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 Litvin6

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of efficacy and safety is frequently necessary because doses specifically for pediatric or neonatal animals are unpublished or unknown.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 anti-inflammatory effects12,13,28,39 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 osteoarthritic pain.5,12,13,33 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.7,20,30,31,36 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 literature29,32 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.
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- to 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 TP500, Gaymar Industries, Orchard Park, NY) and offered DietGel Recovery or 31M (ClearH2O, Westbrook, ME) between feeding times. When not being medicated or having blood collected, all 7- to 14-d-old pups were placed with the dam for nutritional and thermal support.

The experiment was conducted before the next age group. It was also for this reason that after the adults, experiments were conducted in new collection tubes, and immediately frozen at –80 °C for 15 min. Plasma was removed, placed in heparinized plasma (BioreclamationIVT, Chestertown, MD) and offered DietGel Recovery or 31M (ClearH2O, Westbrook, ME) between feeding times. When not being medicated or having blood collected, all 7- to 14-d-old pups were placed with the dam for nutritional and thermal support.

**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 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.

**Dosing procedure.** Meloxicam (dose, 1.34 mg/kg) was administered subcutaneously as close to the intercostal 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.

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**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 quantitative 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-d4 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 \( C_{\text{max}} \) and \( T_{\text{max}} \) in adults were determined. The mean meloxicam concentration was calculated for every time point in each age group; the meloxicam \( C_{\text{max}} \) and \( T_{\text{max}} \) 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 \( C_{\text{max}} \) between adult rats and all other age groups. For this analysis, individual meloxicam plasma concentrations at \( T_{\text{max}} \) were used rather than the calculated \( C_{\text{max}} \), 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 \( C_{\text{max}} \), 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 ASP3005, 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

\( C_{\text{max}} \) and \( T_{\text{max}} \) of meloxicam in adult rats. The mean \( C_{\text{max}} \) of meloxicam was 11.5 ± 2.7 μg/mL at 60 min after a single subcutaneous injection of 1.34 mg/kg meloxicam; \( C_{\text{max}} \) 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 \( T_{\text{max}} \) 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.

\( C_{\text{max}} \) and \( T_{\text{max}} \) in pediatric rats. PND28 rats had a mean \( C_{\text{max}} \) of 13.7 ± 2.6 μg/mL at 30 min after injection. PND21, 14, and 7 rats had \( C_{\text{max}} \) of 16.5 ± 4.3, 16.2 ± 3.3, and 17.0 ± 5.1 μg/mL, respectively, at 15 min after injection. \( C_{\text{max}} \) 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 \( C_{\text{max}} \), but the \( C_{\text{max}} \) and \( T_{\text{max}} \) 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 \( T_{\text{max}} \) in the adult group. Additional time points were omitted for the PND21, PND14, and PND7 groups because the \( T_{\text{max}} \) 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,2,8,11,30 but our data showed no significant differences in plasma concentration between sexes across time. Overall, \( C_{\text{max}} \) was higher in
Plasma concentration of meloxicam in pediatric rats

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

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

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<th>Time (min)</th>
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<th>Female</th>
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<tr>
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<tr>
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<td>7</td>
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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.

5 samples acquired from the PND21 age group at the 24-h time point showed no signs of acute toxicity. Characteristics of acute hepatic toxicity include necrosis and degeneration of hepatocytes and canicular 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, P < 0.01; PND14, P < 0.05; and PND7, P < 0.01).

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.\textsuperscript{22} However,
Table 2. Cₘₐₓ (μg/mL; mean ± 1 SD) and Tₘₐₓ values from male and female rats in each age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>Cₘₐₓ</td>
<td>Tₘₐₓ</td>
<td>Cₘₐₓ</td>
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<tr>
<td>PND70</td>
<td>11.6 ± 2.8</td>
<td>60</td>
<td>11.3 ± 2.7</td>
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<td>PND28</td>
<td>14.7 ± 1.1</td>
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<td>11.4 ± 4.3</td>
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<td>PND21</td>
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<td>PND14</td>
<td>14.9 ± 4.5</td>
<td>10</td>
<td>17.7 ± 2.6</td>
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<td>PND7</td>
<td>18.0 ± 5.4</td>
<td>15</td>
<td>16.0 ± 5.7</td>
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*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. Studies such as the evaluation of the analgesic efficacy of meloxicam at 1.34 mg/kg in pediatric rats are still needed.

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References

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


