Evaluation of Pharmacokinetic-Pharmacodynamic Relationships for BioRelease Meloxicam Formulations in Horses

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ABSTRACT

The present study was designed to evaluate the pharmacokinetic-pharmacodynamic relationships for three controlled release meloxicam formulations in horses so as to select one for continued clinical evaluation. Twelve mature research horses of various light breeds were randomly assigned to one of three treatment groups (n = 4 horses per group). Each horse received two consecutive intramuscular injections at 0 and 72 hours, containing 1,500 mg in 2 mL of one of three experimental BioRelease meloxicam formulations designed to maintain therapeutic blood concentrations (0.15–0.2 µg/mL) for 48 to 96 hours. Blood samples were collected pre and post-treatment. Injection site (0–3) and general well-being (normal or abnormal – with description) were recorded at the time of each blood collection. The plasma concentration-time curve for each horse was analyzed separately to estimate standard non-compartmental pharmacokinetic variables. Results from the present study suggest that potential therapeutic advantages of meloxicam may be enhanced by applying recent advances in biodegradable controlled release drug delivery, allowing single administration products to replace multiple daily treatment protocols.

Keywords: Meloxicam; Nonsteroidal anti-inflammatory drugs; Controlled release formulation

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a chemically heterogeneous group of agents used for the treatment of symptoms of acute pain and chronic inflammatory and degenerative joint diseases, such as osteoarthritis, as well as for their antipyretic, antithrombotic, and antiendotoxic properties. The molecular target for NSAIDs is cyclo-oxygenase (COX). It is now well known that there are two COX isofoms, COX-1 and COX-2, and that the extent of NSAID-associated relative inhibition of COX-1 and COX-2 activities varies among the drugs. Commonly administered NSAIDs in horses are flunixin, phenylbutazone, and ketoprofen, which are relatively non-selective and inhibit both COX-1 and COX-2 to various degrees. Because COX-1-derived prostaglandins play a role in protecting the gastrointestinal mucosa, NSAIDs that inhibit COX-1 have been associated with adverse events such as gastric and intestinal ulcers, gastrointestinal bleeding, and renal injury in horses. 1,2 This has led to development of newer NSAIDs, such as meloxicam and firocoxib, which are more selective for the inhibition of the COX-2 isoenzyme. Meloxicam is a traditional NSAID of the oxicam class, with COX-2 selectivity on the order of 5 to 12 times (depending on blood levels) that of flunixin or phenylbutazone in the horse. 3 On the basis of their observations, Beretta et al 3 suggested that meloxicam seems the best of the tested traditional NSAIDs for use in horses. Meloxicam has most commonly been used for the alleviation of inflammation and relief of pain in both acute and chronic musculo-skeletal disorders or for the relief of pain associated with equine colic. Meloxicam is available for oral administration or intravenous (IV) daily administration and can be administered once daily for a maximum of 14 days. Although meloxicam for horses is not presently registered in the United States, it is licensed for horses in Europe.

Firocoxib, the newest NSAID, has the distinction of being a second-generation coxib, that is, a highly selective COX-2 inhibitor, which is 265 times as selective for COX-2 as it is for COX-1 in the horse. 4 Presently, firocoxib is limited to only oral administration and can be administered once daily for a maximum of 14 days.

Inflammatory joint disorders are a major cause of early retirement of performance horses 5 and a common cause of lameness. Chronically inflamed joints are characterized by pain, synovitis, and progressive deterioration of articular cartilage. 6 Recently, both meloxicam 7 and firocoxib 8,9...
formulated as an oral paste were shown to be effective in reducing lameness when administered to horses. In addition, deGraff et al reported that meloxicam not only alleviated lameness and joint effusion associated with acute severe synovitis, but also reduced synovial fluid concentrations of Prostaglandin E\(_2\) (PGE\(_2\)), substance P, and bradykinin, as well as general matrix metalloproteinase activity as compared with placebo treatment. Meloxicam concentrations in synovial fluid range from 40% to 50% of those in plasma. The free fraction in synovial fluid is 2.5 times higher than in plasma because of the lower albumin content in synovial fluid as compared with plasma. Although the significance of this penetration is unknown, it may account for the fact that it performs exceptionally well in treatment of arthritis in animal models.

