



Pharmacokinetics of pentoxifylline and its 5-hydroxyhexyl metabolite following intravenous administration in cattle

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Abstract

This study investigated the pharmacokinetics of pentoxifylline (PTX) and its 5-hydroxyhexyl metabolite (M-I) after single-dose intravenous (IV) administration (10 mg/kg) of PTX in six healthy cattle. The safety of PTX was evaluated by clinical observation and biochemical analysis. Plasma concentrations of PTX and M-I were simultaneously determined by reverse-phase high performance liquid chromatography. Pharmacokinetic parameters were calculated using non-compartmental methods. Salivation and discomfort were observed for 2 h following the drug administration. Serum direct bilirubin, total bilirubin, and phosphorus levels at 24 h following the drug administration were significantly different from the control values (0 h) ($P < 0.05$). Pharmacokinetic variables of PTX were characterized by a short terminal elimination half-life (1.05 ± 0.19 h), a large volume of distribution (6.30 ± 1.76 L/kg), and high total body clearance (5.31 ± 1.27 L/h/kg). The mean ratio between the area under the concentration-time curves of M-I and PTX was 1.34. These results indicate that single-dose administration of PTX at 10 mg/kg IV in cattle resulted in therapeutic concentrations similar to those observed in humans and horse. However, further studies are necessary to determine the safety and pharmacokinetics following repeated administrations of PTX.

Keywords Pentoxifylline · M-I metabolite · Pharmacokinetics · Intravenous · Cattle

Introduction

Pentoxifylline (PTX), 1-(5-oxohexyl)-3,7-dimethylxanthine, is a non-selective phosphodiesterase inhibitor synthesized from theobromine. PTX is commonly used for hemorheological conditions in humans such as peripheral vascular disease and microcirculation. Hemorheological effects of PTX originate from increased flexibility of erythrocytes, decreased blood viscosity by reducing thromboxane and fibrinogen concentration and increasing fibronolytic activity,

and inhibition of platelet adhesion (Aviado and Porter 1984; Harris et al. 2010). PTX increases the level of intracellular cyclic adenosine monophosphate (cAMP) via inhibition of phosphodiesterase, calcium-dependent phosphoprotein phosphatase and transglutaminase, and magnesium-dependent activation of protein kinase (Aviado and Porter 1984; Bessler et al. 1986). This effect decreases the release of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, and IL-8 (Cakmak et al. 2012). PTX was first used in humans for the treatment of intermittent claudication and then vasculitis, endotoxemia, sepsis, diabetic ulcers, seizure disorders, cancer, and collagen disorders due to its hemorheological and anti-inflammatory properties (Samlaska and Winfield 1994).

PTX is highly metabolized in humans and animals and is converted into its many metabolites. In humans, PTX is metabolized into at least seven metabolites, including active 5-hydroxyhexyl metabolite (M-I, lisofylline) and M-V, which have hemorheological effect (Hinze 1972; Ambrus et al. 1995). However, the inhibitory effect of M-I on platelet aggregation and TNF- α is more potent than that of M-V (Harris et al. 2010). The M-I is reported to be effective in diseases such as sepsis, cancer, and type 1 diabetes (Yang et al. 2005).

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PTX is also used in veterinary medicine as an extra-label drug. PTX is recommended for vasculitis, atopy, contact allergy, dermatomyositis, and systemic lupus erythematosus in dogs (Sykes and Papich 2014) and for cutaneous vasculitis, laminitis, endometritis-placentitis, and foal septicemia in horses (Liska et al. 2006). It may also have potential therapeutic use as a hemorheological agent for some diseases (e.g., vasculitis, laminitis, endometritis-placentitis, and septicemia) in cattle. The pharmacokinetics of PTX has been determined in horse (Crisman et al. 1993; Liska et al. 2006), dog (Marsella et al. 2000), broiler chicken (De Boever et al. 2005), rabbit (Adcock et al. 2007), rat (Italiya et al. 2017), and mice (Honess et al. 1993; Wyska 2010). However, there is no information about PTX pharmacokinetics in cattle. The purpose of the present study was to determine the pharmacokinetics of PTX and M-I in cattle following intravenous administration of PTX at a dose of 10 mg/kg and determine its safety through clinical observation and biochemical analysis.

