Journal of the American Association for Laboratory Animal Science
Copyright 2017
by the American Association for Laboratory Animal Science

Vol 56, No 6
November 2017
Pages 762–767

Plasma Concentration of Meloxicam in Pediatric Rats

Kristina A Pugh,[1,*] Kyle J Reitnauer,[2] Robyn B Lee,[3] William L Wilkins,[4] John H McDonough,[5] M Ross Pennington,[6] and Samantha R Litvin[6]

In this study, we compared the plasma concentrations of meloxicam in pediatric rat pups (ages: 7, 14, 21, and 28 d) with those of young adult rats. Adult rats received 1.34 mg/kg SC meloxicam to determine the target peak plasma concentration (C_{max}) for comparison with the pediatric animals. Pediatric rats received 1.34 mg/kg SC meloxicam, and in all age groups, C_{max} met or exceeded that in adults (11.5 ± 2.7 μg/mL). Plasma concentrations were similar between male and female pups within age groups, and peak plasma concentration was achieved more rapidly in rat pups than adults. The analgesic efficacy of this dose was not evaluated in this study.

Abbreviations: C_{max}, peak plasma concentration; PND, post-natal day; T_{max}, time to peak plasma concentration

When working with pediatric animals, laboratory animal clinicians often extrapolate drug doses of pharmaceuticals based on adult dosages or pediatric dose recommendations from other domestic animal species. Subjective interpretation of efficacy and safety is frequently necessary because doses specifically for pediatric or neonatal animals are unpublished or unknown. Despite the lack of data available, pediatric studies provide important and relevant data to the medical pharmacology and toxicology research industry, and pediatric animals require treatment and pain control just as do adult animals. Analgesia concepts applied to adults, such as dose, schedule, and administration methods, often need to be adjusted or modified to be suitable for pediatrics, with the primary difficulty being the identification of a balance between safety and effectiveness.[13,16] As veterinary medicine pain management has moved to a multimodal approach, NSAID have played an important role in analgesia plans.[17] Meloxicam is a commonly used NSAID in veterinary medicine and in the laboratory animal field. This drug is a preferential inhibitor of cyclooxygenase 2, thus blocking prostaglandin synthesis and leading to antiinflammatory effects[12,13,28,39] with fewer ulcerogenic properties than nonselective NSAID.[5,6,10-13,15,20,25-28,40] In rats, meloxicam is traditionally administered subcutaneously or orally once daily for postoperative, musculoskeletal, or osteoarthritis pain.[6,12,13,33] To assist investigators using meloxicam for postoperative pain control in pediatric rats, we wanted to determine whether a common adult dosage of meloxicam in pediatric rats would meet or exceed the plasma concentration in adults.

Pediatric and neonatal patients have immature organ function and rapid metabolism, compared with adults.[13,18,34] Meloxicam undergoes extensive enterohepatic recirculation and hepatic metabolism.[5,20,24,36] All of the meloxicam metabolites are inactive, and both the unchanged drug and the metabolic products are excreted in the feces and urine.[20,30,31,36] Administering meloxicam to young rats that may not have mature hepatic or renal functionality may result in deleterious effects on the organs.[36] To be safe, we selected a low dose to begin the experiments.

A therapeutic index for subcutaneous meloxicam in rats has not been published, and the pharmacokinetic data that are available still require extrapolation of dosage information for application to an investigator's particular needs.[1,9-11,15,24,27,30,31,36] Recent literature[29,32] has prompted questions regarding the efficacy of NSAID at standard dosages for postoperative pain in rodents, but for the current study, we presumed that the peak plasma concentration (C_{max}) achieved in adults is a therapeutic level. The purpose of our study was to determine a dose of meloxicam for use in pediatric rats that met or exceeded the plasma concentration in adult rats given a common dosage. The experimental strategy was to determine the C_{max} of meloxicam in adult rats (postnatal day [PND] 70) by using standard published dosages of meloxicam (1.0 to 2.0 mg/kg).[6] Once the C_{max} for adult rats was known, we grouped pediatric animals by age (7, 14, 21, and 28 d) and gave them the same dose as used in adult rats to determine C_{max} in the younger animals. We expected that the C_{max} of meloxicam in pediatric rats would be similar to the adults' when given the same dosage.