Colic is a leading cause of death in horses. Strangulating obstruction of the intestines results in disruption of intestinal barrier function, endotoxemia, hypovolemia, and shock.\(^{11}\) Prostaglandin E\(_2\) seems to be important for intestinal repair, and oral COX-1 and COX-2 inhibitors have been shown to interfere with intestinal repair after ischemia in the horse.\(^{12,13}\) However, meloxicam\(^{14}\) or firocoxib\(^{15}\) administered IV did not inhibit mucosal recovery in equine ischemic-injured jejenum. Cook et al\(^{16}\) proposed that systemically administered selective COX-2 inhibitors might inhibit the COX-2 products responsible for pain and inflammation, but allow intestinal recovery because of limited inhibition of COX-1.

Other advantages of selective COX-2 inhibitors, such as meloxicam, are that they may be associated with a decreased risk of adverse effects such as inhibition of platelet function, development of gastrointestinal tract ulcers, and impairment of renal function, as compared with nonselective NSAIDs. However, recent studies have conclusively shown that selective COX-2 inhibitors may tip the natural balance of prothrombotic thromboxane A\(_2\) and antithrombotic COX-2-dependent prostacyclin (PGI\(_2\)), potentially increasing the possibility of a thrombotic cardiovascular event.\(^{16}\) In addition, there are other indications of a protective role for PGE\(_2\) and PGI\(_2\) derived from the COX-2 pathway pertaining to oxidative damage.\(^{17}\) Accordingly, both COX-1- and COX-2-derived PGs seem to have a profound role in the regulation of vascular homeostasis. Therefore, additional factors other than COX-1/COX-2 selectivity, such as the lowest effective dose, pharmacokinetic profile, and frequency of administration, must be considered as important determinants in the prevention of adverse side effects when NSAIDs are used.

At present, The European Agency for the Evaluation of Medicinal Products has approved meloxicam for oral and IV use in horses at a dose of 0.6 mg/kg every 24 hours. However, the clearance of meloxicam in horses is faster as compared with other animals.\(^{18,19}\) Most of the drug will be eliminated during a 24 hour interval with a half-life of approximately 5.5 hours (2.7–8.5 hours).\(^{18,21}\) A drug formulation that can deliver meloxicam through a controlled release formulation at a more continuous but lower rate may maintain the drug concentrations at a more ideal therapeutic level throughout the dosing interval, without increased risk of toxicity. This can be accomplished by applying recent advances in biodegradable controlled release drug delivery systems to allow single administration products to replace multiple daily treatment protocols. Such formulations reduce labor and the associated handling stress to the animals and veterinarians and offer an important means of maintaining effective compliance rates on farms with wide varieties of management systems.

The present study was designed to test this principle of controlled release delivery of meloxicam using this technology. The BioRelease Delivery System used for this study is a proprietary low-viscosity nonaqueous liquid system that uses an easily injectable biocompatible suspension. Our objective was to use a BioRelease Delivery System to prepare several controlled release formulations of meloxicam and use pharmacokinetic principles to compare the plasma concentrations produced. Our hypothesis was that a slow, but controlled release of meloxicam would produce sustained plasma concentrations that would be potentially effective for treating pain and inflammatory conditions in horses, lameness in particular. The results of this study will be used to aid in the selection of one for use in clinical studies to support a potential approval.

**EXPERIMENTAL METHODS**

In the present study, 12 research horses of various light breeds weighing 466 ± 18.4 kg were randomly assigned to one of three treatment groups (n = 4 horses per group), 2 mL (1,500 mg or approximately 3.22 mg/kg) of 3 BioRelease meloxicam formulations Blue (slow release), Green (medium release), and Red (fast release). Formulations were prepared to give a final concentration of 750 mg/mL, and designed to deliver meloxicam for approximately 72 hours after intramuscular (IM) injection. Blood samples were collected before drug administration, and at 1, 3, 6, 12, 24, 36, 48, and 72 hours postinjection. A second injection was administered at 72 hours and continued sampling followed at 73, 75, 84, 96, 108,120, and 144 hours from the initial dose. Plasma samples were harvested and stored at −20°C until assayed for meloxicam by high pressure liquid chromatography (HPLC).

