Evaluation of bioequivalence after oral, intramuscular, and intravenous administration of racemic ketoprofen in pigs

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**Objective**—To assess bioequivalence after oral, IM, and IV administration of racemic ketoprofen in pigs and to investigate the bioavailability after oral and IM administration.

**Animals**—8 crossbred pigs.

**Procedures**—Each pig received 4 treatments in a randomized crossover design, with a 6-day washout period. Ketoprofen was administered at 3 and 6 mg/kg, PO; 3 mg/kg, IM; and 3 mg/kg, IV. Plasma ketoprofen concentrations were measured by use of high-performance liquid chromatography for up to 48 hours. To assess bioequivalence, a 90% confidence interval was calculated for the area under the time-concentration curve (AUC) and maximum plasma concentration (C_{max}).

**Results**—Equivalence was not detected in the AUCs among the various routes of administration nor in C_{max} between oral and IM administration of 3 mg/kg. The bioavailability of ketoprofen was almost complete after each oral or IM administration. Mean ± SD C_{max} was 5.09 ± 1.41 μg/mL and 7.62 ± 1.22 μg/mL after oral and IM doses of 3 mg/kg, respectively. Mean elimination half-life varied from 3.52 ± 0.90 hours after oral administration of 3 mg/kg to 2.66 ± 0.50 hours after IV administration. Time to peak C_{max} after administration of all treatments was approximately 1 hour. Increases in AUC and C_{max} were proportional when the orally administered dose was increased from 3 to 6 mg/kg.

**Conclusions and Clinical Relevance**—Orally administered ketoprofen was absorbed well in pigs, although bioequivalence with IM administration of ketoprofen was not detected. Orally administered ketoprofen may have potential for use in treating pigs. (Am J Vet Res 2008;69:108–113)

Orthopedic problems are common in swine. In Scandinavia, the incidence of lameness is reported to be approximately 10% in young pigs, with a prevalence of 8.8% in loose-housed adult swine. In Australia, fibrinopurulent inflammation is a common finding in lame pigs < 6 weeks old, whereas in Denmark, suppurative arthritis is less common in older lame pigs (3 to 5 months old). In Finland, the most commonly diagnosed clinical condition in lame sows and gilts is osteochondrosis or osteoarthrosis. In Denmark, macroscopic osteochondrotic lesions have been detected at slaughter in 47% of pigs with a history of lameness and 35% of pigs without a history of lameness. Clinical lameness induces an acute-phase reaction in adult swine and finishing pigs. An injection of meloxicam can reportedly alleviate lameness and improve feed intake in pigs with noninfectious locomotor disorders. Alleviation of pain is probably also beneficial when used in combination with administration of antimicrobials to treat pigs with joint infections. A need exists...
for an oral formulation of an NSAID that can be easily administered by swine farmers.

Ketoprofen is an NSAID widely used for the treatment of humans with orthopedic pain. It is also an effective analgesic for conditions such as acute and chronic locomotor disorders in cats and chronic laminitis in horses. Currently, ketoprofen is not licensed for use in food animals in the United States; however, maximum residue limits, including limits for specimens of the muscles and kidneys of swine, have been established for ketoprofen in Canada (muscles, 0.1 µg/g; kidneys, 0.5 µg/g) and the European Union.

Ketoprofen is indicated for use in the treatment of swine with respiratory tract infections and for porcine agalactia syndrome because of its anti-inflammatory, analgesic, and antipyretic actions. The recommended dosage is 3 mg/kg, IM. Although no studies on the efficacy of ketoprofen in alleviating signs of pain associated with orthopedic problems in swine could be found in the literature, ketoprofen may potentially also be used to treat pigs with orthopedic conditions.

Ketoprofen is a racemic mixture of S(+) and R(–) ketoprofen, with each enantiomer having differing pharmacodynamic properties. The pharmacokinetics of ketoprofen are enantioselective in many species, and unidirectional chiral inversion of R(–) to S(+) has been reported with the extent of chiral inversion differing among species. High variation also exists among animal species regarding the bioavailability of ketoprofen after oral administration. For example, the bioavailability is extremely low in horses, whereas it is almost 100% in dogs and cats. In a study performed with 3 pigs, the bioavailability after oral administration of ketoprofen varied from 57% to 100%.

The objectives of the study reported here were to assess the bioequivalence after oral, IM, and IV administration of racemic ketoprofen to pigs and to investigate the bioavailability of oral administration of ketoprofen at 2 dosages. The commercial veterinary ketoprofen products available consist of a racemic (50:50) mixture of the 2 enantiomers, and the maximum residue limits established by Health Canada and the European Agency for the Evaluation of Medicinal Products have been stated for the racemate. We intended to analyze plasma concentrations of racemic ketoprofen in this bioequivalence study.