Materials and Methods

Animals and general husbandry. All procedures were performed in accordance with protocols approved by the US Army Medical Research Institute of Chemical Defense IACUC, and animals were treated in accordance with the *Guide for the Care and Use of Laboratory Animals*.[10]

The US Army Medical Research Institute of Chemical Defense is fully AAALAC-accredited. All animals received species-appropriate environmental enrichment and were socially housed in IVC (Allentown Caging, Allentown, NJ) on corncob bedding (The Andersons Lab Bedding Products, Maumee, OH). Filtered municipal water and a commercial diet (Laboratory Rodent Diet 5001, LabDiet, St Louis, MO) were provided without restriction, and food enrichment consisting of Fruit Crunchies and Bacon Softies (BioServ, Flemington, NJ) were offered on a scheduled rotation. Rooms were maintained on a 12:12-h dark:light cycle at 68 to 79 °F (20.0 to 26.1 °C) and 30% to 70% relative humidity.

Received: 12 Apr 2017. Revision requested: 31 May 2017. Accepted: 30 Aug 2017.
[1]Veterinary Medicine and Surgery Branch, [2]Laboratory Services Branch, [3]Institutional Animal Care and Use Office, [4]Comparative Pathology Branch, [5]Nerve Agent Countermeasures, Neuroscience Branch, and [6]Analytical Toxicology Division, United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland.
[*]Corresponding author. Email: kristina.a.pugh.mil@mail.mil

Sentinel animals were free of Sendai virus, pneumonia virus of mice, rat coronavirus, Kilham rat virus, Toolan H1 virus, reovirus type 3, *Mycoplasma pulmonis*, rat parvovirus, *Helicobacter* spp., lymphocytic choriomeningitis virus, murine adenovirus, and endo- and ectoparasites.

Methods and procedures. Young adult (PND70), male ($n = 18$) and female ($n = 17$) Sprague–Dawley (CRL:CD(SD)) rats (*Rattus norvegicus*; Charles River, Raleigh, NC) received meloxicam (Boehringer Ingelheim Vetmedica, Duluth, GA) at 1.34 mg/kg SC. Timed-pregnant female rats ($n = 12$; 1 to 3 wk pregnant, Charles River) were the source of the pediatric animals used in this project. Pups were sexed at 3 to 5 d of age and tattooed for identification. After processing several litters, pups were tattooed only when they were going to be used for the PND7 or PND14 experiments. On the day of experimentation, all pups were identified on their feet, tails, or backs by using a temporary nontoxic marker. When not being medicated or having blood collected, all 7- or 14-d-old pups were placed with the dam for nutritional and thermal support. All other animals were group housed, provided thermal support with water blankets (model TP500, Gaymar Industries, Orchard Park, NY), and offered DietGel Recovery or 31M (ClearH2O, Westbrook, ME) between blood collections. Rats were weaned at 18 to 21 d of age and group-housed by sex. At study conclusion, all rat pups (PND7 to PND28) were euthanized by means of decapitation (terminal blood collection), and adult animals (PND70) were euthanized by using intraperitoneal injection of sodium pentobarbital (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI) after isoflurane anesthesia. Unused pups, postpartum dams, and adults that did not provide terminal blood samples were transferred to other IACUC-approved protocols.

Dosing procedure. Meloxicam (dose, 1.34 mg/kg) was administered subcutaneously as close to the interscapular region as possible. When dilution was necessary to produce an adequate injection volume, sterile 0.9% NaCl was used. The meloxicam concentration for adult rats was 1.25 mg/mL, PND28 rats received 1.0 mg/mL, and the remaining age groups received 0.5 mg/mL. All dilutions were prepared immediately before use by using aseptic techniques. Low dead-space syringes (Normject, 1.0 mL) were used to eliminate the effect of hub loss. Needle sizes were 25- to 31-gauge, depending on the size of the animal.

Blood sampling and processing. All animals were chemically restrained by using isoflurane gas anesthesia to facilitate safe and timely serial blood sampling or decapitation. The lateral tail veins and ventral tail artery were used for blood collection in PND70 and PND28 rats (one sample obtained from submandibular vein). A warm water bath or contact with a warming pad was used to improve blood circulation and vessel dilation prior to blood collection. Whole blood (0.5 mL) was collected by using sodium heparinized syringes and needles, or directly into a blood tube when rats were decapitated. The maximal volume of blood collected per animal was set at 1% of the body weight over a 24-h period. Because all animals were scheduled for euthanasia after their final blood collection, the 1% limit could be exceeded on the terminal blood draw when needed. Samples were placed on a rocker until they could be centrifuged, but never longer than 180 min after collection. Samples were centrifuged at 2415 × *g* for 15 min. Plasma was removed, placed in new collection tubes, and immediately frozen at –80 °C.