On the basis of previous pharmacokinetic-pharmacodynamic (PK-PD) analysis\(^{20}\) that reported a median effective meloxicam concentration of 0.130 µg/mL for stride length and 0.195 µg/mL for lameness scores, the aforementioned values were used for comparison purposes in the present study.
Injection site assessments were made and recorded at each blood collection. Scores were based on a subjective scale of 0-3 (0 = none, 1 = slight – diameter of swelling 12.5 mm, 2 = moderate - diameter of swelling 12.5 to 25 mm, 3 = significant – diameter of swelling about 25 mm or larger), sensitivity to touch (yes/no), and temperature elevation at injection site (yes/no). Finally, general well-being scores (normal or abnormal – with description) were recorded at each blood collection.

Drug Analysis

Equine plasma samples were analyzed by HPLC, using a method developed and validated in the laboratory at North Carolina State University. Reference standards for meloxicam were purchased from Sigma Chemical (St. Louis, MO, USA). Meloxicam reference standard was weighed and dissolved in 100% HPLC-grade distilled water to a concentration of 1 mg/mL. From this stock solution, further dilutions were made in HPLC-grade distilled water to make up fortifying solutions for plasma so as to prepare quality control samples, calibration curve samples, and for development of these methods. The stock solution was kept at 4°C in a tightly sealed dark vial. The fortifying solutions made from the stock solution were added to blank (control) plasma, to make up 7 calibration standards, including zero (range: 0.0–10 μg/mL).

The mobile phase for HPLC analysis consisted of 40% acetonitrile and 60% 0.05 M sodium acetate buffer. Glacial acetic acid was added to the buffer to adjust the pH to 3.7 to 3.8. Fresh mobile phase was prepared, filtered (0.45 μm), and degassed for each day’s run.

The HPLC system consisted of a quaternary solvent delivery system (Agilent Technologies, Wilmington, DE, USA) at a flow rate of 1 mL/min, an autosampler (1100 Series Autosampler, Agilent Technologies, Wilmington, DE, USA), and Ultraviolet detector set at a wavelength of 365 nm (1100 Series Autosampler, Agilent Technologies, Wilmington, DE, USA). The chromatograms were integrated with a computer program (1100 Series Chemstation software, Agilent Technologies, Wilmington, DE, USA). The column was a reverse-phase, 4.6 mm × 15 cm C8 column (Zorbax Rx-C18, MAC-MOD Analytical, Inc., Chadds Ford, PA, USA), kept at a constant temperature of 40°C. Retention time for the peak of interest was approximately 4.9 to 5.0 minutes.

All plasma samples, calibration samples, and blank (control) plasma samples were prepared identically using solid phase extraction. Solid phase extraction cartridges (Waters Oasis HLB cartridges, Waters Associates, Millford, MA, USA) were conditioned with 1 mL methanol followed by 1 mL distilled water. A plasma sample of 500 μL was added to a conditioned cartridge, followed by a wash step of 1 mL distilled water: methanol (95:5). The drug was eluted with 1 mL 100% methanol and collected in clean glass tubes.

The tubes were evaporated at 40°C for 15 to 20 minutes in an evaporator. Each tube was then reconstituted with 200 μL of mobile phase and were vortexed, and 25 μL of each tube was then injected into the HPLC system. A fresh set of calibration and blank samples were prepared for each day’s run. All calibration curves were linear, with an R² value of 0.99 or higher and the intra-assay precision was <15%. Limit of quantification for this study was 0.01 μg/mL, which was determined from the lowest point on a linear calibration curve. The laboratory used guidelines published by the United States Pharmacopeia.22

Pharmacokinetic Analysis

Each horse was analyzed separately using pharmacokinetic techniques. All horses within each of the three treatment groups were then grouped and averaged (standard two-stage analysis). Visual analysis of the plasma drug concentrations indicated that a noncompartment model that does not assume any compartmental structure was the best approach for this data. Analysis of curves and pharmacokinetic modeling was then performed using a commercial pharmacokinetic program (WinNonlin, Version 5.2, Pharsight Corporation, Mountain View, CA, USA).