Materials and methods

Chemicals

PTX ($\geq 99\%$) and M-I ($\geq 99\%$) analytic standard was obtained from Sigma–Aldrich (St. Louis, Mo., USA). Methanol, sodium acetate buffer, and acetic acid (Merck, Darmstadt, Germany) were used at a purity that was suitable for high-performance liquid chromatography (HPLC). For drug administration to the animals, an aqueous solution of PTX was prepared by dissolving the standard powder with 100 mL of sterile water to prepare the infusion solution of PTX for each cattle at the dose of 10 mg/kg.

Animals

This study used six 28 ± 4 -month-old Holstein cattle weighing 370 ± 50 kg body weight, which had not received any drug administration in the past 2 months. Drug administration was conducted in a special farm (Nigde/Turkey). Animals that had no disease history and were determined to be healthy after examination were included in the study. These animals were kept in a section separate from the other animals at the farm, and individual ear tag numbers were used for differentiating these animals. Before the study, an acclimation period of 1 week was applied for the animals. Cattle were fed with commercially available feed two times a day (07:00 in the morning, 19:00 in the evening) in accordance with their weight and age. In addition, alfalfa hay and water was provided ad libitum. Animals were kept at restraint stations during the 12-h study period. All study protocols were approved by the Ethics Committee of the Faculty of Veterinary Medicine (University of Selcuk, Konya, Turkey).

Drug administration and sample collection

Before administration, catheters were placed into the right and left jugular veins for drug administration and blood sample collection. Animals received PTX at a dose of 10 mg/kg by a slow intravenous (IV) injection over 5-min period using a syringe infusion pump set to 20 mL/min. Blood samples for PTX and M-I analyses (3 mL) were collected into heparinized tubes before drug administration (0 h) and at 0.05, 0.1, 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 h following drug administration. Blood samples for biochemical analyses (3 mL) were collected into gel-containing tubes at 0 h (control) and at 6 and 24 h after PTX administration. Collected blood samples were centrifuged at $4000 \times g$ for 10 min. Plasma and serum samples were stored at -70°C until the time of analysis.

Biochemical analyses

Serum albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (CHOL), creatine kinase (CK), creatinine (CREA), direct bilirubin (DBIL), gamma glutamyltransferase (GGT), glucose (GLU), lactate dehydrogenase (LDH), total bilirubin (TBIL), total protein (TP), urea, calcium (Ca), chlorine (Cl), potassium (K), sodium (Na), and phosphorus (P) levels were measured in an automatic analyzer using commercially available kits (Ilab300, Biomerieux, Italy).

Analytical method

Plasma concentrations of PTX and M-I were determined in a HPLC (Shimadzu, Tokyo, Japan) system according to the method introduced by Marsella et al. (2000) with modifications. The HPLC system consisted of a pump (LC-20 AT with CBM-20A system controller), autosampler (SIL 20 A), degasser (DGU-14A), column oven (CTO 10A), and a SPD-10A UV–VIS detector. The HP PC-controlled LC solution software program (Shimadzu, Japan) was used for data analysis. A total of 300 μL methanol was added to 200 μL plasma and vortexed for 30 s. Then, the mixture was centrifuged at $10000 \times g$ for 10 min. The resulting clean supernatant was transferred to autosampler vials, and 10 μL was injected into the HPLC-UV system. PTX and M-I separation was performed using Gemini™ C18 column (250×4.6 mm; internal diameter, 5 μm ; Phenomenex, Torrance, CA). The column temperature was maintained at 40°C , and the autosampler temperature was maintained at room temperature. The wavelength was set to 275 nm. The mobile phase containing methanol and 0.025 M sodium acetate buffer (40:60 v/v) was transferred to the HPLC with a flow rate of 1 mL/min using a pump.

