Pharmacokinetics of Ketoprofen in Lactating Dairy Cows

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Abstract

The efficacy and economics of antibiotic treatment of clinical coliform mastitis have been questioned. Nonsteroidal anti-inflammatory drugs (NSAID) have received a great deal of attention as an alternative to antibiotic treatment of clinical coliform mastitis. NSAID have been demonstrated to relieve clinical signs of experimentally induced mastitis. NSAID may improve the outcome of clinical coliform mastitis and improve milk out, appetite, and increase food and water consumption.

Ketoprofen is a propionic acid derivative with potent NSAID properties. Ketoprofen has been shown to have a very high therapeutic index and is labeled for veterinary use in horses in the USA. Ketoprofen may be effective in reducing clinical signs of acute coliform mastitis, but has not been studied extensively in cattle. Pharmacokinetic, residue, efficacy, and safety studies are needed before ketoprofen can be used appropriately in cattle.

Ketoprofen was administered to clinically normal lactating Holsteins (1.5 mg/lb). Milk and blood was collected at 0, 5, 10, 15, 25, 40, 60, 90, 120, 240, 360, 480, and 720 minutes after ketoprofen administration. A computer polyexponential curve stripping program was used to fit ketoprofen concentration time data. Pharmacokinetic values for ketoprofen will be presented.

Ketoprofen is a propionic acid derivative with potent nonsteroidal anti-inflammatory drug (NSAID) properties, that functions as a dual inhibitor of cyclooxygenase and lipoxygenase. Ketoprofen has been shown to have a very high therapeutic index and is labeled for veterinary use in horses in the USA. Ketoprofen has been shown to significantly improve circulatory and respiratory function in sheep challenged with endotoxin.[1]

A well designed, blind, placebo-controlled field study was conducted in seven commercial dairies by the Koret School of Veterinary Medicine, Hebrew University of Jerusalem. In this study, ketoprofen treatment significantly improved recovery rates of cows with acute clinical mastitis and significantly improved recovery rates in cows with mastitis caused by gram negative organisms.[2]

In neonatal calves challenged with endotoxin (3 hour infusion of *Escherichia coli* lipopolysaccharide, 2 μg/kg/h), flunixin, ketoprofen, and ketorolac provided similar amelioration of clinical signs and physiologic alterations.[3] In calves challenged with 200 μg of heat-stable *Escherichia coli* enterotoxin, fecal output was reduced with ketoprofen treatment.[4]

The efficacy and economics of antibiotic treatment of clinical coliform mastitis have been questioned. NSAIDs have received a great deal of attention as alternatives to antibiotic treatment of clinical coliform mastitis. Ketoprofen has demonstrated considerable potential in the therapy of acute clinical mastitis, clinical coliform mastitis and other endotoxin-induced disease. Additional pharmacokinetic, residue, efficacy, and safety studies are needed before ketoprofen can be used appropriately in cattle. The purpose of this study was to determine plasma concentration-time data and evaluate the pharmacokinetic properties of ketoprofen in lactating dairy cows.

Materials and Methods

Cows

Holstein cows (n=6) were fed a pelletized concentrate and hay with access to pasture. Cows were (mean±SD) 3.9 ±1.9 years old, weight 1198 ±181 pounds, were 123 ±44 days in milk, and produced 58.4 ±16.5 pounds of milk per day.

Experimental design

Ketoprofen[5] was administered to clincially normal lactating Holsteins (n=6) as an intravenous bolus at the rate of 1.5 mg/pound. Blood was collected at 0, 5, 10, 15, 25, 40, 60, 90, 120, 240, 360, and 480 minutes after ketoprofen administration. Plasma was harvested and assayed for ketoprofen by high performance liquid chromatography (HPLC).

Ketoprofen assay

Ketoprofen was assayed in heparinized plasma by mixing plasma (500 μl) with internal standard (100 μl

of 25 μg/ml ibuprofen in 0.01 M NaOH), 250 μl of 0.6 M H_2SO_4, and 4 ml of isooctane-isopropanol (95:5 v:v). Samples were then centrifuged and the organic layer removed to a new test tube and concentrated until dry under nitrogen gas. The sample was then mixed with 500 μl methanol:acetonitrile (50:50v:v) and analyzed for ketoprofen by HPLC.[6] The mobile phase was composed of 52% acetonitrile, 47.47% H_2O, 0.5% glacial acetic acid, and 0.03% triethylamine.[7] The mobile phase was run at 1.8 ml/min at 20°C through an octadecyl silane reverse phase 30 x 4.6 mm precolumn[8] and 150 x 4.6 mm ID analytic column.[8] All reagents and water used were HPLC quality.[9] Ketoprofen peaks were detected by UV absorbance at 220 nm and were calibrated against internal, analytic grade ibuprofen standard. Ketoprofen and ibuprofen peak areas were calculated by microcomputer.[10]