For the noncompartmental analysis, the area under the plasma concentration versus time curve (AUC) from time 0 to the last measured concentration was calculated using the log-linear trapezoidal method. The AUC from time
Table 1. Pharmacokinetic properties of meloxicam following administration at a dose of 3.22 mg/kg once at time zero, and again at 72 hours

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Red Treatment</th>
<th>Mean</th>
<th>SD</th>
<th>Blue Treatment</th>
<th>Mean</th>
<th>SD</th>
<th>Green Treatment</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal rate</td>
<td>1/hr</td>
<td>0.010</td>
<td>0.003</td>
<td></td>
<td>0.015</td>
<td>0.006</td>
<td></td>
<td>0.019</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Half-life</td>
<td>Hour</td>
<td>79.04</td>
<td>27.25</td>
<td></td>
<td>61.30</td>
<td>43.50</td>
<td></td>
<td>40.11</td>
<td>14.78</td>
<td></td>
</tr>
<tr>
<td>T\textsubscript{max}</td>
<td>Hour</td>
<td>77.25</td>
<td>1.50</td>
<td></td>
<td>80.25</td>
<td>4.50</td>
<td></td>
<td>79.50</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{max}</td>
<td>μg/mL</td>
<td>0.714</td>
<td>0.392</td>
<td></td>
<td>0.346</td>
<td>0.113</td>
<td></td>
<td>0.775</td>
<td>0.411</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>hr·μg/mL</td>
<td>49.97</td>
<td>18.64</td>
<td></td>
<td>22.36</td>
<td>4.52</td>
<td></td>
<td>45.44</td>
<td>18.48</td>
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<tr>
<td>MRT</td>
<td>Hour</td>
<td>107.50</td>
<td>33.89</td>
<td></td>
<td>91.41</td>
<td>62.33</td>
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<td>59.84</td>
<td>21.72</td>
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</tr>
</tbody>
</table>

Terminal rate, terminal rate constant after second dose; half-life, terminal half-life after second dose; T\textsubscript{max}, time to peak concentration after second dose; C\textsubscript{max}, maximum (peak) concentration after second dose; AUC, area under the curve from zero to infinity; MRT, mean residence time after second dose.

a Mean for Green treatment does not include one outlier for all values except T\textsubscript{max} because of extreme values (half-life, >130 hours and AUC, >100 hr μg/mL).

RESULTS

The plasma concentrations of meloxicam are presented in Figure 1. Pharmacokinetic properties for each formulation are presented in Table 1. Figure 1 also shows the values predicted by Toutain and Cester\textsuperscript{20} for effective meloxicam concentrations in horses, which reported a median effective meloxicam concentration of 0.130 μg/mL for stride length and 0.195 μg/mL for lameness score. The values in Table 1 represent the mean values of all four horses in each group, except that one horse in the Green Treatment group seems to be an outlier and the values were not included in Table 1. This horse had a terminal half-life of >130 hours, C\textsubscript{max} of 0.287 μg/mL, mean residence time of 336 hours, and AUC value of >100 μg hr/mL.

Examination of the injection site data indicated that all examination scores for all animals at all time points were 0 or none, therefore statistical examination of the data was considered unnecessary. Similarly, general well-being scores were also recorded as normal at all time points and were not examined statistically.

DISCUSSION

The objective of this study was to examine the feasibility of formulating the NSAID meloxicam in a controlled release formulation that could be administered infrequently IM (once every 3–4 days) and maintain plasma concentrations that are in the effective range for controlling pain and inflammation in horses. Meloxicam has been shown to be an effective analgesic and anti-inflammatory drug in horses by virtue of its registration as a safe and effective drug in Europe by The European Agency for the Evaluation of Medicinal Products. It also improved postoperative pain scores and clinical variables compared with placebo-treated horses.\textsuperscript{14} In a model of inflammation, meloxicam administration in horses decreased markers of joint inflammation.\textsuperscript{7} It has also been shown to have advantages over other NSAIDs because it did not impede recovery of equine ischemia-injured jejunal mucosa compared with flunixin meglumine.\textsuperscript{14} These authors suggested that the COX-2 preferential inhibitor, meloxicam, may permit sufficient COX-1 activity for prostaglandin-mediated recovery of intestinal barrier function, whereas inhibiting the detrimental effects of COX-2 stimulated prostaglandins on clinical signs of endotoxia and pain.\textsuperscript{14}