Materials and Methods

Animals—Eight crossbred pigs (mean ± SD weight, 51.8 ± 8.4 kg at the beginning of the study and 77.9 ± 9.9 kg at the end of the study) were used. The study was performed at the Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki. Pigs were housed separately in pens and fed a standard pelleted diet. Water was available ad libitum.

Study design and procedures—Each pig received ketoprofen 4 times in this randomized crossover study. The washout period between subsequent treatments was a minimum of 6 days. Each pig was treated by oral administration of ketoprofen (3 and 6 mg/kg) or parenteral administration of ketoprofen (3 mg/kg, IM, and 3 mg/kg, IV). The IV administration was via an ear vein other than the one used for collection of blood samples. Oral administration was via a stomach tube. Ketoprofen was mixed with 40 mL of water, which was injected via the stomach tube; the stomach tube was then flushed with 50 mL of water before removal. Feed was withheld from pigs overnight before each treatment, and pigs were fed again 1 to 2 hours after each administration of ketoprofen.

Venous blood samples were collected into heparinized tubes before each treatment; 2, 5, 10, 15, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours after IV ketoprofen administration; and 15, 30, and 45 minutes and 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after PO and IM administrations. Samples were collected via vinyl tubing inserted in an ear vein by use of a nonsurgical method. A soft rope snare was placed around the maxilla of each pig to provide restraint during placement of the tubing. The surface of the ear was cleaned and disinfected. A 13-gauge catheter was inserted into an auricular vein, and approximately 50 cm of vinyl tube (inner diameter, 1.0 mm; outer diameter, 1.5 mm) was threaded through the catheter into the vein. Approximately 50 cm of vinyl tubing was not inserted into the vein to make it easier to collect samples. The 13-gauge catheter was removed, and a blunted, 18-gauge, hypodermic needle hub was inserted into the end of the vinyl tubing; a stopper was then inserted into the needle hub to prevent backflow. The vinyl tubing was filled with heparinized saline (0.9% NaCl) solution (5 U/mL). A zippered pouch affixed to the dorsal portion of each pig’s neck was used to store the external portion of the vinyl tubing and needle hub between sample collections. An adhesive bandage was used to affix the external vinyl tubing to the ear, and the ear was then taped to the pig’s neck to ensure that no part of the vinyl tubing was exposed and to prevent the pig from dislodging the vinyl tubing.

To prevent contamination with heparinized saline solution, the initial 2 mL of blood obtained was discarded. A blood sample was collected into a 10-mL syringe. The tubing was then flushed with heparinized saline solution, and the stopper was replaced in the needle hub.

HPLC analysis—Plasma was separated by centrifugation and stored at −20°C until analyzed. Plasma concentrations of racemic ketoprofen were determined by means of HPLC via a method described elsewhere, with slight modifications. Determinations were conducted on 4 samples in parallel. The HPLC system was equipped with a piston pump, an autosampler, a tunable absorbance UV detector, and workstation. Sample separation was conducted on a 4.6 × 150-mm column packed with 5 µm of reversed-phase silica. Flow rate of the isocratic mobile phase, which consisted of acetonitrile and 0.03% phosphoric acid (1:1), was 1.5 mL/min. The analytic wavelength was 254 nm. The method was validated as recommended for bioanalytic assays by analyzing 6 parallel plasma samples spiked with ketoprofen. The standard curve was found to be linear (r² >
0.998) for the concentration range of 0.36 to 50 mg/L. Mean values at the extremes of the concentration range were 0.39 mg/L (ie, limits of quantitation; coefficients of variation for accuracy and precision were 4.1% and 8.3%, respectively) and 50.8 mg/L (coefficients of variation for accuracy and precision were 4.9% and 1.7%, respectively). No interfering peaks were detected for the plasma blanks.

Pharmacokinetic variables—Pharmacokinetic variables were calculated. The AUC was calculated by use of the trapezoidal method. In each case, AUC$_{0-12}$ was > 80% of the calculated AUC$_{0-\infty}$. Values for C$_{max}$ and T$_{max}$ were determined directly from plasma curves. The t$_{1/2}$ was calculated as 0.693/β. Values for V$_d$ were calculated by use of the area method as V$_d$ = (dose/AUC$_{0-\infty}$), and CL was calculated as dose/AUC$_{0-\infty}$; values for V$_d$ and CL were standardized per kilogram of body weight. The MRT was calculated as the area under the moment curve from time 0 to infinity/AUC$_{0-\infty}$. The MAT was calculated as MRT$_{extravascular}$ - MRT$_{IV}$, where MRT$_{extravascular}$ is the MRT after PO or IM administration and MRT$_{IV}$ is the MRT after IV administration.