Pharmacokinetic and statistical analysis

A computer polyexponential curve stripping program was used to fit ketoprofen concentration-time data and to calculate pharmacokinetic variables.[11] The pharmacokinetic model was selected with a Model Selection Criteria which is a modified Akaike Information Criteria.[11] Concentration-time data from individual cows were fit individually, and then pharmacokinetic variables were averaged. Distribution half-lives ($t_{1/2\alpha}$), elimination half-lives ($t_{1/2\beta}$), plasma ketoprofen concentrations at time zero (C_p^0), slopes (alpha, beta), intercepts (A, B), elimination rate constrants (k_{el}), transfer rate constrants (k_{12}, k_{21}), area under the plasma concentration-time curves (AUC), area under the first moment curves (AUMC), mean residence times (MRT), time of maximal ketoprofen concentration (T_{max}), and maximal ketoprofen concentration (C_{max}) were calculated by use of a computer software program that used standard methods.[11] Apparent volumes of distribution at steady state ($V_{d(ss)}$) were calculated as intravenous ketoprofen dose* AUMC÷AUC^2.[12] Apparent volumes of the central compartment (V_c) were calculated as intravenous ketoprofen dose÷ (A+B).[12] Total clearances (CL_B) were calculated as intravenous ketoprofen dose÷ AUC.[12] Pharmacokinetic data are presented as means and standard deviations (±SD) for coefficients of compartmental models and as harmonic means and ranges for remaining values. AUC were measured from time zero to infinity and were calculated by integration.[11]

Results

Adverse effects were not observed after IV administration of ketoprofen to cows. Mean ketoprofen peak retention time occurred at 3.6 minutes. The limit of quantitation of the assay was 0.1 μg/ml. Plasma ketoprofen concentration-time profiles after IV administration of ketoprofen were determined (Figure 1, Table 1). Data best fit an open two-compartment pharmacokinetic model. Slopes, intercepts, and other pharmacokinetic variables were determined (Table 2).

Figure 1. Mean ketoprofen concentrations in plasma after IV administration of ketoprofen (1.5mg/pounds, n=6).

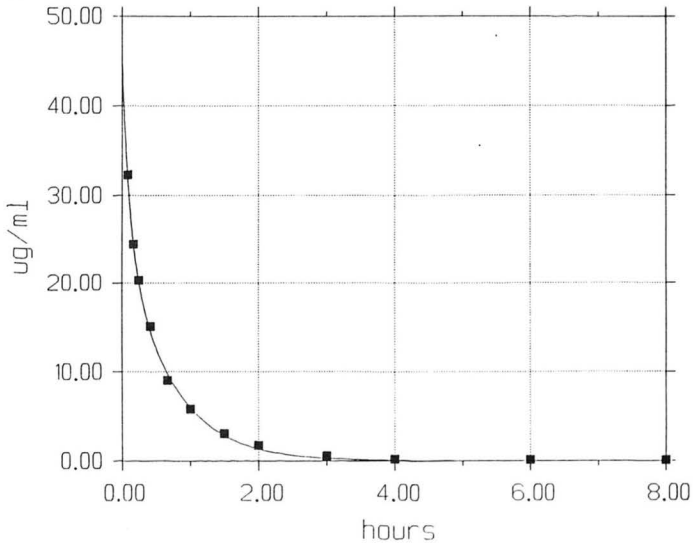

Table 1. Mean ± SD ketoprofen concentration in bovine plasma after IV administration of ketoprofen (1.5 mg/pound) to lactating dairy cows (n=6).