The half-life of meloxicam after IV and oral administration to horses was reported to be 8.54 hours (±3.02) and 21.8 hours (±1.99), respectively.\textsuperscript{21} In another study,\textsuperscript{24} it was reported to be much shorter at 2.7 hours (±0.44). These half-lives are shorter as compared with the half-lives of dogs and require meloxicam to be administered at least once daily in horses to maintain effective concentrations. Less frequent administration of a controlled release formulation would maintain more consistent drug concentrations and obviate the need for frequent administration.

PK-PD studies have allowed for integration of plasma drug concentrations and clinical response.\textsuperscript{25} A PK-PD study in horses with experimentally induced inflammation showed...
that meloxicam can produce improvements in stride length and clinical lameness score at a median effective concentration of 0.13 and 0.195 μg/mL, respectively.\textsuperscript{20} As shown in Figure 1, both the Green and Red formulations developed for this study maintained concentrations at, or above, these levels throughout the dose interval of 72 hours.

Our objective for this study was to examine the possibility of a new formulation that may be administered to horses less frequently with equal or better results, as compared with once-daily oral or IV administration. The following three formulations were developed to control the release of the drug from the matrix by the rate of release: Blue (slow release), Green (medium release), and Red (fast release). These results showed that each formulation produced a similar time to peak plasma concentration (T\textsubscript{max}) (Table 1). However, T\textsubscript{max} does not necessarily indicate the rate of systemic drug absorption. The extent of drug absorption was more apparent among the formulations. Clearly, as shown by the peak concentration (C\textsubscript{max}) and extent of absorption (AUC), the Red and Green formulations were superior to the Blue formulation. The Red and Green formulations appeared to be similar to the Blue formulation, both in consistency and ability to maintain effective levels and should be selected for further study over the Blue formulation. The terminal half-life after the second dose (mean of 79 and 40 hours, respectively, for the Red and Green treatment) was much longer than reported for currently available commercial formulations in horses. We did not perform an IV dose to determine pharmacokinetics. It is not possible to determine the extent of absorption or contribution from “flip-flop” pharmacokinetics without an accompanying IV dose. However, other researchers have shown that IV meloxicam typically has a half-life that is much shorter. Toutain and Cester\textsuperscript{20} reported an average half-life of 5.15 hours and Toutain et al\textsuperscript{21} reported an average half-life of 8.54 hours, whereas Lees et al\textsuperscript{24} reported a half-life in ponies of 2.7 hours. In this study, the half-lives were 40 to 80 hours, depending on the formulation. This supports a pharmacokinetic profile that is most likely described as flip-flop pharmacokinetics in which the terminal half-life is determined from the rate of absorption from the injection site, rather than the rate of elimination. With a long half-life produced from a “flip-flop” effect caused by delayed absorption, a consistent and more sustained plasma concentration can be maintained after injection. The peak concentrations (C\textsubscript{max}) after the controlled release formulation used in this study were 0.775 and 0.714 μg/mL for the Green and Red formulation, respectively. However, a previous PK study\textsuperscript{21} reported treatment with oral administration at an approved dose, meloxicam had a peak concentration of 2.58 μg/mL in horses and it exceeded 10 μg/mL after IV administration.\textsuperscript{21} If adverse effects are associated with the height of the peak concentration, the controlled release formulation developed for this study may have advantages over both IV and oral administration of the commercial formulations.

The animals used in this study were few (only four per group); nevertheless, the results support a proof-of-principle that a long acting formulation can be developed and administered to horses. Given that the results should be considered preliminary and acknowledging the variability noted with each group of horses, the present results and other formulation development issues\textsuperscript{26} support future studies, with the Red formulation using the BioRelease Delivery System evaluated in this study.

REFERENCES