Statistical analysis—To assess bioequivalence, the 90% CI was calculated for AUC$_{0-12}$ and C$_{max}$ (both of which were logarithmically transformed). The 90% CI should be within an acceptance interval of 0.80 to 1.25.

Results
Retention time for the racemic ketoprofen was 4 minutes (Figure 1). Equivalence was not detected for AUC$_{0-12}$ values between 3 mg/kg administered PO and IV (90% CI, 0.75 to 1.02), 3 mg/kg administered IM and IV (90% CI, 0.90 to 1.29), and 3 mg/kg administered PO and IM (90% CI, 0.66 to 0.99). Equivalence was also not detected for C$_{max}$ between 3 mg/kg administered PO and IM (90% CI, 0.53 to 0.80). Bioavailability was almost complete after each IM or PO administration (Table 1). Mean ± SD relative bioavailability (PO vs IM administration) was 89.2 ± 33.1%. Mean plasma ketoprofen concentration time curves after PO, IM, and IV administration were plotted (Figure 2). Pharmacokinetic variables were calculated after each administration.

Comparing the rate of absorption, MAT was longer for 3 mg/kg after PO administration than after IM administration, but there was no significant difference in T$_{max}$ (Table 1). For all treatments, T$_{max}$ was detected approximately 1 hour after drug administration. A second peak was evident for most of the individual plasma ketoprofen concentration profiles, which was also clearly evident at 1.75 hours after administration in the mean curve for the highest dose (ie, 6 mg/kg, PO; Figure 2). Increases in AUC and C$_{max}$ were proportional when

![Figure 1](image1.png)

![Figure 2](image2.png)
the orally administered dose was increased from 3 to 6 mg/kg.

Discussion

Bioavailability of ketoprofen was almost 100% in pigs after PO and IM administration, although bioequivalence was not detected between PO and IM administrations. The $C_{\text{max}}$ was higher after IM administration than after PO administration. Results of the study reported here agree with those of another study performed in 3 pigs and indicate that the oral preparation was adequately absorbed in pigs.

Commercially available veterinary products, including those used in the study reported here, contain a racemic (50:50) mixture of the 2 enantiomers, S(+) and R(–). Therefore, we did not consider it necessary to analyze plasma enantiomer concentrations in a bioequivalence study. Because no published information is available on the pharmacokinetics of ketoprofen enantiomers in pigs, additional studies on the pharmacokinetics of ketoprofen enantiomers in swine are needed.

The second peak evident in plasma ketoprofen concentrations may have been caused by enterohepatic recirculation because ketoprofen can have marked re-circulation, at least in rats. In general, enterohepatic recirculation may prolong elimination of a drug. Increases in AUC and $C_{\text{max}}$ were proportional with the orally administered doses of ketoprofen used in our study (ie, 3 and 6 mg/kg), which makes it easy to plan dosing schedules.

Absorption after oral administration of ketoprofen was noticeably faster in our study than in other studies, in which the peak concentration was reached 2 to 3 hours after oral administration. Mixing the drug with food probably caused the delay in absorption in those other studies, compared with the results for our study in which ketoprofen was mixed with water.

In humans, the effects of food on bioavailability of ketoprofen are somewhat controversial. In 1 study, absorption of ketoprofen was affected by food because $C_{\text{max}}$ decreased, $T_{\text{max}}$ increased, and bioavailability decreased by 40%. However, in another study, food did not alter the extent of absorption of S(+) ketoprofen, although $T_{\text{max}}$ increased and $C_{\text{max}}$ decreased. Bioavailability and systemic concentrations of ketoprofen are not affected by meal composition in humans. In reality, it would probably be easier for a farmer to mix the drug with a small amount of food than to mix it with water for administration. However, in the bioequivalence study reported here, we wanted to ensure that the pigs received the orally administered dose quickly and completely; therefore, we used a stomach tube.

After IM administration, the peak concentration in the pigs of our study was detected at approximately 1 hour, which is similar to that in another report (30 minutes). The extent of distribution, reflected by $V_d$, was low, which was expected because NSAIDs are hydrophilic compounds that are highly ionized at a physiologic pH. Ketoprofen is expected to distribute primarily to the extracellular fluid compartment and to be highly bound to plasma protein. Investigators in another study reported a mean ± SD steady-state $V_d$ of 0.17 ± 0.02 L/kg in pigs. In the study reported here, $V_d$ was calculated by use of a trapezoidal method. Use of this technique when calculating $V_d$ always yields higher numbers than when calculating steady-state $V_d$ which probably explains the slightly higher $V_d$ reported here, compared with the value in that other report.