Time (min)	Mean ±SD (μg/ml)	Time (min)	Mean ±SD (μg/ml)
5	32.3 ±3.42	90	3.05 ±0.70
10	24.5 ±2.57	120	1.74 ±0.55
15	20.3 ±2.21	180	0.58 ±0.20
25	15.1 ±1.74	240	0.23 ±0.080
40	9.03 ±1.02	360	0.15 ±0.070
60	5.73 ±1.06	480	0.095 ±0.030

Discussion

Ketoprofen has a short distribution half-life (4.2 minutes) and a fairly short elimination half-life (29 minutes). The elimination half-life is similar to that of aspirin in cattle and shorter than other NSAIDs commonly used in cattle. Ketoprofen is a weak acid with a pK_a of 5.02, which indicates that ketoprofen will most likely be present in normal milk at very low concentrations.

In neonatal calves challenged with *Escherichia coli* endotoxin, 2.2 mg ketoprofen per kg of body weight ameliorated clinical signs and physiologic alterations.[3]

Table 2. Pharmacokinetic data of ketoprofen in plasma after IV administration of ketoprofen (1.5 mg/pound) to lactating dairy cows (n=6).

Pharmacokinetic variable	Mean ±SD	Pharmacokinetic variable	Harmonic mean (range)
A (μg/ml)	24.0 ±5.55	CL_B ($L \cdot kg^{-1} \cdot h^{-1}$)	0.165 (0.135 to 0.187)
α (h^{-1})	10.0 ±6.16	V_c (L/kg)	0.0694 (0.0541 to 0.088)
B (μg/ml)	23.7 ±5.43	$V_{d(ss)}$ (L/kg)	0.104 (0.093 to 0.123)
ß (h^{-1})	1.42 ±0.326	$t_{½\alpha}$ (h)	0.0693 (0.0367 to 0.155)
C_p^0 (μg/ml)	47.7 ±8.92	$t_{½ß}$ (h)	0.489 (0.397 to 0.665)
k_{el} (h^{-1})	2.42 ±0.614	AUC ($\mu g \cdot ml^{-1} \cdot h$)	19.8 (17.7 to 24.5)
k_{12} (h^{-1})	3.15 ±2.49	AUMC ($\mu g \cdot ml^{-1} \cdot h^2$)	12.1 (9.67 to 19.0)
k_{21} (h^{-1})	5.84 ±3.50	MRT (h)	0.622 (0.526 to 0.842)

In calves challenged with heat-stable *Escherichia coli* enterotoxin, 6 mg ketoprofen per kg of body weight reduced fecal output and 3 mg ketoprofen per kg body weight had no effect on fecal output.[4] Two grams of intramuscular ketoprofen, administered once daily for up to five treatments, improved recovery rates in dairy cattle with acute clinical mastitis.[2] Two grams of ketoprofen administered to an average size Holstein is a dose similar to that used in this study. Ketoprofen appears to have considerable potential as a therapy for endotoxin-induced disease in dairy cattle. Additional studies to determine optimum dosing rates and studies comparing various NSAIDs are needed.

References

1. Sigurdsson GH, Youssef H. Amelioration of respiratory and circulatory changes in established endotoxic shock by ketoprofen. *Acta Anaesthesiologica Scandinavica* 38 (1):33-39. 2. Shpigel NY, Chen R, Winkler M, *et al.* Anti-inflammatory ketoprofen in the treatment of field cases of bovine mastitis. *Research in Veterinary Science* 56 (1):62-68. 3. Semrad SD. Comparative efficacy of flunixin, ketoprofen, and ketorolac for treating endotoxemic neonatal calves. *AJVR* 1993;54 9:1511-1516. 4. Roussel AJ, Dodson SL, Brumbaugh GW, *et al.* Effect of ketoprofen on *Escherichia coli* heat-stable enterotoxin-induced diarrhea of calves. *AJVR* 1993;54 (12):2088-2090. 5. Ketoprofen, 100mg/ml sterile solution, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa. 6. Model 6000A pump, model 717 Autosampler, 486 Tunable UV detector, Waters Assoc, Milford, Mass; 7. Wright MR, Sattari S, Brocks DR, Jamali F. Improved high-performance liquid chromatographic assay method for the enantiomers of ibuprofen. *J Chromatog* 1992;583:259-265. 8. Reverse phase ODS (3), Phenomenex, Torrance, CA 9. Sigma Chemical Co., St. Louis, MO. 10. Millenium 2010 Chromatography Manager, Waters Assoc, Milford, Mass. 11. Rstrip, Micromath Scientific Software, Salt Lake, Utah. 12. Riviere JE. Veterinary clinical pharmacokinetics. Part II. Modeling. *Comp Cont Ed* 1988;10:122-133.