Next, PTX and M-I stock solutions at the concentration of 1 mg/mL were prepared with deionized water. Calibration standards of 0.04–40 $\mu\text{g/mL}$ were prepared by adding PTX

and M-I stock solutions into blank cattle plasma. Low (0.4 µg/mL), moderate (4 µg/mL), and high (40 µg/mL) concentrations of PTX and M-I quality control samples were used to determine recovery, accuracy, and precision values of the HPLC method. Recovery was calculated by comparing the peak areas of the substance, which would be quantified on plasma samples that were evaluated like any sample, with the peak areas of the standards. Recovery for PTX and M-I was 96–103%. The lowest standard solutions of PTX and M-I (0.01–0.1 µg/mL) were loaded into blank plasma samples to determine the limit of detection (LOD) and limit of quantitation (LOQ). On the chromatogram, a concentration with S/G ratio of 3 was determined as the LOD and a concentration with S/G ratio of 6 was determined as the LOQ. The LOD and LOQ values for PTX and M-I were determined as 0.02 µg/mL and 0.04 µg/mL, respectively. Intra-assay and inter-assay repeatabilities were used as a measure of precision. Six repeated analyses were performed for each level of quality control samples at low (0.4 µg/mL), moderate (4 µg/mL), and high (40 µg/mL) concentrations for the detection of intra-assay and inter-assay differences. These levels were repeated in 6 different days. The concentration was quantified in all samples, and the percentage coefficients of variation (CV) were calculated using the concentration in enriched plasma samples. The intra-assay and inter-assay CV for PTX were ≤ 4.90% and ≤ 6.35%, whereas those for M-I were ≤ 3.63% and ≤ 4.02%, respectively.

Pharmacokinetic calculations

For each animal, the concentration-time curves of PTX and M-I were plotted using WinNonlin 6.1.0.173 (Pharsight Corporation, Scientific Consulting Inc., NC, USA) software. Pharmacokinetic variables were quantified using a non-compartment model analysis. In the study, the following pharmacokinetic parameters were determined: terminal elimination half-life ($t_{1/2z}$), mean residence time (MRT), area under the concentration-versus time curve (AUC), volume of distribution at steady state (V_{dss}), and total clearance (Cl_T). The area under the curve was calculated using a linear/log method. $t_{1/2z}$ and $MRT_{0-\infty}$ variables were determined using the following equalities:

$$t_{1/2z} = \frac{0.693}{\lambda}$$

λ is the elimination rate constant.

$$MRT = \frac{AUMC}{AUC}$$

AUMC is the area under the first moment curve; AUC is the area under the plasma concentration-time curve.

For PTX and M-I, peak plasma concentration (C_{max}), and time to reach C_{max} (T_{max}) were quantified by direct observation of the plasma concentration-time curve of each animal. The C_{max} of PTX was the highest concentration observed at the end of the infusion.

Statistical analyses

All values were presented as mean ± SD. The harmonic mean was calculated for $t_{1/2z}$ and MRT values. Biochemical parameters were analyzed using a paired *t* test. SPSS 22.0 software (IBM Corp, Armonk, NY) was used for statistical analysis. $P < 0.05$ was accepted as the limit of significance.

Results

Safety

The effect of PTX administration at a dose of 10 mg/kg IV in cattle on biochemical parameters are presented in Table 1. Following PTX administration in cattle, DBIL, TBIL, and P levels were significantly higher at 24 h in comparison to the control values (0 h) ($P < 0.05$). No significant difference was found in other biochemical parameters ($P > 0.05$). Food and water intake of the animals was normal throughout the study. Except for clinical symptoms, such as salivation and discomfort, which lasted approximately 2 h, no adverse drug reactions were observed in the animals.

