimental treatment groups

Week 1			Week 2	
Group	**Route of administration**	**Dose**	**Route of administration**	**Dose**
1	Subcutaneous	1.0mg/kg	Intramuscular	2.0mg/kg
2	Intramuscular	1.0mg/kg	Subcutaneous	2.0mg/kg
3	Subcutaneous	2.0mg/kg	Control	
4	Intramuscular	2.0mg/kg	Subcutaneous	1.0mg/kg
5	Control (no meloxicam)		Intramuscular	1.0mg/kg

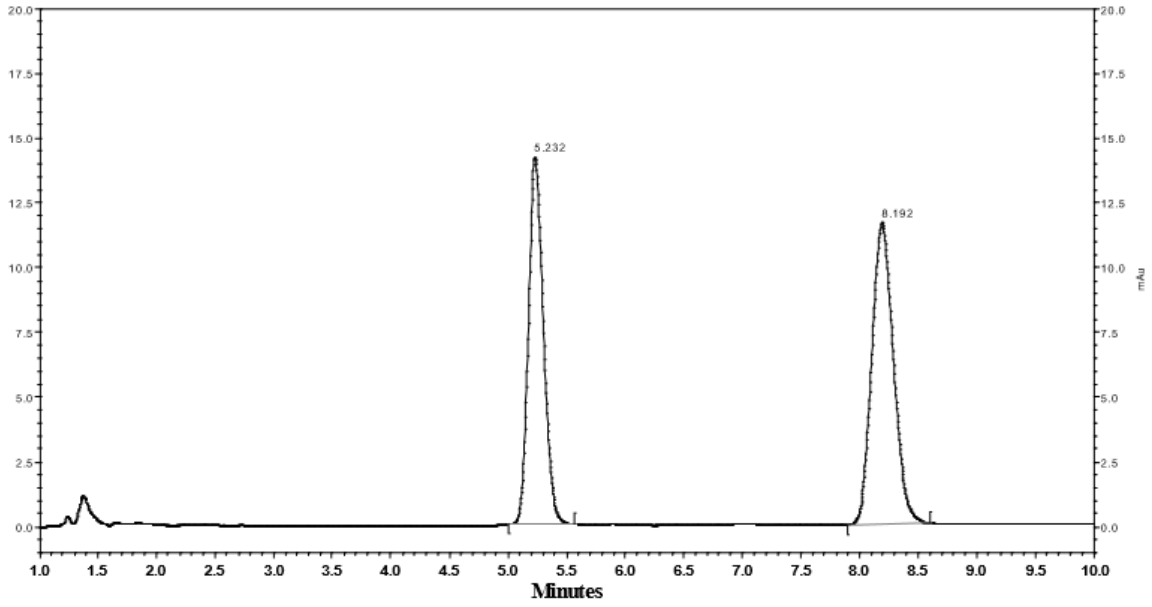


Figure 1. Chromatogram representing drug observation time from HPLC analysis of blank plasma spike with meloxicam (8.5 minutes) and IS (5.5 minutes).