Experimental design. Blood samples were collected from the adult rats at 0, 5, 10, 15, and 30 min and 1, 1.5, 2, 2.5, 3, 6, 12, and 24 h, with a minimum of 4 samples collected at each time point. Blood sample collection times were calculated from the time of meloxicam administration. At estimated weights of 200 g for females and 300 g for males, 5 to 7 blood collections per adult rat were expected over the 24-h period. The sequential blood collection time points for each rat were based on maximizing the rest period between sample collections or rapidly repeated blood sampling, ending with a terminal blood collection so the animal remained under isoflurane anesthesia for the duration (0-, 5-, 10-, and 15-min time points). In light of reviews of similar research in rats and other species, C_{max} was expected to occur 1 to 3 h after injection.[3,4,5,7,8,12,14,19,21-24,31,35,37]

Current dosing instructions for meloxicam in adult rats are once every 24 h,[6] so the 24-h time point was used to determine the mean plasma concentration of meloxicam at the prescribed redosing point, providing additional information to compare between age groups. Once the mean C_{max} was determined in adult rats, it became the target C_{max} for all other age groups. If the C_{max} in a pediatric group was significantly lower than the target C_{max}, the experiment was repeated for that age group with higher doses of meloxicam until the target C_{max} was achieved. If the mean C_{max} was higher than the target C_{max} in any of the pediatric age groups, the experiment was not repeated because the focus of this study was to determine a dose that would meet or exceed the adult C_{max}. Male and female plasma concentration data were analyzed separately to determine whether a sex difference existed. The time to achieve the peak plasma concentration (T_{max}) in each group was used to eliminate or adjust time points before proceeding to the next age group. It was also for this reason that after the adults, experiments were conducted in order of descending age: PND 28, 21, 14, and 7.

Meloxicam assay. Assay validation was completed by using heparinized rat plasma (BioreclamationIVT, Chestertown, MD) to prepare calibration curves and quality-control samples. Plasma was spiked with meloxicam (4000 ng/mL; USP Reference Standard, USP, Rockville, MD) and serially diluted to produce 6 calibrators at 0.0 to 1000 ng/mL. All calibrators were spiked with 40 ng/mL meloxicam-D (deuterated meloxicam; Sigma-Aldrich, St Louis, MO). Meloxicam-D served as the internal standard for this study. The assay was validated according to the FDA guidelines regarding bioanalytical method development.[38] Calibration curves were generated in duplicate and analyzed in triplicate, and a total of 6 calibration curves were used (5 interday and 1 intraday). Quality-control samples were prepared at 900, 180, 18 and 1.8 ng/mL meloxicam and spiked to 15 ng/mL meloxicam-D. Quality-control samples were used to determine intra- and interday variability. Quantification of the quality-control samples was accomplished by running a calibration on each day. A linear least-squares analysis with a 1/y weighting scheme was used to calculate the values for the calibration curve and quality-control samples. Precision (%CV) was calculated by using the formula %CV = (SD/mean) × 100%, and accuracy (% error) was calculated using the formula % error = [(calculated concentration – actual concentration)/actual concentration] × 100%. The %CV for precision and % error for accuracy were below 15% for all validation and quality-control samples.

Plasma for calibrators, quality-control samples, and animal samples were thawed before use. From all animal samples, 5.0 µL was removed and added to 195 µL heparinized plasma. Then 40 µL of meloxicam-D (450 ng/mL) was added to animal samples, calibrators, and quality-control samples, for a final concentration of 40 ng/mL meloxicam-D. Finally, 300 µL 1.0 M acetic acid was added to all samples in preparation for solid-phase extraction.

Solid-phase extraction was performed by using Oasis 1-mL HLB cartridges containing 30 mg sorbent (Waters, Milford, MA).

Each cartridge was prepared by adding 2.0 mL of methanol followed by 2.0 mL of 1.0 M acetic acid. Calibrators, quality-control samples, and 450 µL of animal samples were loaded into the cartridges, followed by a wash with 1.0 mL of 1.0 M acetic acid. Waste tubes were replaced by 15-mL tubes (Falcon, BD Biosciences, Bedford, MA), and the samples were extracted with 2.0 mL of 5% (v/v) ammonia hydroxide in methanol. The eluent was collected in polystyrene tubes and evaporated under a dry nitrogen stream at 40 °C. Samples were reconstituted in 90 µL of 60% acetonitrile, 40% water containing 0.2% formic acid and 10 mM ammonium formate. Solid-phase extraction was performed in duplicate, and the replicates were analyzed by using liquid chromatography-tandem mass spectrometry in triplicate.

Liquid chromatography was performed using a model 1290 Infinity liquid chromatograph (Agilent Technologies, Santa Clara, CA). Separation was performed on a Halo C18 column (2.7 µm, 2.1 mm × 50 mm; Advanced Materials Technology, Wilmington, DE) with an isocratic mobile phase of 60% acetonitrile, 40% water containing 0.2% formic acid and 10 mM ammonium formate at a flow rate of 300 µL/min and an injection volume of 5 µL.