Pharmacokinetic parameters

Semilogarithmic plasma concentration-time curves of PTX and M-I following 10 mg/kg IV PTX administration in cattle are presented in Fig. 1, and plasma concentrations at sampling times are presented in Table 2. After IV administration of PTX at 10 mg/kg in cattle, PTX and M-I were detected at up to 6 h following administration.

Pharmacokinetic parameters of PTX and M-I are presented in Table 3. $t_{1/2z}$, $MRT_{0-\infty}$, $AUC_{0-\infty}$, V_{dss} , and Cl_T values of PTX were 1.05 h, 1.19 h, 1.97 h*µg/mL, 6.30 L/kg, and 5.31 L/h/kg, respectively. $t_{1/2z}$, $MRT_{0-\infty}$, $AUC_{0-\infty}$, C_{max} , and T_{max} values of M-I were 1.51 h, 1.54 h, 2.58 h*µg/mL, 30.01 µg/mL, and 0.10 h, respectively. $AUC_{0-\infty(M-I)}/AUC_{0-\infty(PTX)}$ ratio was 1.34.

Discussion

The efficacy of PTX is dose-dependent, and 10 mg/kg of dose is recommended in horses for hemorheological activity (Liska et al. 2006). In the present study, PTX was administered to cattle at recommended dose for hemorheological effects in microcirculation diseases.

Table 1 Biochemistry parameters of cattle ($n = 6$) administrated intravenously pentoxifylline at a dosage of 10 mg/kg

Parameter	0 h	6 h	24 h
ALB (g/dL)	2.57 ± 0.22	2.70 ± 0.33	2.67 ± 0.18
ALP (U/L)	124.00 ± 28.48	129.83 ± 18.77	124.33 ± 25.39
ALT (U/L)	27.50 ± 5.28	28.83 ± 4.02	27.33 ± 2.66
AST (U/L)	86.00 ± 12.52	91.83 ± 15.48	81.67 ± 9.11
CHOL (mg/dL)	84.00 ± 11.63	89.17 ± 5.85	89.83 ± 7.52
CK (U/L)	368.50 ± 31.26	359.33 ± 23.75	372.00 ± 41.24
CREAT (mg/dL)	0.70 ± 0.04	0.70 ± 0.07	0.69 ± 0.05
DBIL (mg/dL)	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.07*
GGT (U/L)	18.17 ± 5.56	19.50 ± 4.93	19.50 ± 6.02
GLU (mg/dL)	70.83 ± 7.63	76.50 ± 6.89	72.67 ± 6.59
LDH (U/L)	1490.84 ± 158.33	1605.38 ± 147.99	1478.57 ± 29.08
TBIL (mg/dL)	0.07 ± 0.06	0.08 ± 0.06	0.20 ± 0.05*
TPROT (g/dL)	7.77 ± 0.98	8.23 ± 0.77	8.10 ± 0.68
URE (mg/dL)	26.68 ± 5.03	27.50 ± 6.19	30.60 ± 4.50
Ca (mg/dL)	9.63 ± 0.69	9.85 ± 0.80	9.68 ± 0.31
Cl (mEq/L)	97.00 ± 7.13	95.83 ± 4.79	96.67 ± 3.93
K (mEq/L)	3.98 ± 0.48	3.72 ± 0.48	3.95 ± 0.16
Na (mEq/L)	131.62 ± 6.66	127.81 ± 7.69	130.10 ± 4.43
P (mg/dL)	5.20 ± 0.71	5.84 ± 0.59	6.51 ± 0.38*

ALB albumin, ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, CHOL cholesterol, CK creatine kinase, CREAT creatinine, DBIL direct bilirubin, GGT gamma glutamyltransferase, GLU glucose, LDH lactate dehydrogenase, TBIL total bilirubin, TPROT total protein, Ca calcium, Cl chlorine, K potassium, Na sodium, P phosphorus