The $t_{1/2}$ was independent of dose for the oral route of administration, but it was longer after PO administration than after IV administration. Administration of ketoprofen as a powder mixed with a small amount of water in pigs from which food had been withheld could restrict dissolution of the poorly soluble drug in an acidic stomach environment, which could thus affect the rate of bioavailability and result in a decrease in the rate or extent of dissolution. However, although statistically significant, the difference in $t_{1/2}$ was minor and probably not clinically relevant. In another study, also appeared to be noticeably longer after PO administration than after IV administration. In that study, an aqueous solution of ketoprofen was administered with food, although the number of animals was too small for statistical calculations.

Mean ± SD MRT in pigs after IV administration of 3 mg/kg is reportedly 2.32 ± 0.41 hours. That value is slightly lower than the value derived after IV administration in the study reported here.

Serum concentrations of 0.2 to 0.4 µg/mL for the active S-enantiomer of ketoprofen are required for maximal anti-inflammatory effects in rats with experimentally induced arthritis, and concentrations of at least 1 µg of racemic ketoprofen/mL are

<table>
<thead>
<tr>
<th>Variable</th>
<th>3 mg/kg, PO</th>
<th>6 mg/kg, PO</th>
<th>3 mg/kg, IM</th>
<th>3 mg/kg, IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>3.52 ± 0.90</td>
<td>3.22 ± 0.43</td>
<td>2.95 ± 0.21</td>
<td>2.68 ± 0.50</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>5.54 ± 1.28</td>
<td>5.15 ± 0.86</td>
<td>4.49 ± 0.44</td>
<td>3.40 ± 0.51</td>
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<td>MAT (h)</td>
<td>2.15 ± 1.01</td>
<td>1.75 ± 1.11</td>
<td>1.09 ± 0.35</td>
<td>—</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.25 ± 0.90</td>
<td>1.19 ± 0.46</td>
<td>1.08 ± 0.48</td>
<td>—</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>5.09 ± 1.41</td>
<td>12.03 ± 4.81</td>
<td>7.62 ± 1.22</td>
<td>—</td>
</tr>
<tr>
<td>$AUC_{0-12}$ (µg·h/mL)</td>
<td>28.3 ± 6.7</td>
<td>63.4 ± 17.6</td>
<td>34.6 ± 6.5</td>
<td>32.4 ± 7.6</td>
</tr>
<tr>
<td>$AUC_{0-12}$ (µg·h/mL)</td>
<td>32.04 ± 9.20</td>
<td>69.51 ± 19.32</td>
<td>37.14 ± 6.99</td>
<td>33.86 ± 8.45</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>96.7 ± 27.1</td>
<td>104.9 ± 26.8</td>
<td>114.6 ± 29.9</td>
<td>—</td>
</tr>
<tr>
<td>$CL$ (L/h/kg)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.094 ± 0.024</td>
</tr>
<tr>
<td>$V_d$ (L/kg)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.35 ± 0.09</td>
</tr>
</tbody>
</table>

— = Not determined.
necessary for sustained alleviation of orthopedic pain in humans. In calves with carrageenan-induced inflammation, the mean plasma ketoprofen concentration that induced 50% of the maximal effect for inhibition of several prostanoids in serum or exudate varied from 0.06 to 0.12 µg/mL, and the mean plasma ketoprofen concentration that induced 50% of the maximal effect for inhibition of swelling was 0.0003 µg/mL. In the study reported here, those plasma concentrations were achieved in all pigs after each treatment. Mean plasma ketoprofen concentration was at least 1 µg/mL for approximately 10 hours after IM and PO administration at a dosage of 3 mg/kg, >12 hours after PO administration at a dosage of 6 mg/kg, and approximately 8 hours after IV administration at a dosage of 3 mg/kg. However, the analgesic and anti-inflammatory effects of NSAIDs may be prolonged, even after plasma drug concentrations have decreased to less than the minimum effective concentration. In contrast, plasma concentrations of NSAIDs may not always correlate well with clinical analgesic and anti-inflammatory effects because the clinical effects are associated more with tissue concentrations.

Analysis of our results indicated that although bioequivalence was not detected after IM administration, bioavailability of orally administered racemic ketoprofen was high in pigs. Thus, racemic ketoprofen may be potentially useful in the treatment of pigs with pain-inducing or inflammatory disease conditions because it can be easily administered by farmers. The efficacy of orally administered ketoprofen for alleviating signs of pain and inflammation in pigs should be evaluated in clinical trials, probably with the dosage of 3 mg/kg. However, authorization for use differs among countries, and ketoprofen currently is not licensed for use in food animals in the United States.

References