Tandem mass spectrometry was completed by using a model 6500 QTrap triple quadrupole mass spectrometer (Sciex, Ottawa, Ontario, Canada) in positive electrospray mode and with multiple reaction monitoring. The ion source temperature was 700 °C. Capillary voltage was +5500 V, curtain gas setting was 30, and the collision assisted dissociation gas was medium. Ion source gases 1 and 2 were set at 50 and 75, respectively. The declustering potential was 50 V, and the entrance potential was 10 V. The quantifier ion transition was 352.2 to 115.1 Da with a collision energy of 23 eV and collision exit potential of 15 V. The qualifier ion transition was 352.2 to 140.9 Da, with collision energy of 30 eV and collision exit potential of 20 V. The meloxicam-D ion transition was 355 to 115.1 Da with a collision energy of 23 eV and collision exit potential of 14 V. Peak areas were integrated by using Analyst software (Sciex).

Statistical methods. For each age group and both sexes, the mean plasma concentrations at each time point were calculated. Within each age group, plasma concentrations were compared between sexes and across time points by using 2-factor ANOVA. When data did not differ significantly between sexes, male and female data were combined and used for further analyses comparing age groups. The mean, SD, and 95% confidence interval for C_{max} and T_{max} in adult rats were determined. The mean meloxicam concentration was calculated for every time point in each age group; the meloxicam C_{max} and T_{max} were then determined for each age group. The Bartlett test for equal variances was performed to determine the appropriateness of using ANOVA to compare age groups. ANOVA followed by a Dunnett test was used to determine significant differences in C_{max} between adult rats and all other age groups. For this analysis, individual meloxicam plasma concentrations at T_{max} were used rather than the calculated C_{max} means, to obtain the most accurate analysis. Prism version 5.04 for Windows (GraphPad Software, San Diego, CA) was used to perform all statistical tests. For all comparisons, statistical significance was defined as a P value less than 0.05. Prior to experimentation, a sample size of 8 rats (4 males, 4 females) was determined to be sufficient to detect a 50% change from the estimated mean C_{max}, with a coefficient of variation of 33% at an α level of 0.05 and 80% power.

Histologic analysis. Livers and kidneys were sampled from a minimum of 10% of the animals in each pediatric group, with representation from males and females. Samples were processed by using an automated vacuum tissue processor (model ASP300S, Leica Biosystems, Buffalo Grove, IL). Tissue was dehydrated through a graded series of ethanol, and cleared by using Micro-Clear (Micron Environmental Industries, Alexandria, VA) followed by infiltration with Richard–Allan Scientific type 9 paraffin (ThermoFisher Scientific, Austin, TX). Specimens then were embedded and sectioned on a rotary microtome (model RM2255, Leica Biosystems). Routine staining was completed by using a multi-stainer (model ST5020, Leica Biosystems). Slides were stained with Mayer hematoxylin (MHS128, Sigma-Aldrich) and eosin Y with phloxine (HT110332, Sigma-Aldrich).

Liver and kidney sections were examined and graded for signs of acute toxicity using a model BX45 light microscope with 10× magnification eye pieces and 2×, 4×, 10×, 20×, 40×, and 60× magnification objectives (Olympus, Waltham, MA). The condenser was set to bright-field illumination.

Results

C_{max} and T_{max} of meloxicam in adult rats The mean C_{max} of meloxicam was 11.5 ± 2.7 µg/mL at 60 min after a single subcutaneous injection of 1.34 mg/kg meloxicam; C_{max} did not differ between male and female rats (Figure 1), so the data were combined and used for further analyses comparing age groups. At 24 h after injection, female rats had a mean plasma concentration of 6.9 µg/mL, with male rats much lower at 3.3 µg/mL. Initially the experiment was conducted with 15 min as the first time point; however, earlier time points were added to verify that 60 min was T_{max} and that no plasma peaks occurred closer to the injection time. The male and female rats had similar plasma concentrations up until 120 min after injection (Figure 1), so the data were combined for the 0-, 5-, and 10-min blood collections to reduce the number of animals needed. The number of animals sampled at each time point (Table 1), but the minimum of 4, necessary for statistical significance, was maintained for all time points.