* Significantly different from data at 0 h ($P < 0.05$)

Except for approximately 2-h long transient salivation and discomfort symptoms after PTX administration, no local or systemic adverse effect was observed in the animals. Lethargy, eye closing, and deep respiration were reported in chickens following PTX administration (100 mg/kg, IV and oral) (De Boever et al. 2005), and transient side effects, such as sweating, increased heart rate, and fasciculations in muscles, were reported in horses (8.5 mg/kg, IV) (Liska et al. 2006). In our study, PTX caused the increase in DBIL,

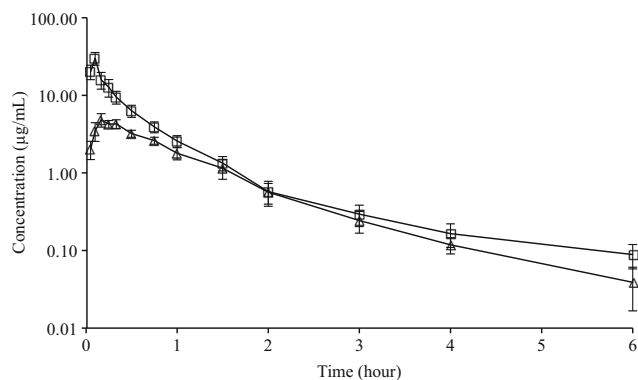


Fig. 1 Semilogarithmic plots of the plasma concentrations to time data of pentoxifylline (triangles) and its 5-hydroxyhexyl metabolite (squares) in cattle ($n = 6$) after intravenous administration of pentoxifylline at a dosage of 10 mg/kg (mean ± standard deviation)

TBIL, and phosphorus levels in cattle. However, this increase was within the reference range reported for cattle (Radostitis et al. 2007). PTX caused no significant change in serum biochemical parameters of horses (8.5 mg/kg, IV) and dogs (8–30 mg/kg, IV and oral) (Rees et al. 2003; Liska et al. 2006).

The $t_{1/2z}$ of PTX in cattle was 1.05 h, which is similar to that reported in chicken (1.05 h, De Boever et al. 2005), shorter than that reported in dog (2.73 h, Rees et al. 2003), and longer than that reported in horse (0.38 h, Liska et al. 2006), rat (0.54 h, Italiya et al. 2017), and humans (0.84 h, Smith et al. 1986). We found that the Cl_T of PTX was 5.31 L/h/kg, which is higher than that reported in chicken (1.9 L/h/kg, De Boever et al. 2005), horse (2.38–3.06 L/h/kg, Crisman et al. 1993; Liska et al. 2006), rat (1.5 L/h/kg, Italiya et al. 2017), and dog (0.03–0.04 L/h/kg, Marsella et al. 2000; Rees et al. 2003). The V_{dss} of PTX was 6.30 L/kg, which is higher than that reported in horse (1.15–2.81 L/kg, Crisman et al. 1993; Liska et al. 2006), dog (1.02–4.1 L/kg, Marsella et al. 2000; Rees et al. 2003), and rat (1.1 L/kg, Italiya et al. 2017). The $t_{1/2z}$ (1.51 h) value of M-I after PTX administration in cattle was longer than that reported in chicken (0.78 h, De Boever et al. 2005) and rat (0.66 h, Italiya et al. 2017). The $t_{1/2z}$ of M-1 in dogs was 1.05–4.78 h (Marsella et al. 2000; Rees et al. 2003). PTX binds to plasma proteins with a rate of 70% in human, and erythrocytes are an important binding

Table 2 Plasma concentrations at the sampling times of pentoxifylline and its 5-hydroxyhexyl metabolite (M-I) following intravenous administration of PTX at a dose of 10 mg/kg in cattle (mean \pm SD)