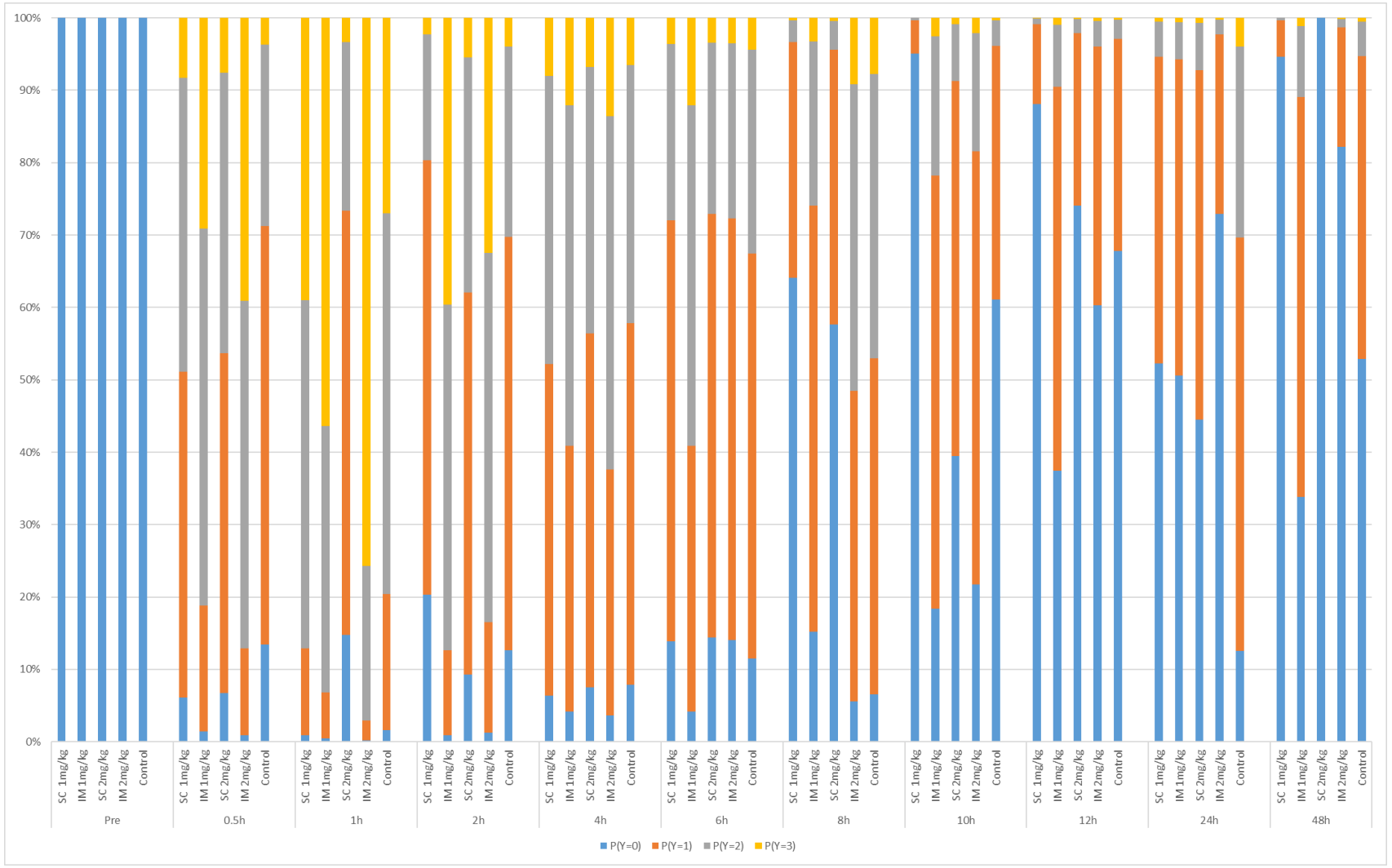
Table 2. NRS used to score behavioural characteristics associated with lameness in sheep injected with oil of turpentine and meloxicam.

Score	Associated behaviour
0	Even distribution across all limbs with no abnormality in gait
1	Mild favouring of limbs, however all limbs used to work
2	Some limping, however all limbs used when waling with reluctance to place limb on ground
3	Severe abnormality of gait demonstrated by limited weight bearing on affected limb, increased lying behaviour and pawing affected limb

Table 3. Predicted means±SEM of skin temperature, limb circumference and limb sensitivity (1.0 = no sensitivity, 0 = highly sensitive) across meloxicam treatment groups and timepoint.