C_{max} and T_{max} in pediatric rats PND28 rats had a mean C_{max} of 13.7 ± 2.6 µg/mL at 30 min after injection. PND21, 14, and 7 rats had C_{max} of 16.5 ± 4.3, 16.2 ± 3.3, and 17.0 ± 5.1 µg/mL, respectively, at 15 min after injection. C_{max} differed significantly between adults and PND21 ($P < 0.01$), PND14 ($P < 0.05$), and PND7 ($P < 0.01$) age groups but not PND28 (Figure 2). Because mean plasma concentration did not differ between sexes in any age group, the data were combined for further analyses. Figure 3 displays the combined mean concentration data across time points for all age groups. All pediatric age groups met or exceeded the adult C_{max}, but the C_{max} and T_{max} did not trend consistently between males and females (Table 2). At 24 h after injection, the mean plasma concentration of meloxicam in the pediatric age groups exceeded that of the adult rats (Figure 3). The 150-, 180-, and 360-min time points for the pediatric age groups had little clinical significance and were only needed to establish the T_{max} in the adult group. Additional time points were omitted for the PND21, PND14, and PND7 groups because the T_{max} occurred so quickly after injection in the PND28 group that it made some of the later time points irrelevant to the objectives of this study (Figure 3). A 24-h time point was obtained for all age groups, to enable comparison of meloxicam plasma concentration at the standard recommended redosing schedule.

Discussion

Several studies in other species have reported differences in meloxicam metabolism between males and females,[2,8,11,37] but our data showed no significant differences in plasma concentration between sexes across time. Overall, C_{max} was higher in

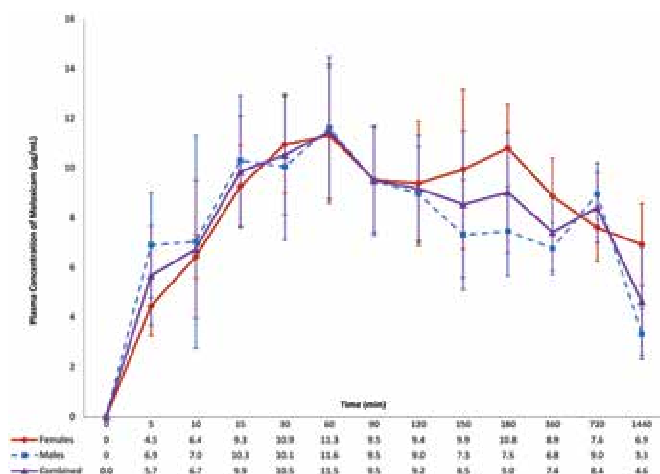

Figure 1. Mean plasma concentration of meloxicam at each time point for male, female, and all adult (PND70) rats.

Table 1. Number of adult (PND70) rats at each time point

Time (min)	Male	Female	Total
0	3	3	6
5	2	2	4
10	3	3	6
15	8	6	14
30	8	9	17
60	10	11	21
90	11	10	21
120	8	10	18
150	8	9	17
180	8	9	17
360	9	6	15
720	6	6	12
1440	7	6	13

The groups for the 0-, 5-, and 10-min time points contained fewer animals because they were added after we decided to combine data from male and female rats. Each group needed to contain at least 4 animals for sufficient statistical power.

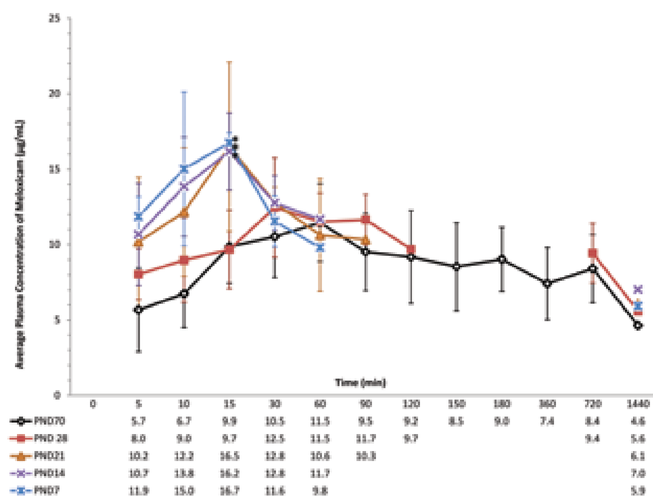

Figure 2. Mean plasma concentration of meloxicam at each time point in pediatric rats. Blanks indicate that data were not collected. C_{max} for the 21-, 14-, and 7-d-old rats differed significantly (*; $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively) from that of adult rats.