Time (h)	Pentoxifylline ($\mu\text{g/mL}$)	5-hydroxyhexyl metabolite (M-I) ($\mu\text{g/mL}$)
0.05	2.02 \pm 0.53	20.22 \pm 4.32
0.1	3.61 \pm 1.23	30.01 \pm 5.58
0.17	4.72 \pm 0.80	15.85 \pm 3.85
0.25	4.26 \pm 0.47	12.73 \pm 3.25
0.33	4.28 \pm 0.56	9.48 \pm 1.76
0.5	3.21 \pm 0.33	6.32 \pm 1.12
0.75	2.61 \pm 0.27	3.91 \pm 0.62
1	1.79 \pm 0.32	2.58 \pm 0.44
1.5	1.15 \pm 0.32	1.34 \pm 0.28
2	0.56 \pm 0.17	0.58 \pm 0.20
3	0.24 \pm 0.08	0.29 \pm 0.09
4	0.12 \pm 0.03	0.17 \pm 0.06
6	0.04 \pm 0.02	0.09 \pm 0.03

target (point) for both PTX and M-I (Harris et al. 2010). Unlike monogastric animals, in ruminants, the reticulo-rumen, which constitutes approximately 20% of the body weight, can change the pharmacokinetics of drugs. The fluid in the reticulo-rumen, which has the great volume and acidic properties (pH 5.5–6.5), may cause to accumulate weakly basic drugs in the reticulo-rumen by the ion-trapping phenomenon and thus change the distribution of the drug (Landoni et al. 1996; Toutain et al. 2010; Jerzsele 2012). The large V_{dss} of PTX in cattle may be associated with the different binding rate of PTX to plasma proteins and erythrocytes, and diffusion into reticulo-rumen by ion-trapping phenomenon. The transformation of PTX into M-I occurs in the liver and erythrocytes via a reduction reaction, whereas its transformation into other metabolites occurs only in the liver via oxidation reactions (Aviado and Porter 1984). PTX binds to erythrocytes with the approximate rate of 45%, and transforms into fast and reversible M-I (Paap et al. 1996). After intravenous administration of PTX, its clearance is higher compared with that in

hepatic circulation, suggesting that erythrocytes are a major site for PTX–M-I interconversion (Nicklasson et al. 2002). As almost all PTX and M-I transform into other metabolites, their urinary elimination are less than 1% (Beermann et al. 1985). While the pharmacokinetics of PTX and M-I do not change in case of renal damage (Paap et al. 1996), the Cl_T value in hepatic damage significantly decreases (Rames et al. 1990). Furthermore, cimetidine inhibited cytochrome (CYP) enzymes and reduced Cl_T of PTX by 37% (Luke et al. 1986). Therefore, hepatic blood flow and metabolic activity are important for the elimination of PTX and M-I. The CYP1A2 enzyme plays an important role in the transformation of PTX and M-I into other metabolites (Peterson et al. 2004). CYP1A2 enzyme activity was reported to be higher in cattle than in dogs and rats (Sivapathasundaram et al. 2001; Szotáková et al. 2004). The higher Cl_T value of PTX in cattle may be caused by different hepatic enzyme activities in cattle in comparison to other species. V_{dss} and Cl_T are the most important parameters that affect $t_{1/2z}$. In our study, the $t_{1/2z}$

Table 3 Pharmacokinetic parameters of pentoxifylline and its 5-hydroxyhexyl metabolite (M-I) in cattle ($n = 6$) after intravenous administration of pentoxifylline at a dosage of 10 mg/kg (mean \pm SD)