Treatment vs. Timepoint	**0**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**24**	**48**
Skin temperature (˚C)											
S/C 1.0mg/kg	19.03±0.94$_{A}$	22.86±0.94$_{B}$	23.93±0.94	24.78±0.94	25.93±0.94$_{A}$	21.91±0.94$_{B}$	23.64±0.94	22.44±0.94	21.50±0.94$_{a}$	18.35±0.94$_{a}$	25.16±0.94
IM 1.0mg/kg	18.8±0.947$_{A}$	22.39±0.94$_{B}$	22.57±0.94	24.48±0.94$_{A}$	28.61±0.94$_{B}$	22.30±0.94$_{A}$	24.82±0.94	22.88±0.94	23.11±0.94$_{ab}$	22.25±0.94$_{ab}$	23.90±0.94
SC 2.0mg/kg	21.03±0.94	22.32±0.94	23.9±0.94	25.76±0.94	26.12±0.94$_{A}$	22.03±0.94$_{B}$	24.62±0.94	22.72±0.94	23.06±0.94$_{ab}$	21.05±0.94$_{Aab}$	24.67 ±0.94$_{B}$
IM 2.0mg/kg	19.08±0.94	22.32±0.94	22.16±0.94	23.55±0.94	25.50±0.94$_{A}$	21.79±0.94$_{B}$	23.07±0.94	21.94±0.94	21.81±0.94$_{a}$	22.45±0.94$_{b}$	24.59±0.94
Control	19.28±1.15$_{A}$	23.97±1.15$_{B}$	22.46±1.15	24.73±1.15	27.64±1.15$_{A}$	23.87±1.15$_{B}$	24.90±1.15	23.87±1.15	25.25±1.15$_{b}$	22.11±1.15$_{Ab}$	25.97±1.15$_{B}$
Limb circumference (mm)											
S/C 1.0mg/kg	13.68±0.31	14.19±0.31$_{ab}$	13.13±0.31$_{A}$	14.12±0.31$_{B}$	14.14±0.31	14.11±0.31	14.14±0.31	14.23±0.31$_{ab}$	13.99±0.31$_{ab}$	13.85±0.31	14.02±0.31
IM 1.0mg/kg	13.53±0.31	13.78±0.31$_{ab}$	12.92±0.31	13.53±0.31	13.97±0.31	13.63±0.31	13.56±0.31	13.70±0.31$_{a}$	13.39±0.31$_{a}$	13.79±0.31	13.90±0.31
SC 2.0mg/kg	13.68±0.31	13.43±0.31$_{a}$	13.56±0.31	13.98±0.31	13.81±0.31	13.86±0.31	13.91±0.31	14.04±0.31$_{ab}$	13.76±0.31$_{ab}$	14.12±0.31	14.04±0.31
IM 2.0mg/kg	14.17±0.31	14.52±0.31$_{b}$	13.74±0.31	14.09±0.31	14.08±0.31	14.12±0.31	14.34±0.31	14.28±0.31$_{ab}$	14.07±0.31$_{ab}$	14.43±0.31	14.65±0.31
Control	13.96±0.42	13.69±0.38$_{ab}$	13.30±0.38	14.05±0.38	14.25±0.38	14.06±0.38	14.39±0.38	14.61±0.38$_{b}$	14.30±0.38$_{b}$	14.43±0.38	14.36±0.38
Limb sensitivity (%)											
S/C 1.0mg/kg	1.0±0.18	0.83±0.18$_{abc}$	0.83±0.18	1.0±0.18	0.83±0.18	0.67±0.18$_{ac}$	0.67±0.18	0.83±0.18	1.0±0.18$_{ac}$	0.67±0.18	0.83±0.18$_{a}$
IM 1.0mg/kg	1.0±0.18	1.0±0.18$_{ad}$	1.0±0.18	1.0±0.18	0.67±0.18	0.5±0.18$_{a}$	0.5±0.18	0.67±0.18$_{A}$	0.17±0.18$_{Bb}$	0.5±0.18	0.17±0.18$_{c}$
SC 2.0mg/kg	1.0±0.18$_{A}$	0.5±0.18$_{Bab}$	1.0±0.18$_{A}$	0.83±0.18	0.67±0.18	1.0±0.18$_{c}$	0.83±0.18	0.5±0.18	0.83±0.18$_{ac}$	0.5±0.18	0.83±0.18$_{a}$
IM 2.0mg/kg	1.0±0.18$_{A}$	0.5±0.18$_{Bab}$	0.83±0.18	0.83±0.18	0.67±0.18	0.67±0.18$_{ac}$	0.5±0.18	0.67±0.18	0.5±0.18$_{ab}$	0.67±0.18	0.5±0.18$_{ac}$
Control	1.0±0.22	1.0±0.22$_{c}$	1.0±0.22$_{A}$	1.0±0.22$_{B}$	0.5±0.22$_{A}$	0±0.22$_{b}$	1.0±0.22	0.5±0.22	1.0±0.22$_{Ac}$	0±0.22$_{B}$	0±0.22$_{bc}$