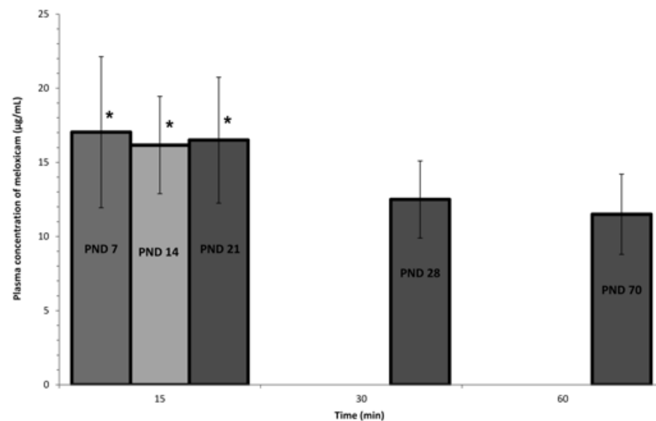

Figure 3. Comparison of C_{max} (with error bars) and T_{max} for each age group. *, C_{max} significantly different from that for PND70 rats (PND21, $P < 0.01$; PND14, $P < 0.05$; and PND7, $P < 0.01$).

younger than adult animals (Figures 2 and 3), but the difference was not significant between adult and PND28 rats. In addition, age and C_{max}/T_{max} did not show a consistent pattern among the pediatric rats, such that the younger the animal, the higher the C_{max} or shorter the T_{max} (Table 2). A complete pharmacokinetic analysis needs to be performed to further characterize the relationship between age, C_{max}, and T_{max} in pediatric rats.

We selected 1.34 mg/kg as the dosage for this study because it was at the lower end of the typically prescribed dosage range (1.0 to 2.0 mg/kg)[6] for adult rats and allowed for simple dilution calculations. Even with diluted meloxicam, the volumes administered were small (0.03 to 0.15 mL), so that any drug loss (on the tip of the needle, in the hub, or on the skin) noted after injection was considered sufficient to alter results. When such loss occurred ($n = 3$), the rats were moved into the next age group, thus ensuring that the washout period from the previous injection was at least 1 wk.

We used light microscopy to screen formalin-fixed livers and kidneys for signs of acute toxicity, but most of our samples ($n = 34$) were from early time points (that is, 5 to 120 min after injection), making it unlikely that degeneration or necrosis within cells would be evident histologically.[22] However, 5 samples acquired from the PND21 age group at the 24-h time point showed no signs of acute toxicity. Characteristics of acute hepatoxicity include necrosis and degeneration of hepatocytes and cannicular epithelium, cholestasis, and hepatic lipidosis. Characteristics of acute renal toxicity include tubular epithelial necrosis and degeneration, glomerular damage, interstitial nephritis, and crystalluria. None of these changes were identified in any samples. Investigators at our institution have used a single subcutaneous injection of 1.0 mg/kg meloxicam postoperatively in hundreds of pediatric rats (PND21 and PND28) with no overt signs of toxicity or postoperative pain while being monitored for 3 to 5 d after injection. Although this evidence is anecdotal only and does not replace a complete safety profile of meloxicam in pediatric rats, our experience may reassure readers that are contemplating the use of this dose in their pediatric rats.

We found that PND21 rat pups that were weaned at 18 d old showed a surprisingly low body weight (average, 37 g; $n = 50$), weighing only slightly more than the PND14 cohort (average 28 g). The original decision to wean at 18 d was to remain consistent with current weaning strategies used in pediatric rat studies at our facility. However, after comparing the weights of the 2 age

Table 2. C_{max} (µg/mL; mean ± 1 SD) and T_{max} values from male and female rats in each age group

Age group	No. of samples obtained/ total no. possible per time point	Male		Female		Combined
		C_{max}	T_{max}	C_{max}	T_{max}	C_{max}
PND70	10/11	11.6 ± 2.8	60	11.3 ± 2.7	60	11.5 ± 2.7
PND28	4/4	14.7 ± 1.1	30	11.4 ± 4.3	90	12.5 ± 2.6
PND21	4/6	14.2 ± 2.4	15	18.0 ± 4.3	15	16.5 ± 4.3[a]
PND14	4/4	14.9 ± 4.5	10	17.7 ± 2.6	15	16.2 ± 3.3[a]
PND7	3/3	18.0 ± 5.4	15	16.0 ± 5.7	15	17.0 ± 5.1[a]

[a]Value significantly ($P < 0.05$) different from that for PND70 rats.

groups, we left the remainder of the PND21 rats in the study (n = 6) with the dam until the time of testing (an additional 3 d) to improve body weight (average, 46 g), blood volume, and hydration status. Statistical analysis was not performed on these data.

To provide the best clinical care for animals, it is ideal to know pharmacokinetic, efficacy, and safety data for the species and age of the animal being treated. Our study used various pharmacokinetic parameters to determine that 1.34 mg/kg SC reached a comparable plasma level in pediatric rats as in adults. The Institute for Laboratory Animal Research has indicated that PND21 rats show a mature response to analgesics and cites other references to indicate that analgesic efficacy studies have been attempted on rat pups younger than 21 d but are more difficult to interpret because their responses to painful or noxious stimuli are generalized and unorganized.[17] Studies such as the evaluation of the analgesic efficacy of meloxicam at 1.34 mg/kg in pediatric rats are still needed.