Parameter	Pentoxifylline	5-hydroxyhexyl metabolite (M-I)
$t_{1/2z}$ (h) (HM)	1.05 \pm 0.19	1.51 \pm 0.21
AUC_{0-6} ($\text{h} \cdot \mu\text{g/mL}$)	1.91 \pm 0.41	2.38 \pm 0.45
$\text{AUC}_{0-\infty}$ ($\text{h} \cdot \mu\text{g/mL}$)	1.97 \pm 0.43	2.58 \pm 0.50
MRT (h) (HM)	1.19 \pm 0.23	1.54 \pm 0.29
Cl_T (L/h/kg)	5.31 \pm 1.27	–
V_{dss} (L/kg)	6.30 \pm 1.76	–
T_{max} (h)	0.17 \pm 0.00	0.1 \pm 0.00
C_{max} ($\mu\text{g/mL}$)	4.72 \pm 0.81	30.01 \pm 5.58
$\text{AUC}_{0-\infty} (\text{M-I}) / \text{AUC}_{0-\infty} (\text{PTX})$	–	1.34 \pm 0.25

$t_{1/2z}$ terminal elimination half-life; $\text{AUC}_{(0-6)}$ area under the plasma concentration-time curve from zero (0) hours to time (t); $\text{AUC}_{(0-\infty)}$ area under the plasma concentration-time curve from zero (0) h to infinity (∞); MRT mean residence time; Cl_T systemic clearance; V_{dss} volume of distribution at steady state; T_{max} time to reach maximum concentration; C_{max} maximal plasma concentration; HM harmonic mean

was not much longer than that in other species, which have high Cl_T despite large V_{dss} .

In cattle, $AUC_{0-\infty (M-I)}/AUC_{0-\infty (PTX)}$ ratio was 1.34. This ratio was lower than that in chicken (1.52, De Boever et al. 2005) and horse (2.40, Liska et al. 2006) and higher than that in dog (0.63, Rees et al. 2003) and horses (1.13, Crisman et al. 1993). PTX metabolism varies between species and even intra-species. PTX has at fewest seven metabolites in humans, six metabolites in horses (Kwong et al. 1989), and three metabolites in dogs (Marsella et al. 2000). In humans, the major metabolites of PTX are M-I and M-V (Nicklasson et al. 2002), whereas, in dogs, M-I is not a major metabolite and M-V was not detected (Marsella et al. 2000). However, in another dog study, M-V was detected as the major metabolite of PTX (Rees et al. 2003). Aldo-keto reductase and CYP2E1 enzymes play an important role in the transformation of PTX into M-I (Raoul et al. 2007; Malátková and Wsól 2014). Furthermore, CYP1A2 is important for the transformation of PTX and M-I into other metabolites. Aldo-keto reductase and CYP enzyme activities are reported to significantly vary between species (Szotáková et al. 2004). The difference of $AUC_{0-\infty (M-I)}/AUC_{0-\infty (PTX)}$ ratio among species may be caused by distinct enzyme activities in different animal species.

The clinical efficacy of PTX is dose-dependent. The therapeutic concentration of PTX in humans is 0.5–2 µg/mL for different clinical conditions (Regenthal et al. 1999) and 1–10 µg/mL in horses for TNF-α inhibition (Barton and Moore 1994). In horses, PTX is reported to inhibit TNF-α at approximately > 1 µg/mL level (Barton et al. 1997). We found that the plasma concentrations of PTX at 1, 1.5, and 2 h were 2, 1, and 0.5 µg/mL, respectively. The C_{max} of PTX was approximately 4.72 µg/mL. In our study, the plasma concentration of PTX reached the therapeutic concentration suggested for humans and horses. However, as PTX is recommended for long-term clinical use in humans and horses, multiple dose administrations are required.

Conclusion

PTX was well tolerated by cattle except for transient salivation and discomfort. In cattle, PTX exhibited the short elimination half-life and the large volume of distribution. The transformation of PTX into the M-I was similar with other species. These results indicate that single-dose administration of PTX at 10 mg/kg IV in cattle resulted in therapeutic concentrations similar to those observed in humans. However, further studies are necessary to determine the safety and pharmacokinetics following repeated administrations of PTX.

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Compliance with ethical standards

The animal experiment was conducted in accordance with the procedure of Committee on Animal Use Ethics (Approval No. 2015/96).

Conflict of interest The authors declare that they have no conflict of interest.

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