*Means in a column without a common subscript (a, b, c) represent significant differences between treatments whilst means in a row without a common subscript represent significant differences between timepoints (A, B) ($p < 0.005$).



463 **Figure 2.** Probabilities of lameness score (Y = 0, 1, 2 or 3) when administered subcutaneously and intramuscularly at 1.0mg/kg and 2.0mg/kg
464 over time.

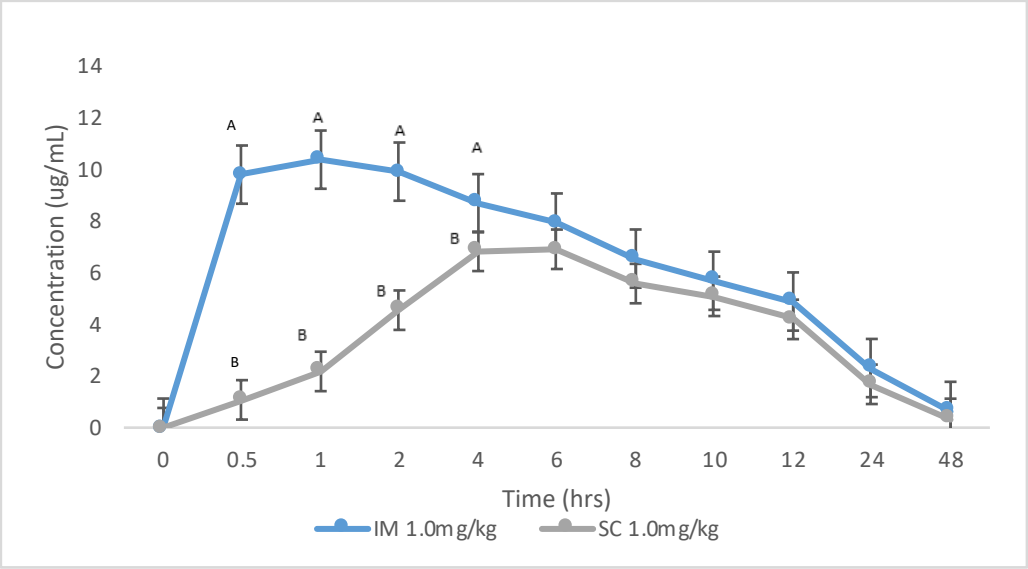


Figure 3. Concentration of meloxicam when administered subcutaneously and intramuscularly at 1.0mg/kg over time. Data points represent predicted mean ± SEM for n = 3 per group. *Subscripts (A,B) represent significant differences between treatments ($p < 0.05$).

Table 4. Mean± SD pharmacokinetic parameters of 1.0mg/kg meloxicam when administered intramuscularly and subcutaneously in sheep.

	IM 1.0mg/kg	**S/C 1.0mg/kg**
Tmax (hrs)	1.17 ± 0.76	4.67 ± 1.15
Cmax (µg/mL)	10.62 ± 1.56	7.23 ± 0.75
AUC0-t (µg/h/mL^{-1})	168.95	121.76
$t_{1/2}$ (hrs)	12.47	10.24
kel	0.0556	0.0677

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