Acknowledgments

We thank David Juarez, Alicia Swails, Jose Leon Cruz, and Pablo Chavez-Hernandez for their technical assistance; Emylee McFarland for sharing her research laboratory knowledge; and Drs Benedict Capacio and Krinon Moccia for their mentorship and guidance. SR Litvin was supported in part by an appointment to the Research Participation Program for the US Army Medical Research and Materiel Command (MRMC) administered by the Oak Ridge Institute for Science and Education through an agreement between the US Department of Energy and US Army MRMC. The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government.

References

1. **Aghazadeh-Habashi A, Jamali F.** 2008. Pharmacokinetics of meloxicam administered as regular and fast dissolving formulations to the rat: influence of gastrointestinal dysfunction on the relative bioavailability of 2 formulations. Eur J Pharm Biopharm **70:**889–894.
2. **Bauer C, Frost P, Kirschner S.** 2014. Pharmacokinetics of 3 formulations of meloxicam in cynomolgus macaques (*Macaca fascicularis*). J Am Assoc Lab Anim Sci **53:**502–511.
3. **Burns PJ, Morrow C, Gilley RM, Papich MG.** 2010. Evaluation of pharmacokinetic–pharmacodynamic relationships for biorelease meloxicam formulations in horses. J Equine Vet Sci **30:**539–544.
4. **Busch U, Englehardt G.** 1990. Distribution of [^{14}C]-meloxicam in joints of rats with adjuvant arthritis. Drugs Exp Clin Res **16:**49–52.
5. **Busch U, Schmid J, Heinzel G, Schmaus H, Baierl J, Huber C, Roth W.** 1998. Pharmacokinetics of meloxicam in animals and the relevance to humans. Drug Metab Dispos **26:**576–584.
6. **Carpenter JW.** 2013. Exotic animal formulary, 4th ed. St Louis (MO): Elsevier Saunders.
7. **Chien KJ, Horng CT, Chao HR, Lee WP, Wang HY, Chen FA.** 2013. The influence of running exercise training on pharmacokinetics of meloxicam in rats. Life Sci J **3:**951–955.
8. **Chinnadurai SK, Messenger KM, Papich MG, Harms CA.** 2014. Meloxicam pharmacokinetics using nonlinear mixed-effects modeling in ferrets after single subcutaneous administration. J Vet Pharmacol Ther **37:**382–387.
9. **El-Awdan S, Al-Shafeey N, Salam O, El-Iraqy W, Kenawy S.** 2015. Modulation of the pharmacological properties of meloxicam by octreotide in rats. J Saudi Chem Soc **19:**123–132.
10. **Engelhardt G, Homma D, Schlegel K, Utzmann R, Schnitzler C.** 1995. Antiinflammatory, analgesic, antipyretic and related properties of meloxicam, a new nonsteroidal antiinflammatory agent with favourable gastrointestinal tolerance. Inflamm Res **44:**423–433.
11. **Engelhardt G, Bogel R, Schnitzler Chr, Utzmann R.** 1996. Meloxicam: influence on arachidonic acid metabolism. Biochem Pharmacol **51:**29–38.
12. **Federal Drug Administration.** [Internet]. 2009. Metacam package insert for dogs. NADA 141–219. [Cited 03 December 2014]. Available at: https://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/DrugLabels/UCM050397.pdf.
13. **Fish RE, Danneman PJ, Brown MJ, Karas AZ.** 2008. Anesthesia and analgesia in laboratory animals, 2nd ed. Cambridge (MA): Academic Press.
14. **Giraudel JM, Diquelou A, Laroute V, Lees P, Toutain PL.** 2005. Pharmacokinetic/pharmacodynamic modeling of NSAIDs in a model of reversible inflammation in the cat. Br J Pharmacol **146:**642–653.
15. **Han HK, Choi HK.** 2007. Improved absorption of meloxicam via salt formation with ethanolamines. Eur J Pharm Biopharm **65:**99–103.
16. **Hunter RP, Isaza R.** 2008. Concept and issues with interspecies scaling in zoological pharmacology. J Zoo Wildl Med **39:**517–526.
17. **Institute for Laboratory Animal Research.** 2003. Guidelines for the care and use of mammals in neuroscience and behavioral research. Washington (DC): National Academies Press.
18. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
19. **Kimble B, Black L, Li KM, Valtchev P, Gilchrist S, Gillett A, Higgins DP, Krockenberger MB, Govendir M.** 2013. Pharmacokinetics of meloxicam in koalas (*Phascolarctos cinereus*) after intravenous, subcutaneous and oral administration. J Vet Pharmacol Ther **36:**486–493.
20. **Kirchgessner MS.** 2006. Meloxicam. J Exotic Pet Med **15:**281–283.
21. **Kreuder AJ, Coetzee JF, Wulf LW, Schleining JA, KuKanich B, Layman LL, Plummer PJ.** 2012. Bioavailability and pharmacokinetics of oral meloxicam in llamas. BMC Vet Res **8:**1–11.
22. **Kumar V, Abbas AK, Fausto N, Aster JC.** 2010. Robbins and Cotran's pathologic basis of disease, 8th ed. Philadelphia (PA): Elsevier Saunders.
23. **Lehr T, Narbe R, Joens O, Kloft C, Staab A.** 2010. Population pharmacokinetic modeling and simulation of single and multiple dose administration of meloxicam in cats. J Vet Pharmacol Ther **33:**277–286.
24. **Miyamoto A, Aoyama T, Matsumoto Y.** 2017. The measurement of meloxicam and meloxicam metabolites in rat plasma using a high-performance liquid chromatography–ultraviolet spectrophotometry method. Chem Pharm Bull (Tokyo) **65:**121–126.
25. **Ogino K, Hatanaka K, Kawamura M, Katori M, Harada Y.** 1997. Evaluation of pharmacological profile of meloxicam as an

antiinflammatory agent, with particular reference to its relative selectivity for cyclooxygenase-2 over cyclooxygenase-1. Pharmacology **55:**44–53.

26. **Ogino K, Saito K, Osugi T, Satoh H.** 2002. Meloxicam (Mobic): a review of its pharmacological and clinical profile. Nippon Yakurigaku Zasshi **120:**391–397 [[Article in Japanese]].
27. **Pairet M, van Ryn J, Schierok H, Mauz A, Trummlitz G, Engelhardt G.** 1998. Differential inhibition of cyclooxygenases-1 and -2 by meloxicam and its 4-isomer. Inflamm Res **47:**270–276.
28. **Quesenberry KE, Carpenter JW.** 2012. Ferrets, rabbits, and rodents: clinical medicine and surgery. St Louis (MO): Elsevier Saunders.
29. **Roughan JV, Bertrand HG, Isles HM.** 2016. Meloxicam prevents COX2-mediated postsurgical inflammation but not pain following laparotomy in mice. Eur J Pain **20:**231–240.
30. **Sadariya KA, Gothi AK, Patel SD, Bhavsar SK, Thaker AM.** 2010. Pharmacokinetic interaction of moxifloxacin and meloxicam following intramuscular administration in rats. Pharma science monitor **1:**27–34.
31. **Schmid J, Busch U, Trummlitz G, Prox A, Kaschke S, Wachsmuth H.** 1995. Meloxicam: metabolic profile and biotransformation products in the rat. Xenobiotica **25:**1219–1236.
32. **Seymour TL, Adams SC, Felt SA, Jampachaisri K, Yeomans DC, Pacharinsak C.** 2016. Postoperative analgesia due to sustained-release buprenorphine, sustained-release meloxicam, and carprofen gel in a model of incisional pain in rats (*Rattus norvegicus*). J Am Assoc Lab Anim Sci **55:**300–305.
33. **Sharp P, Villano JS.** 2012. The laboratory rat, 2nd ed. Boca Raton (FL): CRC Press.
34. **Shimokata H, Kuzuya F.** 1993. [Aging, basal metabolic rate, and nutrition.]. Nippon Ronen Igakkai Zasshi **30:**572–576. [[Article in Japanese]].
35. **Toutain PL, Cester CC.** 2004. Pharmacokinetic–pharmacodynamic relationships and dose response to meloxicam in horses with induced arthritis in the right carpal joint. Am J Vet Res **65:** 1533–1541.
36. **Türck D, Roth W, Busch U.** 1996. A review of the clinical pharmacokinetics of meloxicam. Br J Rheumatol **35 Suppl 1:** 13–16.
37. **Turner PV, Chen HC, Taylor WM.** 2006. Pharmacokinetics of meloxicam in rabbits after single and repeat oral dosing. Comp Med **56:**63–67.
38. **United States Food and Drug Administration**. [Internet]. 2001. Guidance for industry: bioanalytical method validation. [Cited 04 December 2014]. Available at: https://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf.
39. **United States Food and Drug Administration.** [Internet]. 2005. Meloxicam oral suspension: BPCA summary. Efficacy supplement pediatric written request. [Cited 14 December 2014]. Available at: https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM164027.pdf
40. **Villegas I, Alarcon de la Lastra C, La Casa C, Motilva V, Martin MJ.** 2001. Effects of food intake and oxidative stress on intestinal lesions caused by meloxicam and piroxicam in rats. Eur J Pharmacol **414:**79